PARASITE INDUCED BEHAVIOURAL CHANGES IN FISH

by

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Chapter 1

INTRODUCTION

Digenetic larval trematodes occurring in the eyes and brain of freshwater fish belong to the genus *Diplostomum* von Nordmann, 1832 and are generally referred to as diplostomatid flukes. These diplostomatid metacercariae have been reported from many parts of the world and cause pathological effects in fish such as cataracts, blindness and even death (Shariff *et al.* 1980; Dörücü *et al.* 2002; Seppälä *et al.* 2004; Dezfuli *et al.* 2007; Voutilainen *et al.* 2008). The majority of diplostomiasis reports originate from large-scale aquaculture industries and therefore not a lot of research has been conducted in Africa. The studies on African fish parasites in general are very scanty and in some countries the information is non-existent (Khalil and Polling 1997).

In order to cut the economic losses associated with eye diplostomiasis, a lot of scientific attention has been given to infections occurring in Europe and America (Niewiadomska 1996). The absence of obvious clinical signs such as opaque spots within the lens has resulted in less research being conducted on diplostomatids parasitising the brain (Hoffman and Hoyme 1958; Etges 1961). Although *Diplostomum* infections are common, information on the histopathological effects associated with infections and the immune response in host tissues are still extremely limited (Dezfuli *et al.* 2007). Diplostomatid trematodes have a complex life cycle which involves a snail, a fish and a piscivorous bird (Kennedy and Burrough 1977; Locke *et al.* 2010b). Fish act as the second intermediate host and infected individuals need to be eaten by the piscivorous bird host in order for the life cycle to be completed (Seppälä 2005). Many authors have reported that diplostomatids are responsible for changing the behaviour of the fish host in order to enhance trophic transmission and the parasite's own reproductive fitness (Hoffman 1960; Rothschild 1962; Larson 1965; Sweeting 1974; Seppälä *et al.* 2004; Seppälä *et al.* 2005a, b, 2006a, b).

From early in the 20th century numerous authors have indulged in the idea of parasites manipulating the behaviour of a wide variety of hosts. Many of these examples have been reviewed and summarised by Moore (2002) and Thomas *et al.* (2005). Although many of the described changes in behaviour may be the mere result of the pathology associated with infection, some do facilitate parasite transmission. A well known example is that of ants which are infected with *Dicrocoelium dendriticum* (Rudolphi, 1819), a liver fluke of ruminants. Infected ants show abnormal behaviour which

secures successful ingestion of the parasite by the definitive hosts (Jog and Watve 2005; Poulin *et al.* 2005). When the ambient temperature decreases during the evenings and mornings, infected ants migrate and lock their mandibles on the tips of grass, whilst during midday their behaviour is similar to the uninfected ants. Their presence near the top of vegetation during these specific hours facilitates their exposure to the grazing of the definitive hosts and hence enhances the transmission success of the parasite.

The present study aimed to determine if diplostomatid infection results in an increase in the activities of infected fish to a time of day during which piscivorous birds are also active. Fish infected with diplostomatids were sampled from two diverse river basins. The Okavango Panhandle (Botswana) offers a pristine habitat with high ecological integrity and biological diversity. Contrasting to this, the Modder River, situated within the Orange-Vaal River System (South Africa), has been over exploited and much of its natural flow has been limited. This has lead to the destruction of ecosystem functioning and biodiversity. Collected fish were studied and dissected in order to determine:

- The metacercarial types of diplostomatid infections occurring in the eyes and brains of fish in the Okavango River and Modder River catchment areas
- The prevalence and intensity of the infection within different fish species
- The diversity of possible diplostomatid life cycles occurring in these systems, and
- The effect that diplostomatid infection could have on the pathology and behaviour of these fishes

Two sets of behavioural experiments were conducted to determine behavioural changes of infected and uninfected fish exposed to 1) model aerial predators and 2) different light flashes. The null hypothesis (H_0) states that diplostomatid infected fish, from natural populations, do not show dissimilar behaviour from their uninfected conspecifics. The alternative hypothesis (H_1) states that diplostomatid infection, within a natural population, results in changes in behaviour. These behavioural changes could make the hosts more susceptible to predation.

This study forms part of the Okavango Fish Parasite Project which is the novel work of scientists of the Aquatic Parasitology Research Group from the Department Zoology

and Entomology, University of the Free State. For the past 12 years, research has been conducted on the biodiversity, phylogeny, life cycle and parasite host interactions of fish parasites in the Okavango Panhandle and Delta, Botswana. A large variety of topics have been researched such as: the phylogeny, taxonomy and life cycles of myxosporeans, trichodinids, trypanosomes, monogeneans, nematodes and copepods. The role of fish parasites in the decline of the fish populations as well as the water quality and conservation condition of the Okavango Delta have also been studied and reported on. To date nine Master students and three Doctorate students have completed their studies and have presented their work in many publications, conferences and workshops including Erasmus *et al.* (2010) and Grobbelaar *et al.* (in press).

The other discipline which formed part of the present study was the Animal Ethology Research Group. This Research Group specialises in the behaviour and ecology of African vertebrates and has also contributed to numerous scientific outputs. The present study is the first of its kind to act as collaboration between the two disciplines. This combination of Aquatic Parasitology and African Ethology offers a fresh look on parasitism and its effects regarding ecology and animal behaviour.

On completion of this short introduction (Chapter 1) this dissertation will provide a description of each of the two study sites (Chapter 2). This will be followed by the materials and methods used during the present study (Chapter 3). The taxonomy of diplostomatids as well as the confusion and variability present in identification of these parasites will be discussed in Chapter 4. Chapter 5 consists of a description of the anatomical structures and morphometrics for each of the seven diplostomatid metacercarial types sampled from the eyes and brain of fish during the present study. The general life cycle of these diplostomatids are discussed with special reference to the ecology and ecomorphological classification of fish hosts (Chapter 6). This chapter also includes the findings of the present study regarding the prevalence and intensity of infection for fish species belonging to families Mormyridae, Cyprinidae, Characidae, Hepsetidae, Schilbeidae and Cichlidae.

Chapter 7 provides the results and discussion for the pathology observed in the brain and eyes of infected fish. These results are also compared to similar previous studies.

A brief overview of parasite induced changes in host behaviour, with special reference to diplostomiasis in natural and captive fish populations, is provided in Chapter 8. The results found in the present study for the two sets of behavioural experiments conducted on infected and uninfected fish are also discussed. This chapter ends with the concluding remarks regarding parasite induced behaviour in fish. This is followed by the references (Chapter 9), acknowledgements, abstracts and appendices.

Chapter 2

STUDY SITES

LESEDING RESEARCH CAMP

Situated on the western side of the Upper Panhandle, close to the town of Shakawe, is Leseding Research Camp (Figure 2.2 B), the brainchild of the Aquatic Parasitology Research Group (University of the Free State). The Okavango Delta Management Plan (ODMP) recognises this group as specialists on fish ecology, parasites and the livelihood of people in the Panhandle (Varis *et al.* 2008). The campsite is located on the premises of the Krokavango Crocodile Farm next to Samochima Lagoon which is on the outskirts of Samochima Village in the most northern parts of Botswana. Apart from the well-equipped laboratory, Leseding Camp also boasts with aquariums, tented accommodation, ablutions, a kitchen and "braai" facilities, which made it an ideal platform from which research could be conducted.

THE OKAVANGO RIVER SYSTEM

General hydrology

The uniqueness of the Okavango River is due to a variety of features. It is the largest endorheic river system in southern Africa and it forms an inland delta (Figure 2.1 A). Geomorphologically speaking the river terminates into an alluvial fan (Figure 2.2 A), situated in the Kalahari Desert (of Botswana) and not into the ocean like most other rivers (Kgathi *et al.* 2006). The basin spans over three countries, i.e. Angola, Namibia and Botswana and in total covers an area of 192 500 km^2 and a length of over 1 000 km (Kniveton and Todd 2006).

The Delta is maintained by annual pulse flooding originating from heavy rainfall in the highlands of central Angola, which forms a catchment area of about 12 000 km^2. The many tributaries conjoin to form the Cubango and Cuito Rivers which join at the border between Angola and Namibia to form the Okavango River (Mendelsohn *et al.* 2010). The inflow of the catchment delivers a volume of water equal to 10.5 x 10^9 m^3 per year (Kgathi *et al.* 2006) and the tributaries join to form a single broad river which flows in a south-easterly direction, down a narrow waterway through the width of the Caprivi Strip (Namibia). At Mohembo border, it becomes slightly broader and flows into the northern

parts of Botswana where it is known as the Okavango Panhandle. Two faults, parallel to each other are responsible for capturing this massive flood of water and channelling it into the direction of the town of Seronga (Figure 2.1 B).

The two Gumare faults, which lie perpendicular to the first, are responsible for the river to spread out and slow down and form the beginning of the Delta (Mendelsohn *et al.* 2010). Water spills from the main river to form an alluvial fan, which consists of permanent and seasonal floodplains extending up to Maun. Two northeast-southwest aligned faults, namely the Kunyere and Thamalakane, lie parallel to each other and to the Gumare faults and are responsible for ultimately terminating the further south-east flow and extent of the Okavango Delta (Figure 2.1 B). As a result, the whole of the Delta is captured in a bowl-like depression which is surrounded by faults.

The Thamalakane River, which flows past Maun and drains into the Boteti River, defines the lower end of the Delta and therefore is regarded as the most southerly border of the basin. With heavier rainfall (in Angola), the flood is sufficient enough to flow down the Boteti River and fill the Makgadikgadi Pans (Varis *et al.* 2008). For this reason, some authors also include the Okavango System as a sub-basin of the Makgadikgadi Basin. In addition to the Makgadikgadi Pans there are a vast array of fossil drainage lines (such as the Boteti River), floodplains and lakes (Ngami) which sometimes still have active water connections with the system in times of greater rainfall in Angola. These all indicate that the present location of the Okavango Basin is only a smaller remnant of what the historic basin was. Approximately 5 million years ago, the Okavango was connected to the Orange-Vaal River and flowed in a westward direction and terminated in the Atlantic Ocean (Bailey 1998). Geological instability allegedly forced this once great river to change its flow to a more eastern direction and the Okavango then joined with the Zambezi as well as the Limpopo River Systems (Bailey 1998; Kgathi *et al.* 2006; Ramberg *et al.* 2006), whilst the Orange-Vaal River still drained to the west.

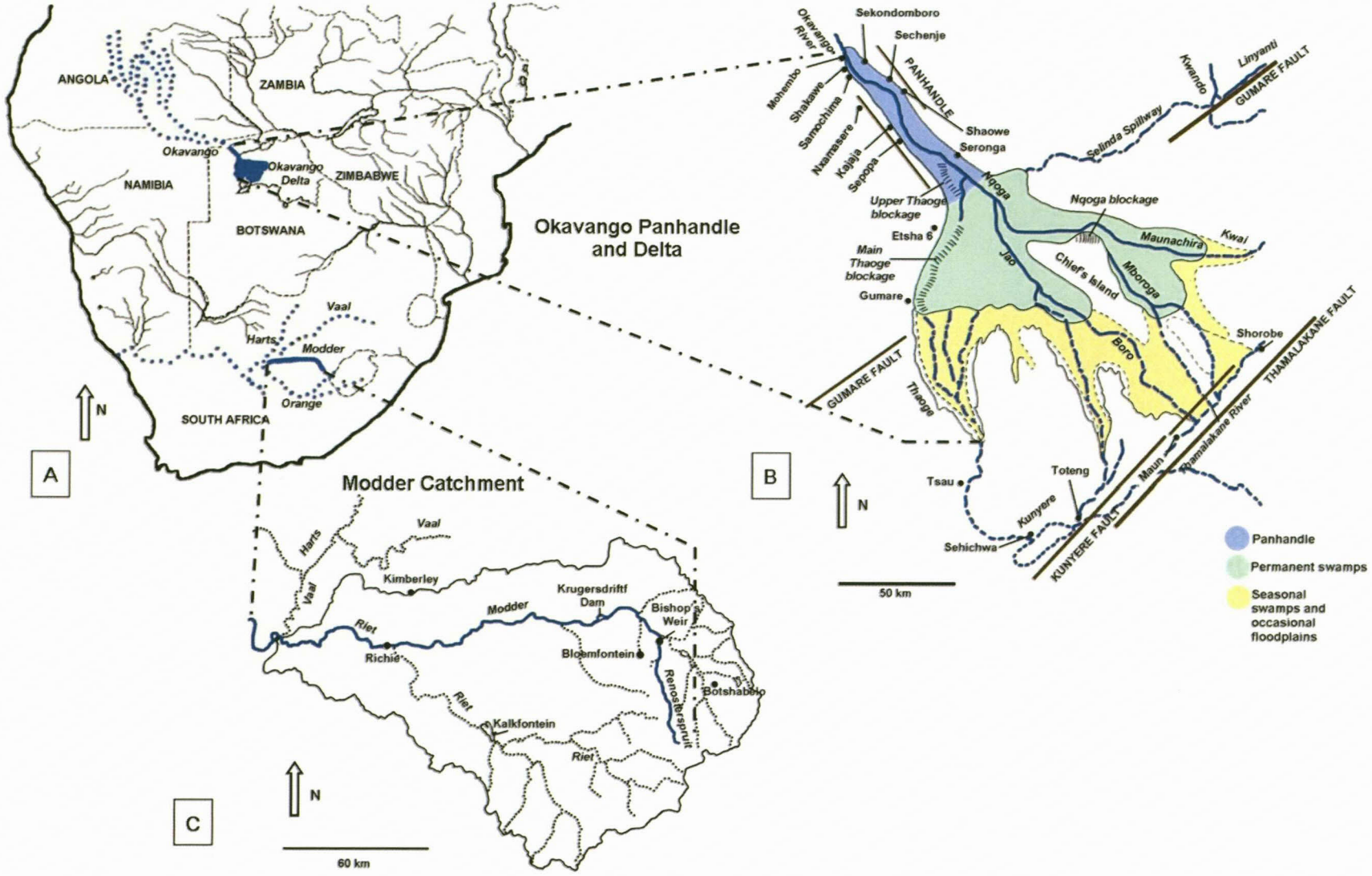


Figure 2.1: Map of (**A**) southern Africa and its major river basins, with special reference to the (**B**) Okavango River and Delta (**C**) Modder River Catchment within the Orange-Vaal Basin (redrawn from Thomas and Shaw 1991, DWAF 2003, Mendelsohn *et al.* 2010).

Rainfall and floods

The driving force that generates stream flow is the rainfall at the top of the catchment, which is about three times higher than in the Delta. According to Mendelsohn *et al.* (2010) the rainfall decreases from over 1 300 mm per year in the catchment's furthest north-west part, to less than 450 mm in the lowest south reaches. The tributaries run from areas with high elevations, of over 1 700 m above sea level and abundant water, to a semi-desert where the lowest reach is 940 m. From the top of the Panhandle to the Thamalakane River at Maun the elevation drops by only 61 m over a distance of 250 km. This extremely low gradient results in the slow pattern of flow and about 95% of the downstream water is lost to evaporation and evapotranspiration (Merron and Bruton 1995). In the southern parts of the catchment, evaporation is the highest in the winter months and gradually decreases towards the north and into the summer (Kgathi *et al.* 2006). The average temperature over the basin is approximately 20°C and it increases to the south (Mendelsohn *et al.* 2010).

The peak of the Angolan summer floods arrives in the northern riverine floodplain of Botswana at about March / April and reaches the most southern drainage rivers of the Delta during the cool, dry season of July / August (Mendelsohn *et al.* 2010). The Okavango Panhandle and Delta is therefore four to six months out of sync with the summer rain which occurred on the Angolan Plato during November / December (Ringrose *et al.* 1988; Bonyongo *et al.* 2000). Due to an increase in average rainfall, record flood levels have been experienced in the Okavango River System since 2006. A gauging station at Mohembo measures the volume of water entering the Panhandle monthly (Figure 2.3). It is hypothesised that this increase in water volume could have an influence on the prevalence and intensity of diplostomatid infection in fish occurring in the river (Chapter 6).

Habitats and vegetation

The Okavango River and Delta is part of the greater Zambezian sub-biome and two major bioregions are also distinguished for this northern part of Botswana. The bioregion which surrounds the Panhandle and Delta is referred to as the

Colophospermum mopane woodland / scrubwoodland, whilst the second refers to the azonal herbaceous swamp and aquatic vegetation (White 1983).

According to Mendelsohn *et al.* (2010) four major habitats are recognised in the Okavango River. The formation of these is based on the seasonal availability of water, which is again linked to the degree of the flood waters spilling across to low-lying parts adjacent to the main, permanent river. These four habitat types grade into one another and include: the Panhandle (riverine swamp), permanent swamps, seasonal swamps and occasional floodplains (Figures 2.2 A - H). As a result these slow-moving waters create a mosaic of lagoons, ox-bow lakes, flooded grasslands and countless islands of dry land (West 2010). Different communities of vegetation are supported by each of these habitats (Bonyongo and Mubyana 2004). Areas with more permanent water availability (Panhandle and perennial floodplains) comprise of papyrus (*Cyperus papyrus*) and reed (*Miscanthus junceus*) associations. Whilst peripheral to the wetter central core the dominant species rather includes grasses such as silver spike (*Imperata cylindrica*) and African bristlegrass (*Setaria sphacelata*) along with trees such as knobthorn (*Acacia nigrescens*) and raintree (*Lonchocarpus capassa*) (Ringrose and Matheson 2001). Around the edges of the Delta and on islands, dry woodlands as well as riverine woodlands are found (Figure 2.2 D).

During times of extensive flooding, the water of the Okavango River and Delta may also push back into fossil rivers and create extremely productive but ephemeral floodplains. An example of one such fossil floodplain is at Nxamasere, south of Shakawe. Riparian forests grow along the edges of this fossil river, whilst in the dry season the centre is covered by green grass. During the present study, the record flood levels (Figure 2.3) resulted in a massive influx of water during July / August and upon the passing of the flood, it dried up and left pools (Figure 2.2 G) with patches of papyrus and water lilies. These provide a refuge for a variety of organisms, including fish species which were collected during the present study. Tectonic activities and a record rainfall in Angola during 2006 possibly also resulted in the former desiccated Lake Ngami receiving water inflow from the Kunyere River. Along with the Nchabe River, which flows through Maun, these two rivers join at the village of Toteng (Figure 2.2 H) and pass as a single channel into Lake Ngami (West 2010).

The perennial and seasonal floodplains are the habitats of greatest value for the Okavango fish communities. The annual fish production in these habitats has been roughly estimated to be some 10 000 tonnes (Høberg *et al.* 2002). With the arrival of the annual flood, the water of the river and channels push over the flat surrounding ground to form broad floodplains. These flooded areas are rich in nutrients and together with the water result in lush vegetation and the emergence of insects and other small animals, all of which make up the diet of many fish species (Mendelsohn *et al.* 2010). The flood-created plains also act as refuge to many young fish against larger predators and consequently these habitats are vital breeding grounds for the fish stocks of the Okavango. With a decrease in the water level, at the end of the flood, the young fish leave the drying floodplains to permanently live in the main streams or permanent backwater pools and channels. Still, a number of fish do get trapped in floodplain pools and hence act as a feast for many birds, people and other predators, at the end of the flood.

Productivity

The abundance of water in the Okavango River and Delta is in direct contrast to the surrounding arid environment and hence forms refuge to a rich biodiversity (Høberg *et al.* 2002). Small topographic changes such as tectonic activity, sediment transport and channel blockages constantly influence the availability and quality of water flow and contribute to this dynamic character of the system. This dynamic shift in flooding patterns results in constant change in the patterns of plant succession and dependant animals and therefore creates a rich species diversity. It is estimated that 1 300 plant-, 33 amphibian-, 64 reptile-, 444 bird- and 122 mammal species occur in the basin (Ramberg *et al.* 2006). Ramberg *et al.* (2006) reports on 71 species of fish (Table 6.2) to be present within the Okavango Panhandle and Delta. The Delta is regarded as one of the World Wildlife Fund's top 200 eco-regions of global significance and is celebrated as one of the world's largest Ramsar sites (Kniveton and Todd 2006). When compared to other river basins of Africa it is definitely one of the least developed hydrological systems and one of the most pristine wetlands in the world (Mendelsohn and El Obeid 2004).

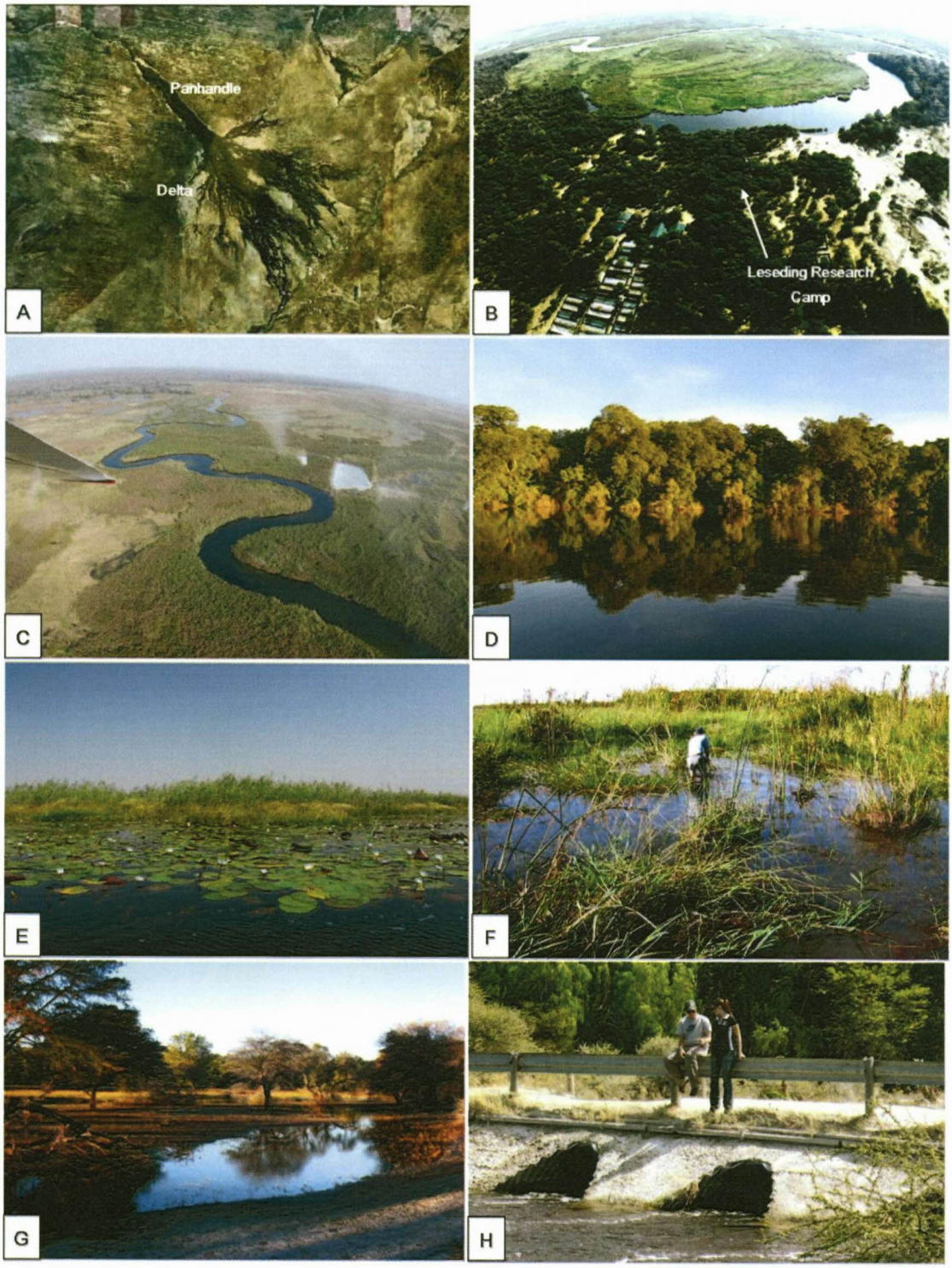


Figure 2.2: Photographs of collection sites in Okavango River and Delta. (**A**) The distinct alluvial fan and Panhandle can be seen from space (Google Earth 2009). (**B**) Leseding Research Camp is next to Samochima Lagoon in the Panhandle. The Okavango River consists of (**C**, **D**) riverine swamps, (**E**) permanent swamps, (**F**) seasonal swamps and (**G**) occasional floodplains. (**G**) Nxamasere is a fossil floodplain and together with (**H**) Toteng Bridge experienced exceptional high flood levels during 2010.

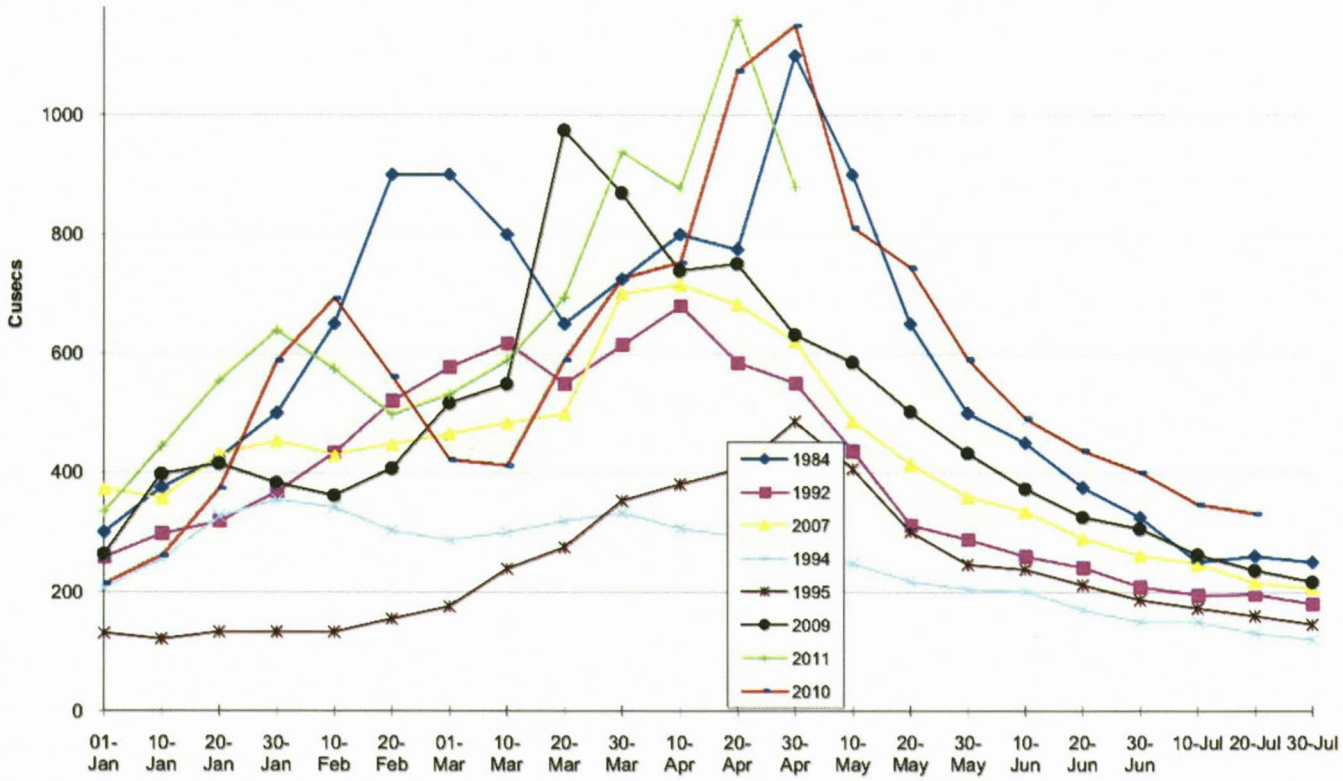


Figure 2.3: Water volume entering the Okavango Panhandle, measured on a monthly basis at a gauging station at Mohembo. Record flood levels have been experienced in the Okavango River System since 2006 (obtained from Aliboats, Maun, Botswana).

The slow-moving character of the water enables the vegetation and sediments to filter out much of the inorganic and organic particles (Mendelsohn *et al.* 2010). Very few suspended and dissolved particles are therefore carried in the water and since the water is low in nutrients, few algae and other planktonic plants occur. The sediments of the Delta and islands, however, act as gigantic nutrient sinks and immensely increase the biomass productivity of the system. The clear, slow-moving water and dense vegetation also contribute to a low oxygen level present throughout the whole system. According to Ramberg *et al.* (2006) the oxygen levels rarely exceed 3 mg / L in the seasonal floodplains and backwaters. At night it can even drop to 1 mg / L which is below the limit needed by most fish species in order to survive. Through evolutionary time, the fish which occur in this system have adapted to the low oxygenated environment. Although there are no endemic fish species, this system's isolation from

other river systems and the selection pressure of low-oxygenated waters, may result in future speciation to occur.

Water extraction and damming

Up to date there are only a few major infrastructures built to extract water from the Okavango River, namely the Eastern National Water Carrier (ENWC) in Namibia, the Mopipi Dam (Kniveton and Todd 2006), as well as pumping stations situated at Mohembo and Sepopa in Botswana. These have a minimal effect on the biological functioning of the system, but it is feared that this system will ultimately be heavily transformed by future water abstraction from planned dams. In 1997 the Delta was designated by the Government of Botswana as a Ramsar site and this boosted the conservation of this wetland and stopped Namibia extracting water through a pipeline to its capital, Windhoek (Ramberg *et al.* 2006). Since this system is shared by so many water-hungry countries and the demand for usable fresh water is rising, it seems inevitable for immense water extraction to start in the nearby future (Anderson *et al.* 2006; Ramberg *et al.* 2006). According to Kgathi *et al.* (2006) this would lead to a reduction in the flow of sediments which could lead to the following ecological impacts: (1) reduction in the rate of switching of channels in the Delta; (2) stabilisation of plant communities with the prevention of the renewal of the ecosystem; (3) eutrophication and (4) ultimately a reduction in biological diversity. An even worse consequence, associated with an increase in irrigation water extraction, is the runoff of insecticides and pesticides from upstream agricultural land in Angola (Mendelsohn *et al.* 2010). A uniform, lifeless and flood-regulated system will be the end result which is very much similar to the current status of the Orange-Vaal River System.

THE ORANGE-VAAL RIVER SYSTEM

General hydrology

The Orange-Senqu River is one of the two main tributaries which form part of the larger Orange-Vaal Drainage Basin. Originally known as the Orange River, this river was named in honour of the Dutch House of Orange by Colonel Robert Gordon, the commander of the garrison of the Dutch East India Company in 1779 (Earle *et al.* 2005). These days the part of the river that originates from Lesotho is called the Senqu and as a result the whole river is sometimes referred to as the Orange-Senqu River System. Since the Senqu River only refers to the part of the river which is located in Lesotho, the rest of the downstream river in South Africa still has the internationally recognised name of Orange River. The basin is shared by four different countries, South Africa, Lesotho, Botswana and Namibia and at approximately 896 368 km^2 is the largest basin south of the Zambezi (Earle *et al.* 2005) (Figure 2.1 A).

The Senqu River originates near Thabana Ntlenyana (3 482 m above sea level) in the Maluti Mountains of the Lesotho Highlands and then flows in a north-westerly direction to join with the other main tributary of the Orange-Vaal Basin, namely the Vaal River. The latter rises from the eastern Highveld escarpment in north-east South Africa and forms the northern border with the Free State Province. In the Northern Cape Province, close to the town of Kimberley, it joins with the Harts River and continues flowing in a westerly direction. At 1 425 km from its most eastern origin, the Vaal-Harts River joins the Orange-Senqu River and then the entire Orange-Vaal River passes through the Karoo and Kalahari. It ultimately forms the southern border with Namibia and enters the Atlantic Ocean at Alexander Bay. The small delta-type wetland, which is formed close to the river mouth, was designated as a Ramsar site in 1992. As a result of many factors, especially due to the prevention of natural flows by upstream dams, the ecological condition of the Orange-Vaal River mouth has deteriorated. The South African portion of the wetland has been placed on the Ramsar Montreux Record, which is a status denoting the need for urgent action to be taken (Coleman and Van Niekerk 2007).

The small Riet-Modder tributary meets the Vaal-Harts River just upstream of Douglas Weir and thereafter the river is joined by the Orange-Senqu. The Modder River originates in the mountainous area of Dewetsdorp, south of Bloemfontein. From an elevation of about 1 500 m it flows in a north-westerly direction and turns westerly until it joins the Riet River at Ritchie (Figure 2.1 C). It flows into the Vaal-Harts River System, south-west to Kimberley. This system, known as the Vaal System, connects to the Orange-Senqu River System, which subsequently flows to Alexander Bay (Seaman *et al.* 2001).

During the present study, fish were collected from Bishop's Weir (26°19'09"E, 28°58'00"S) which is situated in the Modder River at Glen, just east of Bloemfontein (Seaman *et al.* 2001, 2008). This is a tributary of the Riet River and forms part of the Vaal River which ultimately joins the Orange-Senqu River to form the larger Orange-Vaal Drainage System. The Modder River catchment area comprises about 17 360 km^2 (Figure 2.1 C) and the larger part is situated in the south-central Free State Province with a smaller part occurring in the Northern Cape Province.

It therefore forms part of the Upper Orange River catchment area and it is also sometimes erroneously included in the Orange-Senqu River System (Coleman and Van Niekerk 2007, Seaman *et al.* 2008) (Figures 2.4 B and D). It should, however, rather be regarded as a major tributary flowing into the Vaal River System which then forms part of the Orange-Vaal River Basin. Bishop's Weir is situated 2 km upstream of the confluence of a small tributary, the Renosterspruit, and the Modder River at Glen on the eastern outskirts of Bloemfontein (Seaman *et al.* 2001, 2008). It occurs directly downstream of a weir, which consists of a concrete road bridge and a steel train bridge over the river (Figures 2.4 A and B).

Rainfall and floods

Different from the Okavango River System, the Modder River and the rest of the Orange-Vaal River System is not maintained by natural pulse flooding. This is mainly because of the many dams and weirs built to artificially regulate the flow of water. In many regards just enough water is released by these man-made impoundments to

keep the stream flowing slightly. For example, the natural mean annual runoff of the Riet-Modder River is estimated to be [1]407 Mm^3 per year, whilst its ecological reserve is a mere 45 Mm^3 per year ([2]DWAF 2010). The average annual rainfall present in the Modder River area is 550 mm with the average in the east, near Thaba N'chu, being 650 mm and in the west, at Ritchie, 400 mm. The rainfall therefore decreases from east to west, whilst the evaporation increases from east to west. The annual evaporation rate at Dewetsdorp, where the Modder River originates, is 1 500 mm per year and at Ritchie where the Modder River and the Riet River converge, it is 2 100 mm per year (Seaman *et al.* 2001). Most precipitation occurs during summer thunderstorms, with the highest average rainfall occurring in the months of January to March and the lowest in June to August. Bloemfontein has an average summer temperature of 22°C and winter temperature of 10°C.

Habitats and vegetation

In its length the Orange-Vaal River approximately covers 2 300 km (DWAF 2010). As a result of this great length and because it occurs throughout a range of altitudes and climatic zones, various biomes and bioregions occur in this basin. The Modder River catchment specifically occurs within the Dry Highveld Grassland Bioregion (Mucina and Rutherford 2006). The eastern origin of the Modder River catchment is in the Central Free State Grassland (Gh 6) and then as it flows westward it gradually migrates through the Bloemfontein Dry Grassland (Gh 5), the Western Free State Clay Grassland (Gh 9) the North Upper Karoo (NKu 3) and then the Kimberley Thornveld (SVK 4) (Mucina and Rutherford 2006). Bishop's Weir is situated in the Bloemfontein Dry Grassland (Gh 5). Similar to many of the habitats surrounding the Modder River it has been influenced by agricultural practices and urban development. As a result it has mostly been converted to cultivated land and faces various threats such as desertification and pollution.

[1] 1 km^3 per year = 1 000 000 000 m^3 per year = 1 000 Mm^3 per year

[2] Department of Water Affairs and Forestry

Productivity

The overall health of the Modder River is in a poor state. Seaman *et al.* (2001, 2008) described this part of the river to consist of extensive loss in natural habitat, biota and basic ecosystem functions, since it has been exploited to its full capacity (Figures 2.4 C - E). This is attributed to the numerous human influences such as extensive irrigation for agriculture, ploughing of the floodplains, over-grazing and incorrect farming practices. Artificial structures such as road constructions, bridges, weirs and dams have also blocked the natural flow of this river and lead to encroachment of rivers through sediment deposition and alien vegetation overgrowth. These impoundments are also characteristic of the rest of the basin (Skelton and Cambray 1981) and have resulted in the absence of seasonal floods. The Orange-Vaal River Basin therefore has no natural nutrient cycle and hence it is not a very productive ecological system.

Urban development which increased the water abstraction, storm water runoff and treated and untreated sewage discharges have also accelerated changes in the functioning and species composition of the system and resulted in ecological deterioration. Extremely high phosphate (>1 mgP / L) and inorganic nitrogen concentrations have been measured at Bishop's Weir (Seaman *et al.* 2001). Previous nitrate concentrations have been recorded to be six times higher than the average for African rivers. The higher levels of these and other agricultural runoffs (fertilisers and pesticides) contribute to water turbidity, eutrophication and massive algal blooms and the vicious growth of alien reed beds. This high eutrophic production in biomass depletes the dissolved oxygen in the water.

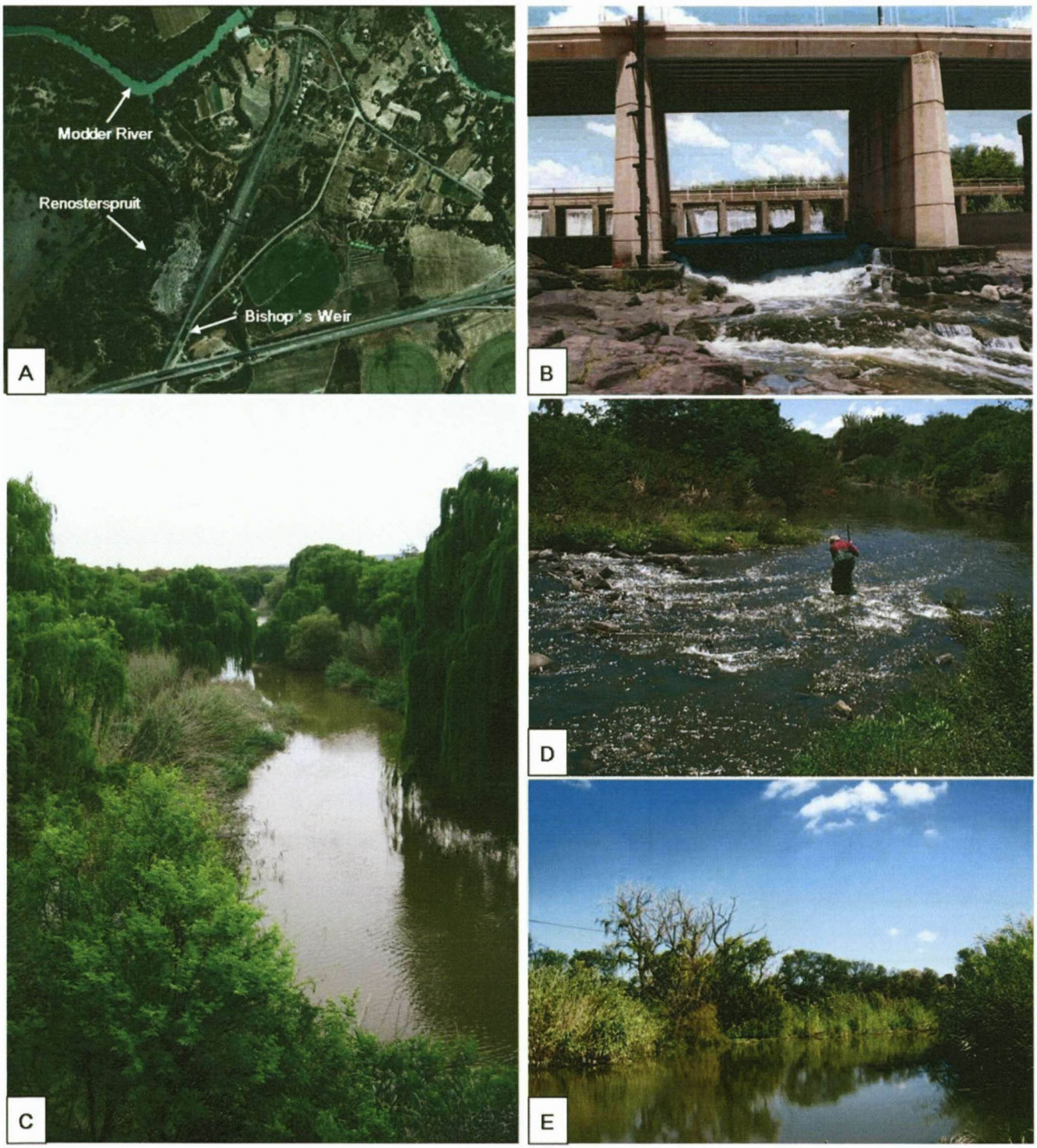


Figure 2.4: Photographs of the sampling sites in the Orange-Vaal River System. (**A**) The main site was at Bishop's Weir, situated just before the (**D**) Renosterspruit's confluence with the Modder River. (**B**) Similar to the rest of the Orange-Vaal System the flow regime is regulated by weirs and dams. (**C**, **E**) As a result rivers and associated tributaries are uniform in appearance with a low ecological diversity.

As a result the oxygen concentrations can get very low and has often been reported to be less than 1 mg / L in the hypolimnion during summer stratification (Seaman *et al.* 2001). The overall water quality of the Modder River is considered to be in a hypertrophic state. This has most probably contributed to the alarming deterioration in the fish numbers and health since 2001 (Seaman *et al.* 2008). Since the species diversity and ecosystem integrity are decreased, it results in lower biodiversity

Although about 20 different fish species (Table 6.2) have been recorded for the Orange-Vaal River System (Skelton 2001), only a few species such as introduced carp, *Cyprinus carpio* Linnaeus, 1758 and the mosquito fish, *Gambusia affinis* (Baird and Girard, 1853), as well as the native sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), dominate in numbers. The endemic Orange River mudfish, *Labeo capensis* (A. Smith, 1841) is also widespread, but other Orange-Vaal endemic species such as largemouth yellowfish, *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913) and small mouth yellowfish, *Labeobarbus aeneus* (Burchell, 1822) are low in abundance. In its natural state the Modder River is a non-perennial river and is naturally associated with intermittent flow (Seaman *et al.* 2008). Indigenous fish species are therefore adapted to survive periods of no-flow or low-flow and associated changes in water quality. The high prevalence of introduced fish species in the Modder River is a result of the increase in its altered flow-regime (Avenant 2000). The increased presence of species such as carp and mosquito fish is indicative of a decrease in stream flow and condition. The acute toxicity and environmental stresses present in the Modder River also contribute to a decrease in biodiversity.

Water extraction and damming

In 2002 the number of people recorded to be dependent on the Orange-Vaal River System was estimated to be 19 million and their associated water demand was calculated at 6.5 km^3 per year. This makes the Orange-Vaal the most developed transboundary river basin in southern Africa (Earle *et al.* 2005). No natural lakes exist within the basin, but numerous water schemes such as dams and other impoundments exist to fulfil in this high demand for water. It is estimated that with every five kilometres a weir is present in the Modder River (Seaman *et al.* 2001). This has had

massive impacts on the water chemistry, sediment transport and average temperatures and has also negatively influenced the aquatic biota as well as the livelihoods of people dependant on the water (DWAF 2010).

The regulation of water flow also results in a daily erratic outflow of the water volume which leaves these impoundments. One of the major effects of such daily fluctuations is the destabilisation of the shallow marginal areas of the river (Skelton and Cambray 1981). These areas are particularly important in the ecology of many fish species, especially regarding their defensive cover, spawning, nursery and feeding sites. Although impoundments cause a more erratic daily outflow of water, the annual flow of an impounded river is more regular and less erratic than the natural flow would have been. This reduces the isolation of cut-off pools and the seasonal fluctuations in temperature. All of the above are important factors regarding the ecology of fishes and other aquatic organisms (Skelton and Cambray 1981).

International laws and regional agreements have been implemented to reduce the impacts and to secure that the environmental flow of water is still incorporated into future management procedures. The South African Ecological Reserve (South Africa National Water Act, Act 36 of 1998) is one such legislation which aims to protect the ecological water reserve, whilst still managing human livelihoods and well-being (Coleman and Van Niekerk 2007). The high water demand needed for agriculture, industries and household use as well as the spatial and temporal variability of rainfall over much of the basin region, leads to an overall low water availability (1 000 m^3 per capita). This places the basin on the border between chronic scarcity (500 - 1 000 m^3 per year) and water stressed (1 000 - 100 m^3 per year) (Earle *et al.* 2005).

The ecological diversity of the pristine Okavango River is in direct contrast to the developed and eutrophic state of the Orange-Vaal River System. The two study sites therefore provided diverse sampling areas regarding their ecological integrity and biodiversity.

Chapter 3

MATERIALS AND METHODS

FIELDWORK

In the time period between December 2008 and August 2010 four field trips to Botswana, which covered different seasons, were conducted. Various techniques (Figures 3.1 A - F) such as gill nets, line fishing, scoop, seine and cast nets were used to collect a variety of fish species. Cast nets proved to be the most successful method for capturing medium to large sized fish, whilst scooping with nets underneath the papyrus and in shallow waters were more effective to catch smaller sized fish species and fingerlings. Large clumps of papyrus were also removed from the river and the roots were then examined for small fish (especially mormyrids). Two motorboats, *Synodontis* and *Labeo*, were used to access remote localities, although the capturing of fish was also conducted from the riverbanks of the main river (Figure 3.1 F). Gill nets were sometimes left overnight at certain lagoon sites and checked in the morning. The casting of nets was also conducted during various times in the evening as well as during the day time. This aided in formulating hypotheses on the influence which diplostomatids could have on the day and night time activities of different fish species (Chapter 6).

The localities sampled included: sites from the main stream, secluded lagoons, backwaters and floodplains such as Samochima lagoon (Figure 3.1 C), Nxamasere Floodplains (Figure 3.1 A, D and E), Lake Ngami and the Nchabe / Kunyere River at the village of Toteng (Figure 3.1 B). Many of the localities are not indicated on a general map available for the Okavango River. A table with GPS coordinates and the locality names created by the Aquatic Parasitology Research Group is summarised in Table 3.1.

Bishop's Weir, which is situated close to Bloemfontein and forms part of the Modder River tributary, was used as sampling site in the Orange-Vaal River System (Figure 2.4). Fieldwork consisted of two expeditions, which were conducted during summer and winter between January 2009 and January 2010. By standing in a canoe or on the river banks cast nets were used to catch fish.

Table 3.1: The habitat types and co-ordinates of the localities in the Okavango and Orange-Vaal Rivers where fishes were collected.

Localities in the Okavango	**Habitat Type**	**South**	**East**
Boro	Riverine swamp	18°59'15.1"	22°33'55.9"
Dead Crocodile Lagoon	Permanent swamps	18°25'00.0"	21°53'00.0"
Drotsky Upstream Temporary Floodplain	Seasonal swamps	18°25'50.1"	21°51'45.7"
Kalatog	Channel	18°24'00.0"	21°56'00.0"
Lake Ngami	Occasional floodplain	20°26'59.9"	22°44'.27.0"
Mohembo Mainstream	Riverine swamp	18°25'49.8"	21°53'46.0"
Mormyrid Marsh	Seasonal swamp	18°25'39.7"	21°54'16.2"
Nxamasere	Seasonal swamp	18°37'34.9"	22°06'24.4"
Phillipa Channel	Channel	18°46'45.7"	22°15'51.8"
Seronga	Floodplain	18°49'45.2"	22°24'40.0"
Shakawe Floodplain	Floodplain	18°26'05.0"	21°54'23.0"
Shakawe Mainstream	Riverine swamp	18°26'05.0"	21°54'23.0"
Toteng Bridge	Seasonal swamp	20°21'33.8"	022°56.47.4'
Locality in the Orange-Vaal	**Habitat Type**	**South**	**East**
Renosterspruit: Bishop's Weir	River tributary	28°58'00.0"	26°19'09.0'

An electro-shocker was used to retrieve small fish hiding beneath rock-covered rapids. This tiny electrical current resulted in fish becoming temporarily paralysed and easy to capture with a hand net. Seaman *et al.* (2001) showed that electro-fishing is an effective method for sampling fish in the Modder River.

The captured fish were kept in a cool box. This was filled with water, aerated through small battery-powered pumps and used to transport the fish back to the laboratories. At the Okavango River, the field laboratory at Leseding Research Camp (Figure 3.2 A), provided an ideal setting for conducting laboratory work. Fish collected from the Orange-Vaal River System at Bishop's Weir were kept in the Aquatic Parasitology Laboratory at the Department of Zoology and Entomology at the University of the Free State, South Africa.

Figure 3.1: Photographs of the various collection methods used during fieldwork. (**A**, **B**) Cast nets, (**C**) collecting and examining the papyrus, (**D**) scoop nets, (**E**) seine net and (**F**) fishing rods.

All of the collected fish were measured in millimeters from the tip of the snout to the end of the caudal fin (total length) and identified using the fish field guide of Skelton (2001). The recorded measurements for the collected and infected fish are supplied in the tables in Chapter 6. Fish were anaesthetised by using MS 222 and killed by the transection of their spinal cord at the back of the head. They were then dissected (Figure 3.2 B) in order to determine the presence and intensity of trematode infections in the eyes and brains. Some of the fish specimens were kept alive in aquariums, in order to be used in later behavioural experiments (Figures 3.2 C - E and 3.4 B - H).

LABORATORY TECHNIQUES

Dissection

A dissection microscope (Nikon SMZ800) was used to examine the eyes and brains of fish, whilst the material was carefully teased apart in a 1% saline solution in search for the larval digeneans (Figures 3.2 A and B). The metacercariae were recovered using a pipette, and transported to a dish containing saline. Some eyes and brains were, however, kept intact, fixed in 10% buffered neutral formaldehyde (BNF) or 40% formaldehyde and sent to the Department of Anatomical Pathology, University of the Free State, where histological work was conducted. During field observations larger numbers of free-moving metacercariae were noted in the brain cavities than could be viewed on the prepared histological sections. This is most probably because the fixation liquid removed the free-moving parasites from the brain surface and also flushed out many of the metacercariae from the brain cavity.

Fixation

A variety of different fixating methods were tested in order to preserve the obtained free-moving and encysted metacercariae. The most effective method to prevent the specimens from contracting was by placing them in lukewarm 70% ethanol or alcohol-formaldehyde-acetic acid (AFA). Due to the soft and very thin bodies of the diplostomatid metacercariae it was not necessary to flatten the material.

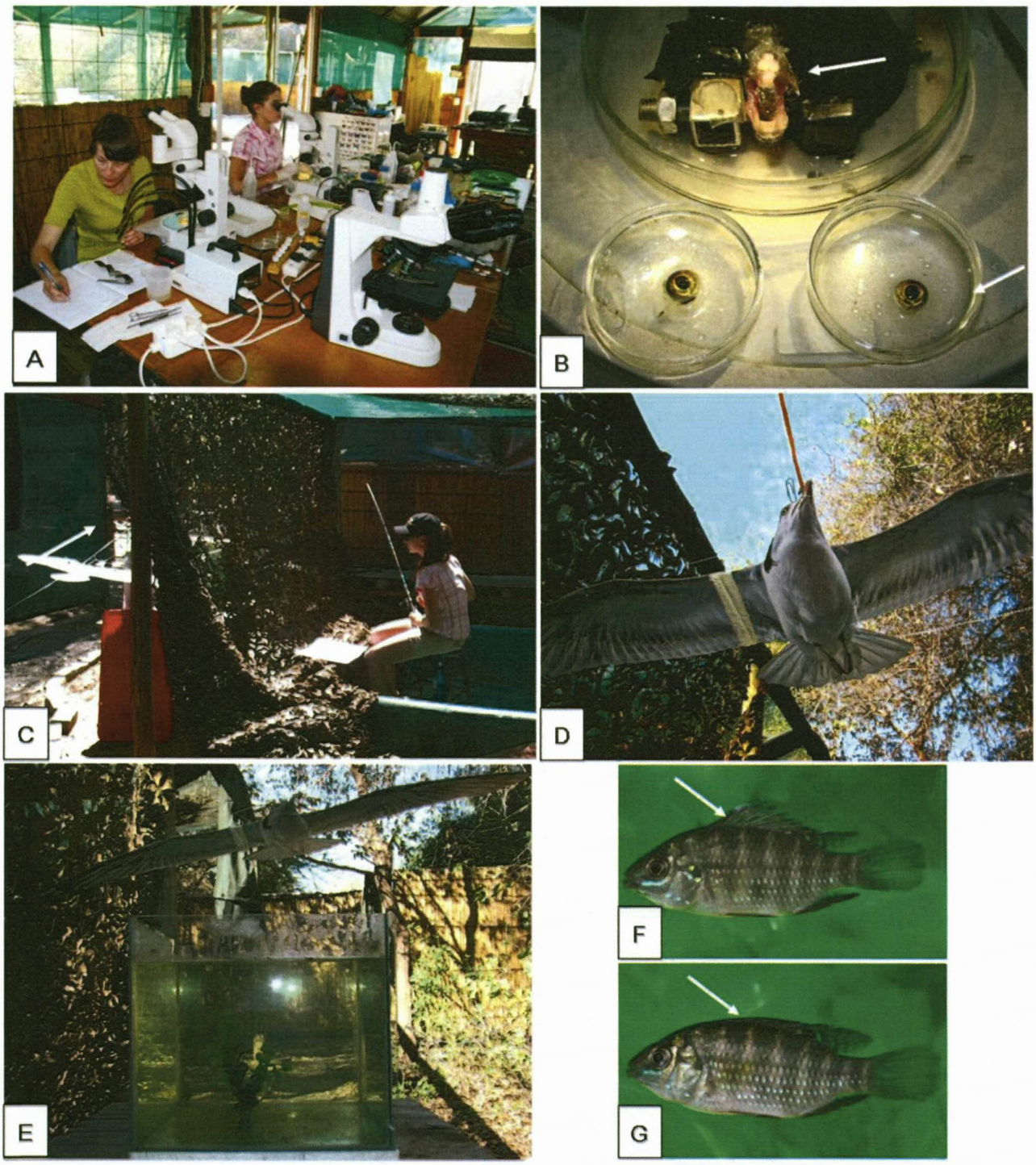


Figure 3.2: Photographs of (**A**) the field laboratory at Leseding Research Camp, (**B**) the dissection of fish eyes and brains and (**C**) the aerial predator detection experiment. The latter was conducted by pulling (**D**) a predator model bird overhead of (**E**) a holding tank and noting the behaviour of (**F**) fish before and (**G**) after this exposure.

All of the above mentioned techniques helped to maintain a true as possible representation of the metacercarial morphology, which is very important when distinguishing between different types (Chapter 4). The relaxed specimens were transferred to vials containing 70% ethanol, labeled and examined back at the laboratory at the Department of Zoology and Entomology (UFS). Attempts were made to carefully excyst the encapsulated metacercariae (cysts) but none proved to be successful since most of the specimens were underdeveloped and disintegrated when the cyst wall was punctured.

Staining and mounting for light microscopy

The method for staining and mounting was adapted with permission from a procedure used for small trematodes by [1]Professor Overstreet and is provided in full detail in Appendix I. It consists of hydrating the material, staining it with Ehrlich's hematoxylin and Van Cleave's hematoxylin and dehydrating it again to be cleared with xylene and mounted with Eukitt (see Appendix II). Microscopy observations were done and photographs were taken of specimens by means of a Nikon Digital Camera DXM1200F mounted onto a Zeiss Aziophot compound microscope. All of the reference material has been deposited in the parasite collection of the Aquatic Parasitology Research Group of the Department of Zoology and Entomology, University of the Free State, South Africa.

Morphological measurement and sketching

By making use of Image-J software the material was digitally measured and different morphological measurements were obtained from each specimen. Figure 3.3 illustrates the various morphometric characters also used in previous taxonomic studies (Niewiadomska 1988; Graczyk 1991b, 1992; McCloughlin and Irwin 1991; Höglund and Thulin 1992; Ibraheem 2000; Chibwana and Nkwengulia 2010). All of the measurements of the present study are in millimeters and are presented as follows: minimum - maximum (mean ± standard deviation) (Tables 5.1 and 5.2). Microscope

[1] Prof. R. Overstreet, Gulf Research Laboratory, Department of Coastal Sciences, The University of Southern Mississippi.

projection drawings of each type of *Diplostomum* were made by making use of a drawing tube attached to a Nikon Eclipse 80 *i* microscope (Chapter 5).

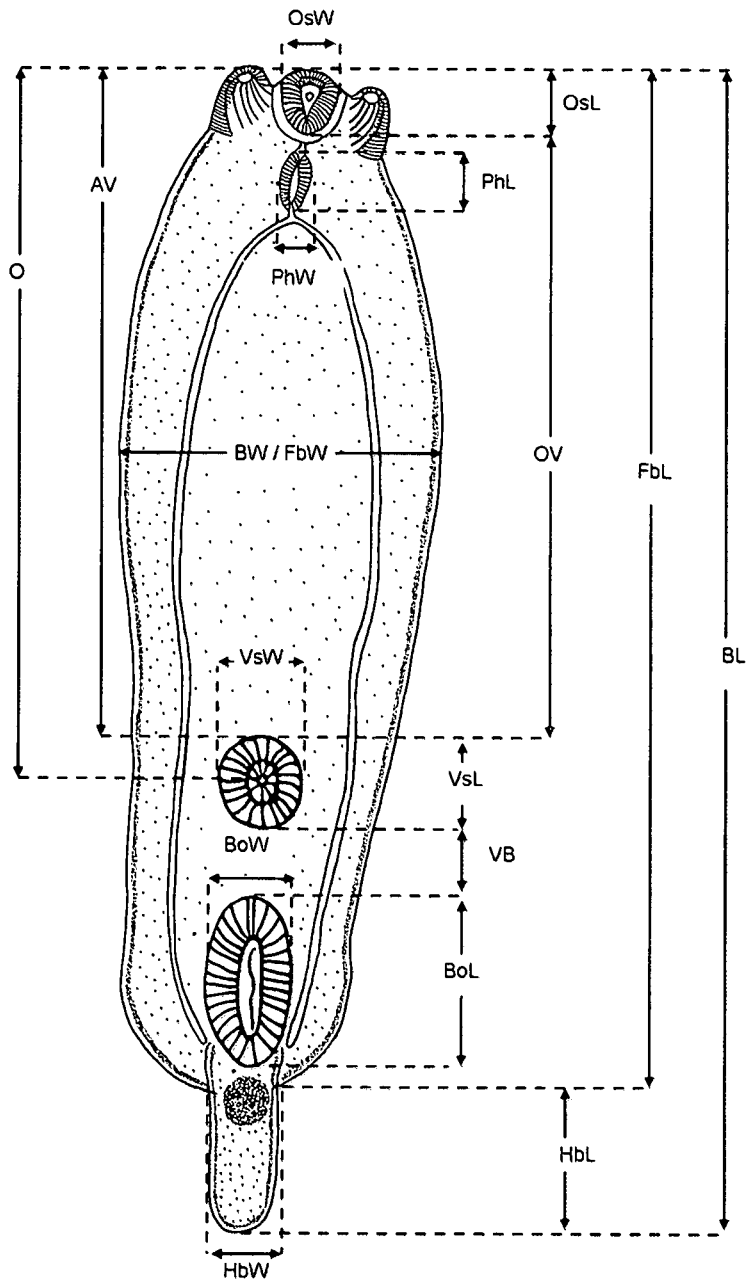
Biometric indices

Apart from comparing morphometrics to try and distinguish amongst diplostomatid species, the ratios of the sizes of the different anatomical structures to each other are also used to form biometric indices. The ten most popular indices used by previous authors (Niewiadomska 1988; Graczyk 1991b; 1992) are described in Table 3.2.

Table 3.2: The descriptions for the diplostomatid morphology biometric indices used during the present study.

b to a of body (%)	width to length of body in percentage
body (ab) / Bo (ab)	length x length of body to width x length of holdfast organ
body (ab) / Vs (ab)	length x width of body to length x width of ventral sucker
Os (ab) / Vs (ab)	length x width of oral sucker to length x width of ventral sucker
Bo (ab) / Vs (ab)	length x width of holdfast organ to length x width of ventral sucker
Os (ab) / Ph (ab)	length x width of oral sucker to length x width of pharynx
O / BL (ab)	distance from mid-ventral sucker to body length
FbW / BL	length of forebody to length of hindbody; width of forebody to body length
Fb (ab) / Hb (ab) (%)	length x width of forebody to length x width of hindbody in percentage

In Chapter 5 the range (mean ± standard deviation) for all the morphometrics (1-18) are provided (Table 5.1). The biometric indices (19-28) measured and calculated for each of the *Diplostomum* types collected during the present study (Table 5.2) as well as the morphological characteristics is summarised (Table 5.3). Remarks on the differences and similarities of the diagnostic and morphometric characteristics of the seven metacercarial types will be given throughout Chapter 5. The majority of animal taxon authorities could be provided in the present study. In rare cases where the date or author and date are omitted, it is because it could not be found from previous published research.



BW: body width
BL: body length
FbW: forebody width
FbL: forebody length
HbW: hindbody width
HbL: hindbody length
OsW: oral sucker width
OsL: oral sucker length
PhL: pharynx length
PhW: pharynx width
VsW: ventral sucker width
VsL: ventral sucker length
BoW: holdfast / Brande's organ width
BoL: holdfast / Brande's organ length
AV: distance from anterior part of body to anterior part of ventral sucker
O: distance from anterior part of body to central part of ventral sucker
OV: distance from posterior part of oral sucker to anterior part of ventral sucker
VB: distance from posterior part of ventral sucker to anterior part of holdfast organ

Figure 3.3: Diagrammatic representation of a metacercaria of *Diplostomum* von Nordmann, 1832 species illustrating the character measurements which were made.

BEHAVIOURAL EXPERIMENTS

General set-up

The experimental set-up included a glass-tank filled with water and placed on a table in the field aquarium of Leseding Research Camp (Botswana) (Figure 3.2 E) and in one of the laboratories at the Department Zoology and Entomology, UFS. Fish were individually allowed to acclimatise for about six hours before any experiments were conducted. The size of the tank consisted of 50 cm (height / depth) x 90 cm (length) x 36 cm (width) and therefore had a volume of 162 000 cm^3 of which about 80% was filled with dechlorinated water.

None of the fish were held in captivity for more than three consecutive days. The water was changed each day and was well aerated. The water temperature ranged between 20°C and 22°C. Observations were conducted from behind a shade-net, situated about a metre from the anterior part of the tank. This served as a one-way screen, since the observer could peep through the holes but the fish could not spot the presence of the observer behind the net (Figure 3.2 C). The rest of the tank was covered with black cardboards to block out any external stimuli except those provided by the experiments. To create a natural as possible environment the bottom of the tank was covered with sand and gravel and plastic plants were also placed in the middle of it. The size of the plants was small, to prevent blocking the view of the observer, but big enough to provide shade and cover for the fish when needed.

Quantification of observations

Small horizontal grid marks were made on the anterior wall 10 cm apart and labelled A (bottom), B, C, D, E and F (surface). This aided in indicating a change in the vertical position of the fish from before and after it was exposed to the experimental stimuli. By conducting very careful observations the observer quantified the rigidness and rate of movement of the different fins, eyes and the body before and after exposure to the external stimuli. A scale, ranging from 0 (none) to 4 (very high), was used to aid in

assigning a value to these descriptive observations which were written down on datasheets (see Tables 3.2 and 3.3). The intensity of each type of response was calculated by subtracting the 'initial quantified scale-value' (before the stimulus exposure) from the 'final quantified scale-value' (after exposure to the stimulus). For example if a fish had a dorsal fin rigidness of 4 (very rigid, Figure 3.2 F) which decreased to 1 (very floppy, Figure 3.2 G) after exposure, then the intensity of its change in dorsal fin rigidness is 4 - 1 = 3. Since every individual was exposed five times (with 15 minute breaks in-between), the mean intensity for behavioural change for each fish could be calculated. Thereafter the fish were anaesthetised and the brain and eyes of each specimen were carefully dissected (Figures 3.2 A and B) to determine the intensity of diplostomatid infection and to compare it with the intensity calculated for behavioural change.

Two different sets of behavioural experiments were conducted respectively in 2009 and 2010. Each made use of exposure to different stimuli, the first of which was noting and comparing the behaviour of infected and uninfected *Tilapia sparrmanii* A. Smith, 1840 exposed to an aerial model predator and the second consisted of exposing infected and uninfected *Tilapia rendalli* (Boulenger, 1896) to different intensities of light flashes.

1) Aerial predator detection experiment

Tilapia sparrmanii was the species selected since it naturally occurs in both the Okavango and Orange-Vaal River Systems and the populations were respectively found to be infected and not infected with diplostomatid eye flukes and cysts. This provided an excellent opportunity to test and determine the difference in the behaviour of control and infected wild populations of the same species of fish. During fieldwork of 2009 a two-dimensional cardboard silhouette of a predatory bird was simulated to fly overhead of the tank containing the fish (Figures 3.2 D and E). This was achieved by means of a pulley system, consisting of a fishing rod and fishing line running across the dorsal side of the tank with the artificial predator suspended from above (Figure 3.2 C). As previously noted, each fish was exposed to this stimulus five times, after each the model was towed back over the tank and kept out of view for 15 minutes. The

estimated minimum and maximum distances the model bird cleared the tank are 20 cm and 40 cm respectively. The intensity of their response towards this form of aerial predation was observed, noted (Table 3.2) and calculated.

Table 3.2: The datasheet used to note the changes in the behaviour of infected and uninfected *Tilapia sparrmanii* A. Smith, 1840 when exposed to an aerial model predator.

Scale: 0 = none, 1 = minimal, 2 = medium, 3 = high, 4 = very high								
Fish Number: Fish Species: Fish Length:						Location: Date: Time:		
Trial number		Dorsal	Caudal	Pectoral	Orientation	Movement	Eyes	Position
	Before							
	After							
General Remarks								

2) Light flash detection experiment

Although it would have been preferable to use the same fish species (*T. sparrmanii*) in the second behavioural experiment, the lack in a big enough sample size during 2010 prompted the use of another cichlid, *T. rendalli*. A large number of infected and uninfected individuals were collected from the Okavango River and their behaviour was individually noted whilst exposing them to different intensities of light flashes. To maximise the effect of the projected light flashes, the tank was placed in a temporarily constructed outdoor darkroom (Figure 3.4 A). Two candles were lit inside the pitch-black room, to enable observation of the behaviour of the fish before exposure. A switch-regulated 1000-candle powered light, shining at an angle of 45° to the water surface, was placed one metre away from the tank. The observer was seated behind a hide, peeping through a small window and could therefore hide in darkness whilst the tank was illuminated (Figure 3.4 B).

Different densities of shade-net were used (80% and 40%) to cover the light source to respectively expose the fish to 20% (Figure 3.4 C, D) and 60% (Figure 3.4 E, F) of the light source. Exposure to 100% (Figure 3.4 G, H) light intensity was conducted by

completely removing the shade-net. Each individual was exposed to the same light intensity three times. Each flash lasted for one second with five minute break intervals in-between each. This was followed by the exposure to three flashes of a bigger light flash intensity (e.g. 20%, 60% and 100%). By making use of datasheets (Table 3.3) the total intensity of each individual's response to each type of light flash intensity could be noted and calculated. Thereafter each fish was also dissected to determine its intensity of diplostomatid brain and eye infection.

Table 3.3: The datasheet used to note the changes in the behaviour of infected and uninfected *Tilapia rendalli* (Boulenger, 1896) when exposed to different intensities of light flashes.

Scale: 0 = none, 1 = minimal, 2 = medium, 3 = high, 4 = very high								
Fish Number: Fish Species: Fish Length:					Location: Date: Time:			
% Light through								
20% / 60% /100%		Dorsal	Caudal	Pectoral	Orientation	Movement	Eyes	Position
	Before							
	After							
General Remarks								

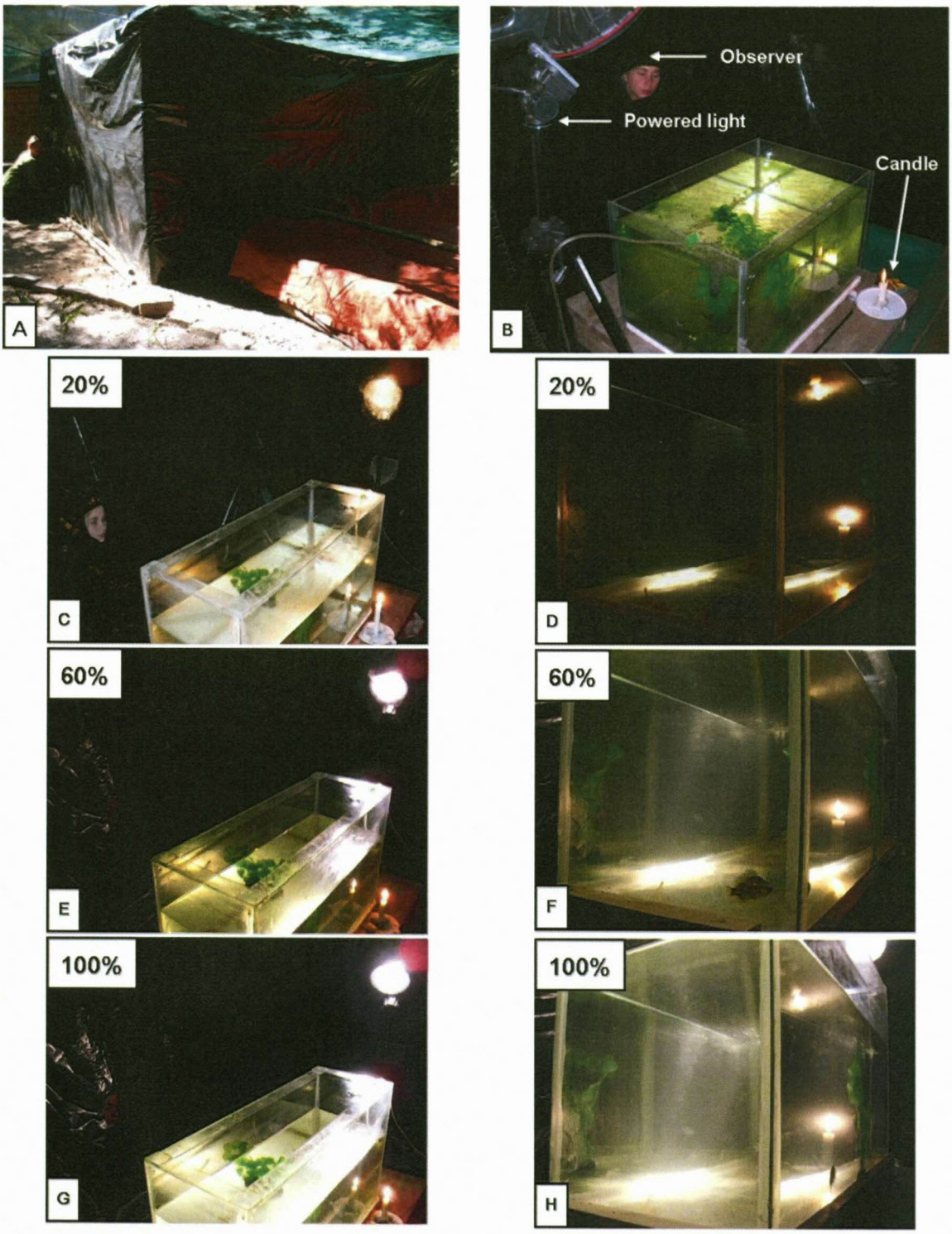
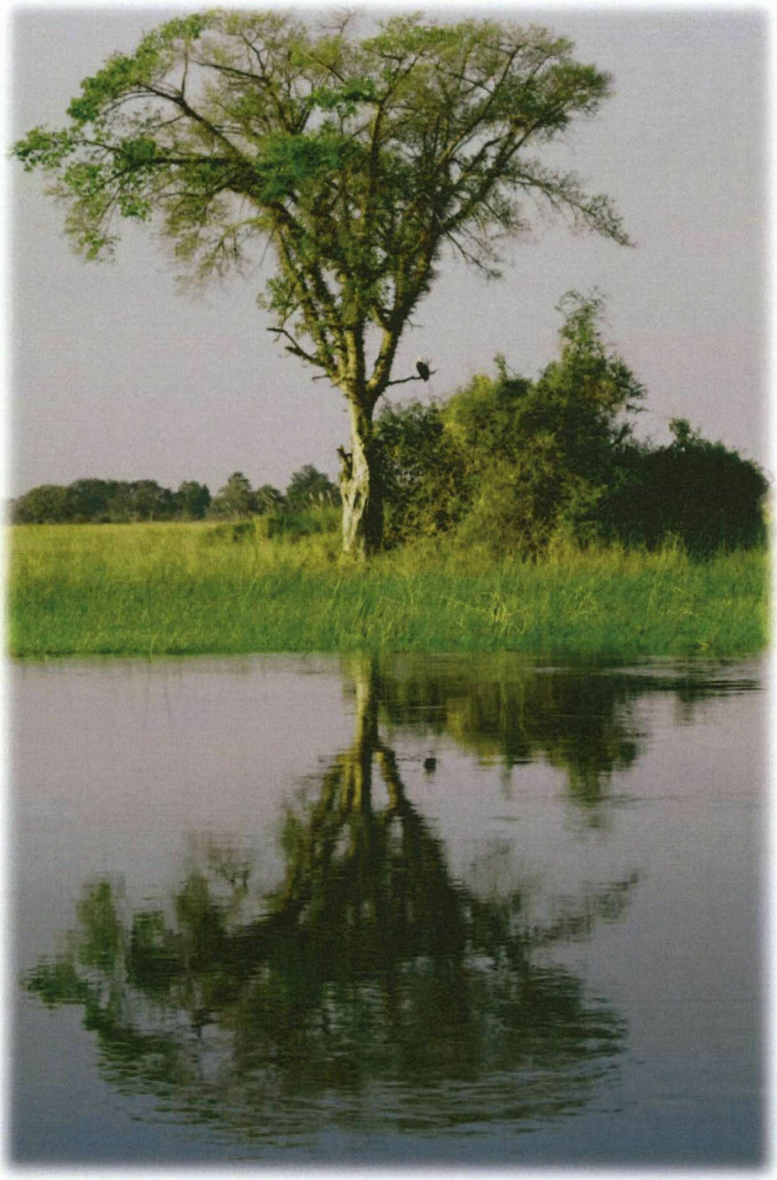


Figure 3.4: (**A**) The outdoor darkroom temporarily constructed at Leseding Research Camp, (**B**) candles lit to illuminate fish placed within a glass tank before exposure to flashes of light, observations conducted on the behaviour of fish individually exposed to (**C**, **D**) 20%, (**E**, **F**) 60% and (**G**, **H**) 100% light.

Chapter 4



TAXONOMY OF DIPLOSTOMATIDS

During previous records of fish eye and brain flukes of the Okavango River, Jansen van Rensburg (2006) used *Diplostomulum* Brandes, 1892 as larval genus name. Since the valid viewpoint of King and Van As (1997) that derived larval generic names complicate changing the genus and species names once the adults were described, the present study uses the adult genus name, *Diplostomum* in naming the sampled metacercariae. Similar to Jansen van Rensburg (2006) numbers are used to distinguish between the different types of metacercariae (*Diplostomum* type 1, *Diplostomum* type 2 and *Diplostomum* type 3), but alphabetical nomenclature is used for the four additional types (*Diplostomum* type a, *Diplostomum* type b, *Diplostomum* type c and *Diplostomum* type d) found in the Okavango and Orange-Vaal River Systems. The morphological measurements and detailed descriptions of each type are provided in Chapter 5.

GENERAL TAXONOMY

Assigning the correct genus or species name to fish eye and brain flukes is difficult and authors differ in their opinion. This discrepancy is fortunately not present with the higher taxonomic classification. Flukes are generally placed in the phylum Platyhelminthes Gegenbaur, 1859 which include bilaterally symmetrical, dorsoventrally flattened worms which consist of four classes, i.e.: Turbellaria Ehrenberg, 1831 (mostly non-parasitic flatworms), Monogenea Carus, 1863 (mainly ectoparasites of fishes), Trematoda Rudolphi, 1808 (endoparasitic flukes) and Cestoda (tapeworms). The class Trematoda is divided into two subclasses, namely Aspidogastrea Faust and Tang, 1936 and Digenea Carus, 1863. The latter refers to flukes with a three-host life cycle, with molluscs acting as primary intermediate and vertebrates as definitive / final hosts (Niewiadomska 2001). Since the larval cercarial representatives of eye and brain flukes are fork-tailed and actively penetrate the next host, these parasites are placed in the order Strigeida Poche, 1926. Niewiadomska (2001) opposes this by arguing that larval characteristics cannot be used for identifying the sexual adult worm to a taxonomic order level. He rather makes use of features of the adults and directly classifies digeneans to the superfamily Diplostomoidea Poirier, 1886. The most

exclusive characteristic of adults and metacercariae of this superfamily is a unique holdfast organ (Figures 5.2 D and E).

The similar morphology shared amongst related genera of this superfamily resulted in different metacercarial forms to be distinguished. Previous studies have mostly made this distinction by using derivatives of the adult genus name when the adult worm was not known. For example it was Brandes (1888) who suggested that the larval genus name *Diplostomulum* should be used as a group larval genus name for the free-moving metacercariae found in fish in the absence of the adult form (*Diplostomum*). Upon the description of the complete life cycle and identification of the adult worms, this larval genus may be replaced with the adult genus, retained or both are used in combination (Hoffman 1960). The present study does not make use of "diplostomulum" as a formal genus name. It is only used as a descriptive term for singular (plural="diplostomula") migrating metacercaria which has not become established within the final tissues of infection.

According to McKeown and Irwin (1995) distinction is sometimes made when referring to the authors of the adults (*Diplostomum* Rudolphi, 1819) and the metacercariae (*Diplostomum* von Nordmann, 1832). This acknowledges the two separate investigators who respectively first discovered and described the adult worm (Rudolphi 1819) present in piscivorous birds and the larvae (metacercariae) in fish (von Nordmann 1832). These combined, contradictory and inconsistent uses of taxon authorities have contributed to the immense confusion regarding *Diplostomum* classification. The present study favours the use of "von Nordmann, 1832" as the author of *Diplostomum* spp. especially when referring to the metacercariae found within fish.

In a recent revision of the classification of the Class Trematoda, Niewiadomska (2001) recognised five metacercarial forms (larval collective groups), within superfamily Diplostomoidea: *Tetracotyle* De Filippi, 1954; *Diplostomulum*; *Neascus* Hughes, 1927; *Prohemistomulum* Ciurea, 1933 and *Neodiplostomulum* Dubois, 1938. The structures of the reserve bladder and excretory system act as the main criteria for the morphological discrimination between these forms of which *Diplostomulum* has the simplest arrangement. Szidat (1969) also suggests an additional larval genus

Tylodelphys Diesing, 1850 which includes metacercariae that are biologically very similar to *Diplostomulum*. His description does not, however, consider the above mentioned criteria and some disagreement exists on whether it should be included as a metacercarial form. Many studies do acknowledge the taxonomic presence of this larval genus and some even elevate *Tylodelphys* to an adult genus (Niewiadomska 2001) and suggest that the larval genus should be referred to as *Tylodelphylus* (Szidat 1969). This use of derived larval generic names has not been implemented for all of the other larval genera, such as *Tetracotyle* and *Neascus,* and this attributes to the many mistakes in Diplostomoidea systematics, which are very difficult to eradicate.

It is characteristic of larval genera such as *Tetracotyle*, *Neascus*, *Prohemistomulum* and *Neodiplostomulum* to form cysts inside the second intermediate host's body. *Diplostomulum* and *Tylodelphys* are, however, the only metacercarial forms which can occur as free-moving flukes as well (Niewiadomska 2001). Some authors treat *Tetracotyle* synonymous with *Diplostomulum* and *Tylodelphys*, but there are marked differences between these larval groups. *Tetracotyle* metacercariae are surrounded by true cyst material of parasite origin, whilst with *Diplostomulum* and *Tylodelphys* it is partially host-induced or absent (Hoffman 1960). The former also has an oval or cup-shaped forebody and a small, rounded hindbody, whilst with the latter two the forebodies are more elongated and foliaceous, ventrally concave and the hindbodies are not as rounded. The present study supports the viewpoint that *Tetracotyle* should be regarded as a subgenus of *Diplostomum*. This is based on the possibility that the differences in larval body morphology and state of encystment are only results of the maturity of the metacercarial developmental stage as well as from the host immunological responses.

A similar conclusion is also made for the genus *Tylodelphys*. Slight morphological differences between *Diplostomum* and the former are only present between the adult worms. It includes the absence of a genital cone and asymmetrical testes (*Diplostomum*) and the presence of a genital cone and symmetrical testes (*Tylodelphys*). Previous studies varied in their conclusions, stating that *Tylodelphys* is similar to *Diplostomum* (Szidat 1969) or that the former is a separate adult genus. The present study supports the viewpoint that representatives of *Tylodelphys* are

transitional forms of the metacercariae of *Diplostomum* and are regarded as a subgenus (Faust 1918; Baer 1957).

Another taxonomic group which has been suggested to represent a separate adult genus, is *Dolichorchis* Dubois, 1961. The adult worms of this group have been described to contain a combination of morphological characters of *Diplostomum* and *Tylodelphys* representatives, such as an asymmetrical testis and a genital cone (Chibwana and Nkwengulia 2010; Zhokhov *et al.* 2010). Some researchers have not accepted the taxonomic status of this group and rather used a combination of names such as *Diplostomum* (*Tylodelphys*) *mashonense* Beverley-Burton, 1963 to describe adults with characteristics of both. Overall this group is considered to have no definite taxonomic status and at most *Dolichorchis* is regarded biologically similar to *Diplostomum* and used as a subgenus.

According to Niewiadomska (2001) the larval genus, *Diplostomulum* generally includes three possible adult genera: *Neodiplostomum* Railliet, 1919; *Alaria* Schrank, 1788 and *Diplostomum*. These adult genera do slightly differ regarding their morphology, but a more distinctive difference is the preference of different second intermediate hosts. The larval stage of *Alaria* is found in anurans, the metacercariae of *Neodiplostomum* may occur in reptiles, whilst mammals can act as paratenic hosts. The metacercariae of *Diplostomum* are the only known diplostomulum-type larval stage which parasitise fish. It is therefore concluded that *Diplostomum* is a compound genus and the only valid genus for metacercariae which can occur as free-moving or encysted strigeoid worms inside of fish. This genus is placed within the Family Diplostomidae to which many authors informally refer to as "diplostomids" or "diplostomatids" (Gibson 1998).

One of the main causes for the taxonomic discrepancy surrounding diplostomatids is the close relation still shared between all of the adult and larval genera. This is due to their common evolutionary origin, which only recently branched out (Szidat 1969). Transitional forms therefore still link the adult as well as the larval stages of different species and genera. This situation is further aggravated with the inconsistency in characteristics and life stages used for species' descriptions.

METHODOLOGY FOR DIPLOSTOMATID IDENTIFICATION

A variety of techniques, such as the identification of eggs, immunological compounds, enzymes and other molecular and histological procedures are currently used in the aspirant identification of parasitic worms. Chappell (1995) depicted modern methods for diplostomatid identification to include: the metacercarial morphology; infected host species and the site occupied within the eye / brain. The occupation site has acted as major character for diplostomatid identification, but the present study concludes that the location of metacercariae varies too much amongst species to fulfil this function. For example Field and Irwin (1995) distinguished between *Diplostomum spathaceum* (Rudolphi, 1819) and *Diplostomum pseudobaeri* (Razmaskin and Andrejak, 1978) metacercariae based on their respective preference in the lens and the retina in the eyes of farmed rainbow trout, *Oncorhynchus mykiss* Walbaum, 1792 in Northern Ireland. On completion of both these species' life cycles similar adult trematodes were, however, identified and therefore a synonymous species was present.

There are various other studies which concluded that similar diplostomatid species may occur in different eye sites as well in the brain tissues of similar or different fish species (Hoffman 1960; Ashton *et al.* 1969; Dubois 1970; Davies *et al.* 1973; Bortz *et al.* 1988). The description of the biological and morphological characters of all the parasite's developmental stages are therefore still regarded as the most effective methods to describe a species (King and Van As 1997; Gibson 1998).

Most authors have based the description of diplostomatid species only on metacercariae recovered from fish. These larvae are, however, only representative of one of the stages of the parasite's life cycle and cannot solely be used for species identification. The variation which may occur in their morphology has also added to the confusion regarding *Diplostomum* species identification (Chappell 1995; Field and Irwin 1995; McKeown and Irwin 1995; Niewiadomska 1996). Even if adult diplostomatid species differ in their morphology they could have morphologically similar-looking cercariae or metacercariae and *vice versa* (Kennedy and Burrough 1977; Burrough 1978; Niewiadomska 1988; Flores and Semenas 2002; Niewiadomska and Laskowski 2002). This has made accurate species separation of *Diplostomum* very difficult and ultimately resulted in conflicting taxonomic keys.

Some authors even conclude that due to its varying nature, morphological characters cannot be used to identify diplostomatid species (Locke *et al.* 2010a). Advanced techniques, such as genetics and other molecular and biochemical probes have been suggested for accurate species' identification (Chappell 1995; Chibwana and Nkwengulia 2010). There is, however, a world-wide lack in reliable species' specific genetic markers, especially in Africa (personal comment, [1]Prof Ernst Swartz and [2]Roger Bills 2009). Similar to the findings of Chibwana and Nkwengulia (2010) the present study concludes that the reason for this lack in genetic knowledge is based on two reasons. Firstly it is due to the absence of high intensity aquaculture practices and associated fish health research and secondly due to the challenges faced when conducting fieldwork in a rural and tropical climate. This is especially true when compared to studies, such as by Chappell (1995) and Niewiadomska (1996), which were conducted in North America and Europe respectively.

Genetic classification practices do have their own limitations regarding accurate species' identification (Niewiadomska and Laskowski 2002). Only a limited number of studies has been conducted on the diplostomatids of African fish and due to the challenges stated above, these descriptions have mostly been based on morphological characters. To ensure comparison with previously described species, the present study also made use of similar characters to describe metacercariae to specific larval type groups (see Chapter 5). These measurements were compiled from studies which focused on morphometrics to aid in species' identification and description (Niewiadomska 1988; Graczyk 1991b, 1992; McCloughlin and Irwin 1991; Höglund and Thulin 1992; Ibraheem 2000; Chibwana and Nkwengulia 2010).

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SHORTCOMINGS OF DIPLOSTOMATID KEYS

The majority of diplostomatid keys are simply based on the morphology and biology of the adult parasite. Only a few keys exist which are devoted to the identification of the metacercarial stage of the life cycle. On the flip side, taxonomic studies which do make use of metacercarial, or any other larval characteristics, mostly exclude the verification and description of the adult worm.

This situation is even further complicated with the absence of keys available in English. Most diplostomatid keys, such as provided by Shigin (1976, 1986) and Sudarikov (1971) are presented in Russian and even expert translators struggle to provide the accurate meaning for all the biological terms. This has overall complicated the ability to relate the cercariae, metacercariae and adults of each species to one another, making it impossible for valid species descriptions (Field and Irwin 1995; McKeown and Irwin 1995; Chibwana and Nkwengulia 2010).

Shigin (1976) was first to propose a key for the classification of 13 species of flukes from the brain, lens and deeper parts of the eye. In 1986 a revised key was given by Shigin which included three additional species as well as the different synonyms for the previously described ones. Niewiadomska (1984) also composed a key in an attempt to include the species of all the metacercariae known to belong to the genus *Diplostomum*. Unfortunately by using all of the above mentioned keys it is known to result in different adult species identification (McCloughlin and Irwin 1991; Niewiadomska 1996). Table 4.1 lists only a few of the numerous examples of keys varying in the use of diagnostic characters, resulting in species' synonymy and overall taxonomic chaos. According to Niewiadomska (1996) the necessity for workers to provide the taxon authority for species, is sometimes also omitted and this decreases the ability to confirm linkages of the works done by the different authors.

Table 4.1: Some of the different diplostomatid species names and synonyms attained when using different keys to identify metacercariae with the same morphology (compiled from Shigin 1976; Höglund and Thulin 1992; Niewiadomska 1984).

Author of key	**Dubois (1970)**	**Sudarikov (1971)**	**Shigin (1976, 1986)**
Species name	*Diplostomum spathaceum* (Rudolphi, 1819)	*Diplostomum baeri* Dubois, 1937	*Diplostomum volvens* von Nordmann, 1832
			Diplostomum helveticum (Dubois, 1929)
			Diplostomum parviventosum Dubois, 1932
Species name	*Diplostomum baeri* Dubois, 1937	*Diplostomum indistinctum* (Guberlet, 1923)	*Diplostomum volvens* von Nordmann, 1832
			Diplostomum helveticum (Dubois, 1929)
			Diplostomum parviventosum Dubois, 1932

MORPHOLOGICAL VARIABLES

Even if a universally accepted key for all metacercarial diplostomatid species could be compiled, the variation in their morphology would most probably prohibit its accurate implementation (Höglund and Thulin 1992). Previous morphological studies as well as the morphometrics of the present study (see Chapter 5) clearly indicate that it is impossible to provide a specific size range for any one of the morphological structures of each metacercarial type and species (Graczyck 1991b; 1992; Höglund and Thulin 1992; Niewiadomska 1996; Ibraheem 2000; Locke *et al.* 2010a, b; Chibwana and Nkwengulia 2010).

Variability in metacercarial size and morphological appearance can be the consequences of four factors: the (1) host species, (2) size of the host, (3) density of infection in the host and (4) age of the parasites (Graczyk 1991b, 1992). These variables are consistently present in natural populations and therefore some researchers suggest that valid verification of metacercarial diplostomatid species may only be conducted through experimental completion of the life cycle (Hoffman 1960;

Bouillon and Curtis 1987). In the present study it was found that a fifth factor also influences the morphometrics and appearance of collected metacercariae, namely the method of specimen fixation and mounting.

1. Host species

As previously mentioned the completion of a trematode's life cycle, using the natural host species, is an important prerequisite for valid species identification (Graczyk 1992; Field and Irwin 1995). Previous studies concluded that the greatest factor which determines metacercarial morphology is the species of fish acting as second intermediate hosts (Khalil 1963; Graczyk 1991a; Field and Irwin 1995; McKeown and Irwin 1995; Chibwana and Nkwengulia 2010). The varying degree of susceptibility towards diplostomatid infection is dependent on the presence of physical barriers to prohibit cercarial penetration as well as the activity of immune responses. Due to variation in ecological and behavioural characters, different fish species are also exposed to different intensities of diplostomatid cercariae (see Chapter 6). During the present study, diplostomatids were collected from the eyes and / or brain tissues of a variety of different fish hosts. This may contribute to the wide range in measurements found for each of the metacercarial types (see Chapter 5). As a result Appendix II includes the morphological measurements and biometric indices for the metacercarial types and cysts sampled from each fish species.

2. Size of host

Fish age and hence body surface, may positively correlate with the intensity of diplostomatid infection within the eyes and brain (Sweeting 1974; Hendrickson 1978; Bouillon and Curtis 1987). This correlation may vary amongst different species of fish, depending on their susceptibility and exposure to diplostomatid cercarial infection. Although not statistically presented, the present study concludes that a general increase in metacercarial numbers was found with an increase in total length of a particular fish species. The increase does not remain linear and reaches a climax

where after it remains relatively constant and therefore large bodied, adult fish are not necessarily found with the greatest infection intensity.

3. Density of metacercarial infection

The size and development of metacercariae may be influenced by the size of the host's infection sites (tissues). Limited space present within small fish could result in an increase in the density of the metacercarial numbers and indirectly lead to a decrease in space for metacercarial growth and development. For example, Graczyk (1991b) found that the density of infection of the lens-inhabiting metacercariae, *D. spathaceum*, is connected with mechanical filling of the fish eye lens. The volume and density of occupation of the available space inside the host can also be directly linked to the availability of food. Nutrition is of particular importance for the developing metacercariae and a limited availability of space will result in lesser developed and smaller intensities of infection. Graczyk (1992) states that with high intensities of *Diplostomum pseudospathaceum* Niewiadomska, 1984 infection within the eyes of fish, the metacercariae were small and lesser developed, whist larger larvae were present if the densities of infection were lower.

4. Age of the metacercariae

After successful penetration and infection, the diplostomula (tailless cercariae) gradually develop into mature metacercariae and hence different metacercarial stages may be present inside the fish host. The majority of previous authors have not taken into account the age of the metacercarial stages when describing the morphology of the larvae. This has contributed immensely to the noting of different sizes in body ratios and anatomical structures of metacercariae collected from the same host (Ashworth and Bannerman 1927; Niewiadomska 1963a; Szidat 1969; Sweeting 1974). Since the date of cercarial penetration is unknown within a natural population of fish, it is also impossible to indicate the precise age of a metacercarial infection. This inhibits

the forming of accurate linkages between metacercarial morphology and diplostomatid species present within wild fish.

Erasmus (1958) suggests a gradual increase in metacercarial body size and that the presence of certain structures such as a hindbody and pseudosuckers are found only during more mature stages. Variation in different species' developmental cycles as well as the influence which environmental factors (e.g. temperature) have on development should also be considered when trying to determine the maturity of metacercariae (Sweeting 1974).

5. Fixation and mounting of metacercarial material

Another huge obstacle prohibiting accurate species identification is the deformation of the small, fragile and soft bodied metacercariae during the specimen preparation process (Hoffman 1960; Graczyk 1991b; Höglund and Thulin 1992; Niewiadomska 1996; Locke *et al.* 2010a). Different and sometimes even similar methods of fixation, staining and mounting, result in different morphological forms of metacercariae belonging to the same species (Rees 1955). Hoffman and Hundley (1957) noted that when adding hot AFA (alcohol-formaldehyde-acetic acid) to mature diplostomatid metacercariae some specimens shrank, some became more extended and in some the body regions were distorted. The present study consistently attempted to use the same fixatives, stains and fixation pressures (Chapter 3), but varying morphometrics were still obtained amongst the metacercariae (see Chapter 5).

Chapter 5

METACERCARIAL TYPES

Large variation and overlap exist in the morphometrics of diplostomatid types. The present study summarises the range (mean ± standard deviation) of the morphometrics (Table 5.1) and biometric indices (Table 5.2) for each *Diplostomum* type collected. Measurements of similar looking metacercariae described by previous authors are not included, but this chapter does give a description of each of the sampled types and compares it with known species. A summary of the characters used to distinguish between diplostomatid types is also provided (see Table 5.3). This is followed by Table 5.4, compiled from Chibwana and Nkwengulia (2010) and the present study, as an example of the variation which can occur within the morphometrics of metacercariae collected from the same host species and from different freshwater bodies. To understand what each of the morphological measurements entail, this chapter will commence with a brief description of the general anatomy known for diplostomatids (Figure 5.1) and this will be followed by the descriptions of the collected metacercarial types.

ANATOMICAL STRUCTURES

Oral sucker: Figures 5.1, 5.2 A and B

The oral sucker consists of the mouth opening, surrounded by lip-like, muscular structures. It is situated terminally or sub-terminally on the anterior part of the metacercarial body. During mature metacercarial developmental stages it contributes to the absorption of nutrients (Erasmus 1958), whilst during earlier stages only the epidermis of the body surface fulfils this function (Ashton *et al.* 1969).

Pseudosuckers: Figures 5.1 and 5.2 A

Since the term "pseudo" wrongly describes these suckers as not being true suckers, the terms "lateral suckers" or "lappets" are also used. These structures vary in appearance, ranging from two inconspicuous to well-developed ear-like protrusions, situated laterally or postero-laterally from the oral sucker (Figures 5.2 A and B). It has been used as diagnostic features for metacercarial species identification but it could be

that these structures are only present during the later, more mature metacercarial stages. The pseudosuckers may also be retracted into pits by means of a series of longitudinal muscles (Brown 1899) or only under pressure, be fully everted (Bibby and Rees 1971). It is therefore difficult to establish the presence and measurements of these structures within a metacercarial type.

There are many different opinions regarding the function of these structures. Due to the thin cuticle lining the pseudosucker and its ample nerve supply, Mataré (1910) suggested that it may function as an organ of taste. Their muscular appearance, with muscle strands inserted into the base of each organ and passing backwards, also assigns the functions of adhesion and suction to it (Rees 1955). Szidat (1969) established the presence of gland cells on the ear-like pseudosuckers of *Diplostomulum mordax* Szidat and Nani, 1951 and suggested that its secretions aid in the penetration of the metacercariae into the tissues of the midbrain and the cerebellum.

Ventral sucker: Figures 5.1 and 5.2 C

This structure is placed more or less in the middle of or just posterior to the ventral side of the body. It consists of a circular opening surrounded by a muscular wall, similar to that of the oral sucker. The term "acetabulum" is also used when referring to this structure. The main function is to attach to substrates and then the metacercariae move forward by contracting rhythmically and undergoing rapid changes of shape, length and width (Brown 1899).

Holdfast organ / Brandes organ: Figures 5.1, 5.2 D and E

A narrow slit, from which tri-, quadric-, or multi-radii may originate, occurs posterior from the acetabulum on the ventral surface of the body. This lumen is lined with a muscular wall of cuticle forming a bi-lobed structure (Ashworth and Bannerman 1927). The surrounding muscles control the size of the lumen and sometimes may also result in the structure being everted as a whole. Other names for it include Brandes organ,

Haftorgan, Haftapparat, tribocytic organ, adhesive organ or the holdfast organ of which the latter terminology is preferred in the present study. The presence of this structure is a unique characteristic of trematodes belonging to the superfamily Diplostomoidea. Its functioning only becomes apparent in adult trematodes, where it is responsible for adhesion as well as secretions to provoke histolysis for the feeding on the host's tissues (Bibby and Rees 1971). Some authors state that even during the younger metacercarial stage it aids in both adhesive and digestive roles (Niewiadomska 2001).

Digestive and excretory systems: Figures 5.1 and 5.2 A, B and F

Although it may vary between species and stage of maturity, the digestive system mainly consists of a short prepharyngeal portion, a more prominent muscular pharynx and an oesophagus which bifurcates into two blind-ending intestinal caeca (Ashton *et al.* 1969; Graczyk and Fried 2001). The degree of visibility of these structures may vary and although sometimes not observable, it may still be present. This system is supplemental to the absorption of nutrients directly through the body surface. The excretory system consists of flame cell units, covering the entire body, which are connected by excretory ducts and terminating into a posterior excretory pore. Some authors regard the patterns formed by the digestive and excretory systems as important taxonomic characters (La Rue 1957). There is no consistency in the flame cell formulas used for species' identification (Graczyk 1991b, 1992) and since it can also only be viewed in live specimens (Ashworth and Bannerman 1927; Szidat 1969), the description and comparisons of these systems are not provided during the present study.

Reproductive system: Figures 5.1 and 5.2 E

Earlier works disregard the presence of any genitalia in the diplostomatid metacercarial stage (Bell and Hopkins 1956). Gonad primordia are present and are mostly viewed as a small mass of darkly stained cells, located in the middle of the body, posterior to the holdfast organ (Chakrabarti 1967). It will only develop into fully functional and large reproductive structures in adult trematodes, after ingestion by the definitive hosts,

during which it will mature into a well-developed hindbody. Depending on the maturity of the metacercarial stage, the genital primordia may vary in size and form. This absence of undifferentiated reproduction organs complicates species' identification, which contributes to the uncertainty regarding metacercarial taxonomy (McCloughlin and Irwin 1991).

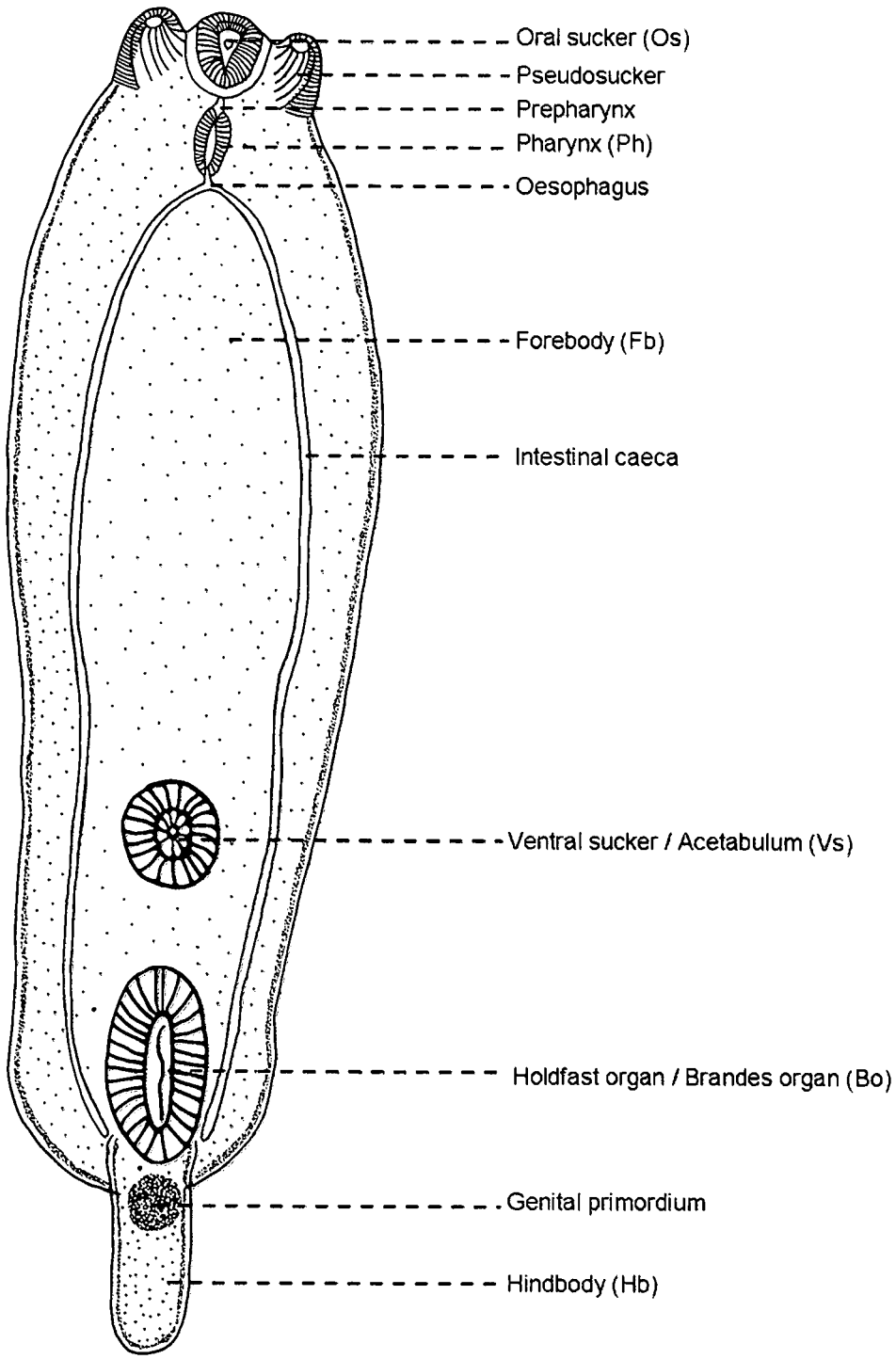


Figure 5.1: Basic morphology and anatomy of a *Diplostomum* von Nordmann, 1832 metacercaria.

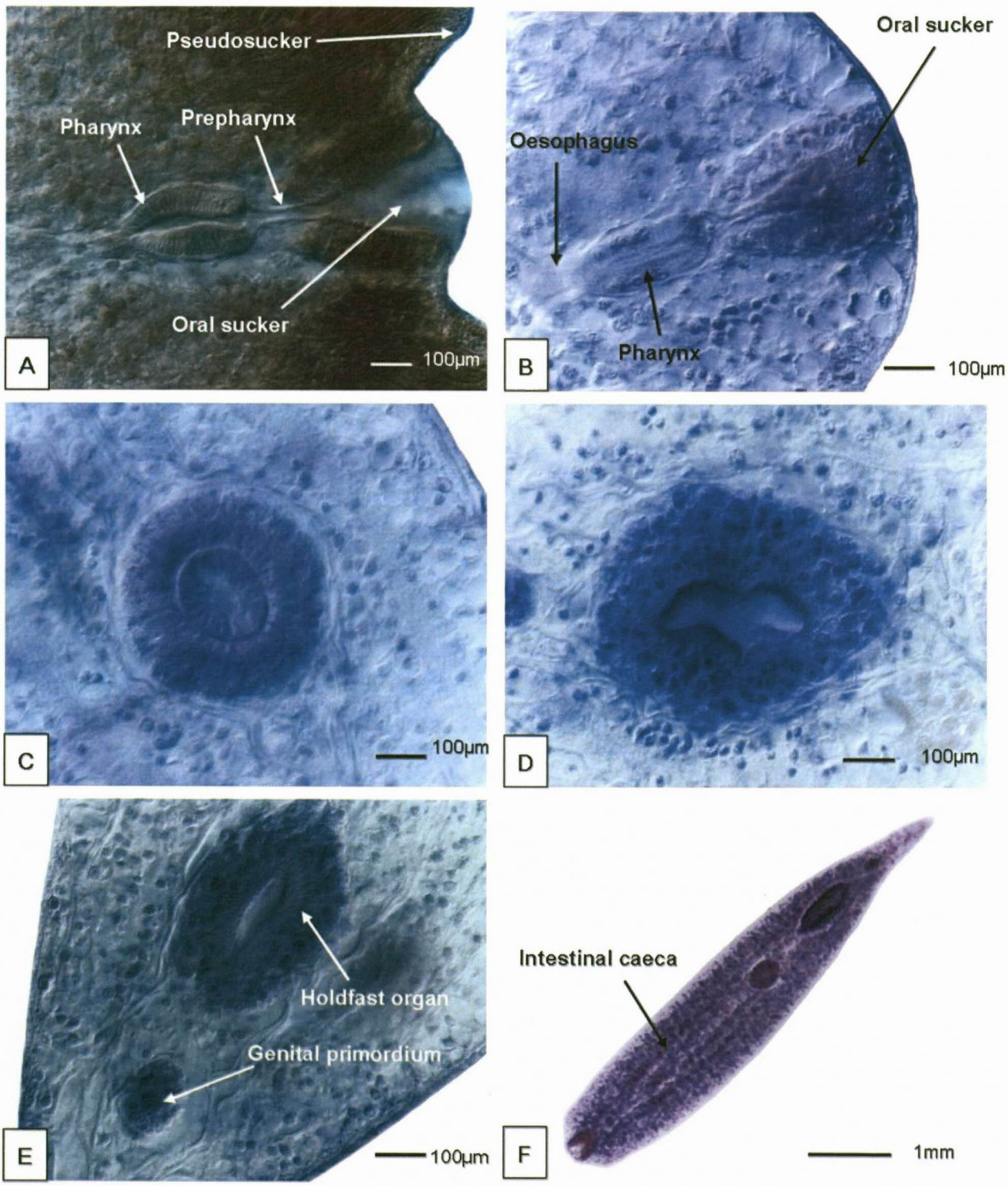


Figure 5.2: Light micrographs of the general morphology and anatomical structures of diplostomatids. (**A**) Oral sucker, pseudosuckers, small prepharynx and pharynx. (**B**) Oral sucker, without pseudosuckers, followed by pharynx and oesophagus. (**C**) Ventral sucker. (**D**) Holdfast organ. (**E**) Holdfast organ followed by genital primordium. (**F**) Intestinal caeca stretching throughout length of the body.

Table 5.1: The range (**mean** ± standard deviation) of different morphological measurements (mm) of the seven different types of *Diplostomum* von Nordmann, 1832 metacercariae collected from the eyes and / or brain of a variety of fish species from the Okavango and Orange-Vaal River Systems. The sample size (n) indicates the number of specimens which were measured to obtain the morphometrics. Features that were not measured are indicated with -.

Diplostomum **type**	**1** (n=12)	**2** (n=292)	**3** (n=19)	***a*** (n=53)	***b*** (n=11)	***c*** (n=15)	***d*** (n=8)
***Diplostomum* type** **Image**							
1. Body length (BL)	0.667 - 1.141 (**0.88** ± 0.156)	0.199 - 0.848 (**0.495** ± 0.109)	0.557 - 1.29 (**0.942** ± 0.226)	0.212 - 3.225 (**0.507** ± 0.397)	0.314 - 1.184 (**0.604** ± 0.308)	0.245 - 0.406 (**0.298** ± 0.053)	0.33 - 0.717 (**0.469** ± 0.13)
2. Body width (BW)	0.604 - 1.153 (**0.858** ± 0.169)	0.048 - 0.212 (**0.112** ± 0.031)	0.329 - 1.177 (**0.796** ± 0.196)	0.066 - 0.625 (**0.127** ± 0.083)	0.086 - 0.272 (**0.147** ± 0.07)	0.097 - 0.248 (**0.144** ± 0.042)	0.141 - 0.293 (**0.223** ± 0.05)
3. Forebody length (FbL)	0.515 - 1.0 (**0.733** ± 0.152)	-	0.464 - 1.039 (**0.774** ± 0.217)	-	0.38 - 1.069 (**0.686** ± 0.302)	-	0.298 - 0.598 (**0.419** ± 0.109)
4. Forebody width (FbW)	0.606 - 1.156 (**0.856** ± 0.168)	-	0.317 - 0.934 (**0.752** ± 0.179)	-	0.077 - 0.278 (**0.181** ± 0.084)	-	0.142 - 0.294 (**0.223** ± 0.05)
5. Hindbody length (Hbl)	0.017 - 0.29 (**0.155** ± 0.068)	-	0.106 - 0.257 (**0.198** ± 0.045)	-	0.042 - 0.155 (**0.099** ± 0.043)	-	0.029 - 0.112 (**0.047** ± 0.027)
6. Hindbody width (HbW)	0.182 - 0.39 (**0.315** ± 0.06)	-	0.115 - 0.377 (**0.269** ± 0.073)	-	0.026 - 0.081 (**0.054** ± 0.021)	-	0.034 - 0.128 (**0.073** ± 0.027)
7. Pharynx length (PhL)	-	0.01 - 0.045 (**0.022** ± 0.006)	0.053 - 0.092 (**0.073** ± 0.016)	0.013 - 0.026 (**0.019** ± 0.004)	0.014 - 0.057 (**0.031** ± 0.019)	**0.019**	0.029 - 0.034 (**0.031** ± 0.003)
8. Pharynx width (PhW)	-	0.006 - 0.049 (**0.013** ± 0.006)	0.022 - 0.039 (**0.031** ± 0.007)	0.01 - 0.014 (**0.012** ± 0.002)	0.009 - 0.032 (**0.018** ± 0.01)	**0.006**	0.017 - 0.021 (**0.019** ± 0.002)

Table 5.1 (continued): Morphological measurements (mm) of *Diplostomum* von Nordmann, 1832 metacercariae.

Diplostomum type	1	2	3	*a*	*b*	*c*	*d*
9. Oral sucker length (OsL)	0.089 - 0.155 (**0.112** ± 0.018)	0.021 - 0.08 (**0.04** ± 0.008)	0.086 - 0.238 (**0.124** ± 0.053)	0.022 - 0.05 (0.0365 ± 0.006)	0.033 - 0.059 (**0.042** ± 0.01)	0.014 - 0.035 (**0.027** ± 0.006)	0.037 - 0.059 (**0.045** ± 0.008)
10. Oral sucker width (OsW)	0.103 - 0.15 (**0.119** ± 0.014)	0.014 - 0.058 (**0.032** ± 0.007)	0.088 - 0.21 (**0.111** ± 0.044)	0.017 - 0.043 (**0.028** ± 0.005)	0.024 - 0.075 (**0.041** ± 0.015)	0.017 - 0.034 (**0.025** ± 0.006)	0.024 - 0.049 (**0.038** ± 0.009)
11. Oral to ventral sucker distance (OV)	0.116 - 0.407 (**0.236** ± 0.081)	0.037 - 0.443 (**0.215** ± 0.054)	0.332 - 0.556 (**0.463** ± 0.087)	0.088 - 0.31 (**0.188** ± 0.045)	0.133 - 0.486 (**0.211** ± 0.114)	0.1 - 0.217 (**0.13** ± 0.039)	0.066 - 0.193 (**0.136** ± 0.065)
12. Ventral sucker length (VsL)	0.1 - 0.162 (**0.135** ± 0.025)	0.025 - 0.075 (**0.041** ± 0.007)	0.088 - 0.201 (**0.13** ± 0.035)	0.022 - 0.248 (**0.041** ± 0.032)	0.032 - 0.1 (**0.048** ± 0.023)	0.031 - 0.036 (**0.033** ± 0.002)	0.022 - 0.046 (**0.033** ± 0.012)
13. Ventral sucker width (VsW)	0.103 - 0.167 (**0.144** ± 0.021)	0.017 - 0.075 (**0.036** ± 0.007)	0.095 - 0.175 (**0.138** ± 0.031)	0.02 - 0.169 (**0.036** ± 0.021)	0.027 - 0.089 (**0.042** ± 0.02)	0.026 - 0.039 (**0.032** ± 0.004)	0.023 - 0.036 (**0.032** ± 0.007)
14. Ventral sucker anterior to anterior body end distance (AV)	0.321 - 0.616 (**0.419** ± 0.09)	0.069 - 0.511 (**0.257** ± 0.057)	0.134 - 0.659 (**0.462** ± 0.21)	0.119 - 1.519 (**0.254** ± 0.195)	0.166 - 0.543 (**0.253** ± 0.12)	0.132 - 0.245 (**0.16** ± 0.038)	0.119 - 0.232 (**0.177** ± 0.057)
15. Mid-ventral sucker to anterior body end distance (O)	0.388 - 0.695 (**0.482** ± 0.096)	0.084 - 0.525 (**0.277** ± 0.059)	0.181 - 0.718 (**0.527** ± 0.216)	0.134 - 1.629 (**0.273** ± 0.208)	0.183 - 0.592 (**0.275** ± 0.131)	0.147 - 0.262 (**0.175** ± 0.039)	0.141 - 0.242 (**0.191** ± 0.051)
16. Holdfast organ length (BoL)	0.074 - 0.227 (**0.14** ± 0.042)	0.029 - 0.107 (**0.063** ± 0.012)	0.1 - 0.263 (**0.186** ± 0.055)	0.037 - 0.41 (**0.064** ± 0.053)	0.03 - 0.143 (**0.072** ± 0.032)	0.035 - 0.07 (0.049 ± 0.013)	0.046 - 0.141 (**0.086** ± 0.034)
17. Holdfast organ width (BoW)	0.183 - 0.3541 (**0.271** ± 0.271)	0.017 - 0.072 (**0.035** ± 0.009)	0.182 - 0.46 (**0.286** ± 0.079)	0.015 - 0.184 (**0.033** ± 0.024)	0.025 - 0.144 (**0.051** ± 0.041)	0.022 - 0.048 (**0.034** ± 0.01)	0.037 - 0.106 (**0.067** ± 0.021)
18. Ventral sucker to Holdfast organ distance (VB)	0.003 - 0.034 (**0.014** ± 0.01)	0.009 - 0.412 (**0.049** ± 0.031)	0.004 - 0.048 (**0.017** ± 0.016)	0.014 - 0.26 (**0.046** ± 0.04)	0.014 - 0.107 (**0.038** ± 0.029)	0.009 - 0.027 (**0.017** ± 0.006)	0.013 - 0.164 (**0.078** ± 0.078)

Table 5.2: The range (**mean** ± standard deviation) of different biometric index ratios of the seven different types of *Diplostomum* von Nordmann, 1832 metacercariae collected from the eyes and / or brain of a variety of fish species from the Okavango and Orange-Vaal River Systems. The sample size (n) indicates the number of specimens which were measured to obtain the morphometrics and 'b' and 'a' are respectively representative of width and length. Features that were not measured are indicated with **-**.

***Diplostomum* type**	**1** (n=12)	**2** (n=292)	**3** (n=19)	***a*** (n=53)	***b*** (n=11)	***c*** (n=15)	***d*** (n=8)
***Diplostomum* type image**							
19. b to a of body (BW:BL)(%)	74.185 - 120.065 (**98.272** ± 14.829)	7.252 - 53.16 (**23.638** ± 7.851)	59.135 - 110.603 (**85.508** ± 15.606)	12.561 - 69.012 (**26.965** ± 12.045)	18.507 - 32.46 (**25.039** ± 4.317)	38.962 - 67.271 (**47.867** ± 8.179)	40.276 - 60.826 (**48.305** ± 6.694)
20. Body (ab) / Bo (ab)	10.109 - 39.734 (**23.988** ± 10.137)	8.946 - 105.555 (**28.033** ± 9.585)	8.852 - 28.16 (**17.413** ± 6.929)	17.94 - 56.925 (**31.233** ± 9.677)	15.565 - 39.67 (**27.768** ± 6.308)	15.483 - 46.567 (**30.308** ± 7.843)	14.067 - 36.171 (**22.15** ± 7.213)
21. Body (ab) / Vs (ab)	23.664 -63.027 (**43.641** ± 13.837)	14.762 - 142.627 (**41.295** ± 16.07)	29.596 - 72.716 (**47.48** ± 14.881)	18.76 - 137.204 (**44.946** ± 21.984)	18.441 - 45.223 (**32.193** ± 10.452)	28.671 - 78.221 (**43.771** ± 16.665)	86.41 - 147.552 (**106.806** ± 35.29)
22. Os (ab) / Vs (ab)	0.431 - 1.754 (**0.797** ± 0.405)	0.28 - 3.707 (**0.903** ± 0.353)	0.275 - 0.99 (**0.537** ± 0.28)	0.5 - 2.563 (**0.899** ± 0.346)	0.401 - 1.172 (**0.845** ± 0.254)	0.161 - 0.989 (**0.657** ± 0.214)	1.549 - 2.649 (**2.16** ± 0.56)
23. BO (ab) / Vs (ab)	0.685 - 3.277 (**2.062** ± 0.753)	0.556 - 4.539 (**1.536** ± 0.549)	1.981 - 5.413 (**3.17** ± 1.154)	0.721 - 3.527 (**0.346** ± 0.522)	0.584 - 1.734 (**1.115** ± 0.438)	0.736 - 3.622 (**1.588** ± 0.93)	3.516 - 6.246 (**4.936** ± 1.368)
24. Os (ab) / Ph (ab)	-	1.549 - 13.504 (**5.377** ± 2.583)	3.218 - 5.528 (**4.191** ± 1.145)	3.649 - 8.479 (**5.745** ± 1.689)	1.699 - 9.204 (**4.68** ± 3.046)	**6.504**	1.981 - 4.795 (**3.469** ± 1.414)
25. O (ab) / BL (ab)	40.605 - 61.512 (**53.725** ± 6.519)	38.458 - 74.816 (**54.373** ± 4.405)	25.304 - 69.358 (**49.274** ± 14.37)	43.325 - 64.946 (**53.005** ± 3.767)	53.324 - 60.923 (**56.731** ± 2.372)	49.521 - 64.413 (**57.583** ± 4.113)	26.57 - 57.821 (**45.064** ± 16.396)
26. FbL (ab) / HbL (ab)	2.867 - 59.404 (**9.094** ± 15.903)	-	2.972 - 5.101 (**3.912** ± 0.689)	-	5.282 - 9.121 (**7.049** ± 1.257)	-	5.332 - 14.605 (**9.804** ± 2.586)
27. FbW (ab) / BL (ab)	0.736 - 1.187 (**0.98** ± 0.148)	-	0.568 - 1.092 (**0.796** ± 0.144)	-	0.176 - 0.272 (**0.232** ± 0.038)	-	0.405 - 0.6 (**0.484** ± 0.065)
28. Fb (ab) / Hb (ab) (%)	6.208 – 37.654 (**4.225** ± 10.539)	-	6.013 - 18.457 (**11.397** ± 3.895)	-	17.503 - 34.87 (**23.185** ± 6.481)	-	12.223 - 55.831 (**32.966** ± 14.254)

Table 5.3: A descriptive summary of the morphological characteristics of the seven different types of *Diplostomum* von Nordmann, 1832 metacercariae collected from the eyes and / or brain of a variety of fish species from the Okavango and Orange-Vaal River Systems. The sample size (n) indicates the number of specimens which were examined to obtain the morphological descriptions.

***Diplostomum* type**	**1** (n=12)	**2** (n=292)	**3** (n=19)	**a** (n=53)	**b** (n=11)	**c** (n=15)	**d** (n=8)
***Diplostomum* type Image**							
Location	Brain	Eyes and brain	Brain	Eyes and brain	Eyes and brain	Eyes and brain	Eyes and brain
Encysted \ free-moving	Encysted	Free-moving	Encysted	Free-moving	Free-moving	Free-moving	Free-moving
Granular bodies	Present	Present	Present	Present	Present	Present	Present
Body shape	Round, flattened with conical posterior part	Elongated to oval, fairly flattened	Round, flattened with conical posterior part	Elongated to oval, fairly flattened	Elongated, fairly flattened	Oval and broad	Oval and broad
Divided in fore- and hindbody	Distinct fore- and hindbody	No distinct division	Distinct fore- and hindbody	No distinct division	Distinct fore- and hindbody	No distinct division	Distinct fore- and hindbody
Anterior end shape	Round	Round, sometimes with small conical horns	Round, with a slight conical head	Round, sometimes with small conical horns	Round, with anterior conical horns	Round, with anterior conical horns	Round, with anterior conical horns
Posterior end shape	Conical	Conical	Conical	Conical	Conical	Less Conical	Conical

Table 5.3 (continued): Morphological characteristics of *Diplostomum* von Nordmann, 1832 metacercariae.

***Diplostomum* type**	**1**	**2**	**3**	**a**	**b**	**c**	**d**
Oral sucker shape / description	Circular	Circular / elliptical	Circular / elliptical	Circular / elliptical	Circular	Circular	Circular / elliptical
Oral sucker location	Sub-terminal	Terminal	Terminal	Terminal	Terminal	Terminal	Terminal
Ventral sucker shape / description	Circular / elliptical	Circular / elliptical	Circular	Circular / elliptical	Circular, but very indistinctive	Circular, but very indistinctive	Circular, but very indistinctive
Ventral sucker location	Just above holdfast organ	Middle of body	Just above holdfast organ	Middle of body	Indistinctive; middle of body	Indistinctive; middle of body	Indistinctive; middle of body
Holdfast organ shape / description	Kidney / bone shaped	Elliptical	Kidney / bone shaped	Elliptical	Elliptical	Elliptical	Elliptical
Holdfast organ location	Posterior in forebody	Just in front of genital primordium	Posterior in forebody	Just in front of genital primordium	Posterior in forebody	Just in front of genital primordium	Just in front of genital primordium
Genital primordium present / visible	Present	Present	Present	Present	Present	Present	Present
Genital primordium shape / description	Irregularly shaped	Circular	Irregularly shaped	Circular	Circular	Circular	Circular
Genital primordium location	Hindbody	Posterior in body	Hindbody	Posterior in body	Hindbody	Posterior in body	Posterior in body
Pseudosuckers present / visible	Present	Absent	Present	Present, but poorly developed	Present, well developed	Present, well developed	Present, well developed
Pseudosuckers shape / description	Large and on lateral sides of oesophagus	Absent but small anterior conical horns may present	Large and alongside oral sucker	Not distinctly developed or on conical horns placed laterally from oral sucker	On conical horns, situated on lateral side of oral sucker	On conical horns, situated on lateral side of oral sucker	On conical horns, situated on lateral side of oral sucker

Table 5.3 (continued): Morphological characteristics of *Diplostomum* von Nordmann, 1832 metacercariae.

***Diplostomum* type**	**1**	**2**	**3**	**a**	**b**	**c**	**d**
Pharynx present / visible	Absent	Present	Present	Present	Present	Present	Present
Pharynx description	Absent	Small (sometimes not visible)	Small, rounded, (sometimes not visible)	Small, (sometimes not visible)	Small, (sometimes not visible)	Small, (sometimes not visible)	Small, (sometimes not visible)
Prepharynx present / visible	Absent	Sometimes visible	Present	Sometimes visible	Present	Absent	Present
Prepharynx description	Absent	Very poorly developed	Short; leads from oral sucker to pharynx	Very poorly developed	Small (sometimes not visible)	Absent	Small (sometimes not visible)
Oesophagus present / visible	Present	Present	Present	Present	Present	Absent	Present
Oesophagus description	From oral sucker, opening into intestinal caeca	From pharynx, opening into intestinal caeca	From oral sucker, opening into intestinal caeca	From pharynx, opening into intestinal caeca	From pharynx, opening into intestinal caeca	Absent	From pharynx, opening into intestinal caeca
Intestinal caeca present / visible	Present	Present	Present	Present	Present	Present	Present
Intestinal caeca description	Up to hindbody	Up to genital primordium	Into hindbody	Up to genital primordium	Up to genital primordium	Up to genital primordium	Up to holdfast organ

Table 5.4: Comparison of mean measurements (mm) of morphologically similar diplostomatid metacercariae *Tylodelphys* Diesing, 1850 sp. 1, *Tylodelphys* Diesing, 1850 sp. 2 recovered from the brain cavity of the sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) from three freshwater bodies in Tanzania (Chibwana and Nkwengulia 2010) and *Diplostomum* von Nordmann, 1832 type 2 from the eyes and brain of different freshwater fish species from the Okavango River, Botswana (**present study**). Morphological similar species *Diplostomum mashonense* Beverley-Burton, 1963 (species type 3) from Tanzania (Chibwana and Nkwenguilia 2010) and *Diplostomum* von Nordmann, 1832 type a from the Okavango River, Botswana (**present study**) are also presented. Features that were not measured are indicated with - and 'b' and 'a' are respectively representative of width and length.

Diplostomatid metacercariae	*Tylodelphys* sp. 1			*Tylodelphys* sp. 2			***Diplostomum* type 2**	*Diplostomum mashonense* (species type 3)			***Diplostomum* type a**
Locality	Lake Victoria	Ruvu River	Kilombero River	Lake Victoria	Ruvu River	Kilombero River	**Okavango River**	Lake Victoria	Ruvu River	Kilombero River	**Okavango River**
1. Body length (BL)	1.022	1.23	1.043	0.495	0.667	0.560	**0.495**	0.818	0.799	0.790	**0.507**
2. Body width (BW)	0.217	0.194	0.214	0.1	0.128	0.111	**0.112**	0.214	0.250	0.224	**0.127**
7. Pharynx length (PhL)	0.04	0.051	0.041	0.023	0.021	0.022	**0.022**	0.039	0.031	0.038	**0.019**
8. Pharynx width (PhW)	0.029	0.043	0.032	0.019	0.019	0.019	**0.013**	0.026	0.02	0.025	**0.012**
9. Oral sucker length (OsL)	0.037	0.061	0.041	0.028	0.031	0.03	**0.04**	0.036	0.04	0.038	**0.0365**
10. Oral sucker width (OsW)	0.037	0.059	0.041	0.028	0.031	0.029	**0.032**	0.036	0.04	0.042	**0.028**
11. Oral to ventral sucker distance (OV)	-	-	-	-	-	-	**0.215**	-	-	-	**0.188**
12. Ventral sucker length (VsL)	0.036	0.044	0.038	0.021	0.021	0.021	**0.041**	0.036	0.036	0.039	**0.041**
13. Ventral sucker width (VsW)	0.036	0.041	0.037	0.021	0.022	0.021	**0.036**	0.037	0.037	0.039	**0.036**

Table 5.4 (continued): Comparison of diplostomatid metacercariae collected from Tanzania and Botswana (**present study**).

Diplostomatid metacercariae	*Tylodelphys* sp. 1			*Tylodelphys* sp. 2			***Diplostomum* type 2**	*Diplostomum mashonense* (species type 3)			***Diplostomum* type a**
Locality	Lake Victoria	Ruvu River	Kilombero River	Lake Victoria	Ruvu River	Kilombero River	**Okavango River**	Lake Victoria	Ruvu River	Kilombero River	**Okavango River**
14. Ventral sucker anterior to anterior body end distance (AV)	-	-	-	-	-	-	**0.257**	-	-	-	**0.254**
15. Mid-ventral sucker to anterior body end distance (O)	0.496	0.792	0.555	0.248	0.366	0.282	**0.277**	0.330	0.302	0.322	**0.273**
16. Brande's organ length (BoL)	0.104	0.163	0.117	0.061	0.06	0.061	**0.063**	0.11	0.092	0.1	**0.064**
17. Brande's organ width (BoW)	0.065	0.062	0.063	0.037	0.022	0.031	**0.035**	0.076	0.070	0.073	**0.033**
18. Ventral sucker to Brande's Organ distance (VB)	-	-	-	-	-	-	**0.049**	-	-	-	**0.046**
19. b to a of body (BW:BL)(%)	21	16	21	21	19	20	**23.638**	26	36	48	**26.965**
20. Body (ab) / Bo (ab)	33.06	24.66	31.68	23.19	66.49	39.92	**28.033**	21.51	27.84	25.39	**31.233**
21. Body (ab) / Vs (ab)	-	-	-	-	-	-	**41.295**	-	-	-	**44.946**
22. Os (ab) / Vs (ab)	1.07	2.18	1.23	1.98	2.55	2.1	**0.903**	1.01	1.22	1.12	**0.899**
23. Bo (ab) / Vs (ab)	5.02	5.87	5.12	5.51	3.47	4.53	**1.536**	6.42	4.94	5.18	**0.346**
24. Os (ab) / Ph (ab)	1.24	1.88	1.38	2.01	2.59	2.17	**5.377**	1.33	2.41	1.91	**5.745**
25. O (ab) / BL (ab)	49	65	53	51	55	50	**54.373**	41	44	41	**53.005**

DESCRIPTION OF METACERCARIAL TYPES

Diplostomum type 1

Hosts (site of infection)

Okavango: *Marcusenius macrolepidotus* (Peters, 1852) (brain); *Petrocephalus catostoma* (Günther, 1866) (brain)

Diagnostic and Morphometric Characteristics: Figures 5.3 A and B and Tables 5.1 - 5.3. Description based on 12 specimens; measurements (mm) represent the length of structures.

Metacercariae not free-moving, encapsulated by thin cyst wall of parasite and / or host origin. Depending on stage of infection, metacercariae can vary in degree of development. Body of developed metacercariae distinctly divided into round, large, broad and flattened forebody 0.515 - 1.000 (0.733 ± 0.152). Hindbody small, slightly conical 0.017 - 0.290 (0.155 ± 0.068), covered with minute spikes. Oral sucker 0.103 - 0.150 (0.119 ± 0.014), circular in outline, situated sub-terminally. Large pseudosuckers on lateral sides of oesophagus. Ventral sucker 0.100 - 0.162 (0.135 ± 0.025), circular or elliptical in outline, situated just above holdfast organ 0.074 - 0.227 (0.140 ± 0.042), kidney- or bone-shaped placed anterior in hindbody. Genital primordium situated in hindbody. Prepharynx and pharynx absent. Oral sucker followed by oesophagus, which bifurcates into intestinal caeca extending into hindbody.

Remarks:

The first record of similar thin-walled encysted metacercariae occurring in the brain of Okavango freshwater fishes was given by Jansen van Rensburg (2006), who named it *Diplostomulum* type 1. The description was based on *Diplostomum*-type I Prudhoe and Hussey, 1977 metacercariae found in the mesenteries of sharptooth catfish, *Clarias gariepinus* from the Limpopo System, South Africa (Prudhoe and Hussey 1977). Previous records from the Okavango River included this larval type from the brain of *M. macrolepidotus*, but the present study is the first report of these diplostomatid metacercariae from the brain of *P. catostoma* (Figure 7.4 A). The present study as well as the study conducted by Jansen van Rensburg (2006) only found the metacercarial

type in the brain of species belonging to the family Mormyridae. This fish family is endemic to Africa and occupy well-vegetated sites in freshwater systems (see Chapter 6).

Prudhoe and Hussey (1977) stated that metacercariae representing the body structure of *Diplostomum* type 1 are frequent parasites of the eyes and central nervous system of freshwater fishes. The formation of the thin walled capsule of connective tissue surrounding it is induced by itself. Self-induced cyst formation is not a characteristic of *Diplostomum* larvae but rather of another larval group namely *Tetracotyle*, although their cyst formation is usually found to be thicker (Niewiadomska 2001).

The morphological characteristics known for *Tetracotyle* larvae such as: an oval, pyriform or ovate-oblong contour, with a ventral compression; a ventral sucker which is often glandular; a pair of muscular pseudosuckers on both sides of the pharynx and well-differentiated genital organs are all present with *Diplostomum* type 1 (Figures 5.3 A and B). Throughout historic literature *Tetracotyle* has been treated as an adult and later as a larval genus, but presently it is regarded as a subgenus of the genus *Diplostomum*. Some authors consider it to be synonymous with the subgenus *Tylodelphys* (see Chapter 4). Although the *Diplostomum* type 1 larvae are not typical representatives of metacercariae of *Diplostomum* species and much literature exist ascribing it to the genus *Tetracotyle*, the present study includes it in the *Diplostomum* genus.

Prudhoe and Hussey (1977) described the similar-looking *Diplostomum*-type I metacercariae to be dorso-ventrally flattened and leaf-like in appearance. The body is divided into an anterior segment (forebody) and a posterior segment (hindbody or caudal process) which contain the genital primordia. The placement of the oral sucker was also found to be sub-terminal with the pseudosuckers, scarcely noticeable and situated as a pair of glandular pads alongside the oral sucker. A transversely oval ventral sucker is present just in front of the rounded holdfast organ, situated in the middle of the body.

This differs from *Diplostomum* type 1 in which the holdfast organ is more kidney- or bone-shaped. Another marked difference is the presence of a prepharynx and

pharynx, noted by Prudhoe and Hussey (1977), but which could not be identified in *Diplostomum* type 1 metacercariae. The oral sucker leads into an oesophagus which immediately bifurcates into the intestinal caeca and runs transversely for a short distance before turning sharply to descend and terminate in the posterior part of the body. Although the similarity in their appearance is apparent, in general the morphometrics of all the body structures of the metacercariae described by Prudhoe and Hussey (1977) were much smaller compared to *Diplostomum* type 1 metacercariae.

Diplostomum type 1 also shows similarities with *Tetracotyle singhi* Pandey, 1970 described from the mesenteries of the visceral organs of spotted snakehead, *Channa punctata* (Bloch, 1793), collected from an Indian fish market (Pandey 1970). The metacercariae are encysted and occur within a dense fluid, apparently from parasitic origin. The body of *Diplostomum* type 1 metacercariae can be clearly divided, whilst with *T. singhi* the fore- and hindbody are not clearly demarcated.

Both their oral suckers are cup-shaped and sub-terminal, but the width of this structure is slightly larger with *Diplostomum* type 1. The ventral sucker of *T. singhi* is larger than that of *Diplostomum* type 1, but in both regards the ventral sucker is still larger than the oral sucker. The pseudosuckers of both types are muscular and located lateral to the oesophagus. A well developed, lobed holdfast organ is situated posterior to the ventral sucker and in both the gonads are represented by a mass of dark staining cells located posterior to the holdfast organ. The thinly encysted larvae of *Diplostomum* type 1 also show a marked similarity to *Tetracotyloides gymnoti* Szidat, 1969 sampled as free metacercariae from the orbit and vitreous humours and brains of banded knifefish, *Gymnotus carapo* Linnaeus, 1758 in South America. The final developmental stage consists of a circular forebody and a short caudal region, which includes the already formed ovary and testes. Lateral to the sub-terminal oral sucker are the pseudosuckers. The holdfast organ is almost circular, lying a short distance behind the ventral sucker and the caeca reach the posterior end of the body. Differences include that the oral and ventral suckers are smaller.

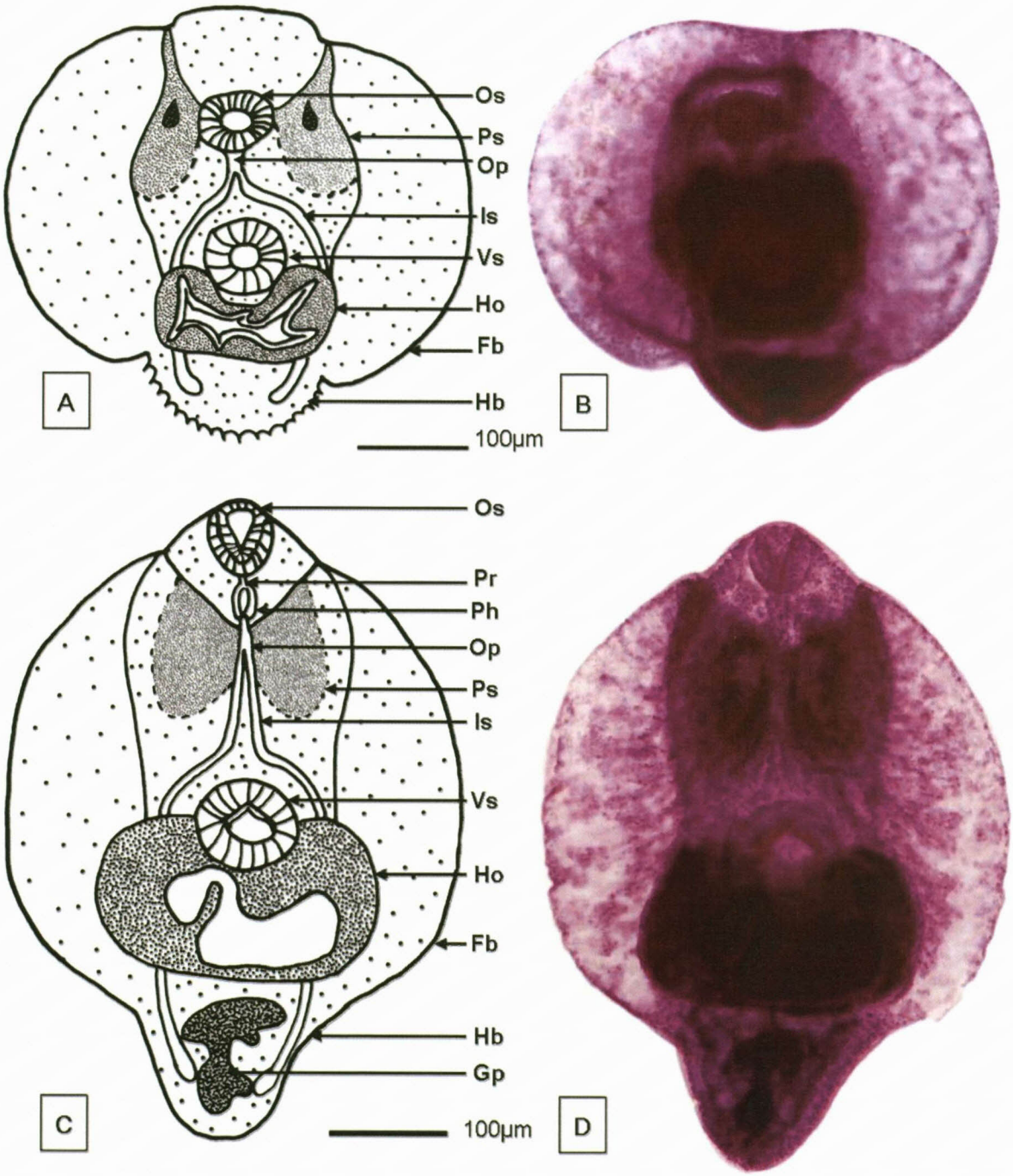


Figure 5.3: (**A**, **C**) Microscope projection drawings (**B**, **D**) and light micrographs of metacercariae of (**A**, **B**) *Diplostomum* von Nordmann, 1832 type 1 and (**C**, **D**) *Diplostomum* von Nordmann, 1832 type 3. **Fb**: forebody; **Gp**: genital primordium; **Hb**: hindbody; **Ho**: holdfast organ; **Is**: intestinal caeca; **Op**: oesophagus; **Os**: oral sucker; **Ph**: pharynx; **Pr**: prepharynx; **Ps**: pseudosucker; **Vs**: ventral sucker.

Many similarities are shared with *Tetracotyle baughi* (Pandey, 1970) collected from the liver and mesenteries from gangetic leaffish, *Nandus nandus* (Hamilton, 1822) bought from an Indian fish market. The body is clearly divided into a fore- and hindbody, with the oval forebody being larger than the slightly conical-shaped hindbody. It differs in that both body divisions of *T. baughi* are larger than those of *Diplostomum* type 1. The oral suckers are similar in size and shape (circular), but with *T. baughi* it is situated terminally, whilst in the present study it was distinctly found sub-terminally. When comparing it to the line drawings of Pandey (1970) this terminal placement could not be verified. It rather illustrates the oral sucker of *T. baughi* metacercariae being placed sub-terminally and the species therefore still sharing similarity with *Diplostomum* type 1. Other similarities include equal sized ventral suckers, circular to elliptical in shape and located almost in the middle of the forebody. Well-developed pseudosuckers are present on the lateral side of the oesophagus, adjacent to the pharynx. The holdfast organ is lobed and is located posterior to the ventral sucker.

Apart from a terminally placed oral sucker, the only main difference is the presence of well-developed gonads within the hindbody of *T. baughi*. This suggests that a terminally placed oral sucker could be characteristic of the anatomy of older developmental stages. *Diplostomum* type 1 specimens collected during the present study could therefore be synonymous with *Diplostomum* type 3 metacercariae, with the latter being a more mature metacercarial stage. The presence of thick-walled cysts and ill-defined morphology might act as further indications of *Diplostomum* type 3 metacercariae being a lesser-developed stage.

Diplostomum type 2

Hosts (site of infection)

Okavango: *Marcusenius macrolepidotus* (eyes and brain); *Petrocephalus catostoma* (eyes and brain); *Pollimyrus castelnaui* (Boulenger, 1911) (eyes and brain); *Barbus poechii* Steindachner, 1911 (eyes); *Brycinus lateralis* (Boulenger, 1900) (eyes and brain); *Rhabdalestes maunensis* (Fowler, 1935) (brain); *Hepsetus odoe* (Bloch, 1794) (brain); *Tilapia sparrmanii* (eyes)

Orange-Vaal: *Labeo umbratus* (A. Smith, 1841) (eyes); *Labeo capensis* (eyes)

Diagnostic and Morphometric Characteristics: Figures 5.4 A and B and Tables 5.1 - 5.3. Description based on 292 specimens; measurements (mm) represent the length of structures.

Metacercariae free-moving, mostly elongated, fairly flattened body 0.199 - 0.848 (0.495 ± 0.109). Some specimens have more oval, flattened appearance. No clear distinction present between fore- and hindbody. Pseudosuckers absent, which act as main distinguishing characteristic from *Diplostomum* type a (Figures 5.4 C and D). Anterior body end round, posterior end conically shaped. Oral sucker 0.021 - 0.080 (0.040 ± 0.008) situated terminally and together with the ventral sucker 0.025 - 0.075 (0.041 ± 0.007) range in outline from circular to elliptical. Holdfast organ 0.029 - 0.107 (0.063 ± 0.012) mostly longitudinal elliptical in shape. Genital primordium situated in posterior third of body. Small pharynx present (sometimes not visible), prepharynx very difficult to distinguish. Pharynx 0.010 - 0.045 (0.022 ± 0.006) leads into oesophagus, bifurcates into intestinal caeca, extend through body up to genital primordium.

Remarks:

Similar to *Diplostomum* type 1, metacercariae of *Diplostomum* type 2 were first identified and named by Jansen Van Rensburg (2006) from the Okavango River found within the eye of *M. macrolepidotus*. The present study is the first report of these diplostomatid metacercariae from the eyes and / or brain of the other above listed fish species collected from the Okavango and Orange-Vaal River Systems. A wide variety of species were found to act as second intermediate host for this metacercarial type ranging from open water fast swimmers (*B. lateralis*), dense vegetation inhabitants (*P. castelnaui*) and bottom dwellers (*L. capensis*). These species also belong to different fish families which greatly vary in natural distribution (see Chapter 6).

Hepsetus odoe is distributed throughout south-central Africa and acts as the sole representative of the family Hepsetidae which is endemic to this continent. Another family endemic to the African fish fauna, also having a tropical distribution, is the family Mormyridae. Three mormyrid fish species (*M. macrolepidotus*, *P. catostoma* and *P. castelnaui*) were collected and found to be infected. The families of the other

infected fish species have a more world-wide distribution, such Characidae (*B. lateralis*, *R. maunensis*) being present in South America and the distribution of the family Cichlidae (see Chapter 6) also including Arabia and India. The family Cyprinidae have a broad distribution which includes Africa, Europe, Asia and North America, but the three representative species (*B. poechii*, *L. umbratus*, *L. capensis*) found to be infected are limited to southern Africa. The infected *Labeo* species were collected from the Orange-Vaal River System, whilst the *T. sparrmanii* population were uninfected in this location. Diplostomatid metacercariae did, however, occur in the eyes of this tilapia species collected from the Okavango River System. The range of fish families, varying in distribution and ecology, infected by this diplostomatid type clearly illustrates its opportunistic nature and non-specificity for second intermediate host species.

The nomenclature used by Jansen van Rensburg (2006) to describe *Diplostomulum* type 2 was based on the free-moving metacercariae, *Diplostomum*-type II Prudhoe and Hussey, 1977 found in the cranial cavity of sharptooth catfish, *Clarias gariepinus* from the northern region of South Africa previously known as Transvaal (Prudhoe and Hussey 1977). Similar to the material of the present study, the body is elongated and dorso-ventrally flattened but it differs in that it consists of a distinct fore- and hindbody. A demarcation between the two body divisions is also not a character of the similar named metacercariae of the present study. The length and width of the metacercarial body and holdfast organ described by Prudhoe and Hussey (1977) are larger compared to the present material. Another difference is the presence of well-developed horseshoe-like testes in the hindbody of *Diplostomum*-type II, whilst in the present material only a small grouping of darker stained cells could be identified as the genital primordium. Similarities include a terminal-placed oral sucker and the ventral sucker and pharynx also being similar in shape, size and placement. A marked difference occurs with the presence of two pseudosuckers situated on the lateral border of the anterior part of the body, adjacent to the oral sucker, whilst *Diplostomum* type 2 metacercariae are characterised by the complete absence of pseudosuckers. Since these structures were not very well-developed, *Diplostomum*-type II metacercariae could be considered morphologically similar to *Diplostomum* type a metacercariae which were recovered during the present study.

Metacercariae collected from the brain, eyes and body cavity of stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) at Lucknow in India was described by Pandey (1970) as a new species, *Diplostomulum ophthalmi* Pandey, 1970. These metacercariae are morphologically similar to *Diplostomum* type 2 regarding the presence of an elongated, flattened body with no clear distinction between a fore- and hindbody, the ventral sucker placed approximately in the middle of the body as well as the absence of pseudosuckers. Since no morphometrics were provided by Pandey (1970) a further comparison between the two types of metacercariae could not be made. A marked difference is present in the oral sucker of *D. ophthalmi* being placed sub-terminally, whilst it is terminal in *Diplostomum* type 2 metacercariae.

Diplostomum type 2 also show similarities with the metacercariae of *Tylodelphys grandis* Zhokhov, Morozova and Tessema, 2010 and an undescribed *Tylodelphys* species, sampled by Chibwana and Nkwengulia (2010). These metacercariae were collected from the cranial cavity and adipose tissue surrounding the brain of *C. gariepinus* from water bodies in Ethiopia and Tanzania. The metacercariae are free-moving, the body shape tongue-like and slightly concave at the ventral side. A rounded anterior end with a terminal-placed oral sucker is present. The absence of both pseudosuckers and a clear demarcation between the fore- and hindbody is also shared. *Tylodelphys grandis* metacercariae differ from *Diplostomum* type 2 metacercariae by being approximately twice in size. The differences in morphometrics between *Diplostomum* type 2 and the two described *Tylodelphys* species are summarised in Table 5.4 and illustrates the high morphometric variation which can occur amongst the same diplostomatid metacercariae sampled from different localities and from different as well as similar fish host species.

A great deal of similarity exists between *Diplostomum* type 2 metacercariae and the free-moving *Diplostomulum cerebralis* Chakrabarti, 1967 metacercariae, described from the cranium of spotted snakehead, *C. punctata* from a fish market in India (Chakrabarti 1967). The most apparent similarities are the absence of pseudosuckers and the fore- and hindbody not being distinguishable. The body is elongated, fairly broad and flattened with the anterior end round, whilst the posterior end can be blunt, pointed or conical depending upon the degree of contraction of the body. The suckers

are similar in shape and size and the holdfast organs are also similar in appearance and location. A prepharynx is not observed, whilst a pharynx is clearly visible and equal in size. Differences do occur such as that the general body size of *D. cerebralis* is bigger than that of *Diplostomum* type 2. With the latter the oral sucker is placed terminally, whilst with the described species it is sub-terminal (Table 5.3).

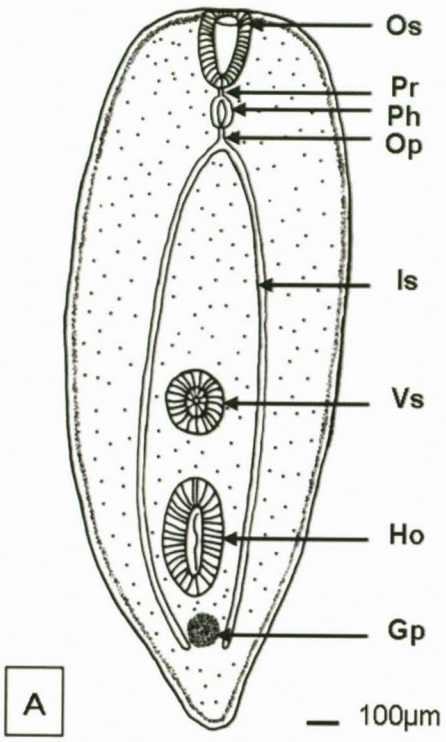
The metacercariae collected during the present study also show similar body and organ measurements to that of *Diplostomum clavatum* von Nordmann, 1832, described from the vitreous humours of various fish hosts belonging to the Cyprinidae and Salmonidae families in North America (Hoffman 1960) and Poland (Kozicka and Niewiadomska 1960). Synonymous species to *D. clavatum* include *Tylodelphys clavata* Diesing 1850 described from European perch, *Perca fluviatilis* Linnaeus, 1758 and adult trematodes described as *Tylodelphys conifera* Dubois, 1937 collected from great crested grebe, *Podiceps cristatus* (Linnaeus, 1758) in Poland (Kozicka and Niewiadomska 1960).

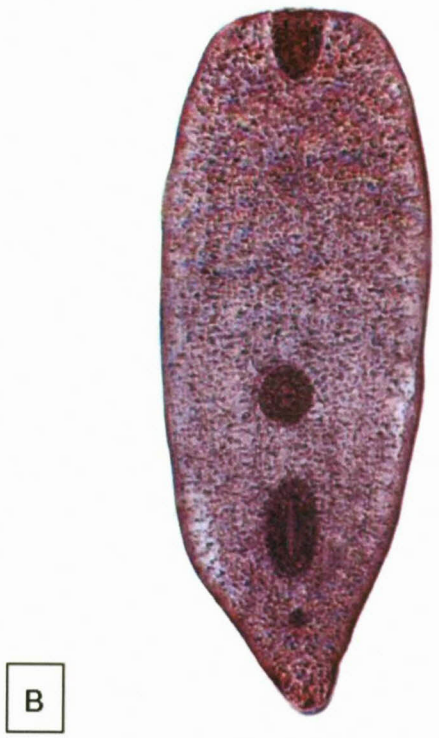
The body shape of *D. clavatum* is elongated with the length more than twice the width whilst the hindbody is not clearly set off from the forebody. The oral sucker is placed terminally followed by a prepharynx and pharynx. The placement of the ventral sucker is slightly posterior to the centre of the body and the holdfast organ is also placed just in front of the posterior narrowing part of the body. Kozicka and Niewiadomska (1960) confirm the absence of pseudosuckers, whilst Hoffman (1960) states its presence lateral to the oral sucker. The only marked difference between *Diplostomum* type 2 and *Diplostomum* type a metacercariae, collected during the present study, is the respective absence and presence of pseudosuckers. It emphasises the possibility of different developmental stages of the same metacercarial type being present.

Variation in morphometrics and morphology throughout metacercarial development has been noted during previous studies. For example Kozicka and Niewiadomska (1960) stated that the youngest stage encountered for the metacercariae of *Tylodelphys clavata* inside the eye of *P. fluviatilis* had morphological features common with the cercariae of the species. The only marked differences were that the metacercarial body dimensions were slightly larger than those of the cercariae, which is a result of the larvae gradually growing in size.

Other morphological differences also develop through time such as: the formation of a more slender posterior part; a distinctive pharynx and the body turning opaque. Hoffman (1960) stated that with another species, *Diplostomulum scheuringi* Hughes, 1929, recorded from the vitreous humour of various North American fish, the pseudosuckers were also only present within the mature metacercariae. The morphology of early metacercarial developmental stages therefore correlates with the metacercariae of *Diplostomum* type 2, whilst the latter corresponds with *Diplostomum* type a.

This variation in morphology during different maturity stages is clearly illustrated by a study conducted by Sweeting (1974). He experimentally infected African clawed toads, *Xenopus laevis* Daudin, 1802 with *D. spathaceum* cercariae and collected the lens-inhabiting metacercariae 30 and 65 days post-infection. The earlier developmental stages showed similar morphology to that of *Diplostomum* type 2 metacercariae. The presence of well-differentiated pseudosuckers and a rounded body of the mature *D. spathaceum* metacercariae, collected 65 days post-infection, were similar to characteristics of *Diplostomum* type c metacercariae. This stresses the fact that the time after cercarial penetration and the stage of metacercarial development need to be taken into account with the identification of diplostomatid metacercariae (see Chapter 4).





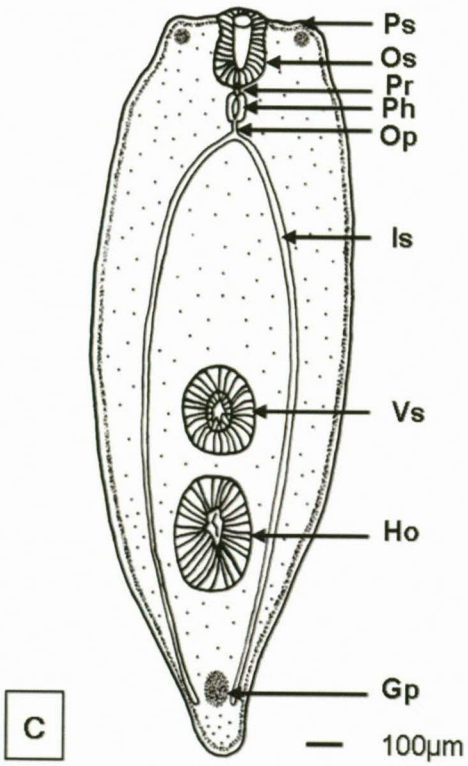


Figure 5.4: (**A**, **C**) Microscope projection drawings and (**B**, **D**) light micrographs of metacercariae of (**A**, **B**) *Diplostomum* von Nordmann, 1832 type 2 and (**C**, **D**) *Diplostomum* von Nordmann, 1832 type a. **Gp**: genital primordium; **Ho**: holdfast organ; **Is**: intestinal caeca; **Op**: oesophagus; **Os**: oral sucker; **Ph**: pharynx; **Pr**: prepharynx; **Ps**: pseudosucker; **Vs**: ventral sucker.

***Diplostomum* type 3**

Hosts (site of infection)

Okavango: *Pollimyrus castelnaui* (brain); *Petrocephalus catostoma* (brain)

Diagnostic and Morphometric Characteristics: Figures 5.3 C and D and Tables 5.1 - 5.3. Description based on 19 specimens; measurements (mm) represent the length of structures.

Body distinctly divided into broad forebody 0.464 - 1.039 (0.774 ± 0.217) and narrower hindbody 0.106 - 0.257 (0.198 ± 0.045). Anterior end of body mainly round, with slight conical head. Terminal part of hindbody conically shaped. Oral sucker 0.086 - 0.238 (0.124 ± 0.053) ranges from round to elliptical in outline, situated terminally. Ventral sucker 0.088 - 0.201 (0.130 ± 0.035), circular in outline. Holdfast organ 0.100 - 0.263 (0.186 ± 0.055), either kidney- or bone-shaped. Genital primordium irregularly shaped, present within hindbody. Pseudosuckers form glandular pads alongside oral sucker. Short prepharynx, rounded pharynx 0.053 - 0.092 (0.073 ± 0.016), both not always observable. Pharynx leads to oesophagus, which bifurcates to intestinal caeca and extends into hindbody.

Remarks:

The present study is the first report of these diplostomatid metacercariae from the brain of *P. castelnaui* from the Okavango River System. The collected encysted metacercariae were, however, underdeveloped and not suitable for morphometrical measurements. The presence of thick cyst material surrounding the specimens also prohibited the observation and identification of internal structures by means of light microscopy. Similar, yet more developed (thinner cyst walled) metacercariae were collected by Jansen van Rensburg (2006) from the brain *P. catostoma.* These specimens were used to obtain morphometrics for *Diplostomum* type 3 metacercariae. Similar to *Diplostomum* type 1, these metacercariae have only been collected from fish belonging to the family Mormyridae. It is, however, possible that *Diplostomum* type 1 is an earlier (lesser) developmental stage of *Diplostomum* type 3 metacercariae. Jansen van Rensburg (2006) differentiated between the two diplostomatid types by means of two main criteria. *Diplostomum* type 3 metacercariae have 1) a terminally placed oral

sucker and 2) the presence of a pharynx. These characters are subjected to the age of the metacercarial development and therefore the possibility of similar types should still be considered. Both types exhibit characteristics of the subgenus *Tetracotyle*, especially regarding the presence of the self-induced cyst walls, well-developed pseudosuckers and the cup-shaped body (Niewiadomska 2001).

Diplostomum type 3 also shows similarities with *Diplostomulum* metacercariae found within the mesenteries of Kerala catfish, *Mystus armatus* (Day, 1865) in India (Rai and Pande 1968). Pseudosuckers are well-developed and placed laterally to the oesophagus, a pharynx is present and the holdfast organ is situated directly behind the ventral sucker. Differences include that the forebody of *Diplostomum* type 3 is markedly broader than the smaller, conical hindbody, whilst with the metacercariae from India, the forebody gradually tapers into the hindbody resulting in a teardrop body shape. Similarities are also shared with *Diplostomum baeri eucaliae* Hoffman and Hundley, 1957, found unencysted in the brain of brook stickleback, *Culaea inconstans* (Kirtland, 1840), North America. Many of these metacercariae were reported to occur in "pseudo-cysts", of host origin, inside the fishes' brains (Hoffman and Hundley 1957). Similarities include a terminally placed oral sucker, an oval-shaped forebody, a conical-shaped hindbody, a large and circular ventral sucker which is placed roughly in the middle of the forebody as well as the presence of a prepharynx and pharynx.

Differences include the presence of minute spines on the forebody of *D. baeri eucaliae* and the circular shape of the holdfast organ, opposed to the kidney / bone shape of *Diplostomum* type 3. The pseudosuckers are smaller, not gland-like and are situated antero-lateral to the oral sucker. These differ from the large glandular pads placed laterally to the oesophagus of *Diplostomum* type 3. Overall the body measurements of *D. baeri eucaliae* are smaller than those of *Diplostomum* type 3. Regarding the shape of the holdfast organ as well as the placement of the pseudosuckers, *D. baeri eucaliae* metacercariae share more morphological similarity with *Diplostomum* type d.

Diplostomum type 3 metacercariae also show similarity with a *Tetracotyle* species described from brook stickleback (Hoffman 1960). Clear distinction is found between the fore- and hindbody, with the anterior part being cup-shaped and the posterior part forming a short, rounded prominence. A pharynx is present, just beneath the terminally

placed oral sucker. The placements of the pseudosuckers of the two types do, however, differ. With the *Tetracotyle* species it is placed on the antero-lateral edges of the oral sucker, whilst with *Diplostomum* type 3 it is laterally alongside the oesophagus.

Diplostomum type 3 also show similarities with encysted *Diplostomulum* metacercariae described from the mesenteries of spotted knifejaw, *Oplegnathus punctatus* (Temminck and Schlegel, 1844) from India (Rai and Pande 1968). Although the pseudosuckers are not glandular, these are situated on either side of the oesophagus and the oral sucker is found terminally. Similarly, the holdfast organ is situated immediately behind the circularly-shaped ventral sucker. A small, conical hindbody and a broad, oval forebody are distinguished. The two types differ in the placement of the ventral sucker. In the case of *Diplostomum* type 3 it is found in the middle of the forebody, whilst in the *Tetracotyle* species it is more anterior. In general, the body size and anatomical structures of *Diplostomum* type 3 are larger than that of the metacercariae described by Rai and Pande (1968).

Diplostomum type a

Hosts (site of infection)

Okavango: *Brycinus lateralis* (eyes and brain); *Hepsetus odoe* (brain); *Tilapia sparrmanii* (eyes)

Orange-Vaal: *Labeo capensis* (eyes)

Diagnostic and Morphometric Characteristics: Figures 5.4 C and D and Tables 5.1 - 5.3. Description based on 53 specimens; measurements (mm) represent the length of structures.

Metacercariae free-moving with narrow, elongated, fairly flattened body 0.212 - 3.225 (0.507 ± 0.397). Some specimens may have a more oval, compressed form. No clear distinction between fore- and hindbody. Anterior body end round and posterior part terminates in cone shape. Oral sucker 0.022 - 0.050 (0.0365 ± 0.006), situated terminally. Ventral sucker 0.022 - 0.248 (0.041 ± 0.032), circular to elliptical. Holdfast organ 0.037 - 0.410 (0.064 ± 0.053), elliptical. Pseudosuckers are difficult to

distinguish, form small projections, antero-lateral side of oral sucker. Genital primordium situated in posterior third of body. Pharynx 0.013 - 0.026 (0.019 ± 0.004) and prepharynx not always easily observed. Pharynx leads into oesophagus, bifurcates into intestinal caeca. Latter extends through body, up to genital primordium.

Remarks:

The present study is the first report of *Diplostomum* type a metacercariae collected from the Okavango and Orange-Vaal River Systems. Similar to *Diplostomum* type 2, the metacercariae were found in diverse fish families and sometimes from both the brain and eyes. The presence of these parasites in a variety of ecological classes of fish, illustrates the opportunistic nature of cercariae to try and penetrate any living tissue. *Diplostomum* type a metacercariae mainly differ from *Diplostomum* type 2 with the presence of pseudosuckers. At most these are very small horn-like structures situated on the antero-lateral side of the oral sucker. In many specimens it is not well-developed and undetectable and therefore *Diplostomum* type a metacercariae could easily be erroneously mistaken for larvae of *Diplostomum* type 2. The possibility also exists for the former type to represent a more mature metacercarial stage of the latter. The similarities shared by *Diplostomum* type 2 and *Diplostomum* type a are also emphasised by the description of Jansen van Rensburg (2006) of *Diplostomulum* type 2 metacercariae collected from *R. maunensis* in the Okavango River. These metacercariae are relatively similar in shape and size to *Diplostomum* type a and also exhibits two earlike protrusions present on the opposite sides of the oral sucker (Jansen van Rensburg 2006).

Diplostomum type a metacercariae share similarities with *Diplostomulum elongates* (Singh, 1957) collected from the mesenteries of banded gourami, *Colisa fasciata* (Bloch and Schneider, 1801); Philippine catfish, *Clarias batrachus* (Linnaeus, 1758) and spotted knifejaw, *O. punctatus* in India (Hoffman 1960; Rai and Pande 1968). The body of the described species is elongated and flattened with an ill-defined division into a fore- and hindbody. Pseudosuckers are visible in mounted specimens and resemble muscular papillae situated lateral to the terminally placed oral sucker. In live specimens the presence of these small suckers are sometimes not distinguished (Hoffman 1960). Doubt therefore arises whether or not pseudosuckers will be

noticeable in all mounted specimens of species and metacercarial types. This again leaves room for discussion regarding *Diplostomum* type a metacercariae being similar to *Diplostomum* type 2. Differences are present between *Diplostomum* type a and *D. elongates* metacercariae such as that the ventral sucker of the latter which is placed more anteriorly. Another difference is the presence of a compact, elongated hold-fast gland, posterior to the holdfast organ in *D. elongates*, whilst such a structure could not be identified in *Diplostomum* type a metacercariae.

Similar morphometrics are also shared between *Diplostomum* type a and larvae of *Diplostomum clavata* (von Nordmann, 1832) Diesing, 1850. This species was described from the vitreous humours of the eyes of various cyprinid and salmonid fish hosts in North America (Hoffman 1960). Pseudosuckers are present in both types and are situated laterally to the terminal-placed oral sucker. Hoffman (1960) noted that these structures ranged in the degree which it could be everted and as a result it cannot be observed sometimes. This further illustrates the possibility that the presence of pseudosuckers can be "overlooked", due to the lack of noticeable elevations in the anterior part of the body. Other similarities included: the elongated body shape, with the length being more than twice the width; a terminally placed oral sucker; the presence of a prepharynx and pharynx and a very ill-defined hindbody. Niewiadomska (1963b) noted the presence of a hindbody with *Tylodelphys excavata* (Rudolphi, 1803), a synonym of *D. clavata*. The present study comes to the same conclusion, namely that the posterior part is easily retractable and extendable and its presence is dependent on the state of contraction when fixed and mounted.

Diplostomum type a metacercariae also share morphological similarities with metacercariae of *Tylodelphys destructor* Szidat and Nani, 1951. According to Szidat (1969) these metacercariae were sampled from the brain cavities and tissues of the midbrain and cerebellum of the Argentinean silverside, *Odontesthes bonariensis* (Valenciennes, 1835), South America. The body is tongue-shaped (elongated and flattened) and a hindbody could not be distinguished, but within the adult worm a distinct hindbody develops. Pseudosuckers are present on both sides of the oral sucker but are hardly visible, even during the latter metacercarial development and adult worm stages. A marked difference does occur regarding the size of the oral and

ventral suckers. With *Diplostomum* type a metacercariae these two structures are approximately equal in size, whilst with *T. destructor* the ventral sucker is much smaller than the oral sucker. No morphometrics were provided by Szidat (1969) and hence no further comparative description could be made.

Diplostomum type a metacercariae show many similarities when compared with *D. spathaceum* metacercariae sampled from the lenses of roach, *Rutilus rutilus* (Linnaeus, 1758) in Northern Ireland (McKeown and Irwin 1995). With both metacercarial types the body is elongated and a fore- and hindbody cannot be distinguished; the oral sucker is placed terminally and well-developed pseudosuckers are situated laterally from it. This description of an elongated body form does not correlate with the common description given by many authors (Ashworth and Bannerman 1927; Rees 1955) for this well-known metacercarial species. In general *D. spathaceum* has an oval body form which appears similar to *Diplostomum* type c metacercariae.

Four African species of metacercariae were found to share morphological similarities with *Diplostomum* type a. The first report was given by Beverley-Burton (1963) who described *Diplostomum* (*Tylodelphys*) *mashonense* metacercariae, found unencysted within the cranial cavity of *C. gariepinus* and blunt-toothed African catfish, *Clarias ngamensis* Castelnau, 1861, respectively from Southern Zimbabwe and Zambia. Similarities include: an elongated, flattened body with a very weak distinction between a fore- and hindbody; the presence of small pseudosuckers; the placement of the rounded ventral sucker approximately in the middle of the body and the presence of an elliptical holdfast organ in the posterior third of the body. Many differences are found such as the oral sucker of *D.* (*T.*) *mashonense* which is placed sub-terminally, the anterior end which is more conical shaped and the overall size which is larger.

Prudhoe and Hussey (1977) as well as Mashego and Saayman (1989) also identified *Diplostomum mashonense* metacercariae from the brain of *C. gariepinus* from the Limpopo River System, South Africa. Both studies failed to provide a detailed and diagrammatical description of the metacercariae. Their species' identification is based on a few characters such as the similar sizes of the oral and ventral suckers. In a study conducted by Chibwana and Nkwengulia (2010) the same diplostomatid species was

found in *C. gariepinus* from various freshwater localities in Tanzania. These authors provided a more satisfactory description stating that the metacercariae have an elongated body, with a vague distinction between a fore- and hindbody and pseudosuckers are placed postero-laterally from the oral sucker. The metacercarial morphometrics of this species as recorded by Chibwana and Nkwengulia (2010) is provided in Table 5.4, proving as an example of the high morphological variation which can occur between metacercariae collected from the same fish species from different localities.

***Diplostomum* type b**

Hosts (site of infection)

Okavango: *Brycinus lateralis* (brain); *Hepsetus odoe* (brain); *Oreochromis andersonii* (Castelnau, 1861) (brain)

Orange-Vaal: *Labeo capensis* (eyes)

Diagnostic and Morphometric Characteristics: Figure 5.5 and Tables 5.1 - 5.3. Description based on 11 specimens; measurements (mm) represent the length of structures.

Metacercariae free-moving with elongated, fairly flattened bodies 0.314 - 1.184 (0.604 ± 0.308). Clear distinction between broad forebody 0.380 - 1.069 (0.686 ± 0.302) and narrow, conical hindbody 0.042 - 0.155 (0.099 ± 0.043). Pseudosuckers well-developed conical outgrowths, situated on lateral sides of oral sucker. Together with oral sucker provide a tri-lobed appearance to anterior end. Oral sucker 0.033 - 0.059 (0.042 ± 0.01) placed terminally and circular in outline. Ventral sucker 0.032 - 0.100 (0.048 ± 0.023) circular and not clearly visible. Holdfast organ 0.030 - 0.143 (0.072 ± 0.032) longitudinal elliptical shaped, situated in posterior part of forebody. Genital primordium in hindbody. Prepharynx difficult to observe, followed by small pharynx 0.014 - 0.057 (0.031 ± 0.019), opens into oesophagus, bifurcates into intestinal caeca, which extends to genital primordium.

Remarks:

The present study is the first record of *Diplostomum* type b metacercariae in southern Africa. It is also the only record of free-moving metacercariae within the brain of *O. andersonii* which belongs to the widely-distributed family Cichlidae. Other infected hosts belong to various other fish families, of which one (Hepsetidae) is endemic to Africa and the other two (Characidae and Cyprinidae) have a more global distribution (see Chapter 6). The metacercariae closely resemble *D. mordax* larvae, found within the brain of Neotropical silversides, *Basilichthys* species in Argentina (Hoffman 1960). Prominent similarities include a clear distinction between a large, elongated forebody and small, conical hindbody as well as the presence of well developed pseudosuckers, representing little horn-like protrusions. These ear-like protrusions are situated lateral to the oral sucker which occurs terminally. In both metacercarial types the presence of a pharynx can be distinguished, whilst the ventral suckers are indistinct. Similar morphometrics are also shared between these two types of metacercariae.

Diplostomum type b is also morphologically similar to *Diplostomum tregenna* Nazmi Gohar, 1932 metacercariae. The larvae were first described by Khalil (1963) from the cranial cavity of the *C. gariepinus* in Sudan. In more recent African studies similar metacercariae were also found in the cerebrospinal fluid of the cranial cavity and olfactory tract of *C. gariepinus* from the Nile River System (Ibraheem 2000) and from Lake Tana in Ethiopia (Zhokhov *et al.* 2010). According to Niewiadomska (2001) the presence of a genital cone, during the adult stage, places it in the genus *Dolichorchis*. Most authors, however, regard the former only as a subgenus of *Diplostomum* (see Chapter 4) and therefore the species name *Diplostomum tregenna* remains valid.

Similarities shared between the described species and *Diplostomum* type b metacercariae include a clear distinction between an elongated, dorsoventrally flattened concave forebody and a small, conical hindbody as well as the presence of well-developed pseudosuckers. These structures form ear-shaped processes and together with the oral sucker provide a tri-lobed shape to the anterior end (Khalil 1963). Other similarities include a prepharynx which is not always visible and the presence of a distinct fringed lip surrounding the oral sucker. Differences do, however, exist: the ventral sucker of *Diplostomum* type b is not as clearly visible and the pseudosuckers

are placed lateral from the oral sucker, whilst in *D. tregenna* it occurs more postero-laterally.

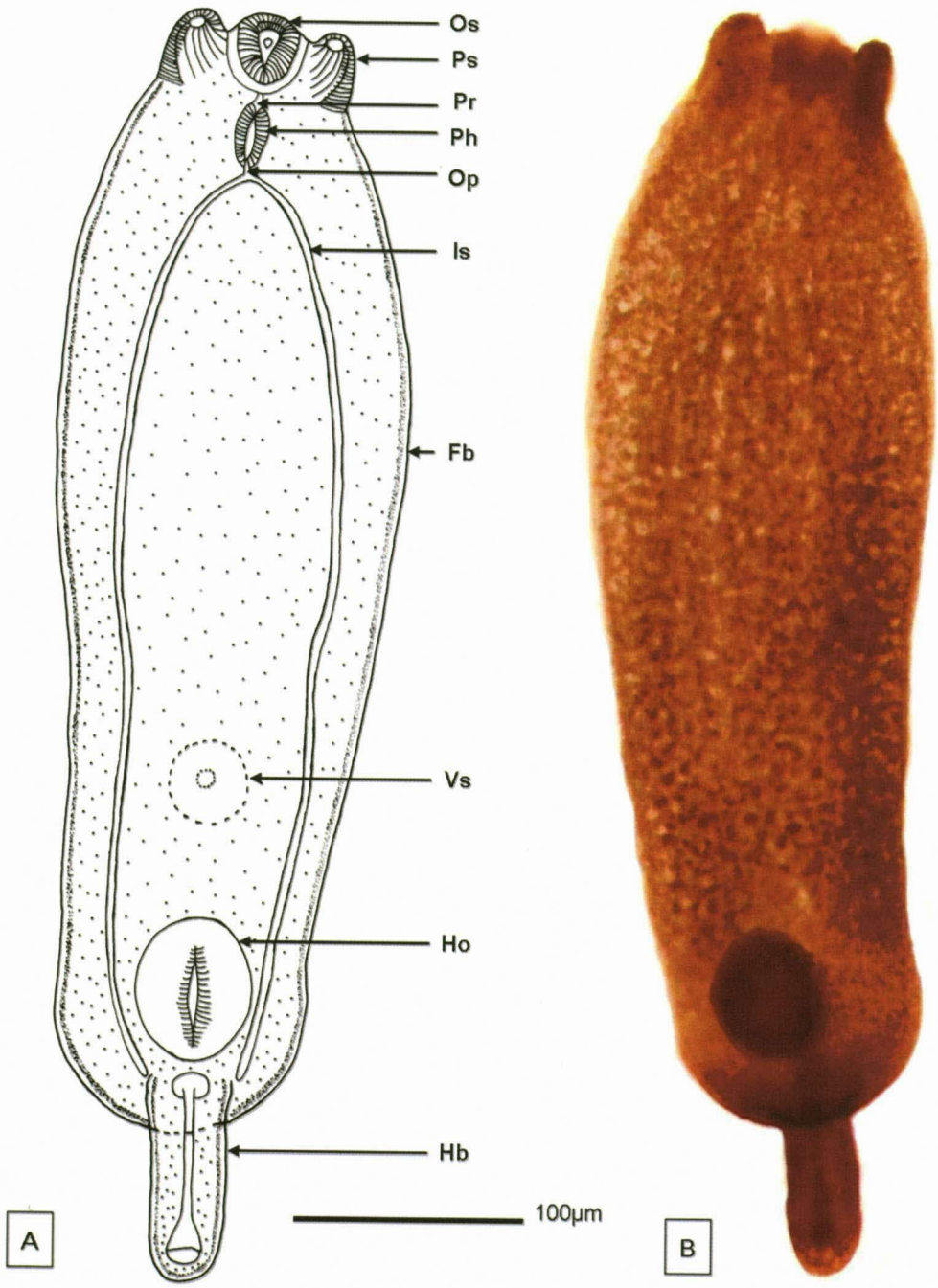


Figure 5.5: (**A**) Microscope projection drawing and (**B**) light micrograph of metacercariae of *Diplostomum* von Nordmann, 1832 type b. **Fb**: forebody; **Gp**: genital primordium; **Hb**: hindbody; **Ho**: holdfast organ; **Is**: intestinal caeca; **Op**: oesophagus; **Os**: oral sucker; **Ph**: pharynx; **Pr**: prepharynx; **Ps**: pseudosucker; **Vs**: ventral sucker.

Important to note is that the above described characters were present in mature metacercariae of *D. tregenna*. During the immature metacercarial stages Khalil (1963) could not distinguish between a fore- and hindbody and the pseudosuckers were underdeveloped, not looking like ear-like structures. This description closely resembles the morphology of *Diplostomum* type a metacercariae. The absence of developed structures, such as a hindbody and prominent pseudosuckers, suggests the possibility that *Diplostomum* type 2 may represent an earlier metacercarial stage of *Diplostomum* type b.

***Diplostomum* type c**

Hosts (site of infection)

Okavango: *Barbus poechii* (eyes); *Brycinus lateralis* (brain)

Orange-Vaal: *Labeo capensis* (eyes)

Diagnostic and Morphometric Characteristics: Figures 5.6 A and B and Tables 5.1 - 5.3. Description based on 15 specimens; measurements (mm) represent the length of structures.

Metacercariae free-moving with oval, broad body shape 0.245 - 0.406 (0.298 ± 0.053). No clear distinction between fore- and hindbody. Well-developed pseudosuckers forming small conical outgrowths present on lateral sides of oral sucker. Oral sucker 0.014 - 0.035 (0.027 ± 0.006) placed terminally and circular in outline. Holdfast organ 0.035 - 0.070 (0.049 ± 0.013), longitudinal elliptically shaped, situated posterior in body. Ventral sucker 0.031 - 0.036 (0.033 ± 0.002) not clearly distinguishable and located posterior in forebody, followed by genital primordium. Pharynx small (sometimes not visible), prepharynx and oesophagus absent. Pharynx bifurcates directly into intestinal caeca which extend through body length to genital primordium.

Remarks:

Except for its oval appearance, another marked difference between *Diplostomum* type c and the other described *Diplostomum* types is the absence of an oesophagus (see Table 5.3). The present study is the first record of *Diplostomum* type c metacercariae

in southern Africa and possibly the only record for Africa as well. Three species, belonging to two families (Cyprinidae and Characidae), collected during the present study, act as hosts. These fish species are limited to southern Africa in their distribution, except *B. lateralis* which also occurs in the Congo River System (see Chapter 6).

Similarities are shared between *Diplostomum* type c and metacercariae of *Diplostomum phoxini* (Faust, 1918). These include a similar sized, oval-shaped body; no marked distinction between the fore- and hindbodies; the presence of well-developed pseudosuckers situated antero-laterally of the terminal oral sucker (Hoffman 1960). *Diplostomum phoxini* metacercariae do exhibit a clearly visible prepharynx and are also larger in general size. According to Barber and Crompton (1997) metacercariae of this diplostomatid species are the most common and widespread parasites of European minnows, *Phoxinus phoxinus* (Linnaeus, 1758) and enormous numbers of the larvae have been recorded from the brain and cranial cavity (Ashworth and Bannerman 1927).

Diplostomum pelmatoides Dubois, 1932 is one of the many synonyms for *D. phoxini*, which Rees (1955) considers to be synonymous with *D. spathaceum*. The latter species has also been named *Diplostomum volvens* (von Nordmann, 1832) and is probably the most infamous of all diplostomatid species. A possible reason for *D. spathaceum* metacercariae having a notorious status is because of their close association with cataract formation, especially in the aquaculture industry of the northern hemisphere. Most probably this is only a result of taxonomic ambiguity. Historically, all diplostomatid metacercariae, obtained from the eyes of freshwater fish have collectively been referred to as *D. spathaceum* and resulted in a species complex rather than a species itself (Field and Irwin 1995).

Tetracotyle phoxini Faust, 1919 is synonymous with *D. phoxini* (Faust, 1918) and therefore also share morphological similarity with *Diplostomum* type c metacercariae. Ashworth and Bannerman (1927) concluded that an oval body shape is only present when *T. phoxini* specimens are contracted after being fixed and mounted. Live specimens were fully extended and showed a more leaf-like body, similar to the body of *Diplostomum* type 2, *Diplostomum* type a and *Diplostomum* type b. This again

illustrates the influence which mounting and other microscopy techniques could have on the morphological appearance and identification of diplostomatid metacercariae. The only measurements for *T. phoxini* metacercariae provided by Ashworth and Bannerman (1927) are the body length and width of mounted specimens, which correlates with the body sizes of mounted *Diplostomum* type c metacercariae. The morphometrics of the oral sucker, ventral sucker and holdfast organ are, however, much larger when compared to *Diplostomum* type c metacercariae. Another difference is the presence of an oesophagus, whilst it is absent in *Diplostomum* type c.

Diplostomum type c larvae share morphological similarities with mounted specimens of *Diplostomum gasterostei* (Williams, 1966). This species was described from the retinal layer (between the retina and choroid) of the eyes of sticklebacks, *Gasterosteus aculeatus* Linnaeus, 1758 in Great Britain. Similarities between mounted specimens include: a terminal oral sucker; the presence of a small pharynx; pseudosuckers present on each side of the oral sucker and an oval body with no distinction between a fore- and hindbody. With live metacercariae of *D. gasterostei* a very small hindbody could be distinguished from the oval forebody (Williams 1966) and therefore show more similarity towards metacercariae of *Diplostomum* type d, which were also collected during the present study.

Diplostomum type d

Hosts (site of infection)

Okavango: *Pollimyrus castelnaui* (eyes and brain); *Schilbe intermedius* Rüppell, 1832 (brain); *Tilapia sparrmanii* (eyes)

Diagnostic and Morphometric Characteristics: Figures 5.6 C and D and Tables 5.1 - 5.3. Description based on eight specimens; measurements (mm) represent the length of structures.

Metacercariae free-moving and distinction made between large, oval forebody 0.298 - 0.598 (0.419 ± 0.109) and small, conical hindbody 0.029 - 0.112 (0.047 ± 0.027). Pseudosuckers well-developed and form small conical outgrowths on

lateral sides of oral sucker. Oral sucker 0.037 - 0.059 (0.045 ± 0.008), terminal in forebody, circular or elliptical in outline. Ventral sucker 0.022 - 0.046 (0.033 ± 0.012), circular, situated posterior in forebody and not clearly observable. Holdfast organ 0.046 - 0.141 (0.086 ± 0.034) longitudinal elliptically shaped, placed in posterior part of forebody. Prepharynx and pharynx 0.029 - 0.034 (0.031 ± 0.003) present, followed by marked oesophagus. Bifurcates into intestinal caeca which extend up to anterior margin of holdfast organ.

Remarks:

The present study is the first record of *Diplostomum* type d metacercariae in southern Africa and most probably a new record for the whole of Africa. It is also the only record of free-moving metacercariae within the brain of *S. intermedius* which belong to the family Schilbeidae. This is a fish family with a widespread distribution in tropical Africa and southeast Asia. Only two schilbeid species are known from southern Africa and only one (*S. intermedius*) occurs in the Okavango and none in the Orange-Vaal River System (see Chapter 6). Other infected fish hosts belong to the family Mormyridae, endemic to Africa, and Cichlidae, which has a far more global distribution.

Diplostomum type d metacercariae share morphological similarity with a species which is one of the earliest records of diplostomatid trematodes found in a vertebrate brain. *Tetracotyle petromyzontis* Brown, 1899 metacercariae were described from the brain of ammocoetes (juvenile lampreys) in freshwater systems in England. A vaguely distinguishable curved hindbody, containing the genital primordium and excretory bladder, occur posterior of the almost rounded forebody. The mean length of the metacercariae is the only morphometrics provided by Brown (1899), but correlates with the length determined for *Diplostomum* type d. On each side of the terminally based oral sucker, two retractile ear-shaped projections (pseudosuckers) are present. A marked difference, however, is that with *T. petromyzontis* the position of the ventral sucker is approximately in the middle of the body, whilst with metacercariae of *Diplostomum* type d it is situated more posteriorly. The intestinal caeca of the described species also reaches beyond the holdfast organ, whilst with *Diplostomum* type d it ends blindly immediately just in front of this structure.

Diplostomum type d metacercariae are also similar to *Diplostomum huronense* (La Rue, 1957) described from the eyes of American yellow perch, *Perca flavescens* (Mitchill, 1814) from Michigan. Similarities include: comparable body measurements; distinction between a club-shaped forebody and a small, conical hindbody; the presence of a pharynx and well-developed, ear-like pseudosuckers placed laterally from the oral sucker. It differs with *D. huronense* metacercariae which exhibit a posterior lip-like structure on the forebody (Hoffman 1960), whilst a comparable structure was not observed during the present study.

Characters such as the presence of a round to oval forebody, with a distinct hindbody and well-developed pseudosuckers are also shared with *Diplostomum volvens* (Shigin, 1986) described from between the retinal and choroid layers of European perch, *P. fluviatilis* from the former USSR (Höglund and Thulin 1992). The general body size of *Diplostomum* type d is, however, smaller than that of *D. volvens*.

Another species described from the retinal layers of fish eyes and sharing morphological similarities with *Diplostomum* type d is *Diplostomum baeri* Dubois, 1937. These metacercariae have been found in-between the retinal layers of *P. fluviatilis* from the former USSR and Poland as well as from lake whitefish, *Coregonus clupeaformis* (Mitchill, 1818) in Canada (Höglund and Thulin 1992). Similarities include: similar body size and overall morphometrics; well-developed pseudosuckers placed laterally from the terminal oral sucker; a broad, oval forebody and conical hindbody. Niewiadomska (1988) noted that in *D. baeri* metacercariae the pseudosuckers are completely or partly retracted and the result is that the anterior end is blunt-shaped. This is in contrast with the prominently everted pseudosuckers, resembling little horns, present in *Diplostomum* type d metacercariae. The position of the ventral sucker is also more to the middle of the body, whilst with *Diplostomum* type d it is situated posteriorly. Although the shapes are similar, the sizes of the ventral sucker and the holdfast organ of *D. baeri* metacercariae are larger.

A diplostomatid species which does have a tri-lobed anterior end, similar to *Diplostomum* type d metacercariae, is *Diplostomulum gymnoti* Szidat, 1969. These metacercariae were found unencysted in the brain of banded knifefish, *G. carapo* in South America (Szidat 1969). Other similarities shared with *Diplostomum* type d is: an

oval, flat forebody; a small, conical hindbody and similar morphometrics regarding the size of the body, oral sucker, pharynx and holdfast organ. The ventral sucker of *D. gymnoti* metacercariae is, however, larger than that of *Diplostomum* type d. *Diplostomum* type d also shares close morphological similarity with *Diplostomulum gigas* Hughes and Berkhout, 1929. These diplostomula were described from the crystalline lenses of white sucker, *Catostomus commersonnii* (Lacépède 1803) from Douglas Lake, North America (Hughes and Berkhout 1929). The main difference is that in general, *D. gigas* metacercariae are far larger than *Diplostomum* type d metacercariae.

Ultimately, the only striking difference between the metacercariae of *Diplostomum* type d and *Diplostomum* type c, is that the former have a well-developed hindbody and a slightly larger body size. Previous research has provided examples of younger metacercarial stages, sharing morphological similarity with *Diplostomum* type c, whilst the mature metacercariae were more similar to *Diplostomum* type d (Erasmus 1958). It is therefore possible that the two types are a similar species and just represent two different metacercarial developmental stages. Fixation and mounting could also have influenced the contraction of the hindbody and the small difference in size.

A clear example of mounting having an influence on morphometrics is provided by Lester and Huizinga (1977) who described *Diplostomum adamsi* Lester and Huizinga, 1977 metacercariae from the retina of American yellow perch, *P. flavescens* in Canada. Live specimens share similar characters with *Diplostomum* type d metacercariae such as: the presence of well-developed pseudosuckers placed laterally from the terminal oral sucker; an oval-shaped forebody and the presence of a conical hindbody. When mounted, the metacercariae shrank in size and the hindbody completely retracted (Höglund and Thulin 1992). This oval body form is characteristic of *Diplostomum* type c metacercariae. This stresses the possibility that metacercariae of *Diplostomum* type c and *Diplostomum* type d could be similar types or species, just representing different ages of metacercarial development.

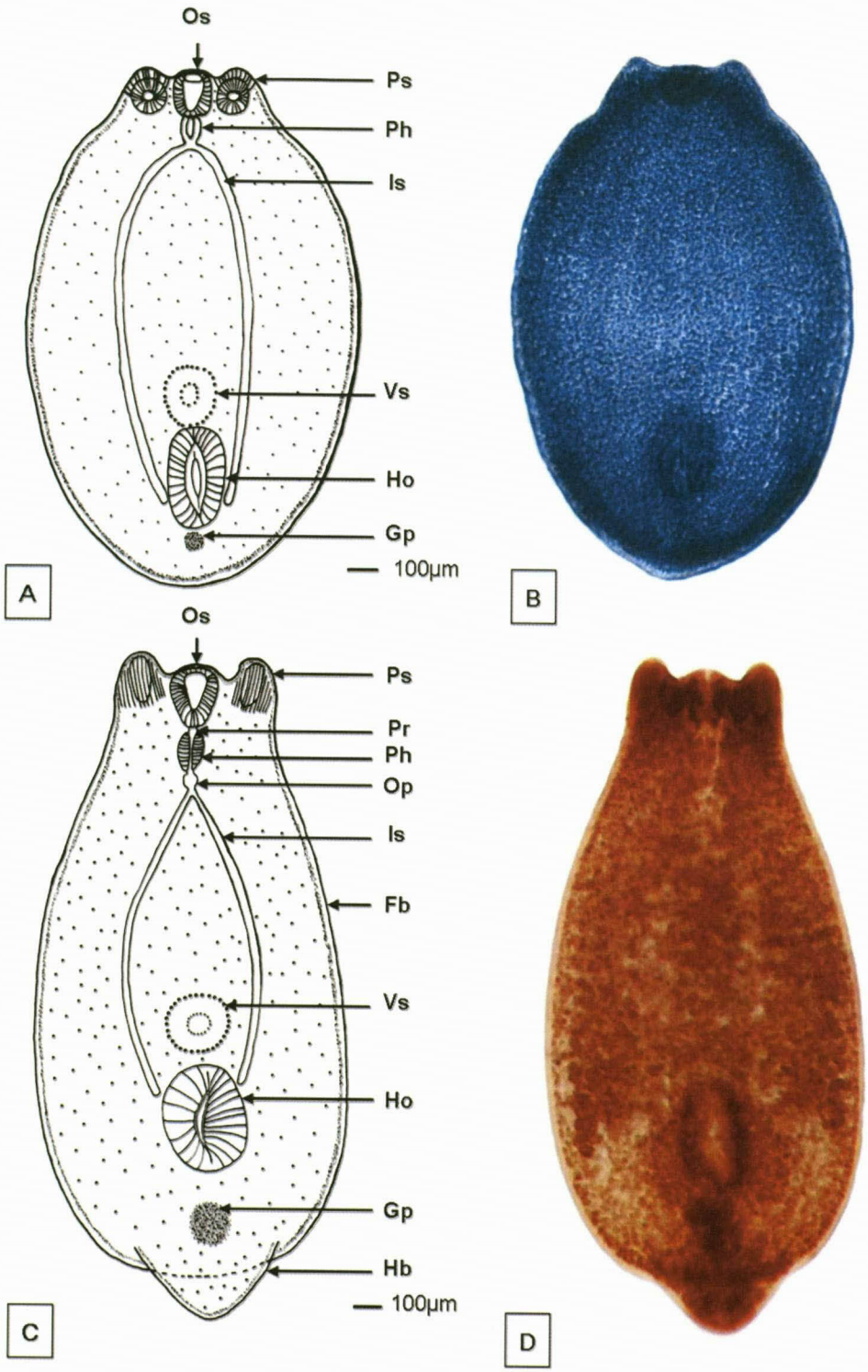


Figure 5.6: (**A**, **C**) Microscope projection drawings and (**B**, **D**) light micrographs of metacercariae of (**A**, **B**) *Diplostomum* von Nordmann, 1832 type c and (**C**, **D**) *Diplostomum* von Nordmann, 1832 type d. **Fb**: forebody; **Gp**: genital primordium; **Hb**: hindbody; **Ho**: holdfast organ; **Is**: intestinal caeca; **Op**: oesophagus; **Os**: oral sucker; **Ph**: pharynx; **Pr**: prepharynx; **Ps**: pseudosucker; **Vs**: ventral sucker.

Cysts

Hosts (site of infection)

Okavango: *Oreochromis andersonii* (eyes); *Oreochromis macrochir* (Boulenger, 1912) (eyes); *Sargochromis greenwoodi* (Bell-Cross, 1975) (eyes); *Serranochromis angusticeps* (Boulenger, 1907) (eyes); *Tilapia rendalli* (eyes); *Tilapia sparrmanii* (eyes)

Diagnostic and Morphometric Characteristics: Anatomical characters and body regions are indistinguishable. Total length 0.772 - 1.601 (1.173 ± 0.17) and width 0.281 - 0.829 (0.48 ± 0.121) were measured from 25 cysts.

Remarks:

Similar to previous studies, such as that of Hoffman (1960), it was not possible during the present study to successfully excyst the encapsulated metacercariae. The cyst wall surrounding the (probably underdeveloped) metacercariae was also too thick for the passage of light needed for light microscopy. Only the length and width of the cysts could be determined, whilst other body regions and structures remained inconspicuous. The absence of general morphometrics and anatomical structures prohibit the identification of the encapsulated metacercariae to an appropriate *Diplostomum* metacercarial type.

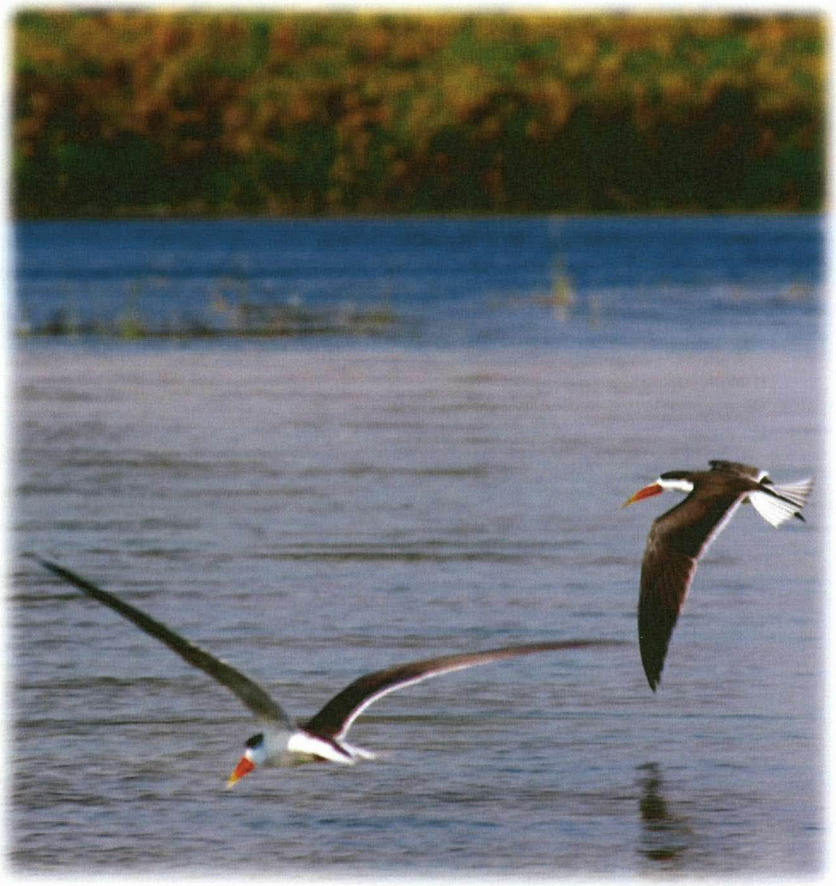
Tetracotyle is regarded as a subgenus of *Diplostomum* and therefore cyst-forming metacercariae do occur within the genus (see Chapter 4). Apart from the *Tetracotyle*-like cysts, named *Diplostomum* type 1 and type 3, found in the brains of the three mormyrid species, thicker walled and oval-shaped cysts were also collected during the present study. These cysts occurred within the eye blood capillaries of fish species belonging to the family Cichlidae. Previous studies have exclusively associated *Diplostomum* metacercariae to be free-moving (unencysted) diplostomatids occurring in immunological privileged sites such as the lens and humours of the eyes and brain of freshwater fish. Since the revised classification by Niewiadomska (2001), non-*Tetracotyle*-like encysted metacercariae (from host origin) have been included in the *Diplostomum* genus. This study therefore concludes that the encysted larvae found within the eye blood capillaries of the cichlid fish were most probably diplostomatids. It is speculated that during migration the free-moving metacercariae became lodged in

the blood capillaries and the evicted host immune response resulted in the formation of cysts. The presence of diplostomatids in sites other than the eye lenses, humours and brain has also been stated by Moravec (1977). In the lattter study diplostomatid cysts were reported from the muscles of *C. gariepinus* from freshwater sites in Egypt. Rai and Pande (1968) also found encysted diplostomatids in the muscles, mesenteries, liver, pericardium and eyes of catla, *Catla catla* (Hamilton, 1822) (Cyprinidae) from freshwater environments in India.

Ukoli (1966) and Fischthal and Thomas (1970) present the possibility of a different trematode family forming cysts within the eyes of fish. These studies report on cysts belonging to the Family Clinostomatidae Lühe, 1901, and specifically the genus *Clinostomum* Leidy, 1856, from the eye sockets of intermediate fish hosts. The encysted metacercariae, *Clinostomum tilapia* Ukoli, 1966, were collected from the eyes of tilapian fish hosts, namely redbelly tilapia *Tilapia zilli* (Gervais, 1848) and mango fish *Sarotheron melanotheron heudelotti* (Duméril, 1861) from Ghana. *Clinostomum* metacercariae are generally known to encyst in fish and amphibian mesenteries and in the lymph spaces between the skin and the muscles (Ukoli 1966). These cysts are also much larger and have a much thinner cyst wall than diplostomatid cysts.

The genus *Clinostomum* is therefore not normally associated with thick-walled cysts infecting sites such as the eyes and brains of fish. The probability of *Diplostomum* metacercariae occurring within the cysts is also increased due to the known high incidence of free-moving diplostomatid flukes found within these sites. Based on these findings the present study concludes that cysts belonging to the genus *Diplostomum* are present within the eyes of cichlid fish in the Okavango River.

Chapter 6



DIPLOSTOMATID LIFE CYCLE:

AN ECOLOGICAL APPROACH

GENERAL LIFE CYCLE AND MEANS OF TRANSMISSION

Characteristic to most digenetic trematodes the life cycle of *Diplostomum* species involves three hosts: a snail, a fish, or very rarely an amphibian which acts as a paratenic host and lastly a piscivorous bird (Kennedy and Burrough 1977) (Figure 6.1). The first two are the intermediate hosts in which the necessary larval stages develop, whilst the latter is termed the final or definitive host, which harbours the sexual adult worm (Gibson 1998). A paratenic host may also host the larval stage, but although it is able to transmit the larvae to the next host, the parasites will not develop and therefore it does not form an essential part of the life cycle.

Previous studies have suggested that due to their multiple-host life cycle, trematode parasites link several different taxa of surrounding animal communities and may act as excellent indicators of other components of community structure, function and biodiversity (Hechinger *et al.* 2007). The adults of different species of trematodes are known to parasitise different regions of the definitive hosts' alimentary system. Adult diplostomatids specifically parasitise and sexually reproduce in the small intestine of piscivorous birds and their eggs are passed out into water via the bird host faeces. Depending on the water temperature, these ovoid eggs will then hatch after one to twenty two days, to release free-moving miracidia.

These free-swimming larvae, covered with cilia, find and penetrate suitable aquatic pulmonate snails, most possibly by sensing physico-chemical and biological stimulants (Lester and Freeman 1976; Saladin 1979; Christensen 1980; Graczyk and Fried 2001). Inside of the digestive gland (Crowden and Broom 1980) and liver (Hoffman and Hundley 1957; Ashton *et al.* 1969) of the snails, a cycle of asexual reproduction results in the formation of mother sporocysts, daughter sporocysts and ultimately fork-tailed cercariae which are then shed from the snail host (Rushton 1938; Rees 1955). Karvonen *et al.* (2004a) suggested that the cercarial shedding follows a light-dark rhythm and occurs mainly during the daytime when the preferred second intermediate host will be most active and susceptible to penetration. However, opposed to this, Lyholt and Buchmann (1996) state that since diplostomatid flukes use aquatic organisms as both first and second intermediate hosts, they do not need light

dependent shedding to optimise their life cycle efficiency. It remains inconclusive whether the first or latter hypothesis is correct.

By means of chemotaxis, thermo receptors and the ability to sense water turbulence (Niewiadomska 1996), these free-swimming cercariae are able to detect the fish host and then actively swim to penetrate it. Penetration is aided by means of the cercarial tail and secretions of the penetration gland cells, which most probably help with the dissolving of the host tissues (Rees 1957; Ratanarat-Brockelman 1974; Haas *et al.* 2007). The diplostomula must reach the preferred sited of infection before the contents of the penetrating glands and the glycogen stores are depleted (Graczyk 1991a). The gills and mouth are known as the preferred sites of cercarial penetration (Erasmus 1958; Ratanarat-Brockelman 1974; Haas *et al.* 2007) but the fins and body surface epidermis may also be penetrated. Direct penetration into the eye cornea is also possible but not as likely (Ferguson 1943b; Hoffman and Hoyme 1958; Shariff *et al.* 1980; Niewiadomska 1996). The penetration is preceded by the tail being discarded and then migration primarily via the bloodstream (Rushton 1938; Khalil 1963; Ashton *et al.* 1969; Bibby and Rees 1971), although other tissues, such as the subcutaneous, muscle and nervous system may also aid in transmission (Ferguson 1943b; Betterton 1974; Hendrickson 1979; Haas *et al.* 2007).

Discrepancy remains whether or not the route of diplostomula migration inside of the host is predetermined (Bouillon and Curtis 1987) or whether the larvae just utilise the passage providing the least mechanical resistance, namely the bloodstream, as a means of transportation. The location within the eyes or brain could therefore be just by chance (Betterton 1974). Haas *et al.* (2007) divided the phases of *D. spathaceum* diplostomula migration into four distinct phases during which the parasite makes use of different host cues. Ferguson (1943b) and Niewiadomska (1996) also stated that diplostomatid metacercariae are pre-programmed to reach the eye or brain and that the reported occurrence within other tissues such as the heart, liver, opercular tissues and gills are just accidental and temporary sites. According to these authors the diplostomula will continue on their migration until the accurate sites are reached.

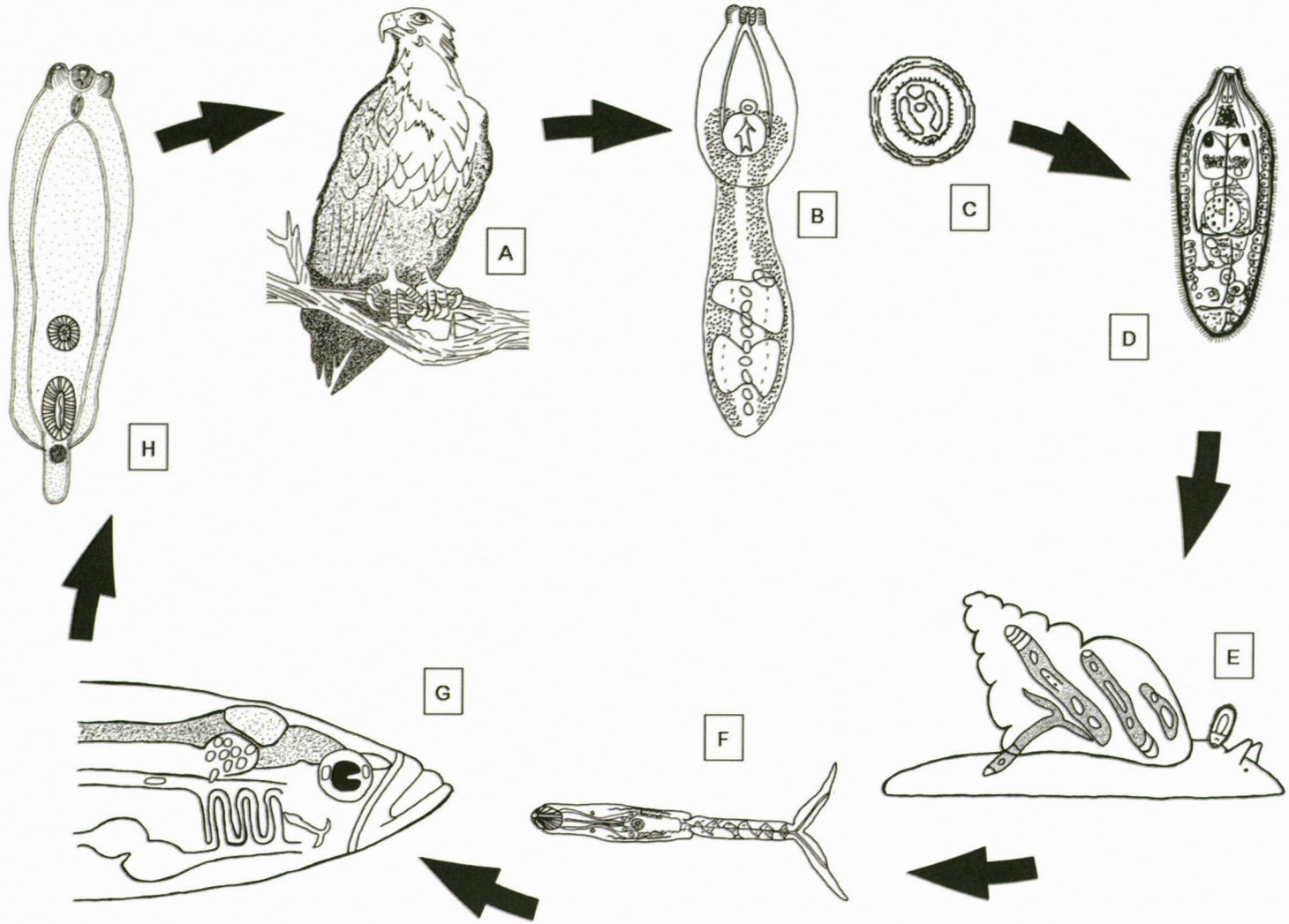


Figure 6.1: The general life cycle of *Diplostomum* von Nordmann, 1832 species consist of (**B**) the adult worms occurring in (**A**) a definitive bird host, which releases (**C**) the eggs and hatch to form (**D**) free-swimming miracidia, which penetrate (**E**) lymnaeid snails, from which (**F**) cercariae are shed, which actively swim to penetrate (**G**) fish. The diplostomula migrate to the eyes and / or brain of the fish and develop into (**H**) mature metacercariae which will be able to infect (**A**) the definitive host (modified from Olsen 1974; Jansen van Rensburg 2006).

The larvae seem to attach to the epithelium lining of the blood vessels and crawl along it (sometimes upstream) and the majority then reach the eye via the optic blood vessels and to a lesser extent via the optic nerves. For metacercariae occurring within the lens, Ashton *et al.* (1969) and Gaten (1987) suggested positive phototaxis to act as cue for the migrating diplostomula. Ferguson (1943b) concluded that the physical presence of the eye tissues is necessary for the successful migration of *Diplostomum flexicaudum* (Cort and Brooks, 1928) to the head region of fish via the body and optic blood vessels. A study by Lester and Huizinga (1977) supports the hypothesis of metacercariae migrating through various tissues to ultimately end up in a predetermined, specific site. Through experimental infection of perch, *P. flavescens* it was determined that some diplostomula of *D. adamsi* migrate and temporarily occur on the surface of the brain, the vitreous humour and eventually the retina and lens.

The present study concurs with the idea that cues may attract the diplostomula to certain body tissues, but it is not regarded as the exclusive possibility, since not enough evidence exists to undoubtedly prove this statement. The majority of earlier literature state that free-moving metacercariae can be found in the eye humours and brains of fish, whilst in the present study encysted trematode larvae were also collected from the other sites such as the blood capillaries within the eye (Chapter 7).

Due to the lack of morphological features these trematode cysts cannot be identified up to species level but could most possibly be *Diplostomum* metacercariae, which had become encysted due to the immunological response of the host. It is hypothesised that the reduced immunological responses present in the lens and humours of the eyes and the brain ventricles are responsible for the metacercariae to remain free-moving (Bell and Hopkins 1956), whilst in other immunological active sites they become encysted. If this hypothesis is true, it is possible that the migration and distribution of diplostomatid infections are indeed random and not site-specific. The only sites where these trematodes will be able to occur in an unencysted form are in sites with a lowered immunological response, such as the humours and lenses of the eyes and in the brain.

Free-moving and encysted metacercariae mature within the different body tissues and the life cycle is completed when infected fish are eaten by the definitive bird host. The mature metacercariae develop into adult trematodes within the piscivorous birds' small

intestines (Figure 6.1). The diplostomula may live, specifically in the lens, for many months and are even able to survive in dead fish (Ashton *et al.* 1969). Since more than one host is present and because some transmission occurs outside of the host body and cercarial development as well as diplostomula migration are temperature dependent (Lyholt and Buchmann 1996), it is not possible to determine the precise time span of the complete life cycle (Berrie 1960; Chappell 1995).

Note that, except for the diagrammatical representations, as part of the general life cycle (Figure 6.1), morphological descriptions of the other diplostomatid larval stages and adult trematodes are not included in the present study. For more detailed descriptions on diplostomatid miracidia, sporocysts and cercariae, previous works such as Rees (1955, 1957); Hoffman and Hundley (1957); Erasmus (1958); Beverley-Burton (1963); Niewiadomska (1963a); Williams (1966); Field and Irwin (1995); McKeown and Irwin (1995), Niewiadomska (1996) and the unpublished M.Sc. dissertation and Ph.D. thesis of Jansen van Rensburg (2001, 2006) are suggested.

First intermediate hosts: molluscs

Hechinger *et al.* (2007) stated that the host specificity of many trematode species is very definite for the first intermediate host and decreases further along the life cycle. It is therefore likely that most diplostomatid infections within a locality, originates from furcocercariae shed by one species of snails, which is sometimes also only restricted to a few individuals, whilst a variety and large number of suitable fish and bird species may become infected (Rees 1957; Niewiadomska 1996). According to Chibwana and Nkwengulia (2010) the natural snail hosts for diplostomatids in Africa are not fully understood. It is, however, concluded from most previous literature reports, that pulmonate snails of the family Lymnaeidae are the favoured, but not the sole, first intermediate hosts of the genus *Diplostomum* (Niewiadomska 1996). Especially the species of the genus *Lymnaea* Lamarck, 1799 have been observed to be infected with mother- and daughter sporocysts, responsible for shedding *Diplostomum* furcocercariae. Jansen van Rensburg (2001, 2006) proposed *Lymnaea natalensis* Krauss, 1848, and to a lesser extent *Biomphalaria pfeifferi* (Krauss, 1848) as the first

intermediate hosts present in the Okavango Panhandle, Botswana. Both species are known to shed diplostomatid furcocercariae and to occur in shallow, well-vegetated waters (Brown *et al.* 1992; Utzinger and Tanner 2000; Appleton *et al.* 2003). According to Chibwana and Nkwengulia (2010) cercariae resembling members of the Diplostomidae have also been reported from *B. pfeifferi* snails in Tanzania. In Zimbabwe, Beverley-Burton (1963) used *Lymnaea natalensis* as experimental hosts.

King and Van As (1997) also proposed *Bulinus tropicus* (Krauss, 1848), present in both the Okavango and Orange-Vaal River Systems, to act as hosts for diplostomatid cercariae. According to Appleton (1996) two other snail species known to shed furcocercariae are *Bulinus africanus* (Krauss, 1848) and *Bulinus globosus* (Morelet, 1866). The first species has been reported from the Okavango and Orange-Vaal River System, whilst the latter is limited to the south to the extreme eastern parts of Limpopo and Mpumalanga Provinces and to a small area of north-eastern KwaZulu-Natal and does not occur in the Orange-Vaal River System (Appleton 1996). *Bulinus globosus* has been reported from the Okavango River System (Jansen Van Rensburg 2001). *Bulinus africanus* have been reported from the Orange-Vaal River. Differing from *L. natalensis* and *B. pfeifferi*, the *Bulinus* Müller, 1781 snail species occur in deeper, open waters. Beverley-Burton (1963) proposed three host snails, namely *L. natalensis*, *Bulinus* spp. and *B. pfeifferi* to act as hosts. She experimentally infected these species with miracidia of *Diplostomum* (*Tylodelphys*) *mashonense*, but found that only *L. natalensis* developed young mother sporocysts.

It appears that the snail host range for diplostomatids in Africa could be wide, which in itself may have implications for the morphological measurements of the resulting metacercariae (Chibwana and Nkwengulia 2010). Table 6.1 provides a summary of snail species and families present within the Okavango and Orange-Vaal River Systems, along with an indication whether the shedding of furcocercariae has been noted in previous studies.

Seasonal variability in cercarial shedding may also have an influence in infection intensity, since collection of data during the present study was not conducted throughout the year. Fish were sampled from the Okavango and Orange-Vaal River

Systems during summer (November - February) and late autumn / winter (June - August). Since diplostomatid metacercariae are able to persist within their fish host throughout the various seasons, including the colder months (Sweeting 1974), it is difficult to obtain clear distinction between infections of successive years and seasons. Most probably the intensity of infection will only increase during times when the water levels decrease, leaving fish captive in shallow pools and exposed to cercariae-shedding snails. High water levels were, however, persistent throughout the time of study and the Okavango experienced new records in flood levels (Figure 2.3).

Second intermediate hosts: fishes

As mentioned, diplostomatids have a much wider range of second than first intermediate hosts. According to Niewiadomska (1996) the former may include lampreys and all groups of freshwater fish and even with successful experimental infections in amphibians such as *Xenopus laevis*. Just less than 60% of the total fish sample size collected from the Okavango and Orange-Vaal River Systems was infected with eye and brain diplostomatids (Table 6.2).

Difference in the susceptibility of various second intermediate host species to diplostomatids is known (Betterton 1974; Sweeting 1974) and is normally attributed to structural, physiological or behavioural differences between the hosts. Betterton (1974) made a detailed study of eye fluke specificity, and postulated that *D. spathaceum* cercariae take longer to migrate and are less successful to infect brown trout, *Salmo trutta* Linnaeus, 1758 than rainbow trout, *Oncorhynchus mykiss*. The behaviour and ecology of fish may also render a specific species more exposed to cercarial penetration. These characters are discussed later in this chapter. According to Betterton (1974) the lower prevalence of *D. spathaceum* metacercariae was a direct result of either a greater impenetrability of the brown trout's tissues or lack of endogenous signals that will orientate diplostomula migration to the eye. This hypothesis has never been tested rigorously, and therefore it remains speculative.

Although a variety of fish host species can be infected, the free-moving metacercariae are very specific in their site selection. Diplostomatid infections are limited to the eyes,

in the lens, humours, retina or choroid and more rarely in the brain, mostly in the ventricles and soft tissue. Whyte *et al.* (1989) stated that by exposing previously unexposed fish to weakened cercariae, it can significantly reduce the numbers of living diplostomula reaching the eyes and brains in subsequent infections. This is due to an increase in immune responses such as macrophage activity and stimulates a kind of immunity towards diplostomiasis in previously exposed fish.

Bortz *et al.* (1988) and Karvonen *et al.* (2005) also suggested that the antibodies formed in certain fish species, during a first light diplostomatid infection, may offer protection against reinfection with cercariae of *Diplostomum* species. Cold-blooded vertebrates have a low natural resistance to the invasion of metazoan parasites and pre-exposure is occasionally ineffective to reduce the severity of infection (Ratanarat-Brockelman 1974). After initial exposure it will also take several weeks for the development of resistance in fish. Sudden heavy cercarial exposure is, however, eminent in aquaculture. Since this time lapse will be too short for individuals to acquire immunity, mass mortalities may follow.

A higher prevalence within certain fish species may also be attributed to differences in the behaviour and ecology of the hosts. These characteristics may render certain species more exposed to cercarial penetration (Burrough 1978). For example, it is expected that fish favouring open waters, would be less likely to come into contact with cercariae than benthic, shallow littoral fish species or species preferring vegetated pools (Brassard *et al.* 1982a; Seppälä *et al.* 2011). This is because the snail hosts, responsible for cercarial shedding, also prefer the well-vegetated, slow-moving waters of the littoral zone (Sweeting 1974; Brassard *et al.* 1982a; Voutilainen *et al.* 2008). These behavioural and ecological differences have even resulted in fish species displaying different morphological features and form so-called ecomorphological classes / groups, as termed by Ramberg *et al.* (2006). However, in the early development of all freshwater fish species, the fry and fingerlings seek out the protection of the calmer, shallow, vegetated waters. It could therefore be speculated that all species are initially, equally susceptible to cercarial exposure (Valtonen and Gibson 1997).

Table 6.1: Species and families of freshwater molluscs present (•) in the Okavango (OK) and Orange-Vaal (OV) River Systems along with previous records (*) of furcocercariae being shed (compiled from Appleton 1996; Appleton *et al.* 2003).

Species name	Family	Known to shed furcocercariae	Presence	
			OK	OV
Class Gastropoda				
Bellamya capillata (Frauenfeld, 1865)	Viviparidae		•	
Bellamya monardi (Haas, 1934)	Viviparidae		•	
Lanistes ovum (Peters, 1845)	Ampullariidae		•	
Pilla occidentalis (Mousson, 1887)	Ampullariidae		•	
Gabiella kisalensis (Pilsbury and Bequaert, 1927)	Bithyniidae		•	
Melanoides victoriae (Dohrn, 1865)	Thiaridae		•	
Cleopatra bulimoides (Olivier, 1804)	Thiaridae		•	
Cleopatra nsendweensis Dupuis and Putzeys, 1901	Thiaridae		•	
Lymnaea columella (Say, 1817)	Lymnaeidae	*	•	•
Lymnaea natalensis (Krauss, 1848)	Lymnaeidae	*	•	•
Lymnaea truncatula (Müller, 1874)	Lymnaeidae	*	•	•
Burnupia spp.	Ancylidae		•	•
Ferrissia spp.	Ancylidae		•	•
Gyraulus costulatus (Krauss, 1848)	Planorbidae		•	
Lentorbis spp.	Planorbidae		•	
Segmentorbis spp.	Planorbidae		•	
Biomphalaria pfeifferi (Kraus, 1848)	Planorbidae	*	•	
Bulinus africanus (Krauss, 1848)	Planorbidae	*	•	•
Bulinus depressus (Haas, 1936)	Planorbidae		•	•
Bulinus forskali (Ehrenberg, 1831)	Planorbidae		•	•
Bulinus globosus (Morelet, 1866)	Planorbidae	*	•	
Bulinus tropicus (Krauss, 1848)	Planorbidae	*	•	•
Physa acuta (Draparnaud, 1805)	Planorbidae			•
Class Bivalvia				
Unio caffer (Krauss, 1848)	Unionidae		•	•
Coelatura kunensis (Mousson, 1887)	Unionidae		•	
Aspatharia pfeifferiana (Bernardi, 1860)	Mutelidae		•	
Corbicula fluminalis (Müller, 1774)	Corbiculidae		•	•
Eupera spp.	Sphaeriidae		•	

Table 6.2: List of the fish families and species present (•) in the Okavango, (below Popa Rapids) (**OK**) and Orange-Vaal (**OV**) River Systems, respectively in Botswana and South Africa (compiled from Skelton 2001). All the fish species examined during field work period (December 2008 - June 2010) are indicated in **bold**. Fish species infected with *Diplostomum* von Nordmann, 1832 metacercariae in their eyes are indicated by blue, whilst brain infection is indicated by pink. Green indicates the fish species infected with both eye and brain diplostomatids. The sample size (**n**) of the fish collected, as well as the prevalence of diplostomatid infection (**P**), are provided.

FISH FAMILIES AND SPECIES	OK	OV	n	P (%)
MORMYRIDAE				
Hippopotamyrus ansorgii (Boulenger, 1905)	•			
Cyphomyrus discorhynchus (Peters, 1852)	•			
***Marcusenius macrolepidotus* (Peters, 1852)**	•		4	100
Mormyrus lacerda Castelnau, 1861	•			
***Pollimyrus castelnaui* (Boulenger, 1911)**	•		21	43
***Petrocephalus catostoma* (Günther, 1866)**	•		4	75
Petrocephalus wesselsi (Kramer and van den Bank, 2000)	•			
KNERIIDAE				
Kneria polli Trewavas, 1936	•			
Parakneria fortuita Penrith, 1973	•			
CYPRINIDAE				
***Barbus afrovernayi* Nichols and Boulton, 1927**	•		10	0
Barbus barotseensis Pellegrin, 1920	•			
***Barbus barnardi* Jubb, 1965**	•		7	0
Barbus bifrenatus Fowler, 1935	•			
Barbus brevidorsalis Boulenger, 1915	•			
Barbus breviceps Trewavas, 1936	•			
Barbus codringtoni Boulenger, 1908	•			
***Barbus eutaenia* Boulenger, 1904**	•		1	0
Barbus fasciolatus Günther, 1868	•			
Barbus haaisianus David, 1936	•			
Barbus kerstenii Peters, 1868	•			
Barbus lineomaculatus Boulenger 1903	•			
Barbus miolepsis Boulenger, 1902	•			
Barbus multilineatus Worthington, 1933	•			
Barbus paludinosus Peters, 1852	•			
***Barbus poechii* Steindachner, 1911**	•		22	41
Barbus puellus Nichols and Boulton, 1927	•			
***Barbus radiates* Peters, 1853**	•		1	0
Barbus tangandensis Jubb, 1954	•			
Barbus thamalakanensis Fowler, 1935	•			
Barbus unitaeniatus Günther, 1866	•			
Coptostomabarbus wittei David and Poll, 1937	•			
Labeo cylindricus Peters, 1852	•			
Labeo lunatus Jubb, 1963	•			

Table 6.2 (continued): List of the fish species present (•) in the Okavango, (below Popa Rapids) (**OK**) and Orange-Vaal (**OV**) River Systems.

Fish families and species	OK	OV	n	P (%)
CYPRINIDAE continued				
Mesobola brevianalis (Boulenger, 1908)	•			
Opsaridium zambezense (Peters, 1852)	•			
Mesobola brevianalis (Boulenger, 1908)	•	•		
Barbus anoplus Weber, 1897		•		
Barbus pallidus A. Smith, 1841		•		
Barbus trimaculatus Peters, 1952		•		
Barbus paludinosus Peters, 1852	•	•		
Labeobarbus kimberleyensis (Gilchrist and Thompson, 1913)		•		
***Labeobarbus aeneus* (Burchell, 1822)**		•	1	0
***Labeo umbratus* (A. Smith, 1841)**		•	13	46
***Labeo capensis* (A. Smith, 1841)**		•	53	42
***Cyprinus carpio* Linnaeus, 1758 (Introduced)**		•	2	50
AUSTROGLANIDIDAE				
Austroglanis sclateri (Boulenger, 1901)		•		
CLARIIDAE				
Clarias dumerilii	•			
***Clarias gariepinus* (Burchell, 1822)**	•	•	1	0
Clarias liocephalus Boulenger, 1898	•			
Clarias ngamensis Castelnau, 1861	•			
Clarias stappersii Boulenger, 1915	•			
Clarius theodorae Weber, 1897	•			
Clariallabes platyprosopos Jubb, 1965	•			
SALMONIDAE (introduced)				
Salmo trutta Linnaeus, 1758 (introduced)		•		
Oncorhynchus mykiss (Walbaum, 1972) (introduced)		•		
CENTRARCHIDAE (introduced)				
Lepomis macrochirus Rafinesque, 1819		•		
Micropterus salmoides (Lacepède, 1802)		•		
PERCIDAE (introduced)				
Perca fluviatilis Linnaeus, 1758		•		
DISTICHODONTIDAE				
Hemigrammocharax machadoi Poll, 1967	•			
***Hemigrammocharax multifasciatus* Boulenger, 1923**	•		6	0
Nannocharax macropterus Pellegrin, 1925	•			
CHARACIDAE				
Brycinus lateralis (Boulenger, 1900)	•		103	92
Hydrocynus vittatus Castelnau, 1861	•			
***Micralestes acutidens* (Peters, 1852)**	•		4	0
***Rhabdalestes maunensis* (Fowler, 1935)**	•		6	0
HEPSETIDAE				
Hepsetus odoe (Bloch, 1794)	•		3	67
CLAROTEIDAE				
Parauchenoglanis ngamensis (Boulenger, 1911)	•			
AMPHILIIDAE				
Amphilius uranscopus (Pfeffer, 1889)	•			
Leptoglanis rotundiceps (Hilgendorf, 1905)	•			
Leptoglanis sp. (Boulenger, 1902)	•			
SCHILBEIDAE				
***Schilbe intermedius* Rüppell, 1832**	•		12	42

Table 6.2 (continued): List of the fish species present (•) in the Okavango, (below Popa Rapids) (**OK**) and Orange-Vaal (**OV**) River Systems.

Fish families and species	OK	OV	n	P (%)
CLARIIDAE continued				
Clariallabes platyprosopos Jubb, 1964	•			
Clarias dumerilii Steindachner, 1866	•			
MOCHOKIDAE				
Chiloglanis fasciatus Pellegrin, 1936	•			
Synodontis leopardinus Pellegrin, 1914	•			
Synodontis macrostigma Boulenger, 1911	•			
Synodontis macrostoma Skelton and White, 1990	•			
***Synodontis nigromaculatus* Boulenger, 1905**	•		**4**	**0**
Synodontis thamalakanensis Fowler, 1935	•			
Synodontis vanderwaali Skleton and White, 1990	•			
Synodontis woosnami Boulenger, 1911	•			
POECILIIDAE				
Aplocheilichthys hutereaui (Boulenger, 1913)	•			
***Aplocheilichthys johnstoni* Günther, 1893**	•		**10**	**0**
***Aplocheilichthys katangae* (Boulenger, 1912)**	•		**1**	**0**
Aplocheilichthys sp. Bleeker, 1863	•			
Gambusia affinis (Baird and Girard, 1853) (introduced)		•		
CICHLIDAE				
Hemichromis elongatus (Guichenot, 1859)	•			
***Oreochromis andersonii* (Castelnau, 1861)**	•		**17**	**18**
***Oreochromis macrochir* (Boulenger, 1912)**	•		**2**	**50**
Oreochromis mossambicus (Peters, 1852)		•		
Pharyngochromis acuticeps (Steindachner, 1866)	•			
***Pseudocrenilabrus philander* (Weber, 1897)**	•		**2**	**0**
***Pseudocrenilabrus philander* (Weber, 1897)**		•	**9**	**0**
Sargochromis carlottae (Boulenger, 1905)	•			
***Sargochromis codringtoni* (Boulenger, 1908)**	•		**1**	**0**
Sargochromis giardi (Pellegrin, 1903)	•			
***Sargochromis greenwoodi* (Bell-Cross, 1975)**	•		**16**	**25**
***Serranochromis altus* Winemiller and Kelso-Winemiller, 1990**	•		**1**	**0**
***Serranochromis angusticeps* (Boulenger, 1907)**	•		**6**	**50**
Serranochromis longimanus (Boulenger, 1911)	•			
Serranochromis robustus (Günther, 1864)	•		2	0
***Serranochromis macrocephalus* (Boulenger, 1899)**	•		**1**	**0**
Serranochromis thumbergi (Castelnau, 1861)	•			
***Tilapia rendalli rendalli* (Boulenger, 1896)**	•		**39**	**92**
Tilapia ruweti (Poll and Thys van den Audenaerde, 1965)	•			
***Tilapia sparrmanii* A. Smith, 1840**	•		**67**	**58**
***Tilapia sparrmanii* A. Smith, 1840**		•	**16**	**0**
ANABANTIDAE				
Microctenopoma intermedium (Pellegrin, 1920)	•			
Ctenopoma multispine Peters, 1844	•			
MASTACEMBELIDAE				
Aethiomastacembelus frenatus (Boulenger, 1901)	•			
Aethiomastacembelus vanderwaali (Skelton, 1976)	•			
TOTAL			**373**	**57**

The behaviour of parent fish to aggregate offspring within a nest may also be a behavioural trait which facilitates contact between the juvenile fish and the cercariae (Machado *et al.* 2005). Since individual diplostomatid infections are known to last for several years within the eyes or brain, in natural fish populations (Ferguson 1943a; Hoffman and Putz 1965; Burrough 1978; Hendrickson 1978) it could be that only juveniles attain infection, which then last throughout adulthood. The longevity and vigour of diplostomatid metacercariae are highlighted by Ashton *et al.* (1969), which collected live *D. spathaceum* metacercariae from the lenses of dead trout from fish markets.

Numerous authors have suggested that metacercariae can accumulate within the eyes and brains of fish throughout their life time (Beverley-Burton 1963; Kennedy and Burrough 1977; Bouillon and Curtis 1987; Burrough 1978; Barber and Crompton 1997; Valtonen and Gibson 1997). Since older, larger fish, have a higher body surface to volume ratio, it can also be deduced that adult fish will host a larger number of metacercariae than juveniles of the same species (Hendrickson 1978). Graczyk (1991a) stated that as a consequence of this accumulation, metacercariae are equally distributed in both eyes. Since blood circulation is the means of transport, no preference exists for the left or right eye. Similar to previous studies such as Kennedy and Burrough (1977), Bortz *et al.* (1988) and Graczyk (1991a), the number of metacercariae per eye were combined and not studied separately during the present study.

According to Kennedy and Burrough (1977) a positive linear relationship does not exist between the number of metacercariae and the age of the fish host. Factors, such as a thicker epidermal layer (Betterton 1974; Ratanarat-Brockelman 1974; Kennedy 1981) and acquired immunology (Sweeting 1974) may increasingly limit the penetration and migration of diplostomula to the eyes and brain of older fish. Since adult fish have a larger volume body mass, this may ultimately result in juvenile fish hosting a larger number of metacercariae per body tissue (Bortz *et al.* 1988; Karvonen *et al.* 2004c). Although not statistically represented, the proposed lower infection intensity present within adult fish correlates with the findings of the present study. The water level of a river system undoubtedly also influences the number of cercariae and the probability of

exposure to them. For example Moody and Gaten (1982) reported on a decrease in cercarial and metacercarial density, when the water level of an aquarium was increased. During the past few years a record flood level was reached in the Okavango River (Chapter 2). It is hypothesised that this lead to a decrease in concentration of cercariae to which fish were exposed and ultimately a decrease in metacercarial infection intensity. A similar hypothesis cannot be formulated for the Orange River System, since this river system is artificially flood regulated.

Final hosts: birds

Since *Diplostomum* infection occurs only in a small number of snail species and then transmits to numerous fish species, it can be concluded that a large variety of different piscivorous bird species, may act as final hosts (Hechinger *et al.* 2007). Seppälä *et al.* (2006b) suggested that diplostomatids are broad generalists regarding their definitive hosts. After ingestion of the fish, the metacercariae settle in the small intestine of the bird, where the adult diplostomatids develop. It is speculated that different diplostomatid species do exhibit specific site selections. For example, previous experimental observations, from authors such as Hoffman and Hundley (1957) and Niewiadomska (1984, 1987), indicated that *D. spathaceum* prefers the middle and rear third of the small intestine, whilst *D. pseudospathaceum* occurs in the first third and in the middle and *Diplostomum paracaudum* and *D. baeri eucaliae* are found in the first third of the small intestine.

The two main criteria for the completion of the life cycle, would be the presence of a piscivorous bird and if it is large enough to successfully catch and eat an infected fish. A wide variety of bird species, fulfilling these criteria, are present in the Okavango and Orange-Vaal River Systems (Table 6.3). The diversity in bird sizes contribute to different sized fish species to successfully act as second intermediate hosts. Another factor which determines successful transmission is whether the trematode eggs will be shed into an aquatic environment which is inhabited with pulmonate snails. Since the diplostomatid eggs pass through the faeces, it is essential that the piscivorous bird remains within the vicinity of a freshwater habitat.

Table 6.3: Species list of piscivorous birds present (•) in the Okavango (**OK**) and Orange-Vaal (**OV**) River Systems. Approximate length (**L**) from tip of bill to tip of tail (cm) and feeding habits are also provided (compiled from Ginn and McIlleron, 1982; Sinclair *et al.* 2002; Sinclair and Davidson 2006).

Species name	Common name	L	Feeding habits	OK	OV
OPEN AND MID WATER PISCIVOROUS BIRDS					
Pelecanus rufescens Gmelin, 1789	Pink-backed Pelican	135	Dives, or scoops fish into pouch beneath bill	◦	
Pelecanus onocrotalus Linnaeus, 1758	Great (Eastern) White Pelican	180	Dives, or scoops fish into pouch beneath bill	•	
Chroicocephalus cirrocephalus Vieillot, 1818	Grey-headed Gull	40	Not clearly understood, but believed to be opportunistic scavengers	◦	◦
Phalacrocorax africanus (Gmelin, 1789)	Long-tailed Cormorant	55	Pursues and catches fish and other aquatic organisms beneath water surface	◦	◦
Phalacrocorax lucidus (Lichtenstein, 1823)	White-breasted Cormorant	90	Pursues and catches large fish and other aquatic organisms (such as frogs) beneath water surface	◦	◦
Anhinga rufa (Daudin, 1802)	African Darter	79	Dives beneath water surface	•	◦
Anastomus lamelligerus Temminck, 1823	African Open-billed Stork	90	Not clearly understood but possibly feeds on fish, frogs or other large freshwater creatures	◦	
Ciconia nigra (Linnaeus, 1758)	Black Stork	100	Feeds on fish	◦	◦
Ciconia episcopus (Boddaert, 1783)	Woolly-necked Stork	85	Feeds on small reptiles, insects, crabs, molluscs and fish	•	
Ephippiorhynchus senegalensis (Shaw, 1800)	Saddle-billed Stork	145	Feeds on various aquatic organisms, but usually on fish trapped in drying pans. Often tosses prey into air and then catches it to swallow head first	•	
Leptoptilos crumeniferus (Lesson, 1831)	Marabou Stork	130	Feeds on fish, aggregated in dried-up pans and especially carrion as well as snakes and rats	◦	
Mycteria ibis (Linnaeus, 1766)	Yellow-billed Stork	95	Probes for food with partially open bills and thrusts head to reach deeper water	•	◦
Chroicocephalus cirrocephalus Vieillot, 1818	Grey-headed Gull	40	Not clearly understood, but believed to be opportunistic scavengers	◦	•
Chlidonias leucopterus (Temminck, 1815)	White-winged Tern	23	Feeds on insects, frogs and possibly fish, collected from or near water surface by dipping	•	◦
Podiceps cristatus (Linnaeus, 1758)	Great Crested Grebe	50	Feeds on or beneath water surface		◦
Podiceps nigricollis Brehm, CL, 1831	Black-necked Grebe	28	Feeds on or beneath water surface		◦
Tachybaptus ruficollis (Pallas, 1764)	Dabchick (Little Grebe)	20	Feeds on or beneath water surface	•	•

Table 6.3 (continued): Piscivorous birds present (•) in the Okavango (**OK**) and Orange-Vaal (**OV**) River Systems.

Species name	Common name	L	Feeding habits	OK	OV
OPEN AND MID WATER PISCIVOROUS BIRDS (continued)					
Alcedo cristata Pallas, 1764	Malachite Kingfisher	15	Perches and then plunges into water to catch fish, frogs and sometimes insects	•	•
Alcedo semitorquata Swainson, 1823	Half-collared Kingfisher	20	Perches and then plunges into water to catch fish, frogs and other aquatic organisms, including insects	•	•
Ceryle rudis (Linnaeus, 1758)	Pied Kingfisher	30	Perches and then plunges into water to catch fish or frogs but also well-known to hover over open water before plunging	•	•
Megaceryle maximus (Pallas, 1769)	Giant Kingfisher	43	Perches and then plunges into water to catch fish or crabs and occasionally hovers over open water before plunging	•	•
Rhynchops flavirostris Vieillot, 1816	African Skimmer	40	Flying over water, inserts tip of lower mandible to detect fish in water	•	
SHALLOW AND SHELTERED WATER PISCIVOROUS BIRDS					
Ardea alba Linnaeus, 1758	Great White Egret	90	Stands motionless or wades very slowly in emergent vegetation or out in open, striking at fish, frogs and aquatic insects	•	•
Ardea cinerea Linnaeus, 1758	Grey Heron	100	Stands motionless in shallow water, then strikes to feed on fish	•	•
Ardea goliath Cretzschmar, 1829	Goliath Heron	140	Stands motionless in medium depth water, then strikes to feed on fish	•	•
Ardea purpurea Linnaeus, 1766	Purple Heron	85	Stands motionless in emergent vegetation, strikes to feed on fish	•	•
Ardeola ralloides (Scopoli, 1769)	Common Squacco Heron	45	Feeds on insects, crustaceans, small frogs and fishes by means of striking after standing or perching motionless in shallow water	•	
Butorides striata (Linnaeus, 1758)	Green-backed Heron	40	Wades; standing in water and remaining motionless, then striking to grab fish, frogs and other aquatic life	•	
Gorsachius leuconotus (Wagler,1827)	White-backed Night-Heron	56	Preys on fish, frogs and aquatic insects	•	
Ixobrychus minutes (Linnaeus, 1766)	Little Bittern	38	Preys on fish, frogs and aquatic insects	•	•
Ixobrychus sturmii (Wagler, 1827)	Dwarf Bittern (Rail Heron)	25	Feeds on insects, crustaceans, small frogs and fishes by means of striking after standing or perching motionless	•	•
Nycticorax nycticorax (Linnaeus,1758)	Black-crowned Night Heron	61	Perches or wades; standing motionless, then striking to grab fish, frogs and other aquatic life	•	•
Egretta ardesiaca (Wagler, 1827)	Black Heron (Egret)	45	Spread wings to form "umbrella" (casting a shadow) which attract fish and then it strikes to grab small fish beneath surface	•	•
Egretta garzetta (Linnaeus, 1766)	Little Egret	70	Hunts in shallow water, by walking or stalking, sometimes stirring mud and then stabbing fish, frogs and insects with its bill	•	•
Egretta intermedia (Wagler, 1829)	Yellow-billed Egret	65	Stands motionless or wades very slowly in emergent vegetation, striking at fish, frogs and aquatic insects	•	•
LARGE PISCIVOROUS BIRDS					
Haliaeetus vocifer (Daudin, 1800)	Fish Eagle	70	Perches and then swoops down to catch open water fish	•	•
Pandion haliaetus (Linnaeus, 1758)	Osprey	68	Birds of prey, catching fish in flight	•	
Scotopelia peli (Bonaparte, 1850)	Fishing Owl	63	Birds of prey, catching fish (of up to 2 kg) in flight	•	

ECOMORPHOLOGICAL CLASSIFICATION OF FISH

Ecological factors such as habitat and food preferences separate fish into four broad categories, namely 1) detrivores, which feed from decaying organic particles present in the water, 2) herbivores, which feed on plant material, 3) predators, actively preying on other fish and 4) omnivores which consume a variety of animal and plant material. These different preferences not only limit species to certain aquatic habitats within a system, it also resulted in the development of different morphological characteristics which aid in their feeding. According to Ramberg *et al.* (2006) the main morphological characteristics which are alike amongst fish exhibiting similar feeding preferences include: body shape, placement of eyes, size and shape of fins as well as the size and shape of the mouth. These morphological structures are used to construct an ecomorphological classification system for fish, ranging from groups A to E (compiled from Ramberg *et al.* 2006). Preferences for a certain habitat and food are also important characters which determine the likelihood for a fish species to become infected with diplostomatids.

Ecomorphological group A: Open- and mid water fast swimmers

Fish belonging to this group have a fusiform body shape, with forked or lunate caudal fins and large, laterally placed eyes. By making use of high speed, representatives of this category hunt, usually in roving shoals, in the upper layer of open water of flowing rivers. An exception to the rule is the African pike, *Hepsetus odoe*. Its morphology places it in this category but it is a solitaire hunter. Individuals occur in the still waters of lagoons, where they stalk and ambush their piscine prey. Fish species infected with diplostomatids and collected during the present study and during the study by Jansen van Rensburg (2006), belonging to this group include: *Barbus poechii*, *Brycinus lateralis*, *Rhabdalestes maunensis* and *Hepsetus odoe*.

Ecomorphological group B: Mid water slow swimmers

Species belonging to this group hover in the open waters and in-between the vegetation of lagoons. Morphological adaptations which assist in remaining motionlessly within the water column include broad fins, which increase the surface area. For example the dorsal fin extends from directly behind the head to the base of the tail stem, with the anterior part consisting of spines and the posterior part with flexible soft rays. They have laterally compressed body shapes and this thin profile assists in the large-mouth breams (*Serranochromis* Regan, 1920) to successfully stalk small fish occurring in the vegetation of lagoons. This morphology also assists tilapias (*Oreochromis* Günther, 1889 and *Tilapia* A. Smith, 1840) and the small-mouth breams (*Sargochromis* Regan, 1920) to select and feed on small invertebrates, plants and detritus along riparian vegetation. Collected fish species, which have been found to be infected with diplostomatid metacercariae, are *Schilbe intermedius*, *Oreochromis andersonii*, *O. macrochir*, *Sargochromis greenwoodi*, *Serranochromis angusticeps*, *Tilapia rendalli* and *T. sparrmanii*.

Ecomorphological group C: Surface feeders

Topminnows or lampeyes of the genus *Aplocheilichthys* Bleeker, 1863 are representative of this group. Four species are present within the Okavango River System but none were collected during the present study. These fish species form small shoals and exhibit several adaptations to feed on invertebrates, such as insects and plankton, on the surface of the water. For example the dorsal fins are flattened which allow it to feed at the water surface, without exposing their bodies to predators from above. To further facilitate its surface feeding lifestyle it also has remarkably large eyes, placed near the top of the heads, with the mouths also facing upward.

Ecomorphological group D: Bottom dwellers

Due to the diverse niches and variety of trophic levels provided by the benthic habitat of a river, the only morphological similarities shared between species of this category are

a dorso-ventrally compressed body and mouths facing face downward. Large quantities of food are available and range from detritus, benthic algae and a wide array of adult and larval insects, snails or even other fishes.

The catfishes (Clariidae) are probably the most well known representatives of this category but during the present study no specimens were collected to determine the prevalence and mean intensity of diplostomatid infection. Another benthic family belonging to this category but not sampled is the Mochokidae, also known as squeakers. Six species of squeakers co-exist in the Okavango and each is adapted to fill a different niche, thereby not competing with each other for resources. The body plan of squeakers indicates that they belong to the bottom feeding group, but a peculiar type of upside down feeding on termites on the surface of the river, has been reported (Ramberg *et al.* 2006). According to Jansen van Rensburg (2006) a whole range of ciliophorans have been recorded to occur in its rectum, probably symbionts assisting in digestion, but no diplostomatids have been observed in the eyes and brain of the dissected specimens.

Another bottom feeder, of which few specimens have been collected from the Orange-Vaal River System, is the invasive *Cyprinus carpio*. This introduced species has been found to yield low diplostomatid infections in the eyes. Other examples of bottom-dwelling fish species collected from the same site, which are infected with diplostomatid metacercariae, include *Labeo umbratus* and *Labeo capensis*.

Ecomorphological group E: Dense vegetation and rocky habitats

The dense foliage and continual darkness provided by papyrus beds in the Okavango River result in a unique habitat which enhances evolutionary adaptations for its inhabitants to find food and avoid predators. The most intriguing fish living in the jungle of dense vegetation belong to the African endemic family Mormyridae. These fish have an unusual morphological appearance with the body laterally compressed, a large anal fin, a forked caudal fin and a pronounced tail stem. Also known as the elephant / bottlenose fishes, they have soft mouths which specialise on the feeding of invertebrates taken from plants. A weak electrical current, which is species' specific, is

discharged by an organ situated on the tail (Ramberg *et al.* 2006). This facilitates in communication and prey detection and provides the family with an adaptive advantage for survival in the dense vegetation. Examples of collected fish species, which have been found to be infected with diplostomatid metacercariae, are *Marcusenius macrolepidotus*, *Pollimyrus castelnaui* and *Petrocephalus catostoma*.

DISCUSSION ON FISH HOST BIOLOGY AND EYE AND BRAIN FLUKE PREVALENCE

Throughout the following discussion, fish species infected with diplostomatids are grouped according to their families. The general biology of each of the families is discussed as well as the probability of its members to become infected with diplostomatids. This will be followed by a summarised description of the species' distribution, general biology and as well as the mean intensity of infection and prevalence of diplostomatids, as determined through fieldwork from 2008 - 2010.

A more comprehensive description of the fish species' habitat preference and ethology will also be given. To summarise the fieldwork from previous excursions (2001 - 2004) (Jansen van Rensburg 2006) and from the present study (2008 - 2010) a table will be provided. All the fish species were redrawn from Skelton (2001). The behavioural characteristics of the fishes were mainly compiled from Skelton (2001) and Froese and Pauly (2010). All reference material is deposited in the Parasite Collection of the Aquatic Parasitology Research Group, Department of Zoology and Entomology, University of the Free State, Bloemfontein.

FAMILY MORMYRIDAE

The family consists of 18 genera and 200 species, endemic to Africa and are commonly known as snout- or elephant fish. This is due to their soft bodies and snouts which often extend to form an unusual proboscis, rendering a dolphin-like appearance. They have a large brain, relative to their body mass, which possibly aids in their communication. Weak electric currents are generated and received to aid in intraspecies' communication and predator detection. Members of this family are mostly shy and mysterious and therefore little is known about their breeding biology. In certain species it is suggested that courtship is complex, the males build nests and guard the larvae (Skelton 2001). The different genera of the family are widespread in the Afro-tropical region but are not present in the Orange-Vaal River System, the rivers of the Cape Provinces and the rivers of the Maghreb (north-western Africa).

Due to their preference for the dense vegetation of the papyrus beds and adaptations to flourish in these dark environments, representatives of this family are grouped in the E ecomorphological group (Ramberg *et al.* 2006). They are constantly exposed to an environment which is also preferred by many snail species such as *Lymnaea natalensis* and *Biomphalaria pfeifferi* (Utzinger and Tanner 2000; Appleton *et al.* 2003), which are known to shed diplostomatid cercariae (Table 6.1). It is therefore clear why a relatively high prevalence of the Okavango mormyrid population was infected with diplostomatids occurring within the eyes and brain (Tables 6.4 - 6.6).

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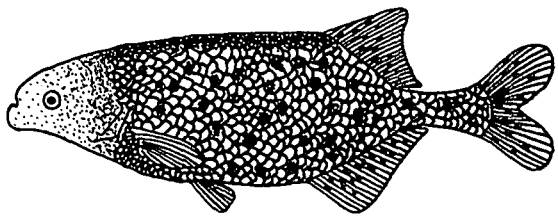
Marcusenius macrolepidotus (Peters, 1852)



Common Name	Bulldog
Distribution	20°S - 31°S; Okavango, Cunene, upper Congo and Zambezi Systems. Also common and widespread in east coastal rivers and lakes from Tanzania south to Umhlatuzi in KwaZulu-Natal
Ecomorphological Classification	Group E - Dense vegetation and rocky habitats
Environment	Freshwater, [1]demersal , [2]potamodromous
Climate	Tropical
Okavango Localities	Nxamasere, Mormyrid Marsh
Collection	Day
Parasite	Metacercariae of *Diplostomum* type 1 and *Diplostomum* type 2
Sites of Infection	Eyes and brain
Prevalence	75% (eye); 100% (brain)
Mean Infection Intensity	2.67 (eye metacercariae); 46.25 (brain metacercariae)
Remarks	Recent studies suggest at least two species

Biology, Ecology and Ethology

The genus *Marcusenius* Gill, 1862 is a relatively large group, consisting of 37 species mostly in tropical Africa. *Marcusenius macrolepidotus* is the sole representative present in southern Africa. The well-developed mental lobe jaw is one of the main characteristics of this shy species. Along with all other Okavango mormyrids, it has a preference for the well-vegetated, muddy-bottomed marginal habitats of rivers and floodplains. Being a nocturnal species, shoals move inshore after dark and feed on a

[1] Organisms which live near bottom and feed on benthic organisms

[2] Organisms which seasonally migrate within streams or in rivers, over distances more than 100 km

wide range of invertebrates. Midge, mayfly larvae and pupae taken from the bottom and off plant stems are a special preference. During the flood season, migrations within the river tributaries have also been recorded but it is not known whether these are for breeding purposes. Breeding does occur during the rainy season, with the female carrying up to 6 000 eggs, and staying hidden in shallow vegetated localities. Electro receptors and electric organs aid in the electro-communication of this very sociable species. The entire head and the ventral and dorsal regions of the body are covered with the receptors, whilst the electric organs are located on the lateral regions and on the caudal peduncle (Froese and Pauly 2010). This species and their electro-communication signals are speculated to play important roles in the annual barbell runs present within the Okavango River System.

Description of metacercarial types (Figures 5.3 A and 5.4 A)

Collection of *M. macrolepidotus* was conducted during the day by means of dragging nets underneath papyrus and other types of peripheral vegetation. Metacercariae of *Diplostomum* type 1 were collected from the brain, whilst *Diplostomum* type 2 metacercariae were collected from the brain and eyes. The morphological description and remarks of each type from various fish species and tissues are provided in Chapter 5. Tables, giving the morphometrics of the different metacercarial types collected from the brain (type 1) and from the eyes as well as the brain (type 2) exclusively from *M. macrolepidotus*, are provided in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.4.

Possible life cycle

The life cycle, of which *M. macrolepidotus* acts as the second intermediate host, includes one of the possible snail hosts (Table 6.1) and piscivorous birds (Table 6.3) present in the Okavango. The shy nature of this fish species, occurring amongst dense vegetation, most probably will result in shallow and sheltered water piscivorous birds acting as definitive hosts. Their preference for well-vegetated, muddy-bottomed

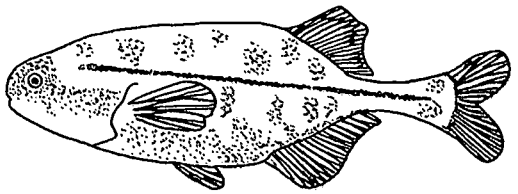
marginal habitats of rivers and floodplains will contribute to cercarial exposure from snails such as *Lymnaea natalensis* and *Biomphalaria pfeifferi*, which also prefer this type of habitat.

Table 6.4: Results of *Marcusenius macrolepidotus* (Peters, 1852) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae in the Okavango River System, Botswana, collected during the present and previous studies. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (**×** = not infected, **na** = data not available).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
2008 - 2010						
Nxamasere	2	145 (140-150)	*2	100	eyes	3 (1-5)
			*2	100	brain	42 (14-70)
Mormyrid Marsh	2	100 (90-110)	1	50	brain	50.5 (1-101)
			*1	50	eyes	2
TOTAL	4	122.5 (90-150)	3	75	eyes	2.67 (1-5)
			4	100	brain	46.25 (1-101)
2001 - 2004						
Boro	3	92.5 (90-95)	×	×	×	**na**
Mohembo Mainstream	9	82.5 (80-90)	7	77.7	brain	**na**
			9	100	eyes	**na**
Toteng Bridge	23	97.2 (90-110)	×	×	×	**na**
Shakawe Mainstream	3	96.6 (95-100)	×	×	×	**na**
TOTAL	38		7	18.42	brain	**na**
			9	23.68	eyes	**na**

* Hosts infected with diplostomatids occurring in both the brain and eyes

Pollimyrus castelnaui (Boulenger, 1911)



Common Name	Dwarf stonebasher
Distribution	11°S - 21°S; Okavango, Cunene, upper Zambezi and Kafue rivers as well as in northern areas of Lake Malawi
Ecomorphological Classification	Group E - Dense vegetation and rocky habitats
Environment	Freshwater; demersal
Climate	Tropical
Okavango Localities	Samochima Lagoon, Mormyrid Marsh, Drotsky Upstream Temporary Floodplain
Collection	Day and night
Parasite	Metacercariae and cysts of *Diplostomum* type 2, *Diplostomum* type 3 and *Diplostomum* type d.
Site of Infection	Eyes and brain
Prevalence	47.37%
Mean Infection Intensity	2.33 (brain metacercariae); 10 (brain cysts); 4.5 (eye metacercariae)
Remarks	It is suggested that marked differences occur between populations and that more than one species may exist

Biology, Ecology and Ethology

The genus *Pollimyrus* Taverne, 1971 consists of about 19 species, widely distributed in tropical Africa, but of which only *P. castelnaui* is found in southern Africa. This species prefers the dense vegetation along the margins of rivers, floodplain lagoons and backwaters and other shallow water just underneath the surface vegetation. These habitat preferences place *P. castelnaui* in the E group of the ecomorphological classification system of Ramberg *et al.* (2006). Little is known about their breeding

biology but the presence of electric organ discharges indicates that this is a very sociable species. Their diet primarily consists of aquatic insect larvae.

Description of metacercarial types (Figures 5.4 A; 5.3 B and 5.6 B)

Collection of *P. castelnaui* was conducted during the day by means of scoop-nets underneath papyrus and other types of peripheral vegetation. Metacercariae of *Diplostomum* type 2, type 3 and type d were collected from the eyes and the brain. The morphological description and remarks of each type (from various fish species and tissues) are provided in Chapter 5. Tables, providing the morphometrics of the different metacercarial types collected from the brain (type 3) and from the eyes as well as the brain (type 2 and type d) exclusively from *P. castelnaui*, are provided in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.5.

Possible life cycle

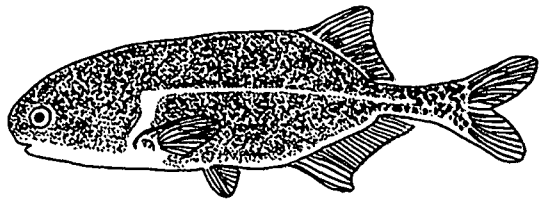
The small size of these fish species and their preference for dense vegetation in shallow water will probably lead to cercarial exposure from snails such as *Lymnaea natalensis* and *Biomphalaria pfeifferi* (Table 6.1). For the same reason capture by piscivorous birds from the Okavango placed in the shallow and sheltered water category (Table 6.3) is also suggested.

Table 6.5: Results of *Pollimyrus castelnaui* (Boulenger, 1911) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae (**M**) and cysts (**C**) in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (× = not infected).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity	Stage
Samochima Lagoon	1	50	×	×	×	×	×
Mormyrid Marsh	13	45.2 (35-66)	8	61.54	brain	2.5 (1-9)	M
			*1	7.69	brain	10	C
			2	15.38	eyes	4.5 (1-9)	M
Mainstream	1	38	×	×	×	×	×
Drotsky Upstream Temporary Floodplain	4	39 (20-51)	1	25	brain	1	M
TOTAL	19	43.6 (20-66)	9	47.37	brain	2.33 (1-9)	M
			*1	5.26	brain	10	C
			2	10.53	eyes	4.5 (1-9)	M

* Hosts infected with diplostomatids occurring in both the brain and eyes

Petrocephalus catostoma (Günther, 1866)



Common Name	Northern Churchill
Distribution	3°S - 27°S; Central and South African eastward flowing rivers. Widespread in Cunene, Okavango and Zambezi Systems south to Save River. Also reported from Zambezi River, Congo River, Great Lakes (Malawi, Tanganyika and Victoria) and East Africa
Ecomorphological Classification	Group E - Dense vegetation and rocky habitats
Environment	Freshwater, demersal, potamodromous
Climate	Tropical
Okavango Localities	Samochima Lagoon, Mormyrid Marsh
Collection	Day and night
Parasite	Metacercariae of *Diplostomum* type 1, *Diplostomum* type 2 and *Diplostomum* type 3
Site of Infection	Eyes and brain
Prevalence	50% (eye); 25% (brain)
Mean Infection Intensity	5.5 (eyes metacercariae); 2 (brain metacercariae)

Biology, Ecology and Ethology

The genus *Petrocephalus* Marcusen, 1854 differs from other mormyrid genera on the presence of unique electro receptor rosette patterns around the eyes. About 20 species occur throughout the Afro-tropical region of which two are found in southern Africa and only one in the Okavango River System. *Petrocephalus catostoma* forms shoals in shallow, muddy and well-vegetated reaches of rivers, floodplains, lagoons, sheltered bays and swampy areas and therefore can be placed in the E ecomorphological group (Ramberg *et al.* 2006). Both sexes are known to be territorial but during the night individuals will gather to form groups which forage together on insect larvae and other small invertebrates. This species has also been found to shoal

with other species which have identical electric organ discharge (EOD) waveforms, supporting the hypothesis of species' recognition through EOD. *Petrocephalus catostoma* is an oviparous species, which possibly migrates upstream during the summer rainy season, where suitable sites are present for breeding. An unconfirmed report claims the males are territorial and build nests.

Description of metacercarial types (Figures 5.3 A, 5.4 A and 5.3 B)

Collection of *P. catostoma* was conducted during the day and night by means of scooping with nets underneath papyrus and other types of riparian vegetation. Metacercariae of *Diplostomum* type 1, type 2 and type 3 were collected from the eyes and the brains. The morphological description and remarks of each type from various fish species and tissues are provided in Chapter 5. Tables, containing the morphometrics of the different metacercarial types collected from the brain (type 1 and type 3) and from the eyes as well as the brain (type 2) are provided in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.6.

Possible life cycle

The life cycle, of which *P. catostoma* acts as the second intermediate host, includes one of the possible snail hosts (Table 6.1) and piscivorous bird hosts (Table 6.3) present in the Okavango. The preference of this species to shoal in shallow, muddy and well-vegetated reaches of aquatic environments will probably result in cercarial exposure from snails such as *Lymnaea natalensis* and *Biomphalaria pfeifferi* and capture by piscivorous birds placed in the shallow and sheltered water category.

Table 6.6: Results of *Petrocephalus catostoma* (Günther, 1866) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae in the Okavango River System, Botswana, during the present and previous studies. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (**na** = data not available).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
2008 - 2010						
Samochima Lagoon	1	60	1	100	eyes	9
Samochima Lagoon	1	70	1	100	brain	2
Mormyrid Marsh	2	50	1	50	eyes	2
TOTAL	4	57.5 (50-70)	2	50	eyes	5.5
			1	25	brain	2
2001 - 2004						
Mohembo Mainstream	4	56 (50-60)	2	50	brain	**na**
Shakawe	10	52.9 (33-65)	4	40	brain	**na**
Shakawe	2	54.5 (50-90)	1	50	brain	**na**
TOTAL	16	53.88 (33-90)	7	43.7	brain	**na**

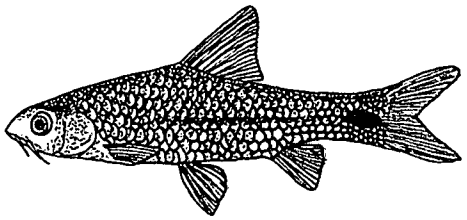
FAMILY CYPRINIDAE

Commonly known as the barbs, yellowfish or labeos, this family consists of eight genera and about 80 species, and is regarded as the largest family consisting of primarily freshwater fish in southern Africa. Their broad distribution includes Africa, Europe, Asia and North America with a total of 275 genera and more than 1 600 species (Skelton 1988, 2001). Family members display a wide range of shapes and sizes and many species have characteristic pigment patterns, of which the full range of variation must be considered when trying to identify specimens up to species level. Diverse life-history styles and habitat preferences are also evident but in general cyprinids are often strong swimmers with distinct modifications to live in strong currents. Characteristics of this family include the presence of teeth on the strong pharyngeal (throat) bones rather than on the jaws and also the absence of a true stomach. Usually, especially in detritus and plant feeders, the gut is extended to aid in

digestion (Skelton 2001). Certain species are of economic importance due to their use in fisheries, aquaculture, aquarium trade and recreational fishing with one of the consequences being that several southern African species are threatened (Skelton 2001).

During the present study four species, belonging to this family, were collected and found to be infected with diplostomatids. *Barbus poechii*, occurs within the Okavango River System and according to the criteria of Ramberg *et al.* (2006), is placed in the A ecomorphological group. These fish prefer open- to mid water environments and are therefore, when compared to mormyrids, not as exposed to snails such as *Lymnaea natalensis* and *Biomphalaria pfeifferi* and their cercarial shedding. *Bulinus* species, which can occur in open waters as well, may also be responsible for this species to become infected with diplostomatids. The nature of this species to actively swim around, searching for prey also decreases their chance for cercarial penetration and prevalence of infection (Table 6.7), especially when compared to the more passive nature of other cyprinids. *Labeo umbratus*, *L. capensis* and *Cyprinus carpio* are cyprinid species collected from the Orange-Vaal River System and were also infected with diplostomatids. These bottom-dwelling species (ecomorphological group D) feed on detritus and hence have a more passive life style. Only two snail species within the Orange-Vaal River System which are known to shed furcocercariae, are associated with their habitat namely *Bulinus tropicus* and *Bulinus africanus*. However, these fishes' inactive nature leaves them more exposed to cercarial penetration and could explain the relatively high prevalence for diplostomatid infected individuals amongst these species (Tables 6.8 - 6.10).

Barbus poechii Steindachner, 1911



Common Name	Dashtail barb
Distribution	11°S - 21°S; Upper Zambezi System and Kafue River; as well as Cunene and Okavango River Systems
Ecomorphological Classification	Group A - Open- and mid water fast swimmers
Environment	Freshwater, [3]benthopelagic
Climate	Tropical
Okavango Localities	Toteng Bridge, Samochima Lagoon, Mormyrid Marsh
Collection	Day and night
Parasite	Metacercariae of *Diplostomum* type 2, *Diplostomum* type c
Site of Infection	Eyes
Prevalence	40.91%
Mean Infection Intensity	5.67 (eye metacercariae)

Biology, Ecology and Ethology

Although there are several widely different barbine cyprinids in Africa, most are included in the genus *Barbus* Cuvier and Cloquet, 1816. Reclassification of African barbs is in progress, since it is now realised that the genus is valid only for certain tetraploid European species and a few species from the Maghreb region of north-west Africa. *Barbus poechii* is categorised to the A ecomorphological group due to its fusiform body shape, forked caudal fins and laterally placed eyes (Ramberg *et al.* 2006). It is a common species in riverine and floodplain habitats, where it feeds on insects and small organisms. It is frequently associated with another ecomorphological

[3] Organisms which are opportunistic feeders and forage on bottom as well as in mid water and near surface

A species, namely *Brycinus lateralis*. These two species are very similar in appearance, with *Barbus poechii* sometimes only distinguishable by the absence of an adipose fin. This suggests mimicry between the species and *B. poechii* obtaining protection from possible threats due to the more aggressive nature of the striped robber.

Description of metacercarial types (Figures 5.4 A and 5.6 A)

Collection of *B. poechii* was conducted during the day by means of cast nets in floodplains and lagoons. Metacercariae of *Diplostomum* type 2 and type c were collected from the eyes. The morphological description and remarks of each type from various fish species and tissues are provided in Chapter 5. Tables, providing the morphometrics of the different metacercarial types collected from the eyes of *B. poechii*, are given in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.7.

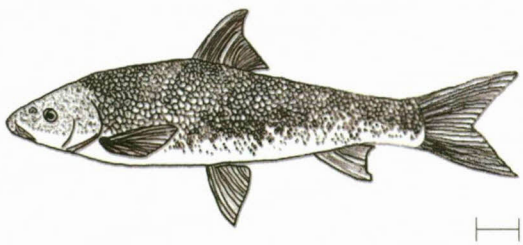
Possible life cycle

The life cycle, of which *Barbus poechii* acts as the second intermediate host, includes one of the possible snail hosts (Table 6.1) and piscivorous bird hosts (Table 6.3) present in the Okavango. As stated earlier *Bulinus* species will most possible act as the first intermediate hosts and source of diplostomatid cercariae. The nature of this fish species to occur in open- to mid waters as well as their relative small size during adulthood most probably will result in open- and mid water piscivorous birds acting as definitive hosts.

Table 6.7: Results of *Barbus poechii* Steindachner, 1911 examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (× = not infected).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
Toteng Bridge	17	83.82 (35-105)	9	52.94	eyes	6.71 (1-16)
Samochima Lagoon	3	72.33 (35-90)	×	×	×	×
Mormyrid Marsh	2	35 (30-40)	×	×	×	×
TOTAL	22	77.82 (30-105)	9	40.91	eyes	5.67 (1-16)

Labeo umbratus (A. Smith, 1841)



Common Name	Moggel
Distribution	23°S - 34°S; within drainage basin of Orange-Vaal as well as in southern and south-western watersheds of Cape coastal region Introduced to Keiskamma and Buffalo Systems (Eastern Cape) as well as Olifants-Limpopo (Mpumalanga) by anglers
Ecomorphological Classification	Group D - Bottom dwellers
Environment	Freshwater, benthopelagic, potamodromous
Climate	Subtropical but survives temperatures below 10°C
Orange-Vaal River Localities	Renosterspruit: Bishop's Weir
Collection	Day
Parasite	Metacercariae of *Diplostomum* type 2
Site of Infection	Eyes
Prevalence	46.15%
Mean Infection Intensity	11.8 (eye metacercariae)
Remarks	Closely resembles *L. capensis* in colouration and in having well developed anterior barbells

Biology, Ecology and Ethology

The genus *Labeo* Cuvier, 1817 is widely distributed in Africa and south-east Asia, with at least 80 species occurring within Africa. In southern Africa there are 12 species, two of which occur in the Orange-Vaal River System and another two in the Okavango River System. *Labeo umbratus* is one of the two *Labeo* species present within the Orange-Vaal River System. The downward facing mouths and dorso-ventral compressed bodies of this species are characteristic of species belonging to the D ecomorphological group (Ramberg *et al.* 2006). It is also recognised as a species of which the adults feed on material provided by the benthic environment such as detritus and mud, whilst the juveniles feed on small invertebrates. Its preferred habitat is not a lotic environment but rather lentic or gently flowing waters which are present in the muddy, shallow areas of impoundments and farm dams (Skelton and Cambray 1981).

Members of this relatively long-lived species breed during the summer after rains and migrate upstream to suitable spawning sites. These sites include flooded grassy banks of rivers or shallow rocky stretches where the highly fecund females will lay as many as 250 000 small eggs. The eggs are sticky and attach to the surrounding grass on rocks where after 40 hours it hatches (Skelton 2001). The newly hatched larvae repeatedly swim to the surface as it is carried by the current into the stream and deeper water. The growth rate is rapid with the young fish reaching about 100 mm standard length after only one year following hatching.

Description of metacercarial types (Figure 5.4 A)

Collection of *L. umbratus* was conducted during the day by means of electro-fishing and cast nets in the Orange-Vaal River at Bishop's Weir. Metacercariae from only *Diplostomum* type 2 was collected from the eyes and the morphological description and remarks are provided in Chapter 5. The morphometrics of the metacercarial types collected from the eyes of *L. umbratus* are given in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.8.

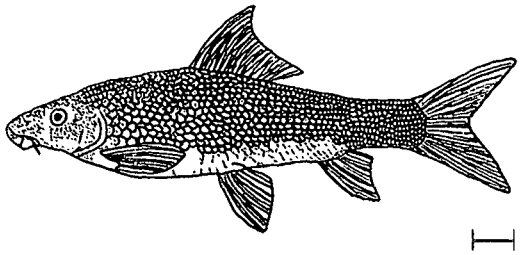
Possible life cycle

Labeo umbratus acts as the second intermediate host for diplostomatid larvae which occur in snail hosts, such as *Lymnaea natalensis* and *Bulinus* species (Table 6.1). Both these species are present in the Orange-Vaal River System. Since higher diplostomatid prevalence occurred in juvenile fishes, which mainly occur amongst rocks and other vegetated shelters, it is most probable that *L. natalensis* will act as first intermediate host. Similar to *L. capensis* the bottom-dwelling nature of *L. umbratus* decreases the possibility of aerial predation. Piscivorous birds belonging to the shallow and sheltered water group could therefore form part of the life cycle (Table 6.3)

Table 6.8: Results of *Labeo umbratus* (A. Smith, 1841) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae at Bishop's Weir in the Orange-Vaal River System, South Africa, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated.

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
Bishop's Weir	13	22.38 (6-45)	6	46.15	eyes	11.8 (1-40)

Labeo capensis (A. Smith, 1841)



50 mm

Common Name	Orange River mudfish
Distribution	24°S - 30°S; possibly restricted to entire Orange-Vaal River System
Ecomorphological Classification	Group D - Bottom dwellers
Environment	Freshwater, benthopelagic
Climate	Subtropical
Orange-Vaal River Localities	Renosterspruit: Bishop's Weir
Collection	Day
Parasite	Metacercariae of *Diplostomum* type 2, *Diplostomum* type a, *Diplostomum* type b and *Diplostomum* type c
Site of Infection	Eyes
Prevalence	42.31%
Mean Infection Intensity	3.5 (eye metacercariae)

Biology, Ecology and Ethology

Labeo capensis is the most common large fish species in the Orange-Vaal River System (Skelton and Cambray 1981). It is reported to occur in a variety of environments such as quiet, well-vegetated backwaters, standing open waters and sandy-rocky stretches. Its preferred habitat, however, is the flowing open waters of rocky channels of large rivers and they also thrive in large impoundments. Although their preferred habitat does not include the benthic environment, *L. capensis* is still placed within the bottom-feeding D ecomorphological group. By means of their downward facing mouths and well-developed complex lips, they graze on algae and organic detritus attached to the firm surface of rocks and plants present on the bottom of water habitats. All labeos are specialised feeders on algae and detritus from the

substratum and have grinding teeth in the pharynx and a very long, coiled intestine in order to ingest and digest this food source. This long-lived species (eight to nine years) breeds during the summer when they gather in large numbers in shallow rocky rapids to lay their eggs. The larvae hatch after three to four days where after the growth is fairly rapid (Skelton 2001).

Description of metacercarial types (Figures 5.4 A, 5.4 B, 5.5 and 5.6 A)

Collection of *L. capensis* was conducted during the day by means of electro-fishing and cast netting in the Orange-Vaal River at Bishop's Weir. Metacercariae of *Diplostomum* type 2, type a, type b and type c were collected from the eyes. The morphological description and remarks of each type from various fish species and tissues are provided in Chapter 5. Tables, giving the morphometrics of the different metacercarial types collected from the eyes from exclusively *L. capensis*, are provided in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.9.

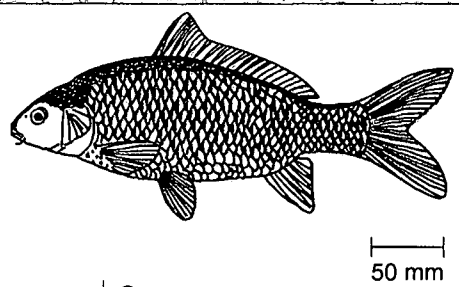
Possible life cycle

The bottom-dwelling nature of this species decreases the possibility of aerial predation. Since higher diplostomatid prevalence occurred in juveniles, which mainly occur amongst rocks and other vegetated shelters, it is possible that shallow and sheltered water piscivorous birds (Table 6.3) will act as definitive hosts. From all the snails species present in the Orange-Vaal System (Table 6.1), *Lymnaea natalensis* and *Bulinus* species will most likely act as the first intermediate hosts and source of diplostomatid cercariae.

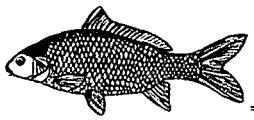
Table 6.9: Results of *Labeo capensis* (A. Smith, 1841) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae at Bishop's Weir in the Orange-Vaal River System, South Africa, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated.

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
Bishop's Weir	52	131.1(38-350)	22	42.31	eyes	3.5 (1-20)

Cyprinus carpio Linnaeus, 1758



Common Name	Carp
Distribution	60°N - 40°N; introduced and widespread throughout southern Africa. Absent from mountain areas and restricted in warmer tropical areas such as lowveld. Natural distribution includes Central Asia, east into China and west into Europe as far as Black Sea and Danube River
Ecomorphological Classification	Group D - Bottom dwellers
Environment	Freshwater and brackish, benthopelagic, potamodromous
Climate	Subtropical
Orange-Vaal River Localities	Renosterspruit: Bishop's Weir
Collection	Day
Parasite	Possibly metacercariae of *Diplostomum* type 2
Site of Infection	Eyes
Prevalence	50%
Mean Infection Intensity	1 (eye metacercariae)



Biology, Ecology and Ethology

Carp were introduced into the Cape in 1896 and today it is the best known and most widely introduced of all fish species in the Orange-Vaal River System as well as in the rest of South Africa (Skelton and Cambray 1981). Some people are even convinced that this species is the national fish and native to South Africa. Presently it does not occur in the Okavango River System but has been introduced to the Gaborone Dam which is connected to the Limpopo System. It causes a very detrimental effect on biodiversity due to its habit of grubbing in the bottom, kicking up sediments and thereby depleting the aquatic environment of oxygen. It is a omnivore, taking mud into its mouth and later ejecting it after food particles have been extracted. This non-specialised feeding technique allows it to feed on a large variety of plant and animal matter, such as aquatic insects, crustaceans, annelids, molluscs, tree seeds, wild rice, aquatic plants and algae. This species is inclined to eat the spawn of other fish and in cultured conditions it will even consume its own eggs. To facilitate in feeding from the benthic habitat of the river, its mouth faces downwards. Consequently the species is placed in the D ecomorphological group of Ramberg *et al.* (2006) which includes other bottom feeders as well.

It can survive between huge ranges of environmental conditions and will even gulp atmospheric air when occurring in an aquatic environment where the dissolved oxygen concentration is low (Hecht and De Moor 2005). The ability of this very hardy and tolerant species to survive adverse ecological conditions and the habit of the adults to uproot and destroy aquatic vegetation make it one of the most detrimental alien fish species in southern Africa. In general the species favours large water bodies with slow-flowing or standing water, soft bottom sediments and abundant vegetation such as large turbid rivers and farm dams.

Breeding occurs during late spring and summer when water temperatures reach about 18°C, with spawning being triggered by the influx of fresh water. A large fecund female is able to lay in excess of a million transparent, adhesive eggs in shallow waters. The sticky eggs sink and stick to submerged vegetation and hatch following four to eight days where after growth of the larvae and fry is rapid (Skelton 2001).

Description of metacercarial types

Collection of *C. carpio* was conducted by means of cast nets at Bishop's Weir in the Orange-Vaal River System during the day. Since only two metacercariae were collected from the eyes of one *C. carpio*, the material was not sufficient to aid in effective metacercarial type identification. The overall body form was noted to be elongated and since *Diplostomum* type 2 metacercariae have been identified from both the other Orange-Vaal River species, namely *L. umbratus* and *L. capensis*, it is suggested that this type could also be present in *C. carpio*. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.10.

Possible life cycle

Similar to *L. umbratus* and *L. capensis,* the bottom-dwelling nature of this species decreases the possibility of aerial predation. Most possibly only the juveniles, which occur amongst rocks and other vegetated shelters, will be caught by shallow and sheltered water piscivorous birds (Table 6.3). The vegetated and bottom-dwelling nature of this species also signifies the possibility that *Bulinus* species and *Lymnaea natalensis* can act as the first intermediate host (Table 6.1).

Table 6.10: Results of *Cyprinus carpio* Linnaeus, 1758 examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae at Bishop's Weir in the Orange-Vaal River System, South Africa, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean intensity of diplostomatid infection are also indicated.

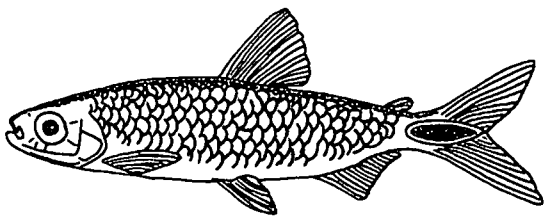
Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
Bishop's Weir	2	11 (9.5-12.5)	1	50	eyes	2

FAMILY CHARACIDAE

The characins form a large and diverse family of fishes which are widespread in tropical South America and Africa but they are absent from the Maghreb region, the Orange-Vaal River System and further down south (Skelton 1988). In the tropical waters of Africa alone there are 18 genera and over 100 species. Six species are present within southern Africa of which four are known from the Okavango Drainage System. The family includes the well-known top-predator tigerfish, *Hydrocynus vittatus* Castelnau, 1861, which exhibits the distinctive characters of most characins: large eyes, bony cheeks, large silvery scales, a short dorsal fin, a long-based sexually dimorphic anal fin, a small adipose fin and sharp-pointed multicuspid teeth on the jaws (Skelton 2001). Except for the recreational and economic benefits provided by tigerfish angling, the rest of the family are not of economic importance. Two species from this family were sampled and observed to be infected with *Diplostomum* metacercariae.

Both *Brycinus lateralis* and *Rhabdalestes maunensis* are species which can be grouped in the A ecomorphological group. These species are active swimmers and prefer open waters, where they hunt for small prey during the night. Due to their preference for hunting in open waters, it is evident why they would be less exposed to snails and cercarial penetration, when compared to the well-vegetated environment of mormyrids. A high prevalence (Table 6.11) and moderate intensity of infection were noted for *B. lateralis* individuals collected during the present study. It is speculated that since the species still retreat to the protection of vegetation cover during the day time, they remain exposed to cercarial shedding of snails such as *Lymnaea natalensis*, *Biomphalaria pfeifferi* and *Bulinus* species.

Brycinus lateralis (Boulenger, 1900)



50 mm

Common Name	Striped robber
Distribution	11°S - 28°S; Okavango, Zambezi System, Cunene and Buzi rivers as well as in the Congo System, Lake Kariba in Zambezi Basin and St. Lucia catchment area in KwaZulu-Natal
Ecomorphological Classification	Group A - Open- and mid water fast swimmer
Environment	Freshwater, pelagic, potamodromous
Climate	Tropical
Okavango Localities	Samochima lagoon, Toteng bridge, Nxamasere
Collection	Day and night
Parasite	Metacercariae of *Diplostomum* type 2, *Diplostomum* type a, *Diplostomum* type b and *Diplostomum* type c
Site of Infection	Brain and eyes
Prevalence	92.23% (brain); 22.33% (eyes)
Mean Infection Intensity	19.26 (brain metacercariae); 2.25 (eye metacercariae)

Biology, Ecology and Ethology

The genus *Brycinus* Valenciennes, 1849 consist of about 30 species in Africa of which two are present in southern Africa. The species is known to form roving groups which hunt in clear, slow-flowing or quiet, well-vegetated waters. Along with other characteristics such as a fusiform body shape, forked caudal fins and large, laterally placed eyes they are typical representative of the A ecomorphological group (open- and mid water fast swimmers) (Ramberg *et al.* 2006). These small, nocturnal fish feed on tiny aquatic and terrestrial organisms. In the Okavango they are also often found together with the dashtail barb (*Barbus poechii).* Having similar dietary preferences and sharing close morphological similarities, mimicry is suggested between them.

Breeding most possibly occurs during the rainy season when this species moves upstream. They are often used by anglers as bait for tigerfish and largemouth bream, but they are also occasionally a nuisance to those whose bait is stripped from the hooks.

Description of metacercarial types (Figures 5.4 A, B, 5.5 and 5.6 C)

Collection of *B. lateralis* was conducted during the day and night by cast nets from boats and edges of the Okavango River. The largest number of infected fish was collected at Toteng Bridge. These fish were caught during the day time, whilst they were shoaling in shallow waters. Metacercariae of *Diplostomum* type 2, type a, type b and type c were collected from the eyes and brains. The morphological description and remarks of each type (from various fish species and tissues) are provided in Chapter 5. Tables, providing the morphometrics of the different metacercarial types collected from the different tissues, exclusively from *B. lateralis*, are provided in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.11.

Possible life cycle

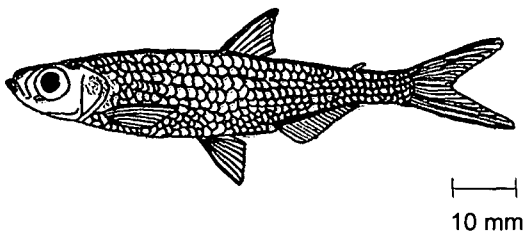
The nature of this species to occur in open- to mid waters as well as their relative small size during adulthood most probably will result that open- and mid water piscivorous birds will act as definitive hosts (Table 6.3). *Bulinus* species which are also known to occur in these mid water habitats could most probably also act as first intermediate hosts (Table 6.1).

Table 6.11: Results of *Brycinus lateralis* (Boulenger, 1900) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated.

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
Samochima Lagoon	40	88.28 (40-105)	34	85	brain	11.02 (1-40)
			*8	20	eyes	2.32 (2-12)
			1	2.5	eyes	1
Nxamasere	2	107.5 (95-120)	2	100	brain	19 (8-30)
		45	*1	50	eyes	1
Toteng Bridge	61	108.11 (90-125)	59	97	brain	24.57 (1-66)
			*13	21.31	eyes	2.142 (1-8)
TOTAL	103	100.35 (40-125)	95	92.23	brain	19.26 (1-66)
			23	22.33	eyes	2.25 (1-12)

* Hosts infected with diplostomatids occurring in both the brain and eyes

Rhabdalestes maunensis (Fowler, 1935)



Common Name	Slender robber
Distribution	11° S - 20° S; natural distribution includes Okavango, Cunene, upper Zambezi and Kafue Systems
Ecomorphological Classification	Group A - Open- and mid water fast swimmer
Environment	Freshwater; pelagic
Climate	Tropical
Okavango Localities	Samochima, Drotsky Upstream Temporary Floodplain
Collection	Day and night
Parasite	Metacercariae of *Diplostomum* type 2
Site of Infection	Brain
Prevalence	Present study : 0%; Previous studies: 37.3%

Biology, Ecology and Ethology

The genus *Rhabdalestes* Hoedeman, 1951 comprises seven species of which only one (*R. maunensis*) occurs in southern Africa. This species can be found in habitats ranging from both swampy to open waters but shoals have mostly been recorded in shallow, vegetated marginal and floodplain habitats. It is regarded to belong to the A ecomorphological group (open- and mid water fast swimmers) (Ramberg *et al.* 2006), since it has the characteristic slender fusiform body shape, forked caudal fins and large, laterally placed eyes. With their multicuspid teeth, placed in a single row on both jaws, they hunt and feed on invertebrates such as small aquatic insects (Ramberg *et al.* 2006). During the flood season (high water periods), these spawners migrate up the rivers to the floodplains to breed.

Description of metacercarial types (Figure 5.4 A)

During the present study the collection of *R. maunensis* was conducted during the day and night but no infected specimens were found (Table 6.2). Jansen van Rensburg (2006) collected metacercariae of *Diplostomum* type 2 from the brain of *R. maunensis* during 2001 – 2004. As a result the metacercariae's morphological description and remarks are compiled from the work of Jansen Van Rensburg (2006). Tables, providing the morphometrics of the metacercarial type collected from the brain of *R. maunensis*, are provided in Appendix II. Remarks and the results of all the metacercariae collected from this fish species are summarised in Table 6.12.

Possible life cycle

The life cycle, of which *R. maunensis* acts as the second intermediate host, includes one of the possible snail hosts (Table 6.1) and piscivorous bird hosts (Table 6.3) present in the Okavango. Similar to *B. lateralis*, the nature of this species to occur in open- to mid waters as well as their relative small size during adulthood most probably will result that *Bulinus* species and open- and mid water piscivorous birds will act as definitive hosts.

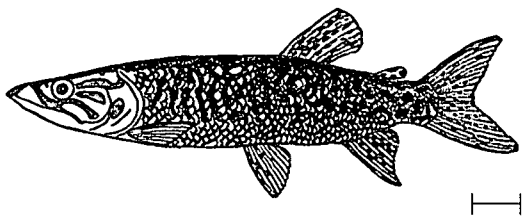
Table 6.12: Results of *Rhabdalestes maunensis* (Fowler, 1935) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae in the Okavango River System, Botswana, during the present and previous studies. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean intensity of diplostomatid infection are also indicated (× = not infected, **na** = data not available).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
2008 - 2010						
Samochima	2	55 (50-60)	×	×	×	×
Drotsky Upstream Temporary Floodplain	4	52.8 (47-56)	×	×	×	×
TOTAL	6	53.5 (47-60)	×	×	×	×
2001 - 2004						
Seronga	30	44.9 (40-55)	8	26.6	brain	**na**
Phillipa Channel	57	37.3 (30-46)	24	42.1	brain	**na**
Shakawe Floodplain	3	40.6 (40-41)	0	×	×	×
Kalatog	7	43 (30-52)	5	71.4	brain	**na**
Lake Ngami	2	44 (43-45)	0	×	×	×
TOTAL	99	40.24 (30-56)	37	37.3		

FAMILY HEPSETIDAE

This monotypic family is endemic to Africa. It has a wide distribution in the tropical and western parts and its most southern locality is the Okavango Delta (Skelton 1988). This predatory fish species is active during the night and easily recognisable by its pointed head and sharp crocodile-like jaws.

Hepsetus odoe (Bloch, 1794)



50 mm

Common Name	African pike
Distribution	Widespread in Africa in Okavango, Cunene, Kafue, Upper Zambezi, Congo and Niger drainage basins. Notably absent from Zambian Congo System and Great Lakes
Ecomorphological Classification	Group A - Open- and mid water fast swimmer
Environment	Freshwater, demersal, potamodromous
Climate	Tropical
Okavango Localities	Samochima Lagoon
Collection	Night
Parasite	Metacercariae of *Diplostomum* type 2, *Diplostomum* type a and *Diplostomum* type b
Site of Infection	Brain
Prevalence	66.67% infected
Mean Infection Intensity	8.5 (brain metacercariae)

Biology, Ecology and Ethology

The morphological appearance of the pike, e.g. the fusiform body shape, forked caudal fin and laterally placed eyes, undoubtedly places it in the A ecomorphological group (Ramberg *et al.* 2006). However, it differs from the similar shaped characins (e.g. tigerfish) regarding its hunting methods. The latter prefers fast flowing currents of the main stream, which is also characteristic of fish belonging to the A ecomorphological group. Adult pike though, rather prefer the quiet, deep waters in channels and the lagoons of large floodplains. Using their fusiform body shape they then stalk and ambush their prey (Ramberg *et al.* 2006). Its prey consists of other fish species, usually up to 30 - 40% of their own size, whilst juvenile and fry pike prey on small invertebrates occurring in well-vegetated marginal habitats. This species is relatively

short-lived (four to five years) but it is a multiple spawner. During the summer months breeding pairs construct and guard a foam nest present on the surface of water, sheltered within dense vegetation of backwaters, lagoons or protected river channels. After hatching the fry will remain suspended in the water, just beneath the nest, until it disintegrates.

Their preference for the quiet waters in channels and lagoons and their passive ambush-type hunting methods, render this species more exposed to snails and cercarial penetration. The aggregation of fry, within the foam nest, also increases the exposure of a large number of juveniles to a single cercarial shedding. These characters all may contribute to the high prevalence of diplostomatid brain infection.

Description of metacercarial types (Figures 5.3 B, 5.4 A and 5.5)

Infected fish were caught during the night by using cast nets from our research boat in lagoons. Metacercariae of *Diplostomum* type 2, type a and type b were collected from the brain. The morphological description and remarks of each type (from various fish species and tissues) are provided in Chapter 5. Tables, providing the morphometrics of the different metacercarial types collected from *H. odoe*, are provided in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.13.

Possible life cycle

Their preference for the well-vegetated backwaters of lagoons will most probably result in snails such as *Lymnaea natalensis* and *Biomphalaria pfeifferi* acting as first intermediate hosts (Table 6.1). The medium to large size of these fish species, as well as their preference for sheltered waters, probably will result in bird species placed in the shallow and sheltered group or large piscivorous bird species to act as definitive hosts (Table 6.3).

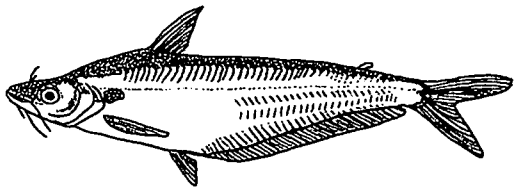
Table 6.13: Results of *Hepsetus odoe* (Bloch, 1794) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated.

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
Samochima Lagoon	3	279.67 (240-310)	2	66.67	brain	8.5 (5-12)

FAMILY SCHILBEIDAE

Members of this family are found widespread in tropical Africa and also occur in southern Asia. The African schilbeids consist of five genera and about 34 species but only one genus (*Schilbe* Oken, 1817) is present in southern Africa. This genus includes 22 species from the tropical African waters, whilst only two species are known from southern Africa of which *Schilbe intermedius* is present in the Okavango Basin (Skelton 2001).

Schilbe intermedius Rüppell, 1832



50 mm

Common Name	Silver catfish; Butter barbell
Distribution	Very wide distribution range: throughout tropical Africa, including entire Niger, Chad and Congo Basins and West Africa to Senegal River, including the Nile. Southwards it occurs in Okavango, Cunene, entire Zambezi and Limpopo Systems up and to Pongola in northern KwaZulu-Natal
Ecomorphological Classification	Group B - Mid water slow swimmers
Environment	Freshwater; pelagic; potamodromous
Climate	Tropical
Okavango Localities	Samochima Lagoon, Nxamasere, Toteng Bridge, Dead Crocodile Lagoon
Collection	Day and night
Parasite	Metacercariae of *Diplostomum* type d
Site of Infection	Brain
Prevalence	41.67%
Mean Infection Intensity	1.6 (brain metacercariae)
Remarks	Previously also known as *Schilbe mystus* (Linnaeus, 1758) and *Eutropius depressirostris* (Peters, 1852)

Biology, Ecology and Ethology

Although the common name 'catfish' generally suggests a bottom-dwelling lifestyle, the silver catfish prefers to form shoals in mid water and even sometimes in surface waters of shallow, slow-flowing sections where adequate emergent or submerged vegetation is present. They migrate mostly during dusk and night to these habitats where they feed on a wide range of food such as pieces of fish, insects, shrimps and plant seeds. It has, however, also been suggested that they are primarily piscivorous when they mature to lengths of 130 - 340 mm. Overall their preference for mid water sections and slow-moving lifestyle places this species in the B ecomorphological group (Ramberg *et*

al. 2006). This is also represented by their unusual body form consisting of a depressed head, a large mouth, short deep abdomen and compressed tapered body with a long anal fin. Their dorsal and pectoral spines end in extremely sharp points, which are mostly the reasons for them to become entangled in nets.

Depending on the locality, this generally long lived species (six to seven years), may be a single or a multiple spawner. Breeding mainly occurs during the rainy season when fairly compact shoals migrate to floodwater pools. Here spawning occurs and the eggs are laid on vegetation and left unguarded. The present study hypothesises that it is mostly during this stage, when the fry are exposed to the surrounding snail population, that they may become infected with cercariae. The adults' preference for mid water sections, cleared from vegetation, will result in less exposure to cercarial shedding and a smaller prevalence of diplostomatid infection (Table 6.14).

Description of metacercarial types (Figure 5.6 B)

Most of the fish were caught during the night by cast nets. Only metacercariae from *Diplostomum* type d were collected from the brain. The morphological description and remarks are provided in Chapter 5. The morphometrics of the metacercarial type collected exclusively from the brain of *S. intermedius* are provided in Appendix II. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.14.

Possible life cycle

The medium to large sizes of these fish species as well as their preference for open, mid waters will probably result in open- and mid water or large piscivorous birds acting as definitive hosts (Table 6.3). Snail species which also prefer less vegetated waters are *Bulinus tropicus* and *Bulinus africanus* (Table 6.1). These snail species are known to shed furcocercariae and could act as first intermediate hosts.

Table 6.14: Results of *Schilbe intermedius* Rüppell, 1832 examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (× = not infected).

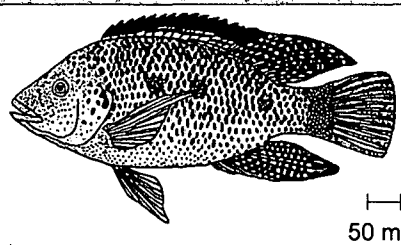
Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
Samochima Lagoon	7	180.62 (125-270)	4	57.14	brain	1.75 (1-4)
Nxamasere	3	116.67 (110-130)	×	×	×	×
Dead Crocodile Lagoon	1	270	×	×	×	×
Toteng Bridge	1	115	1	100	brain	1
TOTAL	12	141.25 (90-270)	5	41.67	brain	1.6 (1-4)

FAMILY CICHLIDAE

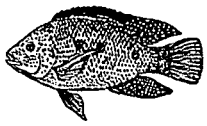
The cichlids are by far the largest fish family in Africa, consisting of 900 species, with many still left undescribed. These fresh and brackish water fish are especially abundant in the Great Lakes, but also occur in South and Central America, Madagascar and parts of Arabia and India. In the south of Africa, their natural distribution extends to the Orange River on the west coast and east to the Bushmans River in the Eastern Cape Province (Skelton 1988). Due to morphological similarity it is sometimes difficult to distinguish between closely related species. With the aid of other diagnostic features, such as internal and skeletal structures and behavioural differences, eight genera and 42 species are identified from southern Africa. Within this area, based on dietary preferences, the following two main lineages are also distinguished: the tilapiines (e.g. *Oreochromis* spp., *Tilapia* spp.), which are chiefly plant or sediment feeders, and the haplochromines (e.g. *Serranochromis* spp., *Sargochromis* spp.), which are predatory species. Some of the typical family features include the dorsal and anal fins which are composed of spinous and soft-rayed sections and the pelvic fins which are situated in a thoracic position. These enlarged fins assist the species to hang motionlessly in the water, until they can strike at their prey. Together with laterally compressed body shapes, these morphological characters place the family in the B ecomorphological group (Ramberg *et al.* 2006).

The preference of the adults for open, mid waters, cleared from vegetation, leaves them less exposed to cercariae. This is portrayed by the prevalence and mean intensity of infection amongst these species (Tables 6.15 - 6.20) to be lower than that of the Family Mormyridae (Tables 6.4 - 6.6). The tendency of some cichlid species' fry to aggregate in constructed nests in shallow water does, however, provide an excellent opportunity for simultaneous infection during one cercarial exposure. This increase in cercarial exposure could explain why the prevalence in *T. rendalli* and *T. sparrmanii* is higher than that of species such as *B. lateralis* (Table 6.11), which do not construct nests. Some cichlid species, such as *O. andersonii*, *O. macrochir*, *S. greenwoodi* and *S. angusticeps*, are mouth breeders. The eggs and embryos are incubated within the mouth of the female and the fry may also even retreat to this safe haven, when they feel threatened in the constructed nest. This additional protection against cercarial penetration could possibly explain the absence of diplostomatid infection in species such as the Southern Mouth-Brooder, *Pseudocrenilabrus philander* (Weber, 1897) (Table 6.2).

Oreochromis andersonii (Castelnau, 1861)



Common Name	Threespot tilapia
Distribution	12°S - 21°S; central west Africa, mainly within upper and middle Zambezi, Kafue, upper Zaire, Cunene and Okavango
Ecomorphological Classification	Group B – Mid water slow swimmers
Environment	Fresh and brackish water; benthopelagic, depth range 0 - 10 m but usually occurs 3 - 6 m
Climate	Tropical
Okavango Localities	Nxamasere, Mohembo Mainstream
Collection	Day
Parasite	Encysted (cysts) and unencysted metacercariae of *Diplostomum* b
Site of Infection	Eyes and brain
Prevalence	11.76% (eyes); 5.88% (brain)
Mean Infection Intensity	4 (eye cysts); 5 (brain metacercariae)



Biology, Ecology and Ethology

Mainly present in tropical, eastern and southern Africa, the genus *Oreochromis* consists of 33 species of which six are indigenous and two introduced in southern Africa and two species are native to the Okavango. *Oreochromis andersonii* is a resilient fish species, able to survive in both fresh and brackish water. Their thin profile, laterally compressed body and extended dorsal fin place them in the B ecomorphological group. Although it may occur in fairly fast-flowing rivers (Ramberg *et al.* 2006), this diurnal, school-forming species rather prefers fairly deep, well-vegetated, slow-flowing or standing water such as in pools, backwaters and floodplain lagoons. Adults occur in the open waters, whilst juveniles seek out protection amongst the inshore vegetation.

It is mostly an opportunistic detrivore, feeding on detritus, zooplankton and algae from the mud surface and not the model predator and omnivore like most other mid-water slow swimmers. Its grazing behaviour is facilitated by features such as fine teeth in several rows on the jaws, fine pharyngeal teeth, a high number of gill rakers and long intestines. In general the diet of this species varies with the availability of food. Larger individuals will act predaciously by taking larger food material such as insects and other invertebrates. Its detrivorous feeding habits are complementary to some other B ecomorphological species such as *Oreochromis macrochir* and *Tilapia rendalli*. The destructive nature of their detrivorous feeding is also the reason why several countries have reported adverse ecological impacts after its introduction and therefore it is mostly regarded as a potential alien pest.

This relatively long lived species (six to seven years) breeds during the warmer months (> 21°C) (Hecht and De Moor 2005). During this period multiple broods are raised but overall it has a relative low fecundity with each female (170 - 260 mm) only having a total of 356 - 567 eggs. It is interesting that this species will not spawn, even in their natural environment, if the water is too deep. The males construct large saucer-shaped nests which they guard, whilst the females are responsible for mouth-brooding the eggs, larvae and fry. After permanently leaving the safety of the mother's mouth, the juveniles will continue to live in the shoal for a period of time.

Description of metacercarial types (Figure 5.5 A)

Infected fish were caught during the day by casting nets from the boats and from the river banks. Unencysted metacercariae of *Diplostomum* type b, were collected and identified from this species' brain and its morphological description and remarks are provided in Chapter 5. The morphometrics of this metacercarial type, specifically collected from *O. andersonii*, is provided in Appendix II together with the mean length and width of the cysts collected from the eyes. These encysted metacercariae do not exhibit any distinguishable morphological characteristics to aid in metacercarial type identification but similar cysts were also found in other fish species, from the family Cichlidae. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.15.

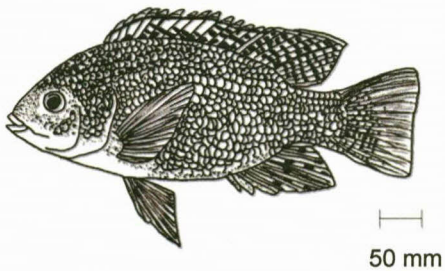
Possible life cycle

The small to medium size of the juveniles (which hide amongst shallow, sheltered waters) most probably will result in bird species, placed in the shallow and sheltered water feeder group, to act as definitive hosts. The larger sized adults, mainly present in the open, mid waters, will rather be caught by bigger bird species which are placed in the open- and mid water or large, piscivorous birds' category (Table 6.3). The diverse habitat preference of this cichlid species exposes it to cercarial shedding from a variety of snail species in the Okavango (Table 6.1) of which *Lymnaea natalensis*, *Biomphalaria pfeifferi* and *Bulinus* species are the most likely first intermediate hosts.

Table 6.15: Results of *Oreochromis andersonii* (Castelnau, 1861) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae (**M**) and cysts (**C**) in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated.

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity	Stage
Nxamasere	13	86.53 (60-160)	2	15.38	eyes	4 (2-6)	C
Mohembo Mainstream	4	396.25 (370-450)	1	25	brain	5	M
TOTAL	17	159.41(60-450)	2	11.76	eyes	4 (2-6)	C
			1	5.88	brain	5	M

Oreochromis macrochir (Boulenger, 1912)



Common Name	Greenhead tilapia
Distribution	5°S - 25°S; Okavango, Cunene, upper Zambezi and Kafue Rivers. Translocation and introduction occurred in Lake Kariba, Buzi River, Zambian Congo System, southern tributaries of Congo, Shashi-Limpopo System and wide across other reaches of Zambia and Zimbabwe
Ecomorphological Classification	Group B - Mid water slow swimmers
Environment	Freshwater, benthopelagic
Climate	Tropical
Okavango Localities	Nxamasere
Collection	Day
Parasite	Encysted metacercariae (cysts)
Site of infection	Eyes
Prevalence	50% (eyes)
Mean Infection Intensity	6 (eye cysts)

Biology, Ecology and Ethology

According to Ramberg *et al.* (2006) tilapias of the genus *Oreochromis* are classified in the B ecomorphological group. *Oreochromis macrochir*, however, does not fit the defined criteria of being a stalking predatory and omnivorous feeder, since they are mostly detrivorous and feed on diatoms, blue-green algae and detritus from the bottom. This detrivorous feeding preference is most possibly the reason for reports of adverse ecological impact after its introduction to foreign habitats. The juveniles feed more on small invertebrates and zooplankton, but lose this predatory tendency with age. Their omnivorous feeding preference renders the species more close to the D ecomorphological group. Their dorso-ventrally compressed bodies and mouths which face downward are characteristic of the B ecomorphological group (Ramberg *et al.* 2006).

Oreochromis macrochir is a diurnal species which prefers quiet, deep and well-vegetated water but occasionally shoals can be found in shallow waters along river margins, backwaters, floodplain habitats and other water impoundments. Multiple spawning may occur during intervals of six to seven weeks in the summer period from October to March. Males play an active role during this breeding period by constructing saucer and mound type nests and in which in some areas the central mounds are strangely star shaped. These nests are often grouped along with several other nests in shallow water areas, thereby aiding in protection by numbers. Females are responsible for mouth-brooding of the eggs, larvae and small fry. For the first 21 days after hatching the juveniles remain in a small school near the mother, re-entering her mouth during times of threat (Hecht and De Moor 2005).

Description of metacercarial types

Infected fish were caught by means of cast nets during the day. Since encysted metacercariae (Chapter 5) do not exhibit any distinguishable morphological characteristics, the material was not sufficient to aid in effective metacercarial type identification. During the present study, similar cysts were found in other fish species,

also from the family Cichlidae. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.16.

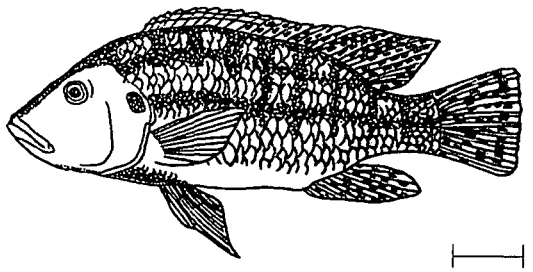
Possible life cycle

The small to medium size of the juveniles, which hide amongst shallow, sheltered waters most probably will result in bird species placed in the shallow and sheltered water feeder group to act as definitive hosts. The adults are much larger in size and they are mainly present in the open, mid waters. As a result they will be caught by larger bird species placed in the open- and mid water or large piscivorous bird category (Table 6.3). Various snail species, such as *Lymnaea natalensis*, *Biomphalaria pfeifferi* and *Bulinus* species can act as first intermediate hosts (Table 6.1).

Table 6.16: Results of *Oreochromis macrochir* (Boulenger, 1912) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 cysts in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean intensity of diplostomatid infection are also indicated.

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity
Nxamasere	2	150 (140-160)	1	50	eyes	6

Sargochromis greenwoodi (Bell-Cross, 1975)



50 mm

Common Name	Deepcheek bream
Distribution	Okavango, upper Zambezi and Kafue Systems (Namibia, Zambia and Botswana)
Ecomorphological Classification	Group B - Mid water slow swimmers
Environment	Freshwater; demersal
Climate	Tropical
Okavango Localities	Nxamasere, Mormyrid Marsh, Samochima Lagoon
Collection	Day and night
Parasite	Metacercariae and cysts
Site of Infection	Eyes and brain
Prevalence	18.75% (eyes); 12.5% (brain)
Mean Infection Intensity	7 (eye cysts); 1 (brain cyst and metacercariae)

Biology, Ecology and Ethology

The genus *Sargochromis* consists of seven species in southern Africa of which four occur in the Okavango River System. Similar to most other cichlid groups the morphological differences between the species (especially in the juvenile stages) are sometimes not very striking and this leads to the difficult identification of species. Overall, the members of the genus are mostly recognised by their robust pharyngeal bones with rounded, molar-like teeth. The species prefers still or slow-flowing water and dense vegetation, suggesting that it can be grouped to the E ecomorphological group. Regarding its morphology, e.g. having a thin profile and a dorsal fin that extends from directly behind the head to the base of the tail stem, it could, however, be placed in the B ecomorphological group (mid water slow swimmers) of Ramberg *et al.* (2006). It differs from other B ecomorphological species (*Tilapia* and *Oreochromis*) in that the mouth is relatively larger. This is used to primarily feed on insects, small

snails, other invertebrates and occasionally small fish. Breeding commences during the summer months and maternal mouth-brooding occurs.

Description of metacercarial types

Fish were collected during the night by using cast nets. Since only a single free-moving metacercaria was collected from the brain of *S. greenwoodi* and encysted metacercariae do not exhibit any distinguishable morphological characteristics, the material was not sufficient to aid in effective metacercarial type identification. Similar cysts and certain free-moving metacercarial types have been identified from *O. andersonii* and *T. sparrmanii* (Chapter 5). These types of metacercariae could therefore also be present in the cichlid, *S. greenwoodi.* Remarks and the results of all the diplostomatids collected from this fish species are provided in Table 6.17.

Possible life cycle

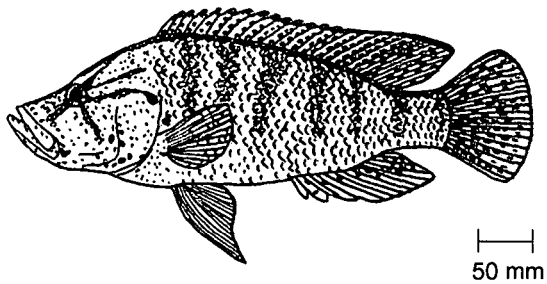
Similar to other cichlids this species may occur in different parts of the river during different stages in their development. As a result various snail species (Table 6.1) such as *Lymnaea natalensis*, *Biomphalaria pfeifferi* and *Bulinus* species may be responsible for their infection with diplostomatid cercariae. The small to medium size of the juveniles most probably will result in the smaller bird species placed in the shallow and sheltered water feeder group to act as definitive hosts. The larger sized adults could be caught by larger birds such as the open- and mid water or large, piscivorous bird species (Table 6.3).

Table 6.17: Results of *Sargochromis greenwoodi* (Bell-Cross, 1975) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae (**M**) and cysts (**C**) in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (× = not infected).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity	Stage
Nxamasere	4	136.25 (120-155)	×	×	×	×	×
Mormyrid Marsh	2	59.5 (53-66)	×	×	×	×	×
Samochima Lagoon	10	120.6 (64-174)	3	30	eyes	7 (4-9)	C
			*1	10	brain	1	C
			1	10	brain	1	M
TOTAL	16	116.875 (53-174)	3	18.75	eyes	7 (4-9)	C
			2	12.5	brain	1	M and C

* Hosts infected with diplostomatids occurring in both the brain and eyes

Serranochromis angusticeps (Boulenger, 1907)



Common Name	Thinface largemouth
Distribution	11°S - 21°S; Okavango, upper Zambezi, Kafue (Angola, Namibia, Botswana, Zambia, Zimbabwe), Luapula-Moeru in Zambian Congo Systems and possible also in coastal rivers north of Cunene in Angola
Ecomorphological Classification	Group B - Mid water slow swimmers
Environment	Freshwater, demersal
Climate	Tropical
Okavango Localities	Dead Crocodile Lagoon, Toteng Bridge, Nxamasere
Collection	Day and night
Parasite	Metacercariae and encysted metacercariae (cysts)
Site of Infection	Eyes
Prevalence	50% (eyes)
Mean Infection Intensity	2.67 (eye cysts); 2 (eye metacercariae)
Remarks	Previously, this species were considered to be the female and humpback largemouth (*Serranochromis altus* Winemiller and Kelso-Winemiller, 1991) to be the male

Biology, Ecology and Ethology

The genus *Serranochromis* includes ten species of serranos or largemouth breams of which seven occur in southern Africa and six in the Okavango River. These species are mostly characterised by a high number of scale rows on the cheeks and a relatively high number of soft rays in the dorsal fin as well as numerous bright egg-spots (ocelli) on the anal fin. It is believed that these 'egg dummies' entice the female, whilst gathering the eggs in her mouth, to the genital region of the male, who then releases the milt. Together with their large bodies and mouths, the thin head-profile is used by these predatory species in stalking and ambushing prey such as small fish, shrimps

and insects. This sudden ambush technique is also facilitated by the ability for it to hover by means of its extended broad fins. All of the above features are characteristic of the B ecomorphological group (Ramberg *et al.* 2006). The habitat of *S. greenwoodi* may include fast-flowing reaches over sand and rocks but generally it prefers well-vegetated lagoons, quiet backwaters and edges of rivers. Breeding starts in spring and reaches a peak throughout summer. Males construct nests by clearing small sandy patches amongst vegetation and the females are known to mouth-brood the fry.

Description of metacercarial types

Fish were collected during the night and day by using cast nets. Since only two free-moving metacercariae were collected from the eyes, the material was not sufficient to aid in morphological type identification. Encysted metacercariae, also collected from the eyes, did not exhibit any distinguishable morphological characteristics to aid in identification. Similar cysts and free-moving metacercarial types have been identified from *O. andersonii* and *T. sparrmanii* (Chapter 5). These types of metacercariae could be present in *S. angusticeps*, which is also a cichlid species. Remarks and the results of all the diplostomatids collected from this fish species are provided in Table 6.18.

Possible life cycle

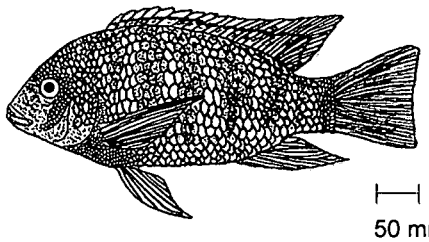
The life cycle, of which *S. angusticeps* acts as the second intermediate host, includes one of the possible snail hosts (Table 6.1) and a wide variety of piscivorous bird hosts (Table 6.3) present in the Okavango. The small to medium size of the juveniles, which hide amongst shallow, sheltered waters most probably will result in bird species placed in the shallow and sheltered water feeder group to act as definitive hosts, whilst the larger sized adults, mainly present in the open, mid waters, will rather be caught by larger birds such as the open- and mid water or large, piscivorous bird species.

Table 6.18: Results of *Serranochromis angusticeps* (Boulenger, 1907) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae (**M**) and cysts (**C**) in the Okavango River System, Botswana, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (× = not infected).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity	Stage
Dead Crocodile Lagoon	2	19.5 (17-22)	1	50	eyes	4	C
			*1	50	eyes	2	M
Nxamasere	1	24	×	×	×	×	×
Toteng Bridge	3	12.17 (9-14)	2	66.66	eyes	2 (1-3)	C
TOTAL	6	16.58 (9-24)	3	50	eyes	2.76 (1-4)	C
			1	16.67	eyes	2	M

* Host infected with cysts and metacercariae in the eyes

Tilapia rendalli (Boulenger, 1896)



Common Name	Redbreast tilapia
Distribution	20°N - 20°S; Kasai drainage (middle Congo River Basin), throughout to upper Congo River drainage as well as in eastern Zaire Basin (Lualaba) and Lakes Tanganyika and Malawi. Eastern coastal areas from Zambezi Delta to Cunene and Okavango Rivers and south to Phongolo coastal lakes and Lake Sibaya. Also occurs in estuaries in Mozambique and in KwaZulu-Natal and Highveld region
Ecomorphological Classification	Group B - Mid water slow swimmers
Environment	Freshwater, brackish, benthopelagic
Climate	Tropical
Okavango Localities	Nxamasere, Samochima Lagoon, Mohembo Mainstream
Collection	Day and night
Parasite	Unencysted and encysted metacercariae (cysts)
Site of Infection	Mostly in the eyes but also found in brain
Prevalence	92.31% (eyes);15.38% (brain)
Mean Infection Intensity	7.46 (eye cysts); 1 (brain cyst); 1.33 (eye metacercariae)

Biology, Ecology and Ethology

Differing from other mouth-brooding cichlids, members of the genus *Tilapia* are substrate spawners, forming firm pair-bond relationships and both parents guarding the brood. *Tilapia* species prefer to feed on coarser foods and they are also smaller than *Oreochromis* species. They retain a distinctive tilapia spot on the dorsal fin during adulthood. The genus *Tilapia* consists of 30 species of which four occur in southern Africa and three in the Okavango. *Tilapia rendalli* is tolerant to a wide range of temperatures and salinities and can therefore occur in a large variety of habitats. Generally it shoals in quiet, well-vegetated water along backwaters or floodplains, river littorals and swamps where it is diurnally active. The feeding style of *T. rendalli* is

predominantly herbivorous with the juveniles feeding on plankton and the adults feeding on higher aquatic plants (Hecht and De Moor 2005). Adults may also prey on aquatic invertebrates and even catch larger prey such as small fish. In order to hover in their open water environment and successfully stalk their prey this species has a small mouth, thin profile, laterally compressed body and broad fins. The above mentioned morphological characters and omnivorous life style places *T. rendalli* in the B ecomorphological group of mid water slow swimmers (Ramberg *et al.* 2006).

It is a relatively long-lived species (seven years) and has a high fecundity in comparison with other cichlids. Each females approximately lays 5 000 to 6 000 eggs during every spawning and several broods are raised during the summer (Hecht and De Moor 2005). The eggs and larvae are protected within nests and in tunnel-like brood chambers. These structures are formed in shallow waters by means of the breeding pair which clear the bottom surface from vegetation. Both parents protect the nest and the female moves the fry from one brood chamber to another after hatching. Juveniles will remain within these chambers up to about 15 mm in (standard) length (Hecht and De Moor 2005). In the past it has been regarded as a useful species for clearing ponds since it rapidly feeds on macrophytes, thereby eliminating weeds. Its ease of reproduction and palatability has also made it a favoured aquaculture species. Hence *T. rendalli* has been introduced to many habitats outside of their natural distribution with adverse ecological impact reports from several countries.

Description of metacercarial types

Using cast nets, fish were caught during the day and night. Since only three free-moving metacercariae were collected from the eyes of *T. rendalli* and encysted metacercariae do not exhibit any distinguishable morphological characteristics, the material was not sufficient to aid in metacercarial type identification. Cysts and certain metacercarial types have, however, been identified from cichlid species such as *O. andersonii* and *T. sparrmanii* (Chapter 5). These metacercariae could therefore also be present in *T. rendalli*. Remarks and the results of all the diplostomatids collected from this fish species are summarised in Table 6.19.

Possible life cycle

Similar to *T. sparrmanii* this species differs in its habitat choice during different stages in its development. The small to medium size of the juveniles will most probably result in bird species placed in the shallow and sheltered water feeder group, acting as definitive hosts. The larger sized adults, mainly present in the open, mid waters, will rather be caught by larger bird species such as open- and mid water or large, piscivorous birds (Table 6.3). Due to their variation in preferred habitat, different snail species such as *Lymnaea natalensis*, *Biomphalaria pfeifferi* and *Bulinus* species, may also act as first intermediate hosts (Table 6.1).

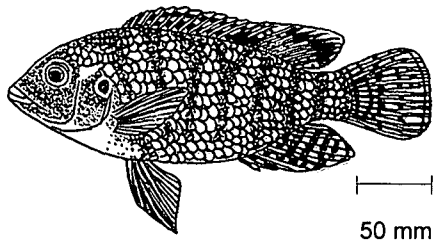
Table 6.19: Results of *Tilapia rendalli* (Boulenger, 1896) examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae (**M**) and cysts (**C**) in the Okavango River System, during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (× = not infected).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity	Stage
Nxamasere	8	22.88 (11-29)	7	87.5	eyes	2.714 (1-10)	C
			**2	25	eyes	1	M
Samochima Lagoon	30	54.6 (7-76)	29	96.67	eyes	84.54 (1-28)	C
			*6	20	brain	1	C
			**1	3.33	eyes	2	M
Mohembo Mainstream	1	280	0	×	×	×	×
TOTAL	39		36	92.31	eyes	7.46 (1-28)	C
			*6	15.38	brain	1	C
			**1		eyes	1.33	M

* Hosts infected with cysts occurring in both the brain and eyes

** Hosts infected with cysts and metacercariae in the eyes

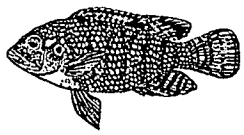
Tilapia sparrmanii A. Smith, 1840



Common Name	Banded Tilapia
Distribution	10°S - 30°S; widespread in Africa: from Orange River and south coast of KwaZulu-Natal up northwards (e.g. Cuanza, Cunene, Okavango, Lake Ngami, Zambezi, Limpopo) to upper reaches of southern Congo tributaries, Lake Malawi and Zambezi System. Extensive translocation lead to its presence south of Orange River, in the Cape
Ecomorphological Classification	Group B - Mid water slow swimmers
Environment	Freshwater, benthopelagic, potamodromous
Climate	Tropical
Orange-Vaal and Okavango River Localities	Orange-Vaal River: Renosterspruit at Bishop's Weir Okavango River: Nxamasere, Toteng Bridge, Samochima Lagoon, Mormyrid Marsh
Collection	Day and night
Parasite	Encysted metacercariae (cysts) and unencysted metacercariae of *Diplostomum* 2, *Diplostomum* a and *Diplostomum* d
Site of Infection	Mostly in eyes, but also occur in brain
Prevalence	Orange River: 0%; Okavango River: 57.83%
Mean Infection Intensity	7.47 (eye and brain metacercariae and cysts)

Biology, Ecology and Ethology

Tilapia sparrmanii is the most wide-spread of all the tilapiines in southern Africa and the only infected fish species to occur in both the Okavango and Orange River study sites. Its natural distribution is as far south as the Orange River but it has been translocated to the extreme south coastal rivers of South Africa (Skelton and Cambray 1981). Overall it is suggested that its distribution is more restricted by high (above 32°C) than by low temperatures.



Tilapia sparrmanii is an omnivorous species, with the juveniles generally feeding on small crustaceans and midge larvae and the adults preferring filamentous algae, aquatic macrophytes, soft, vegetable matter of terrestrial origin (leaves, plants, etc.) and even prey on small invertebrates (e.g. insects) and small fish. A large variety of habitats can be occupied by this species but its preferred habitats include shallow, quiet or standing waters with submerged or emergent plant cover, such as along the edges of rivers, lakes or swamps. Although it generally does not colonise in open waters its morphological adaptations, e.g. broad fins, allows it to hover and stalk its prey in open waters. Its omnivorous feeding preferences as well as the above mentioned morphology places it in the B ecomorphological group (Ramberg *et al.* 2006).

Shoals seasonally migrate upstream during or prior to breeding. The male excavates a simply saucer-shaped nest, within which the eggs are laid, which is then guarded by both parents. Before hatching the eggs and even the larvae may be moved to alternative nests by the parents. The fry become free-swimming after seven to eight days but will remain within a shoal which is guarded by the parents for several weeks.

Description of metacercarial types (Figures 5.4 A, 5.4 B and 5.6 B)

Fish infected with eye diplostomatid metacercariae and cysts were collected during the day and night by using an electro-shocker and cast nets. Metacercariae of *Diplostomum* type 2, type a and type d were collected and identified from the eyes of this species. The morphological description and remarks of each type are provided in Chapter 5. Tables, providing the morphometrics of the different metacercarial types collected exclusively from *T. sparrmanii*, are provided in Appendix II. Since only two free-moving metacercariae were collected from the brain, the material was not sufficient to aid in morphological type identification. Encysted metacercariae (found in the eyes and brains) also do not exhibit any distinguishable morphological characteristics to aid in identification. Similar cysts were collected in other fish species, also from the family Cichlidae. Remarks and the results of all the diplostomatids collected from this fish species are provided in Table 6.20.

Possible life cycle

The life cycle, of which *T. sparrmanii* acts as the second intermediate host, possibly includes snail hosts (Table 6.1) such as *Lymnaea natalensis*, *Biomphalaria pfeifferi* and *Bulinus* species which occur in the Okavango River System. Similar to other cichlid species a wide variety of piscivorous bird hosts (Table 6.3) may act as definitive hosts.

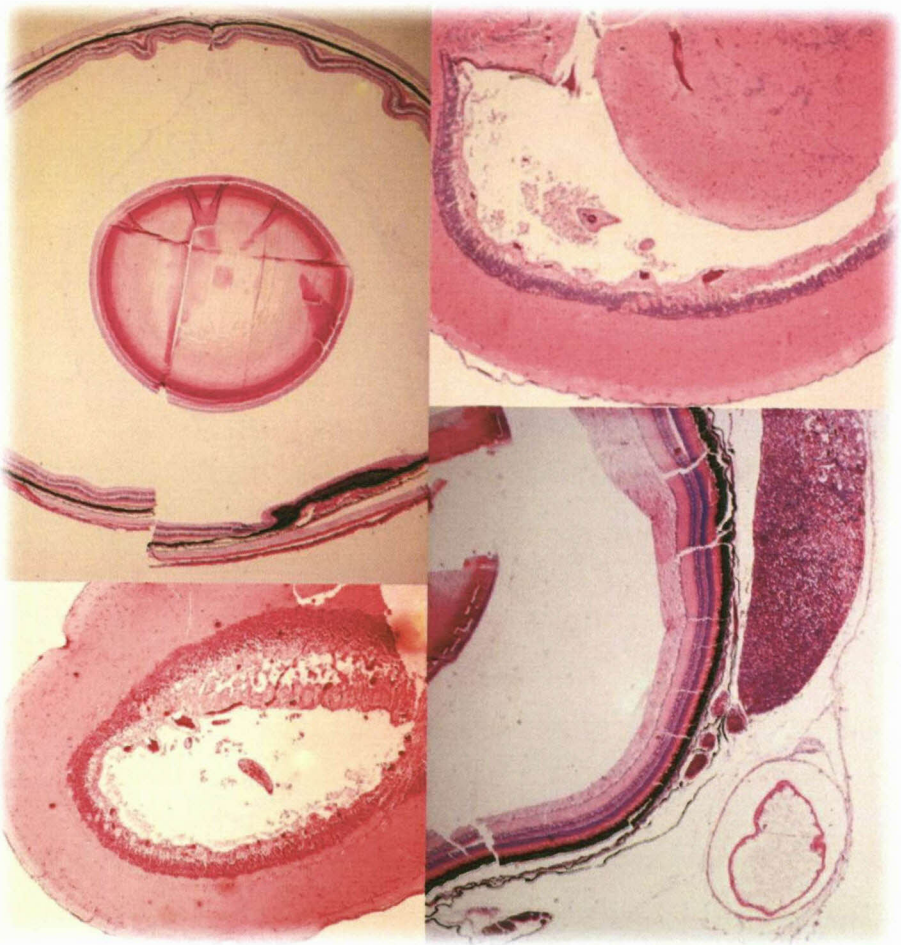
Table 6.20: Results of *Tilapia sparrmanii* A. Smith, 1840 examined for the presence of eye and brain *Diplostomum* von Nordmann, 1832 metacercariae (**M**) and cysts (**C**) in the Okavango (**OK**) and Orange-Vaal (**OV**) River Systems during the present study. The sample size (**n**) of the number of fish examined and their total lengths (mm) are provided. The numbers of infected fish, the prevalence as well as the mean and the range of intensity of diplostomatid infection are also indicated (× = not infected).

Locality	n	Total length	Infected	Prevalence (%)	Organ	Intensity	Stage
Nxamasere	18	104.74 (55-40)	5	27.78	eyes	2 (1-4)	M
			**2	11.11	eyes	1.5 (1-2)	C
			*1	5.56	brain	1	M
			2	11.11	eyes	2.25 (1-4)	C
Toteng Bridge	13	76 (50-100)	8	61.54	eyes	5.71 (2-16)	C
			**1	7.69	eyes	1	M
			*1	7.69	brain	1	C
Samochima Lagoon	32	95.95 (50-230)	22	68.75	eyes	10.52 (1-30)	C
			**1	4.55	eyes	4	M
			*2	9.09	brain	1	C
			**2	9.09	brain	1	M
			1	4.55	eyes	12	M
Mormyrid Marsh	2	57 (55-59)	0	×	×	×	×
Mainstream	2	53.5 (50-57)	0	×	×	×	×
TOTAL OK	83	86.68 (50-230)	48	57.83	eyes and brain	7.47 (1-30)	M and C
Bishop's Weir OV	16	62.63 (35-90)	×	0	×	×	×

* Hosts infected with cysts occurring in both the brain and eyes

** Hosts infected with cysts and metacercariae in the eyes

Chapter 7



SITE OF INFECTION AND PATHOLOGY

This chapter begins by providing different reasons for the site-specific preference of diplostomatids within their fish host. Protection against immunology, in sites such as the brain and eyes, will be elaborated on. To explain the possible pathological effects that eye and brain diplostomatid infection could have on the fish phenotype, the basic functional anatomy of these two organs will be briefly discussed. This will be followed by the results of the histopathology found for encysted and free-moving flukes within the brains and eyes collected during the present study. A discussion regarding the pathology created by the brain and eye flukes will be provided. Changes in host behaviour, as a result of the change in the pathology will be mentioned in this chapter but Chapter 8 provides the full review of parasite induced behaviour and the associated pathology.

SELECTION OF IMMUNOLOGICALLY PRIVILEGED SITES

Free-moving *Diplostomum* metacercariae are reported to be specific in site selection, preferring the ocular and cerebral tissues (Locke *et al.* 2010a, b). The majority of literature refers to different species of diplostomatid flukes to specifically occur within different locations, such as the lens, retina, humours and choroid (Ferguson 1943a; Erasmus 1958; Hoffman and Hundley 1957; Berrie 1960; Williams 1966; Lester and Huizinga 1977; Niewiadomska 1984; Bortz *et al.* 1988; Höglund and Thulin 1992; Chappell 1995; Field and Irwin 1995; McKeown and Irwin 1995; Niewiadomska 1996). Other authors, however, propose that similar species of diplostomatids could be present in all of the different eye sites as well as the brain tissues (Rees 1955; Davies *et al.* 1973; Bouillon and Curtis 1987; Hendrickson 1978; Hoffman and Hoyme 1958; Hoffman 1960; Chibwana and Nkwengulia 2010). If slight morphological differences are present, it could just be a result of ecophenotypic variation which is dependent on the part of the eye or brain in which the metacercariae developed.

The present study concludes that similar types of *Diplostomum* metacercariae may be present within the eye and brain tissues of different and similar fish host species. For example *Diplostomum* type 2 were identified and collected from different fish species, such as: *M. macrolepidotus*, *B. poechii, L. capensis*, *B. lateralis*, *H. odoe* and

T. sparrmanii (see Chapter 5). The route of migration followed by the diplostomula possibly determines the final location of metacercarial development. The only way to determine whether or not diplostomatid species are truly site specific or not, is to conduct experiments on the full completion of the life cycles of different species in separate non-mixed infected host populations. Since metacercariae were collected only from wild fish host populations, the present study remains inconclusive on the site specificity of diplostomatids.

There are various proposed reasons as to why the eyes and brain could be exclusively preferred by flukes. Firstly, it is suggested that since the fish host's skin, external gills, alimentary canal system, visceral organs and musculature have already been successfully colonised by a variety of parasitic species (Dezfuli *et al.* 2007), diplostomatids simply occur within these sites where proportionally fewer other parasitic species are found. This results in a reduction in inter-specific competition for resources and subsequent niche separation between species. Secondly, it may also be that these particular tissues provide the parasite with specific nutritional and / or environmental requirements, such as that the metacercariae embedded within the lens, are granted protection against the digestive secretions of the intestine of the final host (Szidat 1969; Barber and Crompton 1997). The most widely accepted reason for diplostomatids' preferred selection of site, is that it is a method to evade the host's chemical and cellular immune defences (Dezfuli *et al.* 2007; Barber and Crompton 1997; Sitjà-Bobadilla 2008). In mammals it has been verified that the central nervous system (which includes the brain) and the whole anterior chamber of the eye are two of the most consistent and remarkable immunologically privileged sites (IPS) within the body. The lack in immunological responses within these two sites is undoubtedly an evolutionary adaptation to protect these vulnerable organs, which have limited capacity for regeneration, against damage that can be caused by pathogen induced inflammatory responses (Locke *et al.* 2010a).

Due to the absence of blood vessels within the lens, it was initially suggested that immunological responses are completely absent within this site. It was thought that the lack of blood provision provided a physical barrier for the antigens of the immune system (Koornneef *et al.* 1981; Barber and Crompton 1997; Poulin *et al.* 2005) and cell-

mediated immune responses, such as macrophage phagocytosis, could not protect the lens from foreign material (Ratanarat-Brockelman 1974). Some contemporary studies have, however, concluded that antigens actively enter and leave the IPS's, such as the lens, and could therefore induce immune responses (Chappell 1995; Sitjà-Bobadilla 2008). It can therefore be concluded that immune privilege within these sites, is regulated by active processes, rather than by passive physical barriers, such as the absence of blood vessels. According to Sitjà-Bobadilla (2008) the active mechanisms which are responsible for regulating the immune responses within the various immune privileged tissues include:

1) the release of locally produced immune suppressive cytokines, neuropeptides and complement regulatory proteins, which suppress immune responses likely to cause tissue damage,

2) limited expression of the major histocompatibility complex I and II and,

3) cell expression of molecules, such as FAsL (Fas Ligand) and TRAIL (Tumour necrosis factor-related apoptosis-inducing ligand) that can induce apoptosis (cell death) of immune cells.

These mechanisms do not cease but rather only minimise the workings and effects of the immune and inflammatory responses. Therefore these IPS's become Utopia for *Diplostomum* metacercariae. It is speculated that, whilst migrating in the bloodstream or connective tissue, the diplostomula may be the only stage of the diplostomatid life cycle which is exposed to marked fish immunological responses (Whyte *et al.* 1988). Since these endoparasites are known to successfully reach their preferred IPS's within 24 hours (Ratanarat-Brockelman 1974), it is possible that they are able to escape the full activation of the immune response of the body tissues (Chappell 1995). The evasion of fully activated immunological responses is only possible when the number of simultaneously invading cercariae is limited to a few, as is the tendency in nature.

Although advanced immunological responses within the brain and eyes are reduced (Bell and Hopkins 1956), slight inflammation may occur and could be responsible for the encystment of free-moving metacercariae (Matisz *et al.* 2010). Previous studies suggest that due to host cellular secretions, provoked by the structural damage of the

parasites, a middle and outer capsular-like coat is formed, whilst the inner cyst layer originates from the parasite itself (Sitjà-Bobadilla 2008). Since the host immunological response is not able to recognise the metacercariae as foreign material, the formed cysts can remain within the eye and brain for a length of time until it is transmitted to the final host. Only upon digestion, through the gastric and intestinal fluids of the definitive hosts, are the juvenile flukes excysted and migrate to the site of the final host infection, where they develop into adults (Hoffman 1958; Sitjà-Bobadilla 2008). Except for protection against the complete immunological response of the host, an additional benefit of cyst formation may therefore be protection against the initial indigestive processes of the definitive host, until the parasite can attach to the small intestine. Through experimental procedures it has been established that unencysted mature diplostomatid metacercariae have a tolerance to the initial gastric secretions of the definitive host and will successfully infect the small intestine of the bird (Hoffman 1958). Since both free-moving and encysted diplostomatids were sampled from the eyes and brains, it remains inconclusive whether cyst formation forms an essential part of the older developmental stage of metacercariae or if it only occurs by chance, as a side-effect, when the host defensive responses are sufficiently stimulated.

BRAIN ANATOMY

The meninx and cerebrospinal fluid

Different from birds and mammals, the brain and spinal cord of fish are only covered by one layer of connective tissue, namely the primitive meninx and not by a dura mater and pia mater as well (Liem *et al.* 2001). This single connective tissue layer closely encapsulates the central nervous system from which strands extend to form a layer that lines the cranial cavity and vertebral canal. A gelatinous material, termed the cerebrospinal fluid, fills the space between the primitive meninx and the surrounding cartilage and bone. It also circulates in the cavities within the central nervous system. This fluid is secreted by the choroid plexus, a network occurring within and throughout the brain ventricles (Figure 7.1 B). Various brain regions are therefore connected to each other and to the extracellular fluid surrounding the brain (Liem *et al.* 2001). As a

result, if the blood-brain barrier is breached, similar material can occur in different brain regions by means of this inter-connected system.

Basic regions of the teleost brain

According to Liem *et al.* (2001) less is known of the organisation and function of the brains of fishes than of that of mammals, but in general the basic functional anatomy seems to be similar. Three brain extensions, consisting of different regions, can be recognised: (1) a forebrain (prosencephalon), (2) a midbrain (mesencephalon) and (3) a hindbrain (rhombencephalon) (Figure 7.1 A).

1) The forebrain: olfactory tract, cerebrum and diencephalon

The majority of the forebrain consists of the gray matter of the cerebrum with the olfactory bulb and tract occurring at the anterior part and the diencephalon at the caudal part. Previously it was believed that the exclusive function of this region was the reception and motor responses to olfactory cues. It has, however, more recently been established that some visual, lateral line and electrosensory stimuli may also be received and analysed although the input is not extensive (Liem *et al.* 2001).

The posterior part of the forebrain, the diencephalon is subdivided into a dorsal epithalamus, a lateral thalamus and a ventral hypothalamus. The epithalamus includes the pineal-pariental complex which is responsible for photoreception which aids in regulating cyclic behaviours, regarding diurnal and seasonal changes in day length (Liem *et al.* 2001). The thalamus occurs in the wall of the brain, lateral to the third ventricle and is associated with relaying ascending somatosensory, visual, auditory and lateral line information to the midbrain. The major function of the hypothalamus is the coordination of gustatory and other visceral inputs from different receptor cells from the brain and also regulating heart and respiratory rate. It also controls periods of rest and activity as well as the hormonal secretions of the hypophysis (Liem *et al.* 2001).

2) The midbrain: optic lobes

The optic lobes are usually the largest part of the fish brain and also comprise the main region of the midbrain (Liem *et al.* 2001). Neurons occurring in this site are layered with fibers, commencing from the retina, and terminating within the most superficial layers. This allows the reception of external visual inputs and ultimately forms a precise (although inverted) spatial map of the visual field. Within the deeper layers, the optic tectum is also responsible for receiving some auditory, somatosensory and electrosensory cues which help fish to localise visual stimuli as well as to coordinate direct eye and locomotor movements (Liem *et al.* 2001).

3) The hindbrain: cerebellum and medulla oblongata

The cerebellum receives inputs from all the sensory systems throughout the body especially from the vestibular parts of the ear and the lateral line, whilst cues from the olfactory and gustatory receptors are possibly excluded (Liem *et al.* 2001). This region receives auditory and visual cues and enables a fish to orientate itself in its environment regarding its position. It also helps to regulate the coordination of muscle activity. Due to the development of advanced electro-receptive and electro-location systems, it is clear why mormyrid fishes exhibit a very large cerebellum. When unfolded, the brain surface area is about ten times the length of these fish (Liem *et al.* 2001).

Posterior to the cerebellum is the medulla oblongata, which is an anterior extension of the spinal cord. This region contains nuclei that receive sensory input from the receptors of the general cutaneous body surface, the taste (olfactory) buds, the lateral line (including electro receptors) as well as the auditory and vestibular (equilibrium and acceleration) systems. Fish, such as carp, minnows and catfish, with extensive taste receptors on the body surface, have extra sensory nuclei situated within additional lobes such as the facial and vagal lobes (Figure 7.1 A).

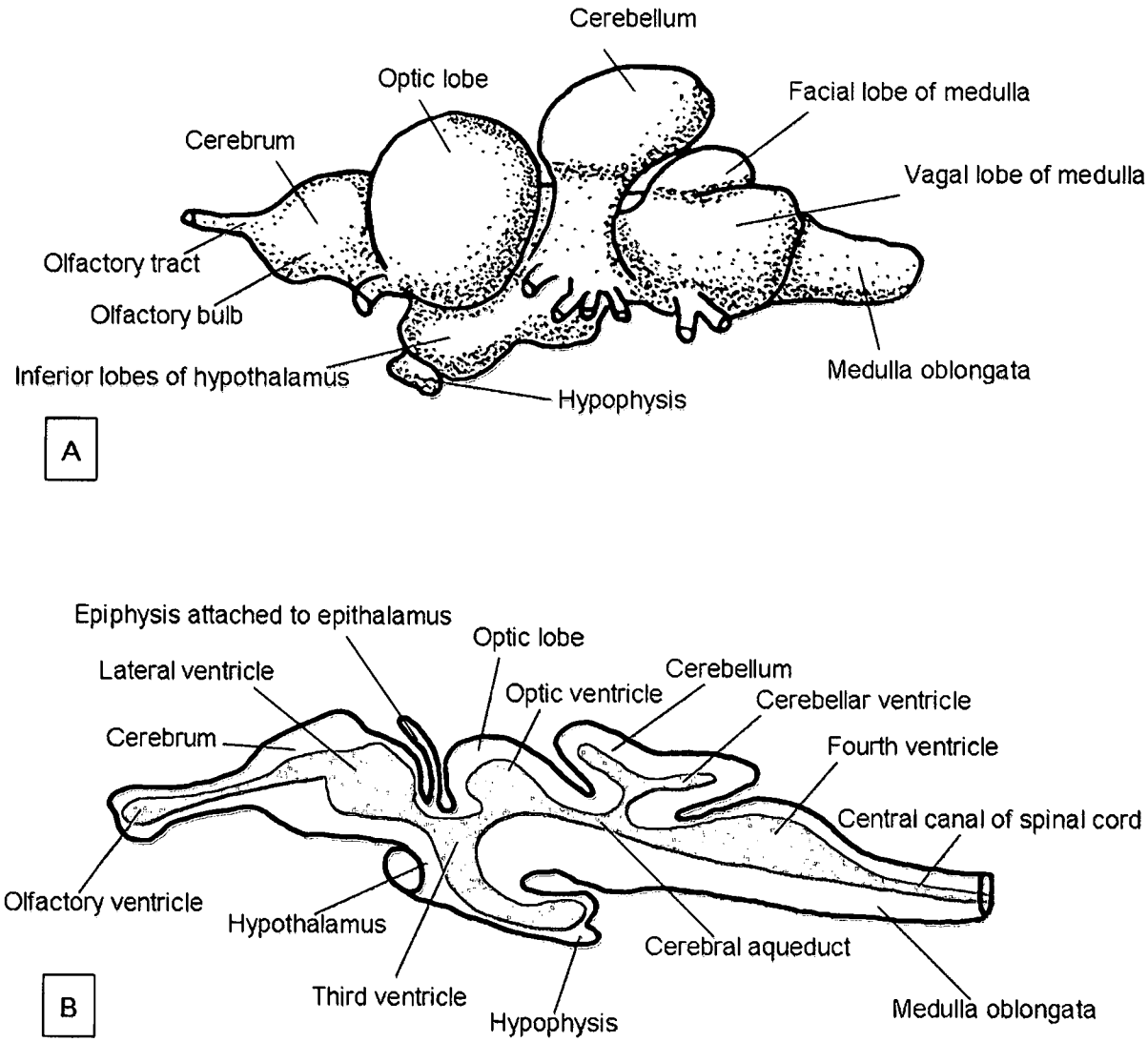


Figure 7.1: Basic anatomy of the (**A**) brain of a carp (teleost) and the (**B**) ventricle system of a dogfish (elasmobranch) (redrawn from Liem *et al.* 2001).

PATHOLOGY OF DIPLOSTOMATID INFECTED BRAINS: PRESENT STUDY

Results of studied brain pathology

Similar to previous studies conducted in Africa (Beverley-Burton 1963; Mashego and Saayman 1989; Ibraheem 2000), free-moving metacercariae were collected from the cranial cavities and ventricles of fish. The behavioural effects which these brain diplostomatids can have on the natural fish populations occurring in the Okavango River System were not experimentally determined during the present study. Small

signs of pathology were, however, noted during the histological sectioning of the brains of *Brycinus lateralis* infected with free-moving flukes (Figures 7.2 A - E) and hence a discussion is warranted.

Only *B. lateralis* provided a large enough sample size to conduct histological work from brains infected with free-moving metacercariae (see Chapter 6). Several other fish species were also found to be infected with a small number of unencysted metacercariae such as: *M. macrolepidotus*, *P. pollimyrus*, *P. catostoma*, *B. lateralis*, *R. maunensis*, *H. odoe*, *S. intermedius*, *O. andersonii*, *S. greenwoodi* and *T. sparrmanii*.

The studied pathology provided clear evidence of free-moving metacercariae within the ventricles of infected *B. lateralis* brain tissue. The presence of the dark-stained morphological structures such as the holdfast organ and genital primordium (Figure 7.2 C) and the oral and ventral suckers (Figure 7.2 D) undoubtedly established the presence of trematodes. This was confirmed by [1]Dr Steyl. Except for small cells surrounding most of the metacercariae, no other evidence of pathological responses were noted. Two kinds of cell types are proposed, namely red blood cells or rodlet cells.

1) Red blood cells

Three possible causes can exist for the presence of red blood cells. Firstly, it originates from the structural damage caused via the ventral and oral suckers of the metacercariae. The lifting and removal of small pieces of host tissues, as a result of attachment, have been noted in previous studies (Dezfuli *et al.* 2007). Secondly, the free-moving metacercariae may actively feed on the brain tissue and cause bleeding. As part of the structural damage done, these parasites can subsequently feed on the proteins in the blood as well as the cerebrospinal fluid present in the brain (Ashworth and Bannerman 1927; Rees 1955). The necessity for the metacercariae to feed is emphasised by their ability to survive for long periods inside of the eyes and brain of fish and the presence of functional anatomical structures aiding in ingestion and digestion (Erasmus 1958).

[1] Dr J. Steyl, Fish Pathologist, Onderstepoort Veterinary Sciences , Pretoria

The ability to feed clearly marks the necessity of the parasite to excrete waste material, which in itself could have possible effects on the physiology of the brain and hence the behaviour of the fish. A third possible explanation for the presence of red blood cells, could be haemorrhages caused by the mechanical damage inflicted by the invading cercariae. Concurring results were found by Ferguson (1943b) which reported on the presence of diplostomula inside of the brain ventricles of rainbow trout, *Oncorhynchus mykiss* and fathead minnows *Pimephales promelas* Rafinesque, 1820 surrounded by masses of blood cells. This provided an explanation for the loss of equilibrium noted with some of the heavily infected fish, whilst during the present study no similar conspicuous behaviour was noted with infected fish.

2) Rodlet cells

Another possibility is that the cells surrounding the metacercariae are rodlet cells. Locke *et al.* (2010b) suggest that rodlet cells are exclusive to teleosts and that it carries out anti-helminth immune responses in somatic and central nervous system tissues. The precise functioning of these responses is still not known (Sitjá-Bobadilla 2008). Dezfuli *et al.* (2007) reported that rodlet cells were the only signs of host cellular reaction found in the brain of *Phoxinus phoxinus* infected with *Diplostomum phoxini* metacercariae. The cells were noticeable in the epithelium lining the ventricles of the optic lobes, in close proximity to the metacercariae and the infected minnows showed a reduced predator avoidance response (Barber and Crompton 1997; Dezfuli *et al.* 2007). Similar to these findings, during the present study the unknown cells were also the only marked change in pathology.

Further specialised staining techniques and electronmicroscopy are needed to determine the precise nature of these cells surrounding the metacercariae. From the conducted histology, it is therefore concluded that the presence of the free-moving metacercariae has no striking pathological effect on the brain but this does not exclude the possibility of structural or hormonal effects. It is speculated that the presence, movement, migration and excretions of the parasites could have possible chemical effects on the hormones present in the brain and result in altered behaviour. An example of trematode induced hormonal (monoamine) changes having an influence on the nervous control of fish is discussed in Chapter 8.

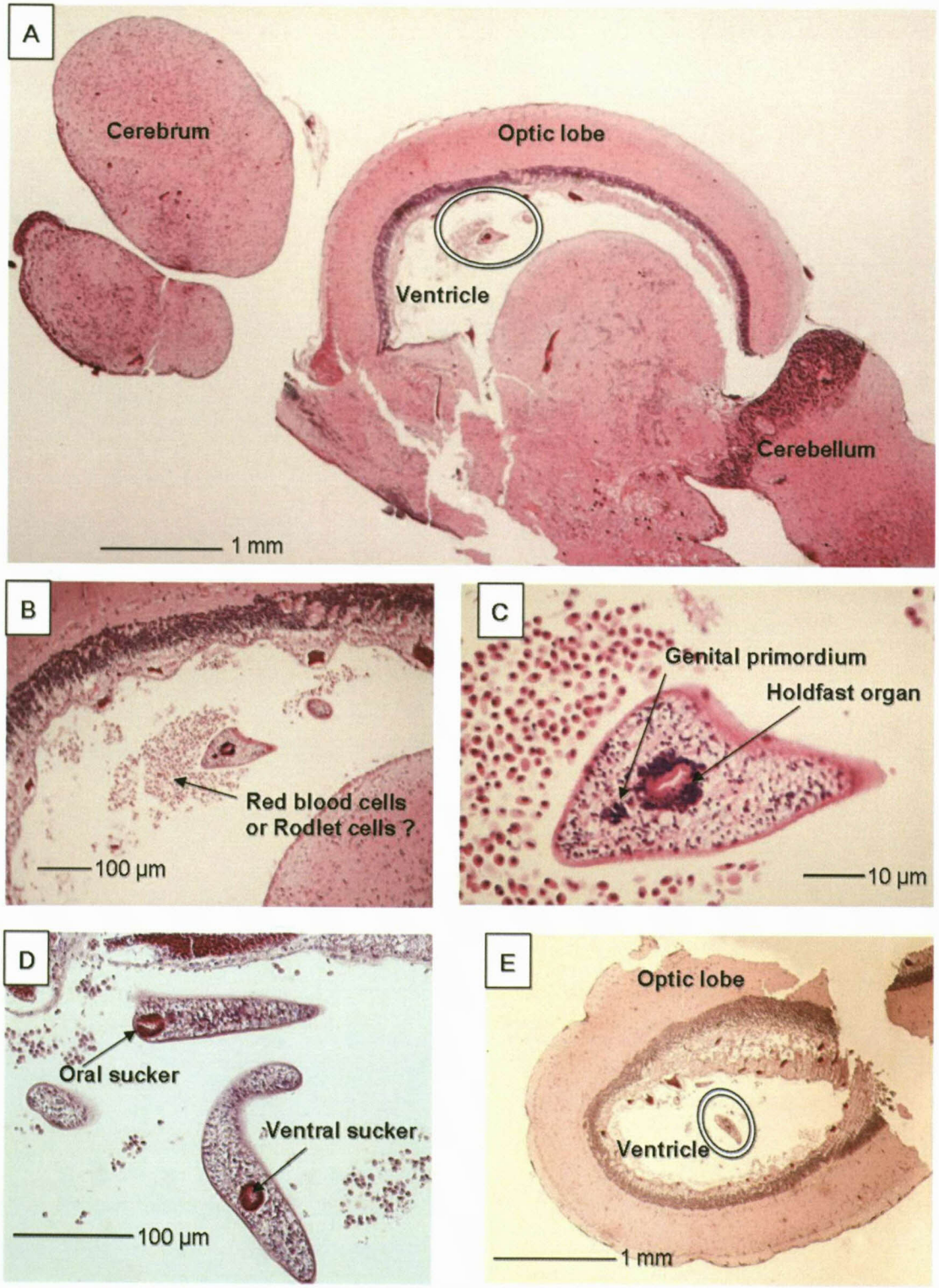


Figure 7.2: Histopathological sections of (**A**, **E**) free-moving diplostomatids (encircled) from the brain ventricles of *Brycinus lateralis* (Boulenger, 1900). The accumulation of (**B**, **C** and **D**) red blood or rodlet cells around the metacercariae is noticeable. Stained morphological structures such as (**C**) the holdfast organ and genital primordium and (**D**) oral and ventral suckers can be observed.

Whilst dissecting fishes form the Okavango River Systems, small numbers of brain encysting metacercariae were also observed (Figure 7.4 A). These fish species included *P. pollimyrus*, *S. greenwoodi*, *T. rendalli* and *T. sparrmanii*. During the present study no associated, altered brain pathology was noted, since the presence of the cysts could not be established through histological sectioning.

PREVIOUS RECORDS OF DIPLOSTOMATID BRAIN INFECTION

The lack of obvious clinical signs such as opaque infected eyes, resulted in less attention given to brain parasite research in the past (Hoffman and Hoyme 1958; Etges 1961; Dezfuli *et al.* 2007). The few behavioural tests conducted on captive fish, specifically infected with brain-dwelling parasites, mostly report on no aberrant behaviour (Barber and Crompton 1997). According to Erasmus (1958) one of the earliest records of diplostomatids within the brain was a brief description given by Gulliver in 1870, on metacercariae from the brain of European brook lamprey, *Lampetra planeri* (Bloch, 1784). These metacercariae are most probably synonymous with *Diplostomulum* (*Tetracotyle*) *petromyzontis* described from the fourth ventricle of the brain of juvenile lampreys from fresh water systems in England (Brown 1899). Little additional information was provided other than that the flukes fed on the brain tissues and although the heavily infected ammocoetes exhibited an inflamed brain appearance, they appeared to live on without any altered behaviour.

Today it is known that a great number of free-moving or encysted digenean metacercariae can occur in the brains of various fish species. The presence of these flukes may result in marked pathology, such as inflammation, but with or without causing noticeable behavioural effects (Sitjà-Bobadilla 2008). Some authors attribute this variation in effect to the intensity of metacercarial numbers and the precise location of brain infection. The possibility of specific brain site infections that lead to altered host behaviour is discussed in Chapter 8.

EYE ANATOMY

Basic anatomy of fish eyes

Due to the complexity involved during the evolutionary development of light detection, the tissues responsible for this function are similar amongst a diversity of organisms. For this reason mammalian eyes show many similarities with fish eyes (Liem *et al.* 2001) and both consist of the same basic functional anatomy (Figure 7.3).

The lens is spherical and suspended by a dorsal ligament and movement is accommodated by means of a muscular retractor lentis (Larson 1965). In the majority of vertebrate eyes the iris, consisting of muscle fibers, regulates the size of the pupil, which permits light passing through the lens to the inner layers of the eye. In most teleosts the pupil is, however, immobile (Munz 1971). The most inner, compound layer is the retina, which includes the light receptor cells, with the rods and cones, respectively responsible for light intensity and colour detection. Following the retina, is a blood rich capillary network, the choroid layer. Folds within the choroid sometimes extend into the vitreous body and in some teleosts it forms the falciform process. Investigators have postulated that by casting a shadow on the retina, these choroid processes help the animal to detect movement of an image across the retina (Liem *et al.* 2001). They speculate that pigment in the choroid, supplementary to the adjacent pigmented retinal layer, prevents the scattering of light and blurring of the image. The choroid layer is followed by the vitreous (posterior of the lens) and aqueous (anterior of the lens) humours, which contain gel and water like substances. Finally the sclera and cornea follow and these two layers are responsible for protecting the eye from the external environment.

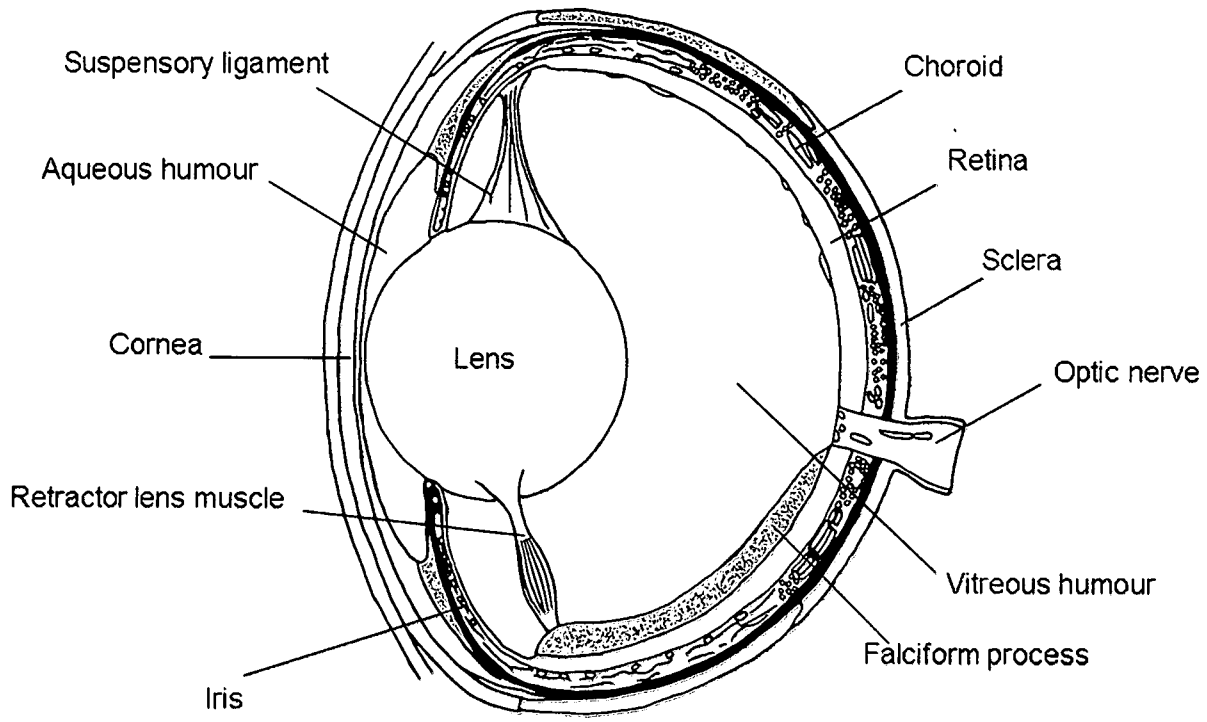


Figure 7.3: Basic anatomy of the teleost eye (redrawn from Liem *et al.* 2001).

PATHOLOGY OF DIPLOSTOMATID INFECTED EYES: PRESENT STUDY

Results regarding eye infection

Free-moving metacercariae (Figures 7.4 B and C) were sampled from the eyes of a variety of fish species present in the Okavango and Orange-Vaal River Systems (Chapter 6). During dissection, these diplostomatids were observed in the gel-like substance of the aqueous and vitreous humours but their presence could not be determined through the histological sections. It is speculated that the intensity of infection was too small to be detected. As a result, the precise location of the free-moving metacercariae is not known. Since a slight perforation of the eye ball mostly resulted in the larvae being squirted out and occurring in a gel-like substance, it is hypothesised that these free-moving flukes occurred within the aqueous and vitreous humours of the eyes. Both humours are sites with reduced immunological responses (Sitjà-Bobadilla 2008; Koornneef *et al.* 1981; Shariff *et al.* 1980). This may also explain why metacercariae occurring within the humours remained free-moving, whilst

encapsulated metacercariae were found in the immunological-active eye blood-vessels. During the present study no free-moving or encysted metacercariae were detected within the lens of any of the fish species in the Okavango and the Orange-Vaal River systems. This noted absence of diplostomatids within the lenses was thought to be very peculiar, especially when compared to the numerous studies conducted on lens-inhabiting metacercariae (Rees 1955; Crowden 1976; Niewiadomska 1996; Seppälä *et al.* 2004, 2005a, b, 2006a, b, 2008). An interesting and possible explanation for this absence of lens inhibiting metacercariae may be found in the geological history of Africa (Chapter 8).

Various histological sections were made from the diplostomatid infected eyes (Figure 7.4 E) of *Tilapia sparrmanii* collected from the Okavango River System. This natural population presented the highest intensity of eye diplostomatid cyst infection during the present study (Chapter 6). Transverse histological sections through the preserved eyeballs, suggested the presence of large, globule-like formations occurring inside the blood vessels. The globules were not mere cell masses, as intricate internal structures were observed (Figure 7.4 D), indicating the presence of trematodes in the cysts.

It is suggested that the diplostomatid cysts, lodged in the eye blood vessels, acted as luminal obstructions. This most probably resulted in the congestion of blood flow and an increase in capillary pressure as suggested by Guyton and Hall (2000). Fluid was increasingly forced across the capillary membrane into the interstitial space and this accumulation of extracellular fluid caused edema and ultimately resulted in ruptures such as retinal or choroid detachments (Figure 7.4 F).

1) Retinal detachment

The pigment (choroid) and the photo receptor cells (retina) are often from different embryological origins. The adhesion between these two layers is therefore relatively weak and is responsible for the most common form of detachments reported from vertebrate eyes (Shariff *et al.* 1980). As a result, the ruptures observed with the histological sections of banded tilapia, *T. sparrmanii* eyes, were initially thought to be

retinal detachments. This was also supported by [2]Dr Esterhuysen who suggested that the infected fish were totally blind due to these detachments found between the layers of the retina and choroid. The separation of these two layers leads to the destruction of adjacent photo receptor cells, such as the rods and cones. Damaged retinal surfaces are not able to receive the light wavelengths of different light intensities and colours and results in a loss of vision. The present study, however, concludes that the damage of a retinal detachment can be localised and do not necessarily extend through the whole eye. Depending on the number of retinal detachments within an eye it could therefore be that infected fish are only partially blind and not, as previously suggested, totally without sight.

2) Choroid detachment

A second opinion, from [3]Dr Labuschagne, provided a different conclusion regarding the analyses of histological sections. He suggested that the separations do not occur between the layers of the retina and the choroid but rather between the choroid and the sclera, resulting in choroid detachments. This small, yet technical difference in detachment site has vast implications for the vision of the fish (Table 7.1). With a choroid detachment, the rods and cones remain intact in the retinal layer and the host will still be able to differentiate between different light intensities and colours. As a result it will still be able to detect movement and distinguish between day and night. The only component which will be detrimentally influenced is the ability of the fish host to correctly view and identify silhouettes. This is attributed to the choroid detachment, which results in an irregular retinal surface, accompanied by an uneven reception of light and image formation.

Similar to retinal detachments, these detrimental effects could possibly occur only adjacent to the choroid detachment. Depending on the intensity of infection and the extent to which the cysts block the blood flow and result in an increase in capillary pressure build-up, the effect of choroid detachment may therefore be localised. The

[2] Dr C. Esterhuysen, Anatomical Pathology, University of the Free State, Bloemfontein.

[3] Dr L. Labuschagne, Medical Ophthalmologist, National Hospital, Bloemfontein.

fish host could only have a limited retinal area which becomes irregularly shaped. The damaging effect which choroid detachments could have, may therefore be restricted to only certain parts of the fish's vision and will not render the host with a completely distorted vision.

Table 7.1: The hypothesised similarities and differences of retinal and choroid detachments, present within tilapia fish species in the Okavango River System.

	Retinal detachment	**Choroid detachment**
Location of cyst infection	Eye capillaries	Eye capillaries
Effect	Congestion of blood flow and increase in capillary pressure	Congestion of blood flow and increase in capillary pressure
Location of detachment	Between retina and choroid Depending on intensity of infection, localised or throughout whole eye	Between sclera and choroid Depending on intensity of infection, localised or throughout whole eye
Pathology	Adjacent or general destruction of retinal layer Localised or general destruction of visual pigments, e.g. cones and rods	Cones and rods remain intact Intact retina but with localised or general irregular surface area
Clinical signs	Loss of light and colour perception in areas adjacent to detachment Partial or total blindness	Loss in ability to correctly view and identify silhouettes in areas adjacent to detachment Partial or total distorted vision

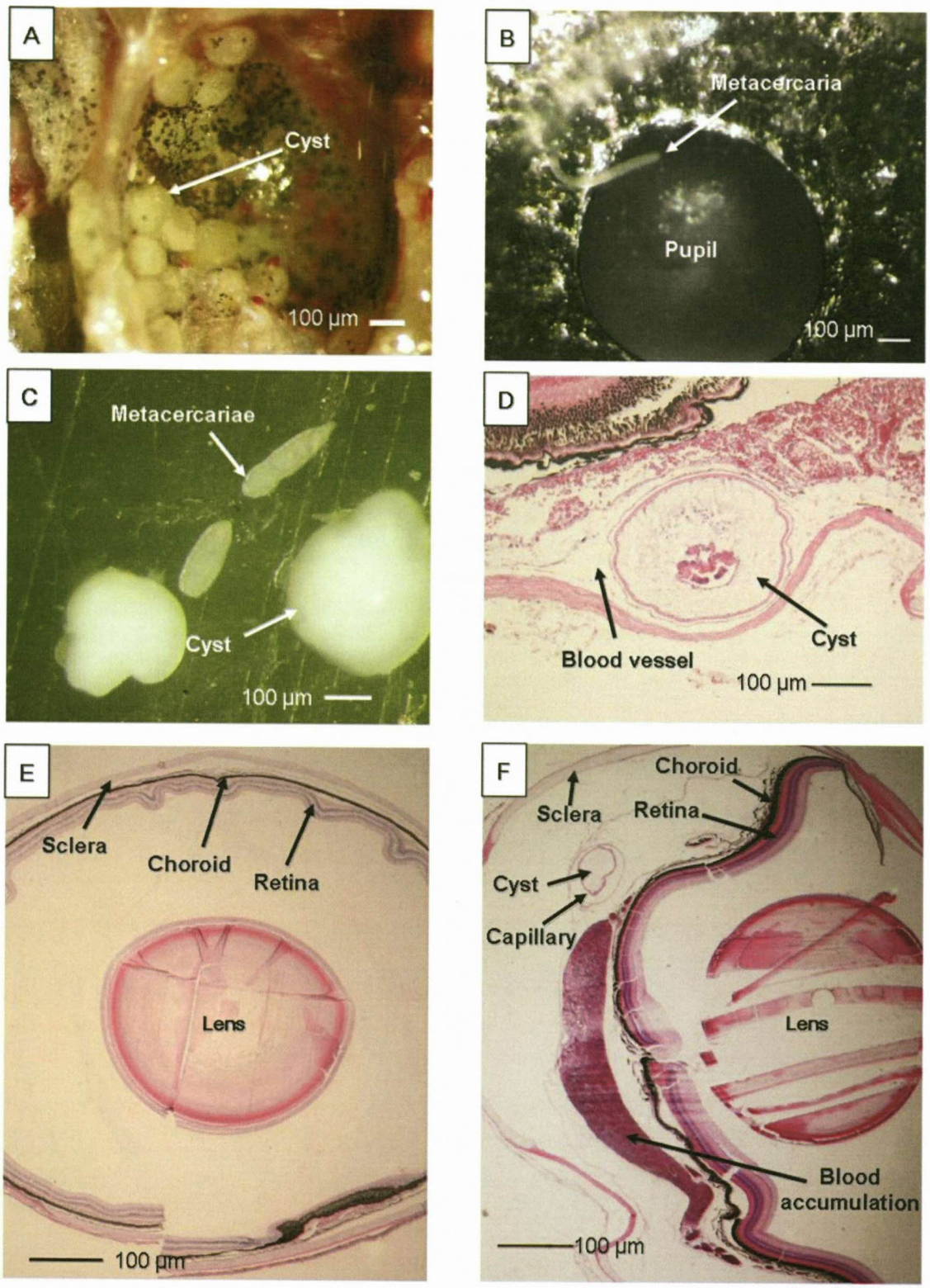


Figure 7.4: (**A - C**) Stereo micrographs of the brain and eye as well as (**D - F**) light micrographs of histopathological sections of eyes of fish species in the Okavango River System. (**A**) Encysted metacercariae beneath (**C**) the brain cranium and (**B**, **C**) free-moving metacercariae in the eyes of fishes. (**D**) Encysted metacercariae were lodged in the eye capillaries, which resulted that the normal eye pathology (**E**) was altered (**F**).

PREVIOUS RECORDS OF DIPLOSTOMATID EYE INFECTION

Lens-inhabiting metacercariae and cataract formation

It is well known throughout the literature that diplostomiasis is a condition which causes cataract formation in the lenses of fish eyes. The popularity of this specific clinical sign is most possibly because cataracts can easily be determined by using a dissecting or ophthalmological microscope prior to dissection (Karvonen *et al.* 2004b). Von Nordmann (1832) was the first to report on diplostomatid metacercariae present within the lenses of fish. He stated that up to 400 specimens of *Diplostomum spathaceum* were collected in the eyes of rudd, *Scardinius erythrophthalmus* (Linnaeus, 1758). This study was the first to suggest the possibility of these parasites to produce cataracts. Initial clinical signs include small, opaque spots in the lens, sometimes also with the metacercariae occurring between the hard core of the lens and the lens capsule (Erasmus 1958). However, a variety of other clinical symptoms are associated with diplostomula induced cataract formation such as blindness, exophthalmia, capsular rupture, lens dislocation and shrinking of the eyes (Larson 1965; Shariff *et al.* 1980).

It is hypothesised that the main reason for the cloudy appearance of the lens is due to the metabolic excretions created by the parasites (Erasmus 1958, Dörücü *et al.* 2002; Seppälä *et al.* 2004; Voutilainen *et al.* 2008), which is an indirect effect of its feeding behaviour. Destruction of the lens material is also associated with the feeding of the metacercariae. The intensity of the cataract formation may therefore correlate with the intensity of the infection (Hughes and Berkhout 1929; Karvonen *et al.* 2004b).

It is further hypothesised that the metacercariae excrete or secrete certain unknown compounds which reduce the normally firm lens tissue to a granular and mushy semi-fluid consistency (Ashton *et al.* 1969). This aids in efficient feeding for the metacercariae, whilst it leads to lens necrosis for the fish host (Shariff *et al.* 1980; Sitjà-Bobadilla 2008). The loosened lens material accumulate, resulting in the unsticking of the lens epithelium and hence also the clouding which may involve all or part of the lens (Niewiadomska 1996) and eventually decreases the host's vision. The ability of these larvae to feed on eye material has been proven by the functional anatomical feeding structures, as well as the presence of lens material found within the intestinal caeca of the parasite (Hughes and Berkhout 1929; Ashton *et al.* 1969). Although the

degenerative, feeding injury to the anterior lens surface initially results in the lens epithelium to become necrotic, Shariff *et al.* (1980) stated that the adjacent cells may migrate to the necrotic area and multiply to form a new epithelial layer. Ashton *et al.* (1969) suggested that proliferation of the lens epithelium, most apparent in areas adjacent to larvae, is a product of the parasite acting as a stimulant. Ultimately this leads to the duplication of the lens capsule in certain locations, increasing the thickness of the lens capsule up to three times its normal size and forming sub-capsular cataracts. The formation of these opaque structures might result in increased blindness and predator susceptibility of the fish host (Seppälä *et al.* 2004).

An interesting study conducted by Larson (1965) reported on the formation of lens herniations in black bullheads, *Ameiurus melas* (Rafinesque, 1820) in North Dakota. It was stated that the herniations were a direct result of pressure exerted by the diplostomatid infection occurring inside the lenses, ranging from 30 to 50 flukes per eye. No other altered pathology was, however, noted and each eye only had one herniation, which consisted of the same material as the lens and formed a continuous structure. The physical pressure exerted during dissection and histological sectioning could therefore be responsible for the occurrence of these singular herniations. The only other account of marked herniations in the presence of diplostomatid infection was given by Davies *et al.* (1973) for rainbow trout (*O. mykiss*) stocked into the lakes of North Park Colorado and infected with an average of 47 *D. spathaceum* metacercariae per eye. Additional pathology such as cataract formation was also noted but surprisingly with 80% of the metacercariae occurring within the retina and only 18% from the lens, 1% from the iris and 1% from the vitreous humour. It was determined that a slight infection of only four to five metacercariae per lens was needed to result in complete opacity of the lens and associated blindness.

Free-moving metacercariae in the humours and retina

Although the histological sections of the present study could not establish the presence of diplostomatids within the retinal layers itself, it is still possible for these types of infections to occur. Previous studies (Hoffman 1960; Davies *et al.* 1973; Betterton

1974; Shariff *et al.* 1980; Bortz *et al.* 1988) have noted the presence of metacercariae originally within the eye humours, which gradually migrated through to the retina. The diplostomula may also reach the retina via the blood capillaries, during which some may become lodged and encysted due to host immunology (Shariff *et al.* 1980). These infections were reportedly accompanied by necrosis and other degenerative retinal changes such as the complete atrophy of the rods and cones and retinal detachments. Depending on the infection intensity, this pathology would most probably lead to partial or total blindness. However, none of these pathological effects were noted during the present study. Another site closely linked to the retina, from which diplostomatid infection has been reported, is the epichorioidal lymph space. This space occurs between the retina and choroid. Bouillon and Curtis (1987) noted high intensities, such as 1 478 diplostomula, within this particular site but with no associated altered behaviour of the fish host (see Chapter 8).

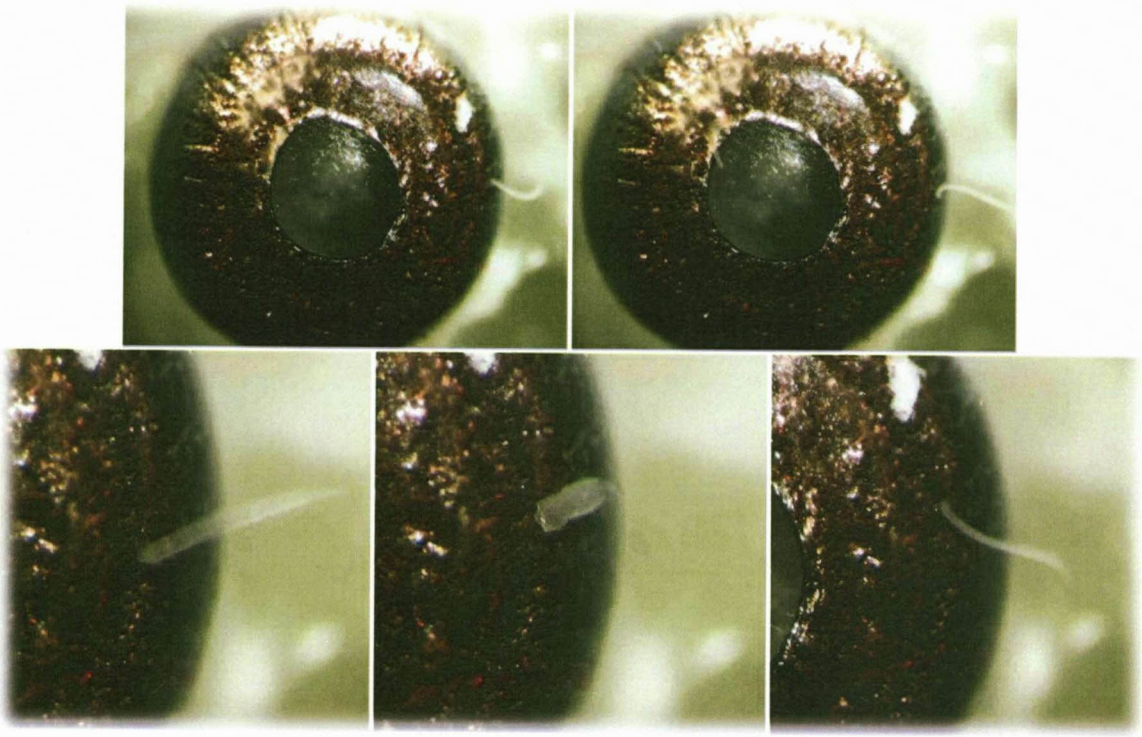
Retinal and choroid detachments

Diplostomatid infections resulting in detachments of the inner eye membranes have been recorded in the literature. When compared to cataracts, the successful diagnosis of this condition is, however, much more complicated since it can only be established through advanced histological sections from fixed eyeballs.

Lester and Huizinga (1977) reported on retinal detachments occurring within the eyes of American yellow perch, *Perca flavescens* from Canada. Clusters of *Diplostomum adamsi* metacercariae occurred between the photo receptor cells of the retina and the epithelium of the choroid. The presence of the metacercariae and the associated provoked macrophages surrounding it, resulted in the adjacent retinal layers to become thin and the misaligning of the cones and rods. As a result cavities were created between the choroid and retina. The associated evoked macrophages and these cavities resulted in the cessation of blood provision to the adjacent parts of the retina and thereby the peripheral rods and cones were destroyed. The infected host therefore had no sensitivity towards light and colour detection within these localised parts of the retina. The behavioural implications of this infection is discussed in Chapter 8.

Membrane detachments have also been noted from rainbow trout infected with diplostomatids in the lens (Chappell 1995). The associated pathological effect, however, differs from the present study in that it was due to the mechanical damage done by free-moving metacercariae feeding on lens fibers and not because of blood-vessel obstruction. The retina was pulled anteriorly by membranes which had formed due to severe inflammatory response in the vitreous humour, caused by the lens material protruding from the damaged capsular lens membrane (Shariff *et al.* 1980). Since lens infection was not present, this could not be regarded as the reason for the observed detrimental pathology in the present study.

Chapter 8



BEHAVIOURAL CHANGES OF DIPLOSTOMATID INFECTION

"In gaining an entrance into their hosts the parasitic worms seem to show the most astounding knowledge of the activities and habits of life of the host. Had they the ability to see, hear and reason it seems doubtful whether they could exhibit a more diabolical cunning way to gain their ends as they do now." - Professor George R. La Rue

To provide a proper background, this chapter will begin with a brief overview of parasite induced changes in host behaviour. This will be followed by the epidemiology of diplostomiasis as well as comments on the prevalence and effect this disease has in natural, aquacultured and experimental fish populations. The final section of this chapter discusses the results found with the two sets of behavioural experiments conducted to determine the parasite induced behavioural change in certain wild fish, infected with diplostomatid eye flukes. The pathology responsible for the alteration in infected hosts' behaviour will be mentioned but is discussed in full in Chapter 7.

PARASITE INDUCED CHANGE IN HOST BEHAVIOUR

From early in the 20th century the idea of parasitic manipulation of the host phenotype (Dawkins 1982) became a fascinating and popular phenomenon for parasitologists to study. This topic offered an opportunity to demonstrate the importance of parasites to the broader scientific community (Thomas *et al.* 2005). Protection from immunology could have been the initial selective pressure for the location of these parasites but one of the possible additional benefits may be the alteration of the host phenotype to increase trophic transmission and reproductive fitness of the parasite (Seppälä 2005). Since successful transmission is usually an unlikely event, any characteristic that enhances parasite transmission may be favoured by natural selection (Seppälä *et al.* 2005a). There are various different ways in which parasites can influence and alter the behaviour of their host (Moore 2002; Thomas *et al.* 2005). These include a reduction in host stamina and activity levels as well as an increase in conspicuousness and disorientation. Larval diplostomatids are considered to physically and physiologically influence the behaviour of the fish host species to make them more conspicuous and thereby acting as a kind of prey delivery service to piscivorous birds (Lafferty and Morris 1996).

Helminth infections are notorious for altering the phenotype of their hosts to increase the probability of the parasites to complete their life cycle (Poulin 1994a). This forms part of the co-evolutionary arms race between hosts and parasites, with the latter constantly evolving new strategies to increase their infectivity, whilst hosts try to resist

and reduce parasite susceptibility (Seppälä 2005). For example, it is a well-known fact that most trematode infections result in the partial or complete castration of the gastropod hosts. Thereby the trematodes can almost exclusively channel the energy of the host to aid in the formation of sporocysts and ultimately cercariae (De Jong-Brink *et al.* 1989; Chappell 1995; Levri 1999; Fredensborg *et al.* 2005). Another example of parasite induced predator susceptibility is of an acanthocephalan species of the genus *Profilicollis* Meyer, 1931 infecting the stalk-eyed mud crab *Macropthalmus hirtipes* (Heller, 1862) in New Zealand. The infected crustacean hosts exhibit less hiding behaviour and are more exposed during low tide than the uninfected conspecifics.

For the past several decades, researchers have, however, been biased in attempting to prove that parasites are the cause of the majority of host behavioural differences and that most of these modifications ultimately result in more successful transmission. It should be noted that although physiological, morphological and behavioural changes are often reported from parasitised animals, the adaptive significance of these changes is not always clear (Poulin 1994a). Caution should be used before the popular deduction of 'manipulation of behaviour to increase own gain' is made (Poulin 1995; Klein 2005). Although not always initially noticeable, certain infected hosts may actually benefit from the parasite induced phenotypic changes.

Behavioural fever helps the host to eliminate its parasites or suicidal behaviour may reduce the risk of infection for the host's kin and population. Alternatively some behavioural and other phenotypic changes may only be the clinical consequences of disease or immunological responses. These induced changes are not necessarily exclusively beneficial to the transmission or reproductive success of either host or parasite but are merely by-products of infection (Thomas *et al.* 2005; Leung and Poulin 2006). Due to the lack of sufficient studies of experimental infections as well as behavioural studies under natural environmental conditions, it remains a fundamental challenge to specifically differentiate between the respective fitness consequences for parasites and hosts (Barber 2007). Guidelines have been provided by Poulin (1995) but it should not be regarded as the absolute criteria to recognise true host manipulation. He proposed that alterations in host phenotype (behaviour) following

infection can only be considered adaptive if they consist of all of the following conditions:

1) the alteration in behaviour should be complex in design and not be mere simple traits. Deciding on the complexity of a behavioural change, however, is very subjective and simple changes in host behaviour may require unnoticeable complex physiological and morphological adaptations in the parasites or hosts

2) the alteration in behaviour should have a specific purpose in design, having an influential function, and not be a simple pathological side-effect of infection

3) behavioural changes are more likely to be adaptations if they have evolved independently in several lineages of hosts and parasites under similar selective pressures and therefore show convergence

4) the behavioural change should ultimately lead to an increase in the parasite's fitness

Up to date it is not clear if diplostomiasis precisely fits in with all of the above criteria. Future experimental studies will hopefully help to elucidate this. One fact which is clear is that the loss of vision, as a result of infection, will most certainly have an influence on behaviours such as food-selection, predator avoidance and shoaling of fish (Boeuf and Le Bail 1999). If by any chance the diplostomatid infection is responsible for these changes in behavioural characters and it predisposes the fish host to definitive bird hosts, the trait will be favoured and selected by natural selection. Goff and Green (1978) proved the importance of fish vision by removing the lenses of radiated shanny fish, *Ulvaria subbifurcata* Storer, 1839. A decrease in their shelter seeking and escape responses were noted at light levels at which potential predators were active and therefore increased their exposure to predation and the reproductive fitness of the parasite.

DIPLOSTOMIASIS: THE DISEASE

Diplostomiasis is a widespread disease of more than 125 reported freshwater and brackish fish species (Bortz *et al.* 1988) and it is speculated that non-official reports will immensely increase the number of species. Although the term generally refers to

diplostomatid eye infection, and associated total or partial blindness (Rushton 1938; Ferguson and Hayford 1941; Karvonen *et al.* 2005), it may also include the infection of the brain, spinal cord and nasal spaces of fish (Niewiadomska 1996; Dörücü *et al.* 2002; Niewiadomska and Laskowski 2002). Due to the nature of some of the well-known diplostomatid species to attack (feed and destroy) the lens, the primary clinical sign of infection is cataract formation. For this reason, the terms "white eye" or "worm star" are sometimes used in the aquaculture industry to describe this type of infection (Chappell 1995).

Fish eyes are the major light receptor organs and are responsible for the detection of changes in light intensities. This function is important regarding the recognition of approaching predators, location of shelter and determining diurnal and seasonal cycles (Liem *et al.* 2001). The disease can have vast impacts on the overall biology of fish hosts (Boeuf and Le Bail 1999). Reports exist of noteworthy decreases in the number of recreational angling fish species (Davies *et al.* 1973) and individuals cultured in fish farming practices (Ashton *et al.* 1969) due to blindness caused by diplostomiasis. The greatest economic impact of this disease has been attributed to lethal cerebral and other tissue haemorrhages caused by the heavy infections of migrating cercariae, and not due to a direct effect of the eye infection and associated blindness (Baylis 1939; Berrie 1960; Lester and Huizinga 1977; Brassard *et al.* 1982b). Infected fish that are not directly killed are often reported to be partially or totally blind, leading to a general decrease in feeding abilities and health and ultimately leading to a reduction and economic loss regarding the market value (Lyholt and Buchmann 1996; Scholz 1999; Karvonen *et al.* 2005).

Many authors have suggested that diplostomiasis' blindness favours the completion of the trematode's life cycle by making infected fish easier prey to the final host, namely piscivorous birds (Baylis 1939; Brassard *et al.* 1982c; Dezfuli *et al.* 2007; Seppälä *et al.* 2004; 2005a; 2006b). The whole pathological effect associated with diplostomiasis, however, needs to be considered in order to determine whether the behavioural changes of the infected prey result from the side effects of general decrease in health, or whether the parasites actively target the host's visual-neuro-endocrine systems (Shaw *et al.* 2009). Barber and Crompton (1997) stated that the accumulation of

parasites in the central nervous system or in the sensory organs such as the eyes, could cause physical damage to the localised tissue and this in turn could affect the sensory physiology or behaviour of the host. Only until recently have the immunopathological and histopathological effects of this kind of infection been considered (Ashton *et al.* 1969; Dezfuli *et al.* 2007). Information relating to the fish host's immune reaction and other histopathological effects therefore remains limited, especially for African fish. There is also little information available on the pathology of diplostomatids inhabiting sites in the eye, such as the humours and retina, other than the lenses (Chappell 1995).

To try and clarify the discrepancy regarding the precise outcome which diplostomatid parasites may or may not have on the behaviour of their fish hosts, an overview is provided of the prevalence and effect this disease has in natural, aquacultured and experimental fish populations.

NATURAL AND EXPERIMENTAL DISPERSION OF LARVAL DIPLOSTOMATIDS

With exposure to many cercariae, numerous lesions occur on the body and fin surface at the sites of cercarial penetration (Ferguson and Hayford 1941). According to Whyte *et al.* (1989) and Chappell (1995) the associated immunological and inflammatory responses may cause the direct death of the fish due to anaphylactic shock. Vast exposure and penetration of cercarial masses is prominent in aquaculture, fish experimentally infected with metacercariae or alien fish species stocked into natural aquatic systems for farming and recreational practices (Davies *et al.* 1973; Hendrickson 1978; Bouillon and Curtis 1987; Crowden and Broom 1980; Machado *et al.* 2005). Most previous reports on diplostomatid infection and associated behaviour make use of these unnatural hosts (Lafferty and Morris 1996). According to Seppälä *et al.* (2011) this is in direct contrast to the over dispersal of parasites in natural systems and therefore may provide a misrepresentation of diplostomiasis occurring in the wild.

A high prevalence but with low infection intensity have been recorded during previous studies from natural fish populations. In a study by Crowden (1976) it was concluded that a prevalence of 100% of *D. spathaceum* infection was present within a natural

population of dace, *Leuciscus leuciscus* (Linnaeus, 1758) collected from the River Thames. The intensity of this infection, occurring within a natural fish population, was low and no correlation was noted between the parasite burden and the health of the fish. Upon experimental cercarial exposure of the same fish host species, greater intensities of metacercariae were noted and these resulted in cataract formations. Due to the associated blindness it led to a decrease in the ability of the dace to detect prey and this increased the time spent feeding, leaving them more susceptible to predation by piscivorous birds. Similar findings were reported by Voutilainen *et al.* (2008) who suggested that infected, hatchery bred Arctic charr, *Salvelinus alpines* (Linnaeus, 1758), spent more time foraging to compensate for the reduced feeding capability. This was also a result of the diplostomatid induced cataract formation.

There have been numerous reports of fish exposed to great cercarial numbers, in aquaculture or experiments, to develop epidermal haemorrhages which were followed by behavioural changes such as restlessness, shivering motion and loss of equilibrium, erratic swimming, loss of vision, deformation of the vertebral column, brain tumours, cellular necrosis and ultimately stunted growth and mass deaths (Betterton 1974; Ratanarat-Brockelman 1974; Crowden and Broom 1980; Whyte *et al.* 1989; Chibwana and Nkwengulia 2010). However, in natural systems, through evolutionary co-development, the over dispersion of parasites results in few digenean parasites simultaneously penetrating a host. This makes sense, as it would not be to the advantage of the penetrating and migrating diplostomula if the host died, since these larval forms are not yet infective for the next trophic stage.

Also different from aquaculture and experimental infections, is that fish in the natural environment are not confined to limited space and they can actively escape from penetrating cercariae. The ability of cercarial evasion has been described for rainbow trout exposed to areas with high densities of *D. spathaceum* cercariae (Karvonen *et al.* 2004c). During experimental infection, where the exposure time is lengthened and fish are restrained within small containers, the probability of contact between the cercariae and the fish increases in relation to time (Barber 2007). The number of diplostomatid metacercariae within naive fish is also more-or-less positively related to the intensity of cercarial exposure. Therefore a massive influx of metacercariae will be present

amongst fish which are contained and exposed to great numbers of cercariae (Brassard *et al.* 1982b).

In some aquaculture practises, fish reared in captivity are provided with a limited range of nutrition. This lack in dietary components can directly reduce the capacity of their immune system to withstand parasite invasion or establishment (Barber 2007). Not only the presence of cercarial masses, but also the presence of reduced immunological responses may result in captive fish providing an inaccurate representation of the effect of diplostomiasis in nature (Voutilainen and Taskinen 2009). In a study conducted by Karvonen *et al.* (2004b), two different infection procedures were used to determine the relationship between diplostomatid infection intensity and the intensity of cataract formation in *O. mykiss*. The first consisted of gradual cercarial exposure with fish placed in natural conditions and the second comprised a single, high-numbered exposure with fish kept in laboratory conditions. It was concluded that the intensity of infection within the laboratory set-up was greater than under natural conditions, given the same passing of time and that the formation of cataracts occurred at a much faster rate. Rees (1955) also reported that if cercarial penetration occurs over a gradual period of time, the number of diplostomula causing fatal haemorrhages will be less and resultantly fewer fish will die as a result of infection. This is generally the case in natural systems, whilst in aquaculture the infection intensity can be immense within a very short period of time. It is therefore possible that natural populations of fish may harbour more metacercariae without displaying abnormal behaviour, whilst their captive conspecifics may exhibit altered behavioural responses at a far lower intensity of infection.

Care should be taken when evaluating proposed phenotypic changes under laboratory conditions. In a study conducted by Seppälä *et al.* (2004, 2005a, b) it was concluded that rainbow trout experimentally exposed to *D. spathaceum* metacercariae, were more susceptible to capture through dip-nets than the uninfected controls. This was attributed to a reduction in vision as a result of parasite induced cataract formation. In a follow-up study by Seppälä *et al.* (2006b) it was puzzling for the researchers to find that the predation vulnerability of similar infected fish did not differ from the controls, when wild birds were allowed to feed on the fish. It was suggested that the

experimental set-up of the latter study, consisting of experimentally infected fish placed in floating cages in a lake, provided an unnatural and easy platform for piscivorous birds to catch the control and infected fish. This most probably reduced the distance needed to strike and therefore lead to decrease in movement and shadow stimuli observed by the fish.

A limited number of reports exist on pathological effects caused by heavy diplostomatid infection within a natural population of fish hosts (Erasmus 1958; Crowden and Broom 1980; Barber and Crompton 1997). These are considered to be extreme cases since the theory of natural parasite dispersion states that only a few individuals of the host population will shelter many parasites, whilst many will host much lesser and most may not even become parasitised and will not show any altered behaviour (Machado *et al.* 2005). The most severe effects caused by elevated intensities of infection will be confined to a few individuals and have little effect on the natural population as a whole. It may also be that the few fish individuals which are indeed severely infected do not survive for as long, as they would be unable to avoid the attacks of predators. According to Seppälä *et al.* (2011) they are removed from the natural population before pathology or altered behaviour can be noted.

Little is known of the effect which the intensity of diplostomatid infection will have over a range of levels, especially in natural populations (Crowden 1976; McCloughlin and Irwin 1991). There is an overall need for modern studies to focus on naturally infected hosts and their susceptibility. The present study sampled fish from natural fish populations from the Okavango and Orange-Vaal Rivers and focused on the pathological effect the diplostomatid infections have on the brain and eyes. Due to the complexity of noting the behaviour of fish in the wild and in order to determine the exact intensity of infection, an experimental set-up was constructed (see Figure 3.4) whilst naturally infected fish were used in the behavioural studies. The intensity of infection of each fish was established through dissection, whilst their behavioural changes were noted beforehand.

PREVIOUS RECORDS OF DIPLOSTOMATID BRAIN INFECTION

Infection in specific brain sites

Since the general viewpoint, that diplostomula migrate to the brain in the bloodstream, passing into the cerebral cavity (cerebrum) and ventricles via the choroid plexus (Barber and Crompton 1997), there should be no reason to expect any particular brain region to be more easily invaded than others. As stated in Chapter 7, the entire teleost brain is connected by means of the cerebrospinal fluid occurring within the ventricles and other cranial spaces and therefore migration to various sites is easily achievable.

Numerous previous studies report on diplostomatids occurring exclusively within specific brain sites (Hendrickson 1979; Shaw *et al.* 2009), which are accompanied with specific altered behavioural responses. According to Dezfuli *et al.* (2007), *Diplostomum phoxini* metacercariae exhibit organ region selection within the brains of infected *Phoxinus phoxinus*. The examination of a sample of fish from a naturally infected population revealed that the heaviest infections were found within the cerebellum, medulla oblongata and optic lobes. The fish cerebellum is linked to a variety of cognitive functions, whilst the medulla oblongata is known to control a range of involuntary responses and the optic lobes enable vision (Dezfuli *et al.* 2007). This site-specific distribution in the brain could therefore aid as a mechanism to alter the nervous control of the fish. This in turn could result in aberrant swimming behaviour, leaving the fish more conspicuous to the definitive host (Shirakashi and Goater 2001, 2002).

Ashworth and Bannerman (1927) collected *D. phoxini* metacercariae from the brain, mainly in the optic lobe ventricles and the fourth brain ventricle of *P. phoxinus* in Britain. Some fish were infected with 250 or more metacercariae and there was extensive proliferation of the epithelial lining the ventricles but no modification in their reactions or swimming movements were noted. This is in contrast with the findings of Barber and Crompton (1997) who also collected *D. phoxini* in the brain of *P. phoxinus* but within specific sites and tissues, such as the medulla oblongata, the cerebellar cavity, the optic lobes and the superior lobe of the cerebellum. It was argued by these authors that the specific site infections resulted in a change in sensory systems, motor control and antipredator behaviour of the infected European minnows.

Examples do exist of specific site infections not resulting in noticeable altered behaviour. Hoffman and Hoyme (1958) determined the presence of *Diplostomum baeri eucaliae* metacercariae within the brain of brook stickleback fish, *Culaea inconstans* in North America. With moderate metacercarial infections (335 per stickleback brain) the feeding effect of these parasites, localised specifically within the choroid plexus, optic lobes and ventricles of the brain, resulted in hyperplasia and the accumulation of cells appearing to be macrophages and columnar epithelium and led to the formation of large tumor-like structures within the lobes. Although a considerable loss of brain tissue occurred in heavily infected fish, no impairment of the reflexes was however noted. Hoffman and Hoyme (1958) reported that death, as a result of the parasite infection, was rather only due to the extensive cellular damage and subsequent hemorrhages and inflammation caused by the heavy, experimental cercarial infection and not by any other brain pathology.

Migration during metacercarial development to specific brain sites

Further motivation, that the site of infection may determine the behavioural change of the host is provided by a study of Szidat (1969). *Diplostomulum mordax* were identified as free-moving metacercariae occurring under the ependymal epithelium lining the ventricles of the brain of the Argentinean silverside, *Odontesthes bonariensis*. This initially caused no alteration in the behaviour of the host, whilst in later infection stages it was noted that the location and effect of these parasites changed during their development. The more mature metacercariae, which are more infective, migrated to various and specific brain tissues, especially the midbrain, cerebellum and optic lobes. Only then did the penetration and feeding of the more developed metacercariae result in the destruction of the optic centre in the midbrain and the cerebellum. The respective behavioural changes included blindness and loss of equilibrium and movement, rendering the fish host more susceptible to aerial predation (Szidat 1969).

Mashego and Saayman (1989) also reported on the initial concentration of juvenile *Diplostomum mashonense* metacercariae detected around the anterior part of the brain cavity of *Clarias gariepinus* in South Africa. Over time the infection seemed to move

more posterior to the medulla oblongata. Poulin (1994b) and Barber (2007) supported the viewpoint that with certain infections only the mature, infective metacercariae are responsible for alteration in host behaviour by means of migrating to specific sites. This seems plausible, since the premature death of the second intermediate host will not benefit parasite transmission, if the metacercariae are not developed enough to become established within the final host. It is therefore possible that a parasite can only be regarded as a true manipulator parasite species when it can alter the host behaviour once it becomes infective to the definitive host (Leung and Poulin 2006).

Not many studies have been conducted to determine the time needed for juvenile metacercariae to become mature but Hoffman and Hundley (1957) suggested that between 13 and 23 post-infection days are needed by *D. baeri eucaliae* metacercariae to become mature enough. These metacercariae occur in the ventricles of the optic brain lobes of brook stickleback, *C. inconstans* found in North Dakota. A different time span may, however, be needed for different diplostomatid and fish species, as well as for infections occurring in different parts of the brain and eyes (Sweeting 1974). For example Seppälä *et al.* (2005a) determined the time span for *D. spathaceum* infecting the eye lenses of *O. mykiss* to be between 17 and 21 days.

When altered behaviour is displayed before the parasitic stage is infective, the behaviour is more likely to be a mere side-effect of the pathology associated with the infection. This seems to be exactly the case with mature, infective metacercariae of *Ornithodiplostomum pthychocheilus* Faust encysting within the brain of fathead minnows, *Pimephales promelas* in Canada. Shirakashi and Goater (2005) reported on the mature metacercariae occurring in the meninx and causing conspicuous enlargement of the cranium of naturally infected juvenile fathead minnows, without any altered behaviour. In a similar study by Radabough (1980), it was concluded that these parasites did indeed affect the behaviour of the fish hosts when the juvenile metacercarial stages were still migrating to specific sites such as the optic lobes and cerebellum. The sensory systems and motor control were influenced and led to the formation of less compacted shoals and thereby increased the fish's individual predator susceptibility.

Shirakashi and Goater (2005) provided the possible explanation for the difference in results by establishing that this altered behaviour of infected *P. promelas* was only noticeable with juvenile metacercariae, at two and four weeks post infection. During this time interval the developing parasites showed a maximum growth rate and therefore had the greatest pathological effect on the brain with associated behavioural influences. These metacercariae were not yet mature enough to successfully infect the final bird host. Only after ten weeks post-infection, when the metacercariae had finished their migration and had encysted in the optic lobes, were they fully developed but then abnormal shoal formations ceased. Since the altered phenotype occurred during a period when the parasite was non-infective, this change in compact shoal formation is seen as an inevitable result of the pathology associated with the developing metacercariae. This cannot be regarded as a form of parasite 'manipulation' to increase successful transmission.

It is therefore essential to consider the preferred site of infection and the age of the metacercarial infection, before drawing conclusions regarding the effect on the host phenotype. Whilst experimental infection enables an accurate determination of the time span of post-infection, the same is not possible for metacercariae collected from natural fish populations. This is especially true since the same diplostomatid infection can exist for several years within a fish host. The accumulation of metacercariae over several seasons could also result in various developmental stages being present within a single host. The question can be asked, if only less-infective metacercariae are responsible for altered host behaviour, could these individuals decrease their own direct fitness in order to secure the successful transmission of more mature metacercariae?

African brain diplostomatids and other reports of brain cavity infections

In contrast to specific site location, Beverley-Burton (1963) speculates that the presence of flukes in the open spaces within the cranial cavity, such as the ventricles, will result in less of a convenience for the fish host. Many reports exist on the presence of free-moving diplostomatid metacercariae within the ventricles of fish brains, without

causing noticeable alteration in behaviour. Rees (1955) determined the presence of *D. phoxini* metacercariae mainly within the fourth brain ventricle of *P. phoxinus*, which did not show any altered behaviours or pathological effects. Again, only with heavy infections (> 1 297) did cerebral haemorrhages result in the death of the host. Infection of similar fish host species, reported by Mataré (1910) and Ashworth and Bannerman (1927), were respectively noted to host a maximum of 500 and 247 metacercariae within the brain but also with no associated altered behaviour present.

In Africa there are three records of unencysted metacercariae sampled from the brain cavities of *Clarias gariepinus*, without any noticeable altered behaviour being reported. In southern Zimbabwe as many as 15 000 metacercariae of *Diplostomum* (*Tylodelphys*) *mashonense* occurred in cranial cavity of a single fish. Despite the enormous mechanical pressure which this worm burden must have exerted on the brain, no sign of distress or abnormal behaviour were noted (Beverley-Burton 1963).

Also, from nine different sampling sites in Lebowa, South Africa, the mean intensity of the *D. mashonense* infection was determined to be 2 391 metacercariae per *C. gariepinus* brain (Mashego and Saayman 1989). Twenty five to several hundred *Diplostomum tregenna* were recorded from the cerebrospinal fluid of the cranial cavity and olfactory tract of *C. gariepinus* in the Nile River system (Ibraheem 2000), also without any noticeable altered behaviour. The unseen but possible hormonal effects which these brain infections could have on the fish host, will be discussed.

INDUCED BEHAVIOUR OF DIPLOSTOMATID INFECTED BRAINS

Results from the present study

Since no experimental observations were conducted, the present study remains inconclusive on the precise effect which diplostomatid brain infection will have on the behaviour of the collected fish from the Okavango. It was, however, observed that the infected *Brycinus lateralis* were collected during the day-time, whilst it is known that this species is mainly nocturnal. Through dissection it was determined that a relatively high intensity of diplostomatid infection occurred within the brains of these individuals (see Chapter 6). The same peculiar day-time activity was also noted during previous

fieldwork, for another Characidae family member, i.e. *Rhabdalestes maunensis*, found to be infected with brain diplostomatids (Jansen van Rensburg 2006). Being exclusively visual predators of fish, piscivorous birds mainly forage by day. It is therefore hypothesised that, to enhance trophic transmission, the diplostomatid brain infection could be responsible for the uncanny preference for the two fish species' day-time activity. The precise mechanism used by the parasite to enhance the fishes' preference for diurnal activity is not known but a change in the neuro-endocrine control has been established in diplostomatid infected fish (Seppälä *et al.* 2005a).

Hormonal effect of diplostomatid brain infection

According to Shaw *et al.* (2009) it seems plausible that metacercariae might secrete molecules or excrete metabolic waste that could modify the brain monoamine activity of infected fish. For example, the cysts of the trematode brain parasite *Euhaplorchis californiensis* (Martin, 1950) affects specific locomotory behaviours of infected California killifish, *Fundulus parvipinnis* Girard, 1854 by increasing dopamine and decreasing serotonin. This alteration in neuro-transmitting hormones results in suppression of fish stress response and leads to a decrease in their freezing behaviour. In many fish species, this behavioural response is the primary avoidance behaviour towards danger. Infected killifish showed an increase in conspicuous swimming behaviour by as much as four times and this rendered them 10 - 30 times more susceptible to aerial predation, when compared to uninfected individuals (Shaw *et al.* 2009).

The presence of such hormones cannot be established through the basic histological procedures which were used during the present study. Further specialised staining techniques are needed in this regard. It is, however, possible that hormonal change, induced by the diplostomatids, could be responsible for the increase in day-time activity of the characins observed during the present study. Further specialised neurological experiments and observations to prove specific pathological effects, which could be responsible for this odd behaviour, are needed.

PREVIOUS RECORDS OF DIPLOSTOMATID EYE INFECTION

Lens-inhabiting metacercariae and cataract formation

The feeding and excreting of diplostomatid metacercariae within the lenses of fish are known to cause cataract formation and a variety of other clinical symptoms (Chapter 7). The most reported condition of diplostomiasis within the lenses of fish is the formation of cataracts (Hughes and Berkhout 1929; Erasmus 1958; Dörücü *et al.* 2002; Karvonen *et al.* 2004b; Voutilainen *et al.* 2008). A build-up of cataracts will ultimately lead to a decrease in vision and an increase in the infected fish's predator susceptibility (Davies *et al.* 1973; Niewiadomska 1996; Seppälä *et al.* 2004). A positive relationship regarding metacercarial numbers and cataract formation can therefore be expected.

As mentioned, for completion of the life cycle, it is necessary for the metacercariae to be mature enough to infect the final host, a piscivorous bird. Seppälä *et al.* (2005a) noted that with *D. spathaceum* infection within the eyes of *O. mykiss*, the induced cataract formation was initially slow. The build-up of metabolic waste material however, increased, the formation of opaque spots covering the eyes of the infected fish. As a result infected fish became more readily catchable with dip-nets at a time when the parasites were mature enough to become infective to birds.

Three years later, another study by Seppälä *et al.* (2008), concluded that the induced cataract formation also resulted in a decrease in the ability of the infected rainbow trout to join and / or keep up with shoals. A general conclusion that diplostomatid infection will always result in less cohesive group formation and other odd fish behaviour should not, however, be made. It could be that infected fish, differing in their behaviour, may have exhibited this character prior to infection. The presence of strange behaviour might have been the original reason for greater exposure to free-swimming cercariae (Sweeting 1974) and not *vice versa*. It is therefore difficult to distinguish between the two hypotheses of diplostomatid infection, namely firstly that odd behaviour results in an increase in cercarial exposure or secondly, that cercarial exposure results in an increase in peculiar behaviour.

Free-moving metacercariae in the humours and retina

In a study by Bouillon and Curtis (1987) great numbers of diplostomatids (1 478 per eye) were noted within a site linked to the retina, namely the epichorioidal lymph space (Chapter 7). These flukes originally occurred within the humours of the eye and gradually migrated to the retina. The infected fish hosts did not exhibit any altered behaviour. It is argued that the distribution of the majority of the metacercariae (99%) in the epichorioidal lymph space permitted the free passing of light onto the retinal surface layer. The remainder (1%) of the diplostomula which were recorded from the eye humours were too low in numbers to have a noteworthy blocking of light or pathological effect on the eye and the host's vision (Bouillon and Curtis 1987).

Free-moving diplostomatids were also observed from the eye humours of various fish species during the present study (Chapter 6). Similar to the study by Bouillon and Curtis (1987) the small numbers of flukes present within the humours most probably attributed to the fishes' vision to remain unaffected.

Retinal and choroid detachments

Previous reports on diplostomatids causing retinal detachments in fish include a study by Lester and Huizinga (1977). *Diplostomum adamsi* metacercariae were collected from the eyes of American yellow perch and were responsible for the destruction of rods and cones in localised parts of the fishes' retinas. Lester and Huizinga (1977), however, concluded that no noticeable change could be observed amongst the yellow perch. This was explained by their findings that only the retina cells adjacent to the metacercariae were influenced. The majority of metacercariae occurred only in the peripheral retinal cells and therefore the retinal detachments were localised and did not result in total blindness. As a result, peculiar behaviour was not noted amongst infected fish.

The effect which retinal as well as choroid detachments could have on the quality of a fish's vision may therefore be very small (Chapter 7). It would ultimately depend on the intensity of the infection and the extent to which the cysts block the blood flow and result in an increase in capillary pressure build-up. This localised detrimental effect

which diplostomatid cysts may have on the vision of the fish may explain the results of the behavioural studies conducted during the present study.

INDUCED BEHAVIOUR OF DIPLOSTOMATID INFECTED EYES

To determine the effect which diplostomatid eye infection has on the vision of fish hosts, two sets of behavioural experiments were conducted during the present study. During the first, the behavioural responses of infected and uninfected *Tilapia sparrmanii* were noted, whilst exposed to a predator model bird pulled overhead. The second experiment consisted of noting the behavioural responses of infected and uninfected *T. rendalli*, whilst exposed to different light flashes.

1) Aerial predator detection

The first set of behavioural experiments was inspired from a study originally conducted by Seppälä *et al.* (2004). In this study experimentally infected rainbow trout were exposed to black model plates, which represented predatory birds, drawn over the water surface. The escape responses were noted, focusing on changes in the vertical position of the fish in the water column. The study made use of *D. spathaceum* cercariae which, when established as metacercariae, caused cataract formation and blindness in the infected fish. It is therefore not surprising that infected *O. mykiss* did show a decrease in the intensity of their escape response behaviour when the artificial predator was pulled overhead. The reduced vision of infected fish was also demonstrated by a follow-up experiment by Seppälä *et al.* (2005b), where it was found that the cataract formation reduced the ability of *O. mykiss* to notice a change in background colour and light intensity and therefore to adjust their skin colouration (crypsis). Impairment of their camouflage, left the infected individuals more conspicuous and susceptible to aerial predation. This has important implications for fish occurring in the littoral zones where the intensity of light may change rapidly due to the substrate composition and weather.

Present study: Results and discussion

The present study also made use of a bird model which was "flown" overhead, whilst the behavioural responses of infected and uninfected fish host were noted (see Chapter 3). A former study conducted by Lafferty and Morris (1996) suggested the use of multiple regression analysis (r^2) to compare the mean intensity behavioural responses of infected fish to that of uninfected fish. It was concluded that similar behavioural responses ($r^2 = 0.69$) were present amongst infected (from the Okavango) and uninfected *T. sparrmanii* specimens (from the Orange-Vaal) (Figure 8.1 A). This indicates that *T. sparrmanii* individuals infected with diplostomatid cysts within their eyes were still able to detect the aerial predator model.

By making use of histopathology the presence of membrane (retinal or choroid) detachments were verified in these fish (Chapter 7). It was concluded that the diplostomatid cysts only caused localised retinal and / or choroid detachments and therefore had no notable effect on the aerial predator detection capability of the fish. Through general observation it was also noticed, that no change occurred in the crypsis of the infected fish.

2) Light flash detection

The second set of behavioural experiments conducted during the present study was also adapted from Seppälä *et al.* (2004). In their study the preference of fish regarding their vertical location in plastic tubes consisting of a light-dark gradient was determined. The bottom of each tube was covered with black gravel and each tube was illuminated from above with a 40-W lamp. The vertical location of infected and uninfected fish were recorded before and after a black plate (momentarily blocking the light incidence from above) was moved across the surface of the tubes. It was concluded that the change in light intensity was detected more readily by the uninfected fish but that it did not show any preference for a certain vertical position within the light-dark gradient tubes.

Present study: Results and discussion

During the present study, this change in light intensity was modified by making use of light flashes from an electrical light source. Infected and uninfected *T. rendalli* were exposed to three light flashes, each of which yielded a greater intensity (see Chapter 3).

It was concluded that with an increase in light flash intensity (20%, 60% to 100%) there was also an increase in the mean intensity of the behavioural responses of the infected and uninfected *T. rendalli* (Figure 8.1 B). This suggests that both infected and uninfected red breasted tilapia were able to observe and respond to increasing light flash intensities. Multiple regression analysis (r^2) was again used to compare the mean intensity of the overall change in an individual fish host's behaviour to that of the intensity of infection found within the eyes. It was concluded that no relationship exists between the number of diplostomatids in the eye and the intensity of the fish's behavioural response, even when the fish were exposed to different light flash intensities (Figure 8.2).

Analyses of these observations conclude that fish infected with diplostomatid eye cysts are still able to detect light flashes and that they are also able to distinguish between different intensities. It is hypothesised that the diplostomatid cysts present within the eyes of *T. rendalli* only caused localised retinal and / or choroid detachments (Chapter 7). Infected fish would therefore still be able to detect light flashes as well as changes in its intensity.

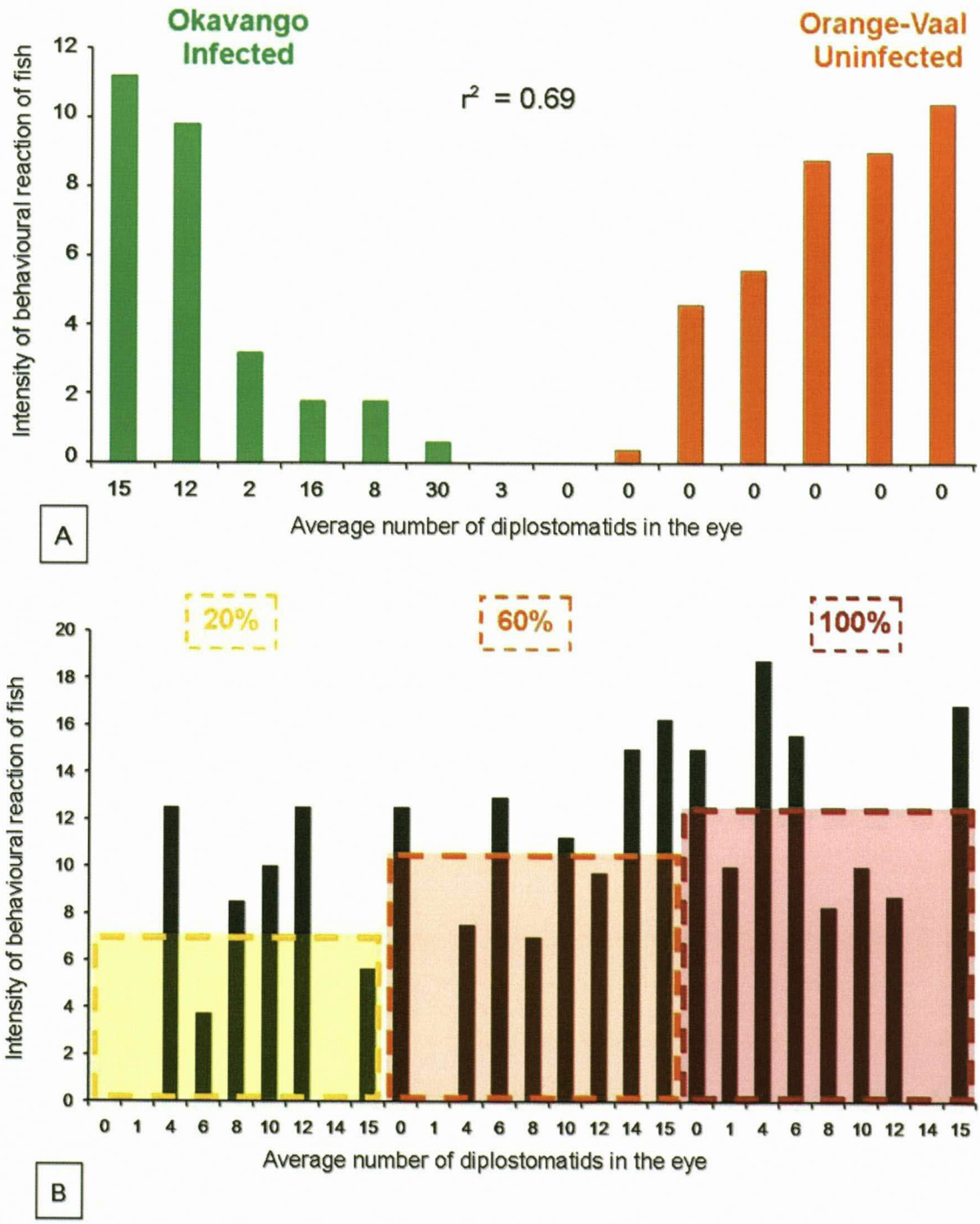


Figure 8.1: (**A**) Mean intensity of the behavioural reactions, of *Tilapia sparrmanii* A. Smith, 1840, infected with different numbers of eye diplostomatids (Okavango) as well as uninfected individuals (Orange-Vaal), when exposed to an aerial predator model. (**B**) Intensity of the behavioural reactions of *Tilapia rendalli* (Boulenger, 1896), infected with different numbers of eye diplostomatids, when exposed to different light flashes of 20%, 60% and 100%. Coloured histograms indicate the increase in mean intensity of behavioural reaction during the three increasing light flash intensities.

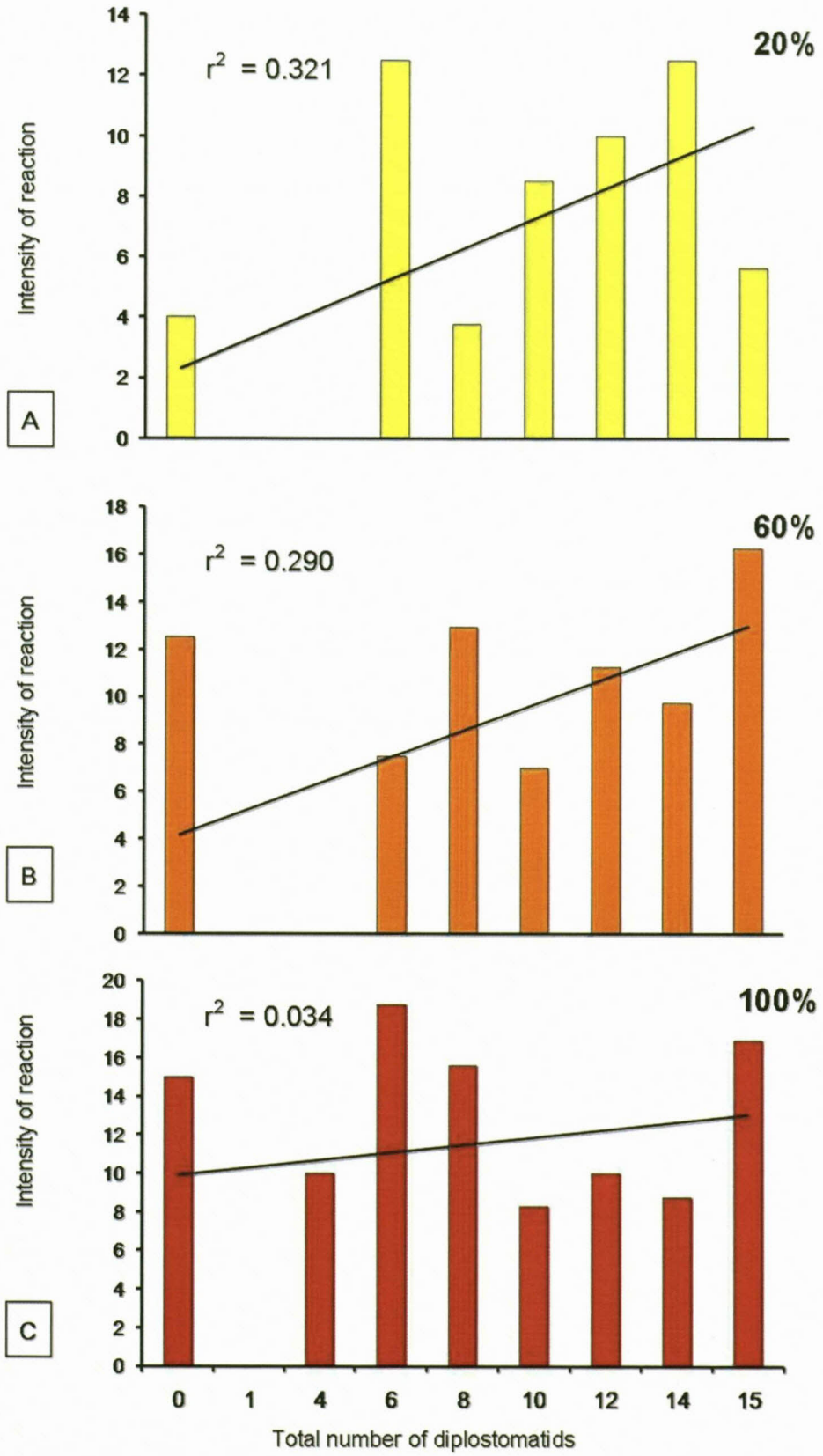


Figure 8.2: Correlation between the intensity of reaction of infected *Tilapia rendalli* (Boulenger, 1896) and that of the intensity of its diplostomatid eye infection at (**A**) 20%, (**B**) 60% and (**C**) 100% light intensities.

AFRICAN INFECTION - A CONTROVERSIAL HYPOTHESIS

As mentioned, no diplostomatid metacercariae were observed to parasitise the lenses of fish during the present study. The absence of flukes within the lenses will be very peculiar, especially when compared to the numerous studies conducted on lens-inhabiting metacercariae in other countries (Rees 1955; Crowden 1976; Niewiadomska 1996; Seppälä *et al.* 2004, 2005a, b). A controversial hypothesis to explain the probable absence of lens inhabiting metacercariae may be found in the geological history of Africa.

Szidat (1969) stated that the more evolutionary developed a parasite species is, the more able it will be to infect and survive in immunological privileged sites within the host. This is possible because the parasite had a longer evolutionary time to co-develop with its host species. Through time and natural selection this could have led to diplostomatids obtaining access to sites with the least pathological responses, such as the lens. The site shows a marked difference in immunology when exposed to foreign material, especially when compared to reactions in the rest of the body. This is due to the absence of blood vessels and the lens being impermeable to leucocytes. Szidat (1969) therefore stated that trematodes, mostly found in the brain and humours of the eye (and not the lens), from fish in South America are species which had a shorter evolutionary development than their counterparts from Europe and North America. These latter continents predominantly yield exclusive trematode-lens infections (Niewiadomska 1996).

The same hypothesis may also be applied for diplostomatids found in the Okavango and Orange-Vaal River Systems, as well as the rest of Africa. Together with other landmasses, such as South America and India, it once formed the supercontinent Gondwana, whilst continents such as Europe and North America were clubbed together in Laurasia. The hypothesis of Szidat (1969) fits well with the results obtained during the present study, in which no metacercariae were found within the eye lens and infection only occurred within the brain and eye humours. Could the absence of metacercariae from this immunologically privileged site be attributed to African diplostomatids sharing a shorter period of co-evolutionary development with their hosts?

If a preference for the lens is truly a characteristic of evolutionary-developed diplostomatid infection, it can be speculated that the original selective pressure for site selection may be protection against immunological activity. Other selective benefits, such as increased predator susceptibility, may therefore have been secondarily derived. Due to its adaptive value it is, however, still favoured by natural selection (Seppälä *et al.* 2004; Locke *et al.* 2010b). Another possible advantage is protection of metacercariae, lodged within the lens material, against the digestive secretions of the intestine of the final host. The chemical composition of the lens ensures that gastric secretions in the intestine do not attack the lens material and hence the metacercariae are also protected (Szidat 1969).

Since protection against digestion is considered to be an important component of successful establishment of diplostomatid infection within bird hosts, it may explain the encysted flukes found within the eye capillaries. The present study could not conclude whether the development of the cyst layers were due to host immunology or parasite secretion (Hoffman and Putz 1965) but the possibility of both playing part is very probable.

It is therefore concluded that, in the present study, diplostomatid infection occurred in sites with decreased immunological responses, such as the brains and eyes. It is also speculated that diplostomatid species, with longer co-evolutionary relationships with their fish hosts, will specifically seek out sites with the least immunological response, such as the lens.

Hence, it is concluded that the diplostomatid infection present in the Okavango and Orange-Vaal River Systems (and possibly in the rest of former Gondwana) shares a shorter evolutionary history with their fish hosts and it could contribute to the absence of induced fish host behaviour. It may, however, be a different scenario for diplostomatid types which have had a longer evolutionary time to co-develop with their fish hosts and parasitise inside of the eye lens, resulting in cataract formation.

INTENSITY OF INFECTION

Previous studies

Since cataract formation is an effect of the feeding and metabolic waste production of metacercariae, it can be linked to the number of larvae present within the humours and specifically the lens. It is therefore hypothesised that the intensity of cataract formation has a positive correlation with the intensity of metacercarial infection and can ultimately result in a positive correlation with altered behaviour (Owen *et al.* 1993; Poulin 1994b). Some studies support this positive correlation between parasite intensity and the extent of parasite alteration of host behaviour (Lafferty and Morris 1996). Crowden and Broom (1980) found that the number of *D. spathaceum* eye flukes was positively correlated with a decrease in the feeding efficiency of infected dace, *Leuciscus leuciscus*. As a result of a decrease in their visual accuracy, heavily infected fish had to spend more energy and time to obtain food from the surface waters. The increase in infection intensity ultimately led to an increase in the time the fish were left exposed to aerial predation at the surface waters.

Seppälä (2005) stated that the low water depth of only 30 cm used in the study of Crowden and Broom (1980) created unnatural conditions. This could have been responsible for the increase in time spent near the water surface by the infected dace. Seppälä (2005) therefore conducted his own set of experiments, with a tank depth of two meters and concluded that infected rainbow trout did not prefer surface layers of the water column more than the controls. In a study by Karvonen and Seppälä (2008) the feeding efficiency of another fish species, European whitefish *Coregonus lavaretus* (Linnaeus, 1758) was also determined. This species was experimentally infected with *D. spathaceum* metacercariae and it was found that with an mean infection intensity of 46.5 flukes per fish, 100% cataract coverage occurred. Due to the associated decline in feeding efficiency, the mass of the infected fish decreased significantly. A positive correlation between the diplostomatid infection intensity and the feeding efficiency of the fish were therefore established. Karvonen *et al.* (2004b) suggested a minimum number of 20 *D. spathaceum* metacercariae per eye to cause cataracts to cover the entire eye and completely reduce the vision of rainbow trout. Davies *et al.* (1973) suggested that only four to five *D. spathaceum* metacercariae per lens were needed to

result in complete opaqueness of the eye of minnows and trout in the lakes in Colorado.

The link between diplostomatid infection intensity and altered behaviour is not straightforward, since the relationship between parasite intensity and the expression of altered host behaviour itself is poorly understood (Shirakashi and Goater 2002). It is possible that even heavily infected fish reared in aquaculture conditions may receive other cues from less-infected conspecifics in the group, which can aid in their general behaviour such as food location, feeding initiation or predator avoidance. The group as a whole can therefore initiate active movement towards the surface when food is introduced or to the bottom when predators fly overhead (Karvonen and Seppälä 2008). The size of the fish host may also have a profound influence on the number of parasites needed to cause an effect, since small fish, such as juveniles or smaller species, have small eyes and brains and therefore can host fewer diplostomatids in these small volumes (Seppälä 2005).

Present study

During the present study a variety of fish species from the Okavango and Orange-Vaal River Systems were found infected with a relatively high prevalence of diplostomatids (Table 6.2). The mean intensity was low and did not exceed 50 free-moving flukes per organ (Figure 8.3). The overall highest mean intensities were noted from the Okavango River System with *Marcusenius macrolepidotus* and *Brycinus lateralis*, infected with free-moving metacercariae inside their brains (Chapter 6). The mormyrid species, *Petrocephalus catostoma* and *Pollimyrus castelnaui*, were both infected with flukes in the eye humours. The highest intensity of eye flukes was recorded from *Labeo umbratus* from the Orange-Vaal River System (Figure 8.3). The author concludes that the numbers of free-moving metacercariae in the eye humours and brain (and not the lens) are too small to result in any obvious pathological changes.

Encysted metacercariae were collected from the eye capillaries of various Okavango cichlid fish species (Figure 8.3). The mean infection intensity observed from all the species is equal or less than 10 eye cysts per fish (Chapter 6). Since the extent of the

eye membrane detachment is dependent on the number of cysts creating obstructions to blood flow, this mean intensity is considered too low to create a huge eye membrane detachment. The author concludes that the induced retinal and choroid detachments are localised to certain parts of the fish eye and will therefore have no noticeable effect on the ability of infected fish to detect aerial predation (Figure 8.1 A) and changes in light flash intensities (Figures 8.1 B and 8.2).

CUL-DE-SAC PREDATORS

If increased predator susceptibility is a result of behavioural manipulation, it may be that infected hosts are also rendered more susceptible to unsuitable predators. Non-piscivorous predators are dead-ends and will not contribute to the completion of the parasite's life cycle (Mouritsen and Poulin 2003; Seppälä *et al.* 2004). Altogether more rigorous studies, in the laboratory and especially in the field, are needed to determine the precise mechanisms and conditions which facilitate parasite transmission to the definitive host.

In studies conducted by Seppälä, *et al.* (2004) and Seppälä (2005) it was noted that rainbow trout experimentally infected with cataract-causing *D. spathaceum* were more susceptible to aerial predation. This was, however, not the case when similar infected rainbow trout were exposed to Northern pike, *Esox lucius* Linnaeus, 1758 during a follow-up study by Seppälä *et al.* (2006a). The reason for the decrease in susceptibility of the rainbow trout to the piscivorous fish is unclear but Seppälä *et al.* (2006a) suggested that the behaviour of the pike was responsible. In contrast to fish-eating birds which actively search for their prey, pike and most predatory fish such as the cichlids, remain motionless until striking distance, where after they ambush their prey.

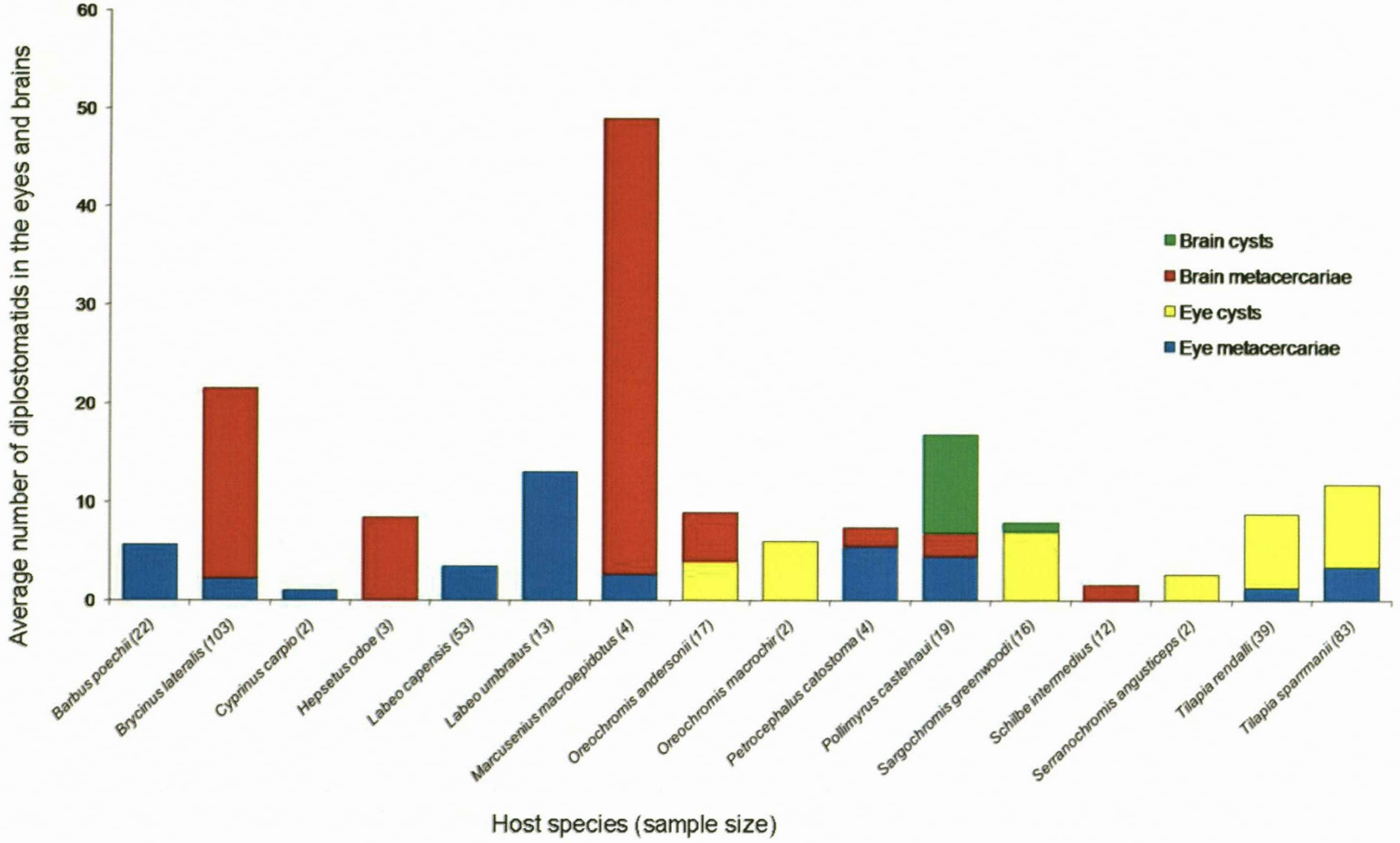


Figure 8.3: Different species of fish, collected from the Okavango and Orange-Vaal River Systems infected with diplostomatids. The average intensity of infection for free-moving and encysted diplostomatid metacercariae found within the eyes and brains varied amongst the different fish species. Numbers in brackets refer to the sample size of the fish species which were collected during the present study.

It is therefore possible that if the diplostomatid parasitic infection leads to an overall reduction in the activity and movement of its fish host, it could result in a lower contact probability with the ambush-type predator such as pike (Seppälä *et al.* 2006a). Infected fish then only have an increased exposure to active-hunting avian predation. Since fish infected in their eyes are still able to use their lateral line and sense of smell to detect under water predators, which are non-suitable diplostomatid hosts, it may also aid in their ability to escape under-water predation. With aerial predation only its vision can be utilised and if it is impaired through diplostomiasis it would lead to an increase in aerial avian predator susceptibility.

There are, however, examples of infected fish being equally susceptible to suitable as well as cul-de-sac host predation. In a study conducted by Brassard *et al.* (1982c) it was found that heavily experimentally-infected guppies *Lebistes reticulates* (Peters, 1859) remained immobile near the surface or on the bottom of the holding tank, which left them more susceptible to predation by brook trout, *Salvelinus fontinalis* (Mitchill, 1814). Many investigators, however, argue that the potential fitness-gains of diplostomiasis, namely increased susceptibility to the final piscivorous bird host, still outweigh the fitness-costs of susceptibility to non-suitable hosts. Ultimately it will be still worthwhile for certain metacercariae to pay the price and continue with the manipulation of the host's behaviour, even if some do end up in the wrong definitive host (Mouritsen and Poulin 2003; Poulin *et al.* 2005; Seppälä 2005; Seppälä *et al.* 2006a). This does not, however, mean that the cost is negligible and may as well be the major reason why host manipulation has not evolved in a larger number of parasitic species.

HUMAN INFECTION

Violating all contemporary ethical codes, some earlier researchers have conducted experiments to determine the possibility and prevalence of diplostomatids to infect humans. Ferguson (1943a) used a pipette to drip diplostomatid flukes into the eyes of hosts such as tadpoles, chicks, turtles, ducks, mice, rats, rabbits and guinea pigs. He

determined that a smaller number of flukes were needed in warm-blooded animals than in fish to cause similar severe ocular damage and blindness.

The few reports of alleged trematode larvae found within the eyes of humans are sketchy and not very convincing but still prompts interest and the need for investigation. The first report was in 1833 when during the post-mortem examination of a five-month old child, live "Distoma" was found in the lens (Niewiadomska 1963a). Opaque spots, associated with the flukes, were observed whilst the child was still alive. Another report was given in 1907 when two trematode larvae were noted in the cortex of the cataractous lens of a 55 year old fisherman.

In a study by Lester and Freeman (1976) the ability of the lens-inhabiting *D. spathaceum* and the retina-inhabiting *D. adamsi* diplostomatids to penetrate and successfully lodge inside the eyes of small and large mammals, were tested. The cercariae of the two diplostomatid species were directly dripped into the eyes of small and large, unanaesthetised rabbits and cold-stored, enucleated human eyes by using a pipette. It was observed that the cercariae of *D. adamsi* were not able to penetrate or develop in the eyes of any of the mammals. The success of the establishment of *D. spathaceum* metacercariae, however, varied according to the thickness of the cornea. With the thinner cornea, present in the small rabbits, some *D. spathaceum* diplostomula were able to penetrate through it and crossed the anterior chamber and entered the lens. After two to three weeks, the metacercariae died and became semi-permanent amorphous cataracts. The cercariae that were not able to migrate through the cornea remained within it and formed stromal nebulae, which were visible for at least three months.

The cornea of the larger rabbits and humans proved to be too thick for successful migration for any of the diplostomula and none reached the lens. It was, however, reported that occasionally the diplostomatid exposure did result in temporary inflammation of the conjunctiva and more persistent stromal nebulae (Lester and Freeman 1976). Since diplostomatid infection is known to occur throughout the entire body surface and then migrate via the bloodstream to the humours of the eye, cercariae were also injected directly into the aqueous humours of the large rabbit and human eyes. Successful establishment within the lenses and retina were not achieved.

In any regard it is also possible that the skin epidermis of mammals is too thick to facilitate the penetration of diplostomatid cercariae.

It is therefore concluded that the thick cornea, skin epidermis and possible ocular secretions may inhibit the incidence of diplostomatid infection in humans and other large mammals (Ferguson 1943a). The possibility of superficial pathological changes to occur does, however, exist. An example of this is known as swimmer's itch which has mostly been described from people, after swimming in aquatic environments, experiencing a rash on their skin (Lester and Freeman 1976).

CONCLUDING REMARKS

No marked altered pathology was observed in the brains of infected fish species during the present study. Detachments were prominent in the eyes of fish infected with diplostomatid cysts. The two sets of behavioural experiments were conducted on uninfected fish and individuals infected with eye cysts. The null hypothesis (H_0) stated that infected fish, from natural populations, do not show dissimilar behaviour from their uninfected conspecifics, whilst the alternative hypothesis (H_1) stated that diplostomatid infection within a natural population, results in changes in behaviour. The results from the present study therefore reject the alternative hypothesis (H_1) and confirm the null hypothesis (H_0) and therefore the author concludes that diplostomatids do not cause induced behaviour in natural populations of fish hosts. This natural over dispersion of fish parasites also confirms the pristine status and good water condition of the Okavango at present.

Chapter 9

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REFERENCES

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Dr Johan Steyl: For his advice regarding the histopathology of fish eyes and brains.

ABSTRACT

Numerous studies have been conducted on the hypothesis of parasites influencing the behaviour of their hosts. Many authors have been bias in trying to prove that this is a form of manipulation and that parasites increase their transmission success by changing host behaviour. Not much research has been conducted in Africa regarding parasite induced behaviour. During the present study fish were collected from the Okavango and Orange-Vaal River Systems, respectively situated in Botswana and South Africa. Within these ecologically diverse systems a wide variety of fish were found to be infected with diplostomatid metacercariae occurring inside their eyes and / or brains. These digenetic trematodes are known to cause blindness and other pathological effects, known as diplostomiasis, especially in fish reared in aquaculture. Diplostomatids have a three-host life cycle which involves different species of snails, fishes and piscivorous birds. Fish act as the second intermediate hosts and need to be eaten by a piscivorous bird in order for the diplostomatids to be trophically transmitted and the life cycle to be completed. Seven different metacercarial types and cysts were identified and some were collected from both the eyes and brains of infected fish species. The results signified a moderate prevalence of these parasites amongst the different fish species, whilst the intensity of infection proved to be low. No marked altered pathology was observed in infected fish brains but noticeable histopathological changes were noted within eyes infected with diplostomatid cysts. To determine the effect these infections have on the behaviour of natural populations of fish, two behavioural experiments were conducted. The results indicate that infected fish show similar behaviour than uninfected fish and therefore the null hypothesis (H_0) is accepted.

Keywords: Okavango River System, Orange-Vaal River System, diplostomatid, metacercariae, life cycle, pathology, fish behaviour

OPSOMMING

Verskeie studies rakende die hipotese dat parasiete die gedrag van gashere kan beïnvloed is uitgevoer. Baie van die outeurs was bevooroordeeld deur te probeer bewys dat dit 'n vorm van manipulasie is en dat parasiete die sukses van hul oordrag verhoog deur die gedrag van die gasheer te verander. Min navorsing is al in Afrika ten opsigte van parasiet geïnduseerde gedrag gedoen. Visse is gedurende die huidige studie vanuit die Okavango en Oranje-Vaal Rivierstelsels, onderskeidelik geleë in Botswana en Suid-Afrika, versamel. Diplostomatid metaserkarieë is in die oë en / of breine van 'n wye verskeidenheid van visse vanaf hierdie ekologies uiteenlopende stelsels, gevind. Hierdie digenetiese metaserkarieë is bekend daarvoor om blindheid, asook ander patologiese effekte, in veral vis wat in akwakultuur aangehou word, te veroorsaak en staan as diplostomiase bekend. Drie gashere vorm deel van die diplostomatid lewenssiklus en sluit verskillende slak-, vis- en visvretende voëlspesies in. Visse, wat die tweede tussengashere is, moet deur visvretende voëls geëet word om te verseker dat die diplostomatid metaserkarieë na die volgende trofiese vlak oorgedra word en sodoende die lewenssiklus voltooi. Sewe verskillende tipes metaserkarieë en siste is geïdentifiseer en sommiges is vanuit beide die oë en breine van geïnfekteerde visse versamel. Die resultate dui aan dat hierdie parasiete 'n matige voorkoms in die verskillende visspesies het, terwyl die intensiteit van die besmetting laag is. Geen merkwaardige patologiese veranderinge is in die breine van geïnfekteerde visse gevind nie, maar opsigtelike histopatologiese veranderinge was wel in die oë wat met verteenwoordigers van diplostomatid siste geïnfekteerd was, opgemerk. Twee gedragseksperimente, om die effek van hierdie besmettings op die gedrag van natuurlike visbevolkings te bepaal, is uitgevoer. Die resultate dui aan dat geïnfekteerde visse dieselfde gedrag as ongeïnfekteerde visse toon en dus word die nul hipotese (H_0) aanvaar.

Sleutelwoorde: Okavango Rivierstelsel, Oranje-Vaal Rivierstelsel, diplostomatid, metaserkarieë, lewenssiklus, patologie, visgedrag

APPENDIX I

The procedure used for the staining and mounting of small trematodes was adapted (with permission) from the method used by [1]Professor Robin Overstreet

a. Pipette trematode specimens into 70% ethyl alcohol in a small shell vial ½ full.

b. Add a few drops of distilled water to the vial whilst twirling it with the other hand. Specimens covered with debris can be cleaned by using the pipette and squirting part of the solution over the specimens.

c. To ultimately completely replace the alcohol with distilled water first bring the level of the fluid up to 4 / 5 full with 3 - 4 groups of 3 - 10 drops each of distilled water. Since diplostomatid specimens are small and have thin teguments, the time period between adding drops can be as short as 2 - 3 minutes.

d. Under view of a dissecting microscope, when the vial is about 4 / 5 full (about 45% alcohol), remove enough fluid so that that level is again about ½. Continue to slowly bring the level to 4 / 5 and then remove some fluid until only water is present. For the small diplostomatid specimens the entire hydration process should take about 1 - 1 ½ hours.

e. Remove all the water except enough to cover the specimens when the vial is held at an angle and then add some filtered Van Cleave's hematoxylin. Use less than one pipette full of stain so that the level of the stain is about 1/6th. To produce a slightly darker stain 1 - 2 drops of Ehrlich's hematoxylin must be added to the 3 - 4 ml of Van Cleave's stain.

f. Cover the shell vial with a 5 ml plastic beaker to prevent evaporation, tipping of the vial, and spilling. Since diplostomatids are small in size, it is the best to let the specimens remain in the stain overnight.

[1] Robin Overstreet, Gulf Research Laboratory, Department of Coastal Sciences, The University of Southern Mississippi

Formula for [2]Ehrlich's hematoxylin		Formula for Van Cleave's hematoxylin	
Haematoxylin	2 gm	Delafield's haematoxylin	1 ml
Ethyl alcohol (95%)	100 ml	Ehrilch's haematoxylin	1 ml
Glycerine	100 ml	Aluminum ammonium sulphate	6.5 gm
Distilled water	100 ml	Distilled water	100 ml
Glacial acetic acid	10 ml		
Potassium alum ($K_2SO_4Al_2(SO_4)_324H_2O$)	3 gm		

g. The following day, remove the stain and replace with 1 - 2 exchanges of water so that the level of clear water is about ¾ full. Add 1 - 3 drops of 40% alcohol for 1 - 2 minutes, where after the excess fluid should be removed. This step should be repeated every 10 minutes for 45 minutes. Thereafter the alcohol level must be increased to 50 % by again dropping 1 - 3 drops of 50% alcohol, removing the excess every 10 minutes for 45 minutes. This step must also be repeated with 60 %, 70 % and 80% alcohol and for each it should take 45 minutes or more.

h. When the specimens are in 80% alcohol, 3 - 6 drops of lithium carbonate must be added and the specimens should remain in the solution for about 5 minutes. Then add two or more drops of 0.1M butylamine so that the pH is above 7.0. Leave the specimens in the solution for 10 or more minutes. If when adding any of the above bases you should notice a white precipitate, remove the solution quickly and force the precipitate off with squirts of 70% alcohol or 35% alcohol. The carbonate is not very soluble in alcohol but it is in water. Adding the bases should prevent future staining after the mounts are several years old.

i. Take the specimens up to 80 and 85% alcohol and then replace with 95% alcohol. Never allow the specimens to come in direct contact with air. They should always be covered with, at least, a small amount of solution. Replace the 95% with 100% and replace the 100% with a second ½ vial of 100% alcohol. Specimens should remain in each absolute treatment for 5 minutes.

[2] Allow to ripen for several weeks or artificially ripen with addition of 0.2 gm sodium iodate.

j. As quick as possible, to avoid taking up atmospheric water, swirl the solution in the vial so that the specimens are free in the fluid. Transfer the fluid and specimens to a small stender dish.

k. Remove with pipette all the alcohol from the tipped dish except a minimal amount covering the worms. Quickly pour 1/5 to ¼ dish of Xylene and keep the worms submerged with a 000 brush.

l. After the worms remained submerged by themselves and they are cleared, they are nearly ready to mount. They should be cleaned by brushing any detritus or air bubbles off with a 000 brush. A minute pin on a wooden applicator can also be helpful.

m. Extract the specimens from the Xylene solution and carefully place them on a clean and dry microscope slide by means of two (000) brushes.

n. Remove any excess Xylene from the slide, surrounding the specimens, by wiping it with tissues. Be sure to work swiftly, to ensure that the specimens do not dry out.

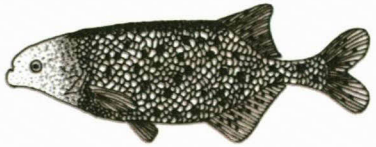
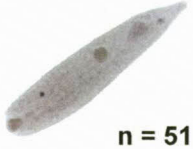
o. Carefully pipette one drop of Eukitt solution next to the specimens, onto the slide, and by making use of a pair of forceps and a dissecting pin, delicately place a coverslip onto the slide, whilst ensuring that no air bubbles are formed.

p. The Eukitt solution should cover the whole of the coverslip, with the specimens then mounted within the solution.

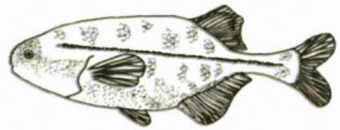
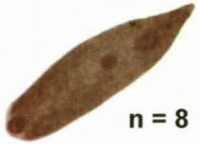
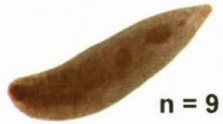
q. Store the slide horizontally for about two days, where after it may be studied with the aid of a microscope.

APPENDIX II

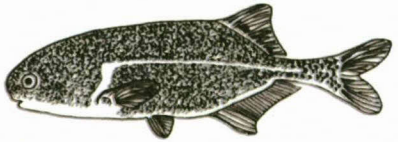
Range (**average** ± standard deviation) of different morphological measurements (mm) (1-18) and biometric indices (19-28) of *Diplostomum* von Nordmann, 1832 metacercariae collected from the eyes or brains of specific fish species from the Okavango and Orange-Vaal River Systems. The sample size (n) indicates the number of specimens which were measured to obtain the morphometrics. Features that were not measured are indicated with **-**.

Fish species	 ***Marcusenius macrolepidotus***		
Metacercarial type	***Diplostomum* type 1 Brain**	***Diplostomum* type 2 Brain**	***Diplostomum* type 2 Eye**
Image and sample size	 **n = 5**	 **n = 51**	 **n = 10**
1. Body length (BL)	0.680 - 1.023 (**0.847** ± 0.135)	0.201 - 0.749 (**0.505** ± 0.099)	0.300 - 0.627 (**0.430** ± 0.106)
2. Body width (BW)	0.730 - 1.153 (**0.931** ± 0.177)	0.067 - 0.212 (**0.118** ± 0.027)	0.08 - 0.175 (**0.112** ± 0.029)
3. Forebody length (FbL)	0.558 - 1.000 (**0.749** ± 0.184)	-	-
4. Forebody width (FbW)	0.726 - 1.156 (**0.928** ± 0.181)	-	-
5. Hindbody length (HbI)	0.017 - 0.218 (**0.118** ± 0.071)	-	-
6. Hindbody width (HbW)	0.182 - 0.368 (**0.287** ± 0.071)	-	-
7. Pharynx length (PhL)	-	0.01 - 0.045 (**0.024** ± 0.008)	**0.017**
8. Pharynx width (PhW)	-	0.006 - 0.049 (**0.014** ± 0.01)	**0.012**
9. Oral sucker length (OsL)	0.089 - 0.12 (**0.107** ± 0.011)	0.026 - 0.066 (**0.04** ± 0.007)	0.021 - 0.048 (**0.037** ± 0.009)
10. Oral sucker width (OsW)	0.103 - 0.139 (**0.117** ± 0.015)	0.02 - 0.055 (**0.032** ± 0.007)	0.023 - 0.045 (**0.033** ± 0.008)

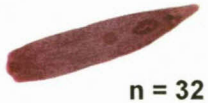
Fish species	*Marcusenius macrolepidotus*		
Metacercarial type	***Diplostomum* type 1 Brain**	***Diplostomum* type 2 Brain**	***Diplostomum*type 2 Eye**
11. Oral to ventral sucker distance (OV)	0.116 - 0.299 (**0.213** ± 0.066)	0.145 - 0.408 (**0.228** ± 0.053)	0.06 - 0.24 (**0.179** ± 0.063)
12. Ventral sucker length (VsL)	0.100 - 0.162 (**0.139** ± 0.025)	0.025 - 0.045 (**0.037** ± 0.004)	0.029 - 0.041 (**0.035** ± 0.004)
13. Ventral sucker width (VsW)	0.122 - 0.167 (**0.143** ± 0.02)	0.023 - 0.05 (**0.035** ± 0.006)	0.025 - 0.044 (**0.035** ± 0.006)
14. Ventral sucker anterior to anterior body end distance (AV)	0.339 - 0.452 (**0.393** ± 0.05)	0.186 – 0.470 (**0.271** ± 0.053)	0.108 - 0.285 (**0.217** ± 0.065)
15. Mid-ventral sucker to anterior body end distance (O)	0.396 - 0.533 (**0.454** ± 0.052)	0.203 – 0.495 (**0.288** ± 0.053)	0.126 - 0.301 (**0.234** ± 0.066)
16. Holdfast organ length (BoL)	0.074 - 0.159 (**0.124** ± 0.038)	0.044 – 0.097 (**0.062** ± 0.01)	0.043 - 0.064 (**0.052** ± 0.066)
17. Holdfast organ width (BoW)	0.183 - 0.331 (**0.239** ± 0.056)	0.026 – 0.072 (**0.039** ± 0.011)	0.025 - 0.048 (**0.038** ± 0.009)
18. Ventral sucker to Holdfast organ distance (VB)	0.003 - 0.023 (**0.011** ± 0.008)	0.026 – 0.126 (**0.059** ± 0.02)	0.017 - 0.109 (**0.058** ± 0.03)
19. b to a of body (BW:BL)(%)	85.621 - 120.065 (**110.269** ± 14.043)	10.885 - 42.276 (**23.949** ± 6.516)	14.594 - 53.16 (**27.665** ± 10.544)
20. Body (ab) / Bo (ab)	11.826 - 39.734 (**29.882** ± 10.622)	16.267 - 54.043 (**28.357** ± 7.57)	13.747 - 41.376 (**26.262** ± 9.993)
21. Body (ab) / Vs (ab)	23.664 - 63.027 (**42.568** ± 17.335)	28.172 - 94.744 (**51.112** ± 13.963)	24.469 - 65.036 (**41.716** ±12.339)
22. Os (ab) / Vs (ab)	0.483 - 0.942 (**0.657** ± 0.189)	0.428 - 1.712 (**1.005** ± 0.249)	0.658 - 1.667 (**1.002** ± 0.307)
23. BO (ab) / Vs (ab)	0.685 - 2.183 (**1.535** ± 0.583)	1.045 - 4.539 (**1.889** ± 0.647)	0.855 - 2.677 (**1.725** ± 0.564)
24. Os (ab) / Ph (ab)	-	1.549 - 13.504 (**5.049** ± 3.026)	**4.212**
25. O (ab) / BL (ab)	49.383 - 60.034 (**54.059** ± 4.766)	50.757 - 69.538 (**55.39** ± 3.776)	40.924 - 57.728 (**51.613** ± 6.46)
26. FbL (ab) / HbL (ab)	2.943 - 59.404 (**16.098** ± 24.278)	-	-
27. FbW (ab) / BL (ab)	0.851 - 1.187 (**1.098** ± 0.14)	-	-
28. Fb (ab) / Hb (ab) (%)	6.59 - 376.536 (**87.623** ± 161.645)	-	-

Fish species	 *Pollimyrus castenaui*				
Metacercarial type	***Diplostomum* type 2 Eye**	***Diplostomum* type 2 Brain**	***Diplostomum* type 3 Brain**	***Diplostomum* type d Eye**	***Diplostomum* type d Brain**
Image and sample size	 **n = 8**	 **n = 9**	 **n = 9**	 **n = 1**	 **n = 1**
1. Body length (BL)	0.445 - 0.738 (**0.576** ± 0.113)	0.301 - 0.57 (**0.46** ± 0.089)	0.588 - 1.169 (**0.879** ± 0.194)	**0.538**	**0.717**
2. Body width (BW)	0.063 - 0.157 (**0.114** ± 0.028)	0.059 - 0.166 (**0.119** ± 0.038)	0.512 - 1.177 (**0.824** ± 0.228)	**0.258**	**0.293**
3. Forebody length (FbL)	-	-	**0.464**	**0.498**	**0.598**
4. Forebody width (FbW)	-	-	**0.556**	**0.258**	**0.294**
5. Hindbody length (Hbl)	-	-	**0.156**	**0.049**	**0.112**
6. Hindbody width (HbW)	-	-	**0.212**	**0.082**	**0.128**
7. Pharynx length (PhL)	0.013 - 0.023 (**0.018** ± 0.007)	0.021 - 0.024 (**0.023** ± 0.002)	-	**0.034**	-
8. Pharynx width (PhW)	0.009 - 0.01 (**0.009** ± 0.0007)	0.011 - 0.017 (**0.014** ± 0.004)	-	**0.021**	-
9. Oral sucker length (OsL)	0.036 - 0.043 (**0.04** ± 0.004)	0.032 - 0.048 (**0.042** ± 0.006)	-	**0.041**	**0.05**
10. Oral sucker width (OsW)	0.028 – 0.043 (**0.032** ± 0.006)	0.021 - 0.039 (**0.032** ± 0.006)	-	**0.034**	**0.039**
11. Oral to ventral sucker distance (OV)	0.184 – 0.243 (**0.211** ± 0.027)	0.187 - 0.232 (**0.201** ± 0.015)	-	-	-
12. Ventral sucker length (VsL)	0.032 – 0.041 (**0.039** ± 0.008)	0.03 - 0.046 (**0.039** ± 0.006)	**0.088**	-	-
13. Ventral sucker width (VsW)	0.031 - 0.041 (**0.035** ± 0.004)	0.018 - 0.048 (**0.036** ± 0.010)	**0.095**	-	-
14. Ventral sucker anterior to anterior body end distance (AV)	0.226 - 0.282 (**0.25** ± 0.024)	0.160 - 0.291 (**0.23** ± 0.039)	**0.134**	-	-
15. Mid-ventral sucker to anterior body end distance (O)	0.243 - 0.293 (**0.27** ± 0.026)	0.173 - 0.318 (**0.25** ± 0.044)	**0.181**	-	-

Fish species	*Pollimyrus castenaui*				
Metacercarial type	***Diplostomum* type 2 Eye**	***Diplostomum* type 2 Brain**	***Diplostomum* type 3 Brain**	***Diplostomum* type d Eye**	***Diplostomum* type d Brain**
16. Holdfast organ length (BoL)	0.041 - 0.067 (**0.057** ± 0.009)	0.036 - 0.076 (**0.059** ± 0.011)	**0.198**	**0.103**	**0.141**
17. Holdfast organ width (BoW)	0.029 - 0.055 (**0.04** ± 0.01)	0.025 - 0.045 (**0.033** ± 0.007)	**0.182**	**0.063**	**0.106**
18. Ventral sucker to Holdfast organ distance (VB)	0.035 - 0.099 (**0.058** ± 0.023)	0.029 - 0.068 (**0.045** ± 0.013)	**0.024**	-	-
19. b to a of body (BW:BL)(%)	12.761 - 29.475 (**20.329** ± 6.321)	10.759 - 64.4 (**25.465** ± 5.154)	62.667 - 110.603 (**93.612** ± 13.664)	**47.961**	**40.817**
20. Body (ab) / Bo (ab)	16.233 - 37.973 (**28.701** ± 7.533)	14.763 - 41.238 (**28.905** ± 9.507)	**8.852**	**21.346**	**14.067**
21. Body (ab) / Vs (ab)	28.749 - 57.232 (**45.105** ± 10.259)	16.15 - 55.603 (**41.441** ± 16.967)	**37.934**	-	-
22. Os (ab) / Vs (ab)	0.701 - 1.234 (**0.912** ± 0.234)	0.625 - 1.559 (**0.948** ± 0.295)	-	-	-
23. BO (ab) / Vs (ab)	1.009 - 1.771 (**1.567** ± 0.284)	0.922 - 2.264 (**1.46** ± 0.444)	**4.285**	-	-
24. Os (ab) / Ph (ab)	4.446 - 9.888 (**7.167** ± 3.848)	3.607 - 5.709 (**4.658** ± 1.486)	-	**1.981**	-
25. O (ab) / BL (ab)	48.466 - 54.641 (**51.87** ± 2.041)	49.784 - 57.638 (**54.576** ± 2.594)	**30.759**	-	-
26. FbL (ab) / HbL (ab)	-	-	**2.972**	**10.226**	**5.332**
27. FbW (ab) / BL (ab)	-	-	**0.94 5**	**0.478**	**0.411**
28. Fb (ab) / Hb (ab) (%)	-	-	**7.794**	**32.004**	**12.223**

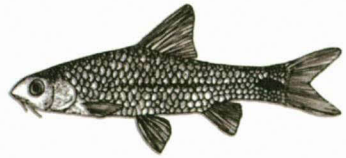
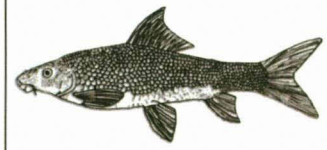
Fish species	 *Petrocephalus catostoma*			
Metacercarial type	***Diplostomum* type 1 Brain**	***Diplostomum* type 2 Brain**	***Diplostomum* type 2 Eye**	***Diplostomum* type 3 Brain**
Image and sample size	 **n = 7**	 **n = 4**	 **n = 1**	 **n = 10**
1. Body length (BL)	0.667 - 1.141 (**0.903** ± 0.176)	0.423 - 0.626 (**0.488** ± 0.093)	**0.691**	0.557 - 1.290 (**0.999** ± 0.246)
2. Body width (BW)	0.604 - 1.111 (**0.806** ± 0.155)	0.116 - 0.151 (**0.135** ± 0.015)	**0.167**	0.329 - 0.933 (**0.771** ± 0.171)
3. Forebody length (FbL)	0.515 - 0.930 (**0.721** ± 0.139)	-	-	0.468 - 1.039 (**0.805** ± 0.201)
4. Forebody width (FbW)	0.606 - 1.104 (**0.804** ± 0.15)	-	-	0.317 - 0.934 (**0.772** ± 0.176)
5. Hindbody length (Hbl)	0.14 - 0.29 (**0.182** ± 0.054)	-	-	0.106 - 0.257 (**0.202** ± 0.045)
6. Hindbody width (HbW)	0.263 - 0.39 (**0.336** ± 0.047)	-	-	0.115 - 0.377 (**0.275** ± 0.074)
7. Pharynx length (PhL)	**0.049**	0.018 - 0.023 (**0.021** ± 0.003)	-	0.053 - 0.092 (**0.073** ± 0.016)
8. Pharynx width (PhW)	**0.054**	0.007 - 0.014 (**0.01** ± 0.005)	-	0.022 - 0.037 (**0.031** ± 0.007)
9. Oral sucker length (OsL)	0.093 - 0.155 (**0.116** ± 0.023)	0.037 - 0.048 (**0.043** ± 0.006)	-	0.086 - 0.238 (**0.124** ± 0.053)
10. Oral sucker width (OsW)	0.108 - 0.15 (**0.264** ± 0.098)	0.027 - 0.033 (**0.031** ± 0.003)	-	0.088 - 0.210 (**0.111** ± 0.044)
11. Oral to ventral sucker distance (OV)	0.2 - 0.407 (**0.264** ± 0.098)	0.2 - 0.288 (**0.235** ± 0.046)	-	0.332 - 0.556 (**0.463** ± 0.087)
12. Ventral sucker length (VsL)	0.1 - 0.154 (**0.13** ± 0028)	0.033 - 0.044 (**0.038** ± 0.006)	-	0.112 - 0.201 (**0.136** ± 0.087)
13. Ventral sucker width (VsW)	0.103 - 0.157 (**0.145** ± 0.024)	0.026 - 0.054 (**0.039** ± 0.014)	-	0.099 - 0.175 (**0.144** ± 0.028)
14. Ventral sucker anterior to anterior body end distance (AV)	0.321 - 0.616 (**0.445** ± 0.118)	0.245 - 0.338 (**0.281** ± 0.05)	-	0.154 - 0.659 (**0.509** ± 0.175)
15. Mid-ventral sucker to anterior body end distance (O)	0.388 - 0.695 (**0.511** ± 0.127)	0.265 - 0.355 (**0.298** ± 0.049)	-	0.205 - 0.718 (**0.576** ± 0.178)
16. Holdfast organ length (BoL)	0.098 - 0.227 (**0.153** ± 0.043)	0.057 - 0.071 (**0.064** ± 0.007)	-	0.1 - 0.235 (**0.185** ± 0.059)
17. Holdfast organ width (BoW)	0.222 - 0.354 (**0.297** ± 0.059)	0.027 - 0.054 (**0.039** ± 0.013)	-	0.210 - 0.460 (**0.299** ± 0.074)
18. Ventral sucker to Holdfast organ distance (VB)	0.003 - 0.034 (**0.017** ± 0.013)	0.034 - 0.089 (**0.059** ± 0.028)	-	0.004 - 0.048 (**0.016** ± 0.017)

Fish species	*Petrocephalus catostoma*			
Metacercarial type	***Diplostomum* type 1 Brain**	***Diplostomum* type 2 Brain**	***Diplostomum* type 2 Eye**	***Diplostomum* type 3 Brain**
19. b to a of body (BW:BL)(%)	74.185 - 98.754 (**89.703** ± 8.128)	18.504 - 33.446 (**28.544** ± 6.792)	**24.162**	59.135 - 109.473 (**78.215** ± 14.012)
20. Body (ab) / Bo (ab)	10.109 - 26.733 (**19.077** ± 7.18)	16.211 - 36.994 (**27.978** ± 10.661)	-	10.106 - 27.449 (**18.483** ± 6.565)
21. Body (ab) / Vs (ab)	33.133 - 57.718 (**44.716** ± 11.288)	23.031 - 86.4 (**52.158** ± 31.991)	-	29.596 - 72.716 (**48.845** ± 15.524)
22. Os (ab) / Vs (ab)	0.43 - 1.754 (**0.971** ± 0.563)	0.627 - 1.841 (**1.062** ± 0.676)	-	0.275 - 0.990 (**0.537** ± 0.333)
23. BO (ab) / Vs (ab)	2.091 - 3.277 (**2.589** ± 0.49)	1.421 - 2.335 (**1.762** ± 0.499)	-	1.980 - 5.413 (**3.011** ± 1.147)
24. Os (ab) / Ph (ab)	**6.453**	3.126 - 12.137 (**7.632** ± 6.372)	-	3.218 - 5.528 (**4.190** ± 2.120)
25. O (ab) / BL (ab)	40.605 - 61.512 (**53.391** ±8.523)	56.683 - 62.636 (**59.965** ± 3.023)	-	25.304 - 69.358 (**51.919** ± 13.253)
26. FbL (ab) / HbL (ab)	2.867 - 4.848 (**4.09** ± 0.795)	-	-	3.339 - 5.101 (**4.006** ± 0.648)
27. FbW (ab) / BL (ab)	0.736 - 0.991 (**0.896** ± 0.084)	-	-	0.568 - 1.092 (**0.781** ± 0.142)
28. Fb (ab) / Hb (ab) (%)	6.208 - 12.324 (**9.836** ± 2.292)	-	-	6.0129 - 18.457 (**11.757** ± 3.907)

Fish species	 *Brycinus lateralis*					
Metacercarial type	***Diplostomum* type 2 Eye**	***Diplostomum* type 2 Brain**	***Diplostomum* type a Brain**	***Diplostomum* type a Eye**	***Diplostomum* type b Brain**	***Diplostomum* type c Brain**
Image and sample size	 **n = 4**	 **n = 120**	 **n = 32**	 **n = 33**	 **n = 1**	 **n = 10**
1. Body length (BL)	0.228 - 0.65 (**0.381** ± 0.185)	0.199 - 0.665 (**0.464** ± 0.106)	0.212 - 3.225 (**0.525** ± 0.502)	0.268 - 0.526 (**0.437** ± 0.118)	**0.437**	0.245 - 0.299 (**0.269** ± 0.02)
2. Body width (BW)	0.048 - 0.119 (**0.079** ± 0.03)	0.052 - 0.198 (**0.110** ± 0.032)	0.077 - 0.625 (**0.121** ± 0.094)	0.066 - 0.137 (**0.108** ± 0.031)	**0.086**	0.097 - 0.137 (**0.12** ± 0.011)
3. Forebody length (FbL)	-	-	-	-	**0.38**	-

Fish species	*Brycinus lateralis*					
Metacercarial type	***Diplostomum* type 2 Eye**	***Diplostomum* type 2 Brain**	***Diplostomum* type a Brain**	***Diplostomum* type a Eye**	***Diplostomum* type b Brain**	***Diplostomum* type c Brain**
4. Forebody width (FbW)	-	-	-	-	**0.077**	-
5. Hindbody length (Hbl)	-	-	-	-	**0.058**	-
6. Hindbody width (HbW)	-	-	-	-	**0.026**	-
7. Pharynx length (PhL)	**0.018**	0.014 - 0.023 (**0.019** ± 0.003)	0.017 - 0.02 (**0.019** ± 0.002)	-	-	**0.019**
8. Pharynx width (PhW)	**0.012**	0.01 - 0.013 (**0.012** ± 0.002)	0.010 - 0.014 (**0.013** ± 0.003)	-	-	**0.006**
9. Oral sucker length (OsL)	0.031 - 0.034 (**0.033** ± 0.002)	0.022 - 0.08 (**0.04** ± 0.009)	0.022 - 0.046 (**0.035** ± 0.006)	0.028 - 0.04 (**0.036** ± 0.005)	**0.034**	0.002 - 0.035 (**0.029** ± 0.005)
10. Oral sucker width (OsW)	0.028 - 0.038 (**0.033** ± 0.007)	0.014 - 0.055 (**0.031** ± 0.008)	0.017- 0.034 (**0.027** ± 0.004)	0.023 - 0.032 (**0.026** ± 0.004)	**0.03**	0.018 - 0.029 (**0.023** ± 0.004)
11. Oral to ventral sucker distance (OV)	**0.037**	0.09 - 0.309 (**0.199** ± 0.048)	0.088 - 0.244 (**0.178** ± 0.041)	0.107 - 0.224 (**0.183** ± 0.052)	**0.193**	0.1 - 0.12 (**0.109** ± 0.008)
12. Ventral sucker length (VsL)	**0.044**	0.025 - 0.075 (**0.042** ± 0.009)	0.022 - 0.248 (**0.044** ± 0.039)	0.028 - 0.035 (**0.032** ± 0.003)	**0.032**	0.031 - 0.034 (**0.033** ± 0.001)
13. Ventral sucker width (VsW)	**0.039**	0.017 - 0.075 (**0.037** ± 0.009)	0.020 - 0.169 (**0.037** ± 0.026)	0.026 - 0.037 (**0.030** ± 0.005)	**0.027**	0.026 - 0.033 (**0.03** ± 0.003)
14. Ventral sucker anterior to anterior body end distance (AV)	**0.069**	0.124 - 0.365 (**0.241** ± 0.052)	0.119 - 1.519 (**0.258** ± 0.242)	0.136 - 0.269 (**0.221** ± 0.059)	**0.235**	0.132 - 0.147 (**0.138** ± 0.006)
15. Mid-ventral sucker to anterior body end distance (O)	**0.084**	0.138 - 0.399 (**0.261** ± 0.052)	0.134 - 1.629 (**0.279** ± 0.259)	0.151 - 0.283 (**0.060** ± 0.059)	**0.244**	0.147 - 0.163 (**0.153** ± 0.006)
16. Holdfast organ length (BoL)	**0.044**	0.029 - 0.09 (**0.062** ± 0.013)	0.037 - 0.41 (**0.066** ± 0.067)	0.037 - 0.072 (**0.060** ± 0.016)	**0.056**	0.035 - 0.042 (**0.038** ± 0.003)
17. Holdfast organ width (BoW)	**0.039**	0.017 - 0.058 (**0.033** ± 0.009)	0.015 - 0.184 (**0.034** ± 0.030)	0.026 - 0.035 (**0.032** ± 0.004)	**0.027**	0.022 - 0.032 (**0.026** ± 0.004)
18. Ventral sucker to Holdfast organ distance (VB)	**0.082**	0.009 - 0.098 (**0.039** ± 0.015)	0.018 - 0.26 (**0.043** ± 0.043)	0.014 - 0.057 (**0.03** ± 0.02)	**0.030**	0.009 - 0.025 (**0.015** ± 0.006)

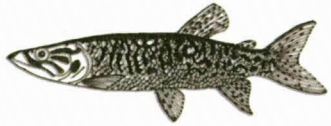
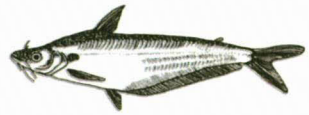
Fish species	*Brycinus lateralis*					
Metacercarial type	***Diplostomum* type 2 Eye**	***Diplostomum* type 2 Brain**	***Diplostomum* type a Brain**	***Diplostomum* type a Eye**	***Diplostomum* type b Brain**	***Diplostomum* type c Brain**
19. b to a of body (BW:BL)(%)	11.726-45.827 (**25.884** ±15.708)	10.378 - 53.068 (**24.812** ± 8.332)	15.876 - 40.988 (**24.653** ± 6.250)	20.711 - 27.649 (**24.765** ± 2.97)	**19.626**	38.962 - 52.622 (**44.813** ± 4.929)
20. Body (ab) / Bo (ab)	**31.217**	13.309 - 55.263 (**28.46** ± 8.37)	19.318 - 56.925 (**31.737** ± 10.911)	18.429 - 30.575 (**24.484** ± 5.003)	**24.792**	25.187 - 46.567 (**33.842** ± 7.784)
21. Body (ab) / Vs (ab)	**28.671**	14.762 - 116.748 (**37.221** ± 13.92)	25.218 - 137.204 (**41.713** ± 19.498)	22.421 - 67.138 (**50.102** ± 19.277)	**42.981**	28.671 - 41.343 (**33.6** ± 4.3584)
22. Os (ab) / Vs (ab)	**0.682**	0.28 - 3.707 (**0.875** ± 0.498)	0.500 - 2.563 (**0.847** ± 0.382)	0.826 - 1.125 (**0.971** ± 0.124)	**1.172**	0.567 - 0.817 (**0.664** ± 0.094)
23. BO (ab) / Vs (ab)	**0.918**	0.563 - 3.63 (**1.357** ± 0.489)	0.721 - 1.997 (**1.294** ± 0.382)	1.217 - 2.838 (**2.014** ± 0.675)	**1.734**	0.736 - 1.233 (**1.026** ± 0.211)
24. Os (ab) / Ph (ab)	-	5.813 - 8.777 (**7.138** ± 1.275)	4.153 - 6.520 (**5.281** ± 1.188)	-	-	**6.504**
25. O (ab) / BL (ab)	**60.416**	43.329 - 66.678 (**53.956** ± 3.934)	45.735 - 57.326 (**52.721** ± 2.527)	52.338 - 56.497 (**54.308** ± 1.723)	**55.742**	49.521 - 60.416 (**56.447** ± 3.822)
26. FbL (ab) / HbL (ab)	-	-	-	-	**6.522**	-
27. FbW (ab) / BL (ab)	-	-	-	-	**0.176**	-
28. Fb (ab) / Hb (ab) (%)	-	-	-	-	**19.653**	-

Fish species	 *Rhabdalestes maunensis*	 *Barbus poechii*		 *Labeo umbratus*
Metacercarial type	***Diplostomum*** **type 2 Brain**	***Diplostomum*** **type 2 Eye**	***Diplostomum*** **type c Eye**	***Diplostomum*** **type 2 Eye**
Image and sample size	 **n = 48**	 **n = 9**	 **n = 9**	 **n = 14**
1. Body length (BL)	0.411 - 0.646 (**0.54** ± 0.054)	0.395 - 0.848 (**0.597** ± 0.158)	0.278 - 0.406 (**0.362** ± 0.057)	0.37 - 0.635 (**0.523** ± 0.109)
2. Body width (BW)	0.062 - 0.167 (**0.103** ± 0.022)	0.085 - 0.210) (**0.158** ± 0.038)	0.171 - 0.248 (**0.205** ± 0.032)	0.102 - 0.149 (**0.124** ± 0.019)
3. Forebody length (FbL)	-	-	-	-
4. Forebody width (FbW)	-	-	-	-
5. Hindbody length (Hbl)	-	-	-	-
6. Hindbody width (HbW)	-	-	-	-
7. Pharynx length (PhL)	0.019 - 0.031 (**0.029** ± 0.003)	-	-	**0.02**
8. Pharynx width (PhW)	0.009 - 0.023 (**0.018** ± 0.004)	-	-	**0.015**
9. Oral sucker length (OsL)	0.032 - 0.057 (**0.042** ± 0.005)	0.026 - 0.035 (**0.028** ± 0.003)	0.014 - 0.026 (**0.022** ± 0.006)	0.032 - 0.064 (**0.045** ± 0.012)
10. Oral sucker width (OsW)	0.026 - 0.04 (**0.032** ± 0.003)	0.025 - 0.038 (**0.028** ± 0.006)	0.017 - 0.034 (**0.028** ± 0.008)	0.03 – 0.058 (**0.042** ± 0.011)
11. Oral to ventral sucker distance (OV)	0.151 - 0.274 (**0.225** ± 0.035)	0.13 - 0.443 (**0.224** ± 0.144)	0.114 - 0.217 (**0.172** ± 0.053)	0.151 – 0.303 (**0.235** ± 0.06)
12. Ventral sucker length (VsL)	0.031 - 0.063 (**0.043** ± 0.005)	0.028 - 0.05 (**0.034** ± 0.009)	0.031 - 0.036 (**0.034** ± 0.006)	0.042 – 0.054 (**0.047** ± 0.005)
13. Ventral sucker width (VsW)	0.024 - 0.045 (**0.036** ± 0.005)	0.028 - 0.044 (**0.031** ± 0.007)	0.027 - 0.039 (**0.033** ± 0.006)	0.034 – 0.05 (**0.042** ± 0.007)
14. Ventral sucker anterior to anterior body end distance (AV)	0.184 - 0.326 (**0.271** ± 0.033)	0.229 - 0.511 (**0.29** ± 0.123)	0.146 - 0.245 (**0.201** ± 0.05)	0.185 - 0.345 (**0.278** ± 0.063)

Fish species	*Rhabdalestes maunensis*	*Barbus poechii*		*Labeo umbratus*
Metacercarial type	***Diplostomum* type 2 Brain**	***Diplostomum* type 2 Eye**	***Diplostomum* type c Eye**	***Diplostomum* type 2 Eye**
15. Mid-ventral sucker to anterior body end distance (O)	0.205 - 0.347 (**0.292** ± 0.034)	0.243 - 0.525 (**0.303** ± 0.122)	0.156 - 0.262 (**0.215** ± 0.054)	0.204 - 0.369 (**0.3** ± 0.065)
16. Holdfast organ length (BoL)	0.054 - 0.105 (**0.07** ± 0.009)	0.044 - 0.107 (**0.058** ± 0.026)	0.058 - 0.007 (**0.064** ± 0.005)	0.058 - 0.079 (**0.069** ± 0.009)
17. Holdfast organ width (BoW)	0.025 - 0.045 (**0.034** ± 0.006)	0.023 - 0.051 (**0.033** ± 0.009)	0.044 - 0.048 (**0.047** ± 0.002)	0.027 - 0.042 (**0.035** ± 0.006)
18. Ventral sucker to Holdfast organ distance (VB)	0.011 - 0.082 (**0.042** ± 0.013)	0.047 - 0.412 (**0.111** ± 0.156)	0.011 - 0.027 (**0.018** ± 0.008)	0.03 - 0.093 (**0.057** ± 0.024)
19. b to a of body (BW:BL)(%)	10.418 - 37.919 (**19.081** ± 5.81)	18.692 - 32.819 (**27.198** ± 7.264)	49.288 - 67.271 (**57.311** ± 8.476)	17.885 - 32.533 (**24.332** ± 5.467)
20. Body (ab) / Bo (ab)	11.188 - 35.522 (**23.425** ± 6.193)	27.531 - 105.555 (**47.831** ± 30.019)	15.483 - 29.673 (**25.25** ± 6.684)	14.556 - 39.872 (**27.927** ± 9.116)
21. Body (ab) / Vs (ab)	18.099 - 70.975 (**36.058** ± 10.037)	44.33 - 142.627 (**78.53** ± 37.649)	56.08 - 78.221 (**66.107** ± 11.217)	21.61 - 47.763 (**34.429** ± 11.847)
22. Os (ab) / Vs (ab)	0.501 - 1.382 (**0.849** ± 0.169)	0.598 - 1.42 (**0.862** ± 0.385)	0.161 - 2.81(**0.989** ± 0.418)	0.672 - 1.374 (**0.967** ± 0.262)
23. BO (ab) / Vs (ab)	0.904 - 3.465 (**1.535** ± 0.465)	1.224 - 2.437 (**1.684** ± 0.476)	2.173 - 3.622 (**2.81** ± 0.74)	0.739 - 2.19 (**1.324** ± 0.563)
24. Os (ab) / Ph (ab)	2.213 - 5.447 (**4.851** ± 0.960)	-	-	**4.817**
25. O (ab) / BL (ab)	47.66 - 57.941 (**53.019** ± 3.141)	38.458 - 74.816 (**53.15** ± 14.539)	56.183 - 64.413 (**60.727** ± 4.182)	55.201 - 60.289 (**57.263** ± 2.051)
26. FbL (ab) / HbL (ab)	-	-	-	-
27. FbW (ab) / BL (ab)	-	-	-	-
28. Fb (ab) / Hb (ab) (%)	-	-	-	-

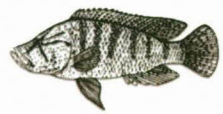
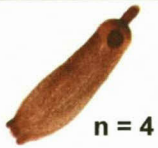
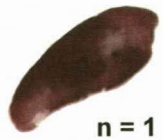
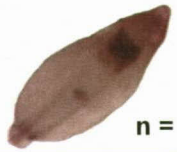
Fish species	 *Labeo capensis*			
Metacercarial type	***Diplostomum*** **type 2 Eye**	***Diplostomum*** **type a Eye**	***Diplostomum*** **type b Brain**	***Diplostomum*** **type c Eye**
Image and sample size	 **n = 5**	 **n = 10**	 **n = 4**	 **n = 1**
1. Body length (BL)	0.309 - 0.774 (**0.523** ± 0.132)	0.414 - 0.663 (**0.512** ± 109)	**0.381**	**0.338**
2. Body width (BW)	0.071 - 0.14 (**0.098** ± 0.025)	0.083 - 0.147 (**0.109** ± 0.020)	**0.115**	**0.137**
3. Forebody length (FbL)	-	-	**0.042**	-
4. Forebody width (FbW)	-	-	**0.044**	-
5. Hindbody length (Hbl)	-	-	-	-
6. Hindbody width (HbW)	-	-	-	-
7. Pharynx length (PhL)	0.013 - 0.02 (**0.019** ± 0.005)	0.013 - 0.017 (**0.015** ± 0.003)	**0.014**	-
8. Pharynx width (PhW)	0.012 - 0.017 (**0.014** ± 0.003)	0.009 - 0.012 (**0.011** ± 0.001)	**0.009**	-
9. Oral sucker length (OsL)	0.033 - 0.043 (**0.037** ± 0.004)	0.033 - 0.045 (**0.039** ± 0.004)	**0.381**	**0.033**
10. Oral sucker width (OsW)	0.023 - 0.042 (**0.03** ± 0.006)	0.025 - 0.039 (**0.031** ± 0.005)	**0.115**	**0.028**
11. Oral to ventral sucker distance (OV)	0.147 - 0.373 (**0.25** ± 0.062)	0.208 - 0.310 (**0.219** ± 0.050)	0.034 - 0.04 (**0.037** ± 0.001)	**0.128**
12. Ventral sucker length (VsL)	0.038 - 0.052 (**0.042** ± 0.004)	0.032 – 0.050 (**0.039** ± 0.007)	0.031 - 0.04 (**0.038** ± 0.004)	**0.033**
13. Ventral sucker width (VsW)	0.028 - 0.050 (**0.035** ± 0.006)	0.024 – 0.042 (**0.034** ± 0.006)	0.133 - 0.2 (**0.159** ± 0.002)	**0.037**
14. Ventral sucker anterior to anterior body end distance (AV)	0.179 - 0.418 (**0.293** ± 0.063)	0.213 – 0.351 (**0.263** ± 0.051)	0.038 - 0.05 (**0.046** ± 0.001)	**0.169**
15. Mid-ventral sucker to anterior body end distance (O)	0.194 - 0.44 (**0.313** ± 0.064)	0.234 – 0.363 (**0.280** ± 0.050)	0.031 - 0.04 (**0.038** ± 0.004)	**0.186**
16. Holdfast organ length (BoL)	0.051 - 0.077 (**0.061** ± 0.009)	0.043 – 0.082 (**0.063** ± 0.011)	0.166 - 0.24 (**0.199** ± 0.002)	**0.053**
17. Holdfast organ width (BoW)	0.025 - 0.048 (**0.033** ± 0.007)	0.024 – 0.036 (**0.030** ± 0.004)	0.183 - 0.25 (**0.219** ± 0.008)	**0.03**
18. Ventral sucker to Holdfast organ distance (VB)	0.023 – 0.077 (**0.053** ± 0.019)	0.025 – 0.114 (**0.050** ± 0.025)	0.03 - 0.059 (**0.05** ± 0.001)	**0.023**

Fish species	*Labeo capensis*			
Metacercarial type	***Diplostomum* type 2 Eye**	***Diplostomum* type a Eye**	***Diplostomum* type b Brain**	***Diplostomum* type c Eye**
19. b to a of body (BW:BL)(%)	7.252 - 36.496 (**20.442** ± 8.52)	12.561 - 49.838 (**23.034** ± 10.263)	27.669 - 32.46 (**29.026** ± 2.295)	**40.638**
20. Body (ab) / Bo (ab)	19.073 - 43.556 (**29.429** ± 7.995)	17.940 - 44.574 (**30.556** ± 7.642)	28.234 - 39.67 (**33.657** ± 4.837)	**29.331**
21. Body (ab) / Vs (ab)	22.851 - 67.014 (**38.629** ± 13.736)	18.760 - 87.723 (**44.736** ± 18.979)	18.441 - 40.635 (**26.089** ± 10.43)	**37.794**
22. Os (ab) / Vs (ab)	0.576 - 0.949 (**0.779** ± 0.147)	0.636 - 1.813 (**0.964** ± 0.328)	0.656 - 1.092 (**0.839** ± 0.213)	**0.761**
23. BO (ab) / Vs (ab)	0.556 - 1.951 (**1.41** ± 0.468)	0.936 - 1.968 (**1.439** ± 0.379)	0.584 - 1.166 (**0.769** ± 0.267)	**1.289**
24. Os (ab) / Ph (ab)	1.907 - 5.339 (**3.717** ± 1.889)	7.609 - 8.479 (**8.044** ± 0.615)	-	-
25. O (ab) / BL (ab)	50.68 - 60.639 (**56.502** ± 2.702)	49.660 - 64.946 (**55.296** ± 5.162)	53.324 - 60.923 (**57.431** ± 3.166)	**54.964**
26. FbL (ab) / HbL (ab)	-	-	**9.121**	-
27. FbW (ab) / BL (ab)	-	-	**0.272**	-
28. Fb (ab) / Hb (ab) (%)	-	-	**23.652**	-

Fish species	 *Hepsetus odoe*			 *Scilbe intermedius*
Metacercarial type	***Diplostomum* type 2 Brain**	***Diplostomum* type d Brain**	***Diplostomum* type b Brain**	***Diplostomum* type d Brain**
Image and sample size	 **n = 8**	 **n = 2**	 **n = 2**	 **n = 5**
1. Body length (BL)	0.294 - 0.623 (**0.538** ± 0.115	0.518 - 0.451 (**0.485** ± 0.048)	0.471 - 0.593 (**0.532** ± 0.02)	0.330 - 0.476 (**0.393** ± 0.071)
2. Body width (BW)	0.073 - 0.143 (**0.107** ± 0.031)	0.102 - 0.106 (**0.104** ± 0.002)	0.087- 0.141 (**0.114** ± 0.06)	0.181 - 0.224 (**0.192** ± 0.033)
3. Forebody length (FbL)	-	-	**0.499**	0.298 - 0.443 (**0.354** ± 0.070)
4. Forebody width (FbW)	-	-	**0.142**	0.142 - 0.225 (**0.193** ± 0.032)

Fish species	*Hepsetus odoe*			*Scilbe intermedius*
Metacercarial type	***Diplostomum* type 2 Brain**	***Diplostomum* type d Brain**	***Diplostomum* type b Brain**	***Diplostomum* type d Brain**
5. Hindbody length (Hbl)	-	-	**0.094**	0.029 - 0.044 (**0.034** ± 0.006)
6. Hindbody width (HbW)	-	-	**0.041**	0.034 - 0.066 (**0.058** ± 0.013)
7. Pharynx length (PhL)	0.015 - 0.021 (**0.018** ± 0.003)	0.017 - 0.020 (**0.018** ± 0.002)	0.014 - 0.026 (**0.02** ± 0.01)	**0.029**
8. Pharynx width (PhW)	0.009 - 0.013 (**0.01** ± 0.003)	0.011 - 0.014 (**0.012** ± 0.002)	0.011 - 0.013 (**0.012** ± 0.002)	**0.017**
9. Oral sucker length (OsL)	0.026 - 0.059 (**0.041** ± 0.011)	0.037 - 0.043 (**0.040** ± 0.004)	0.033 - 0.034 (**0.034** ± 0.001)	0.037 - 0.043 (**0.04** ± 0.003)
10. Oral sucker width (OsW)	0.025 - 0.05 (**0.035** ± 0.008)	0.025 - 0.034 (**0.029** ± 0.006)	0.032 - 0.034 (**0.033** ± 0.001)	0.024 - 0.049 (**0.038** ± 0.013)
11. Oral to ventral sucker distance (OV)	0.119 - 0.253 (**0.217** ± 0.048)	0.171 - 0.218 (**0.195** ± 0.033)	**0.209**	0.15 - 0.193 (**0.172** ± 0.031)
12. Ventral sucker length (VsL)	0.034 - 0.056 (**0.045** ± 0.007)	0.039 - 0.040 (**0.039** ± 0.0002)	**0.033**	0.022 - 0.030 (**0.026** ± 0.006)
13. Ventral sucker width (VsW)	0.028 - 0.049 (**0.04** ± 0.008)	0.029 - 0.0291 (**0.029** ± 0.001)	**0.037**	0.023 - 0.035 (**0.029** ± 0.009)
14. Ventral sucker anterior to anterior body end distance (AV)	0.106 - 0.308(**0.252** ± 0.066)	0.217 - 0.218 (**0.218** ± 0.0004)	**0.245**	0.181 - 0.232 (**0.206** ± 0.036)
15. Mid-ventral sucker to anterior body end distance (O)	0.121 - 0.331 (**0.275** ± 0.069)	0.229 - 0.235 (**0.232** ± 0.005)	**0.263**	0.191 - 0.242 (**0.217** ± 0.036)
16. Holdfast organ length (BoL)	0.050 - 0.086 (**0.067** ± 0.012)	0.054 - 0.055 (**0.055** ± 0.001)	0.057 - 0.069 (**0.063** ± 0.002)	0.046 - 0.081 (**0.062** ± 0.017)
17. Holdfast organ width (BoW)	0.02 - 0.043 (**0.034** ± 0.011)	0.025 - 0.030 (**0.027** ± 0.003)	0.029 - 0.045 (**0.037** ± 0.01)	0.037 - 0.071 (**0.056** ± 0.014)
18. Ventral sucker to Holdfast organ distance (VB)	0.031 - 0.062 (**0.049** ± 0.012)	0.033 - 0.056 (**0.044** ± 0.017)	**0.038**	0.013 - 0.056 (**0.035** ± 0.031)
19. b to a of body (BW:BL)(%)	12.153 - 24.961 (**20.329** ± 4.857)	20.376 - 22.683 (**21.529** ± 1.631)	18.507 - 23.842 (**21.174** ± 1.045)	40.276 - 60.826 (**49.304** ± 7.817)
20. Body (ab) / Bo (ab)	20.346 - 43.22 (**30.067** ± 8.006)	28.666 - 39.189 (**33.928** ± 7.441)	24.945 - 27.02 (**25.982** ± 1.05)	18.129 - 36.171 (**25.628** ± 7.582)
21. Body (ab) / Vs (ab)	21.836 - 40.44 (**31.781** ± 6.54)	40.372 - 47.989 (**44.181** ± 5.386)	**33.704**	86.456 - 147.552 (**117.004** ± 43.202)
22. Os (ab) / Vs (ab)	0.685 - 0.906 (**0.795** ± 0.0802)	0.941 - 1.097 (**1.019** ± 0.110)	**0.89**	2.282 - 2.649 (**2.465** ± 0.259)
23. BO (ab) / Vs (ab)	0.821 - 1.374 (**1.141** ± 0.239)	1.225 - 1.408 (**1.316** ± 0.130)	**1.351**	2.649 - 6.246 (**4.881** ± 1.93)
24. Os (ab) / Ph (ab)	8.26 - 10.853 (**9.556** ± 1.834)	4.669 - 5.708 (**5.189** ± 0.735)	4.063 - 6.078 (**5.07** ± 1.357)	**3.632**

Fish species	*Hepsetus odoe*			*Scilbe intermedius*
Metacercarial type	***Diplostomum* type 2 Brain**	***Diplostomum* type d Brain**	***Diplostomum* type b Brain**	***Diplostomum* type d Brain**
25. O (ab) / BL (ab)	41.071 - 57.622 (**50.635** ± 5.954)	45.376 - 50.723 (**48.050** ± 3.781)	**55.847**	50.802 - 57.821 (**54.312** ± 4.963)
26. FbL (ab) / HbL (ab)	-	-	**5.282**	8.193 - 14.605 (**10.472** ± 2.444)
27. FbW (ab) / BL (ab)	-	-	**0.24**	0.405 - 0.6 (**0.495** ± 0.075)
28. Fb (ab) / Hb (ab) (%)	-	-	**18.238**	18.457 - 55.831 (**37.3448** ± 14.959)

Fish species	 *Oreochromis andersonii*		 *Oreochromis macrochir*	 *Sargochromis greenwoodi*	 *Serranochromis angusticeps*
Metacercarial type	***Diplostomum* type b Brain**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**
Image and sample size	 **n = 4**	 **n = 2**	 **n = 1**	 **n = 5**	 **n = 4**
1. Body length (BL)	0.415 - 1.184 (**0.904** ± 0.337)	1.382 - 1.601 (**1.491** ± 0.016)	**1.209**	0.96 - 1.309 (**1.096** ± 0.143)	0.892 - 1.295 (**1.167** ± 0.185)
2. Body width (BW)	0.114 - 0.272 (**0.215** ± 0.075)	0.551 - 0.560 (**0.556** ± 0.006)	**0.494**	0.288 - 0.561 (**0.404** ± 0.115)	0.401 - 0.518 (**0.444** ± 0.055)
3. Forebody length (FbL)	0.887 - 1.069 (**0.952** ± 0.101)	-	-	-	-
4. Forebody width (FbW)	0.201 - 0.278 (**0.251** ± 0.043)	-	-	-	-
5. Hindbody length (Hbl)	0.121 - 0.155 (**0.134** ± 0.019)	-	-	-	-

Fish species	*Oreochromis andersonii*		*Oreochromis macrochir*	*Sargochromis greenwoodi*	*Serranochromis angusticeps*
Metacercarial type	***Diplostomum* type b Brain**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**
6. Hindbody width (HbW)	0.059 - 0.081 (**0.072** ± 0.011)	-	-	-	-
7. Pharynx length (PhL)	0.042 - 0.057 (**0.051** ± 0.009)	-	-	-	-
8. Pharynx width (PhW)	0.026 - 0.032 (**0.029** ± 0.004)	-	-	-	-
9. Oral sucker length (OsL)	0.04 - 0.059 (**0.052** ± 0.009)	-	-	-	-
10. Oral sucker width (OsW)	0.024 - 0.075 (**0.052** ± 0.022)	-	-	-	-
11. Oral to ventral sucker distance (OV)	0.164 - 0.486 (**0.325** ± 0.227)	-	-	-	-
12. Ventral sucker length (VsL)	0.034 - 0.001 (**0.067** ± 0.047)	-	-	-	-
13. Ventral sucker width (VsW)	0.03 - 0.089 (**0.06** ± 0.041)	-	-	-	-
14. Ventral sucker anterior to anterior body end distance (AV)	0.208 - 0.543 (**0.376** ± 0.237)	-	-	-	-
15. Mid-ventral sucker to anterior body end distance (O)	0.227 - 0.592 (**0.409** ± 0.259)	-	-	-	-
16. Holdfast organ length (BoL)	0.062 - 0.143 (**0.103** ± 0.033)	-	-	-	-
17. Holdfast organ width (BoW)	0.026 - 0.144 (**0.089** ± 0.049)	-	-	-	-
18. Ventral sucker to Holdfast organ distance (VB)	0.039 - 0.107 (**0.073** ± 0.048)	-	-	-	-
19. b to a of body (BW:BL)(%)	20.438 - 27.438 (**24.339** ± 3.248)	34.997- 39.89 (**37.444** ± 3.46)	**40.818**	28.544 - 42.86 (**36.468** ± 16.104)	32.093 - 45.131 (**38.502** ± 5.487)
20. Body (ab) / Bo (ab)	15.565 - 28.894 (**23.517** ± 5.754)	-	-	-	-
21. Body (ab) / Vs (ab)	31.281 - 45.223 (**38.252** ± 9.858)	-	-	-	-
22. Os (ab) / Vs (ab)	0.401 - 0.938 (**0.67** ± 0.38)	-	-	-	-
23. BO (ab) / Vs (ab)	1.194 - 1.565 (**1.38** ± 0.262)	-	-	-	-
24. Os (ab) / Ph (ab)	1.7 - 2.354 (**1.351** ± 0.463)	-	-	-	-

Fish species	*Oreochromis andersonii*		*Oreochromis macrochir*	*Sargochromis greenwoodi*	*Serranochromis angusticeps*
Metacercarial type	***Diplostomum* type b Brain**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**
25. O (ab) / BL (ab)	54.664 - 57.872 (**56.268** ± 2.268)	-	-	-	-
26. FbL (ab) / HbL (ab)	6.874 - 7.47 (**7.124** ± 0.309)	-	-	-	-
27. FbW (ab) / BL (ab)	0.202 - 0.271 (**0.235** ± 0.035)	-	-	-	-
28. Fb (ab) / Hb (ab) (%)	17.503 - 34.87 (**25.855** ± 8.702)	-	-	-	-

Fish species	 *Tilapia sparrmanii*				 *Tilapia rendalli*
Metacercarial type	***Diplostomum* type 2 Eye**	***Diplostomum* Type Eye a**	***Diplostomum* Type d Eye**	***Diplostomum* cyst Eye**	***Diplostomum* cyst Eye**
Image and sample size	 **n = 1**	 **n = 4**	 **n = 1**	 **n = 6**	 **n = 1**
1. Body length (BL)	**0.396**	0.367 - 0.547 (**0.431** ± 0.081)	**0.53**	1.009 - 1.327 (**1.151** ± 0.136)	**1.209**
2. Body width (BW)	**0.14**	0.239 – 0.281 (**0.253** ± 0.020)	**0.271**	0.342 - 0.726 (**0.451** ± 0.16)	**0.494**
3. Forebody length (FbL)	-	-	**0.479**	-	-
4. Forebody width (FbW)	-	-	**0.269**	-	-
5. Hindbody length (Hbl)	-	-	**0.046**	-	-
6. Hindbody width (HbW)	-	-	**0.086**	-	-
7. Pharynx length (PhL)	**0**	**0.026**	**0.03**	-	-
8. Pharynx width (PhW)	**0**	**0.014**	**0.018**	-	-

Fish species	*Tilapia sparrmanii*				*Tilapia rendalli*
Metacercarial type	***Diplostomum* type 2 Eye**	***Diplostomum* cyst Eye**	**Metacercarial type**	***Diplostomum* type 2 Eye**	***Diplostomum* cyst Eye**
9. Oral sucker length (OsL)	**0.04**	0.047 - 0.050 (**0.049** ± 0.003)	**0.059**	-	-
10. Oral sucker width (OsW)	**0.041**	0.028 - 0.043 (**0.035** ± 0.011)	**0.043**	-	-
11. Oral to ventral sucker distance (OV)	**0.166**	**0.168**	**0.066**	-	-
12. Ventral sucker length (VsL)	**0.059**	**0.027**	**0.066**	-	-
13. Ventral sucker width (VsW)	**0.043**	**0.039**	**0.066**	-	-
14. Ventral sucker anterior to anterior body end distance (AV)	**0.207**	**0.225**	**0.066**	-	-
15. Mid-ventral sucker to anterior body end distance (O)	**0.232**	**0.237**	**0.066**	-	-
16. Holdfast organ length (BoL)	**0.059**	0.060 - 0.066 (**0.063** ± 0.004)	**0.066**	-	-
17. Holdfast organ width (BoW)	**0.034**	0.049 – 0.056 (**0.052** ± 0.005)	**0.066**	-	-
18. Ventral sucker to Holdfast organ distance (VB)	**0.014**	**0.159**	**0.066**	-	-
19. b to a of body (BW:BL)(%)	**35.342**	43.798 - 69.012 (**60.211** ± 11.339)	**51.138**	26.412 - 54.748 (**38.891** ± 11.216)	**40.818**
20. Body (ab) / Bo (ab)	**27.901**	35.424 - 40.788 (**38.106** ± 3.793)	**17.121**	-	-
21. Body (ab) / Vs (ab)	**21.775**	**124.947**	**86.41**	-	-
22. Os (ab) / Vs (ab)	**0.643**	**1.228**	**1.549**	-	-
23. BO (ab) / Vs (ab)	**0.780**	**3.527**	**5.047**	-	-
24. Os (ab) / Ph (ab)	-	**3.649**	**4.795**	-	-
25. O (ab) / BL (ab)	**58.615**	**43.325**	**26.57**	-	-
26. FbL (ab) / HbL (ab)	-	-	**10.518**	-	-
27. FbW (ab) / BL (ab)	-	-	**0.507**	-	-
28. Fb (ab) / Hb (ab) (%)	-	-	**32.781**	-	-