CHAPTER 1

GENERAL INTRODUCTION

There are about 3000 species of freshwater teleosts in Africa (Khalil & Polling, 1997; FAO, 2002). Fish provide a comparatively cheap source of animal protein and attention is now being focussed on fish production, both from natural waters and from aquaculture (Coche *et al.*, 1994; Khalil & Polling, 1997). Fish proteins are essential and critical in the diets of some densely populated countries where the total protein intake may be low (FAO, 2002). Three of the world's largest lakes – Victoria, Tanganyika and Malawi – are found on the African continent, and so are three of the world's largest rivers – the Nile, Congo and Niger, and so Africa has a considerable potential for fish production (Crul, 1998)

With the declining catch from marine capture fisheries in recent years, many countries have sought to use aquaculture to increase the production of fish and other aquatic food resources (Coche *et al.*, 1994; Sugunan, 2000). Sub-Saharan Africa lags behind in this respect compared to other regions; FAO statistics for 2000 show that the region produced about 16 000 tonnes of finfish by aquaculture, while North and North-East Africa produced 70 000 t, North America 230 000 t, South America, 50 000 t, Europe 500 000 t, and Asia 6.7 million t (FAO, 2002). In Zimbabwe, non-governmental organisations such as the United Nations Development Programme (UNDP), the UN Food and Agricultural Organisation (FAO) and the Canadian International Development Agency (CIDA) have been funding aquacultural development projects since 1988 through government departments like National Parks (Henderson and Nyanga Research Stations), Research and Specialist Services (DRSS) and the University of Zimbabwe (Lake Kariba Research Station) (Coche *et al.*, 1994).

Man-made impoundments are becoming important as sources of fish production (Sugunan, 2000). In an impoundment, the fish stocks of the river will form the stock of the dam although new species may later be introduced to produce a stock with the desired species composition (Lagler, 1969). The riverine stock does not always adapt to the new lacustrine environment since river populations are normally low in plankton-feeding fishes while the opposite is true for lacustrine populations (Lagler, 1969; Allanson *et al*., 1990). A good example of this are the cyprinid fish species of the Manyame River in Zimbabwe, such as *Barbus* sp., several of which failed to adapt to lacustrine conditions when the river was dammed to form Lake Chivero (formerly Lake McIlwaine). Only *B. paludinosus* Peters, 1852, is the most widespread and occurs where streams flow into the lake (Marshall, 1982). A number of riverine fish species have also failed to adapt to the damming of the Zambezi River (Begg, 1974).

Fish are hosts to many protozoan, helminth and arthropod parasites (Branson and Southgate, 1992). In many instances, they harbour larval forms of these parasites as intermediate or paratenic hosts, the adults of which occur in amphibians, reptiles, birds or mammals, the definitive hosts. It is important to study fish parasites because they are part of the total ecology of an aquatic ecosystem and can thus exert some influence on the rest of the environment (Hoffman and Bauer, 1971). Some parasites inflict damage on the hosts, sometimes causing gross mortalities, which can lead to great losses in commercial fisheries and aquaculture (Paperna and Zwerna, 1976; Roberts, 2001). In some cases, parasitized fish look so unsightly that they are rejected by consumers (Paperna, 1996; Dick and Choudhury, 1995). Studying the parasites contributes towards a better understanding of the ecology of a system, and also helps to develop methods of controlling them (Hoffman and Bauer, 1971). It can also yield information that would make

certain parasites usable as biological tags; in South Africa, for example, research is already under way on how fish parasites can be used as indicators of water pollution (Avenant-Oldewage, 2001). According to Hoffman and Bauer (1971) there also is a remote possibility of controlling unwanted fish using parasites.

In many parts of the world, especially developed countries, studies on fish parasites have advanced dramatically because of technological advances in microscopy and molecular systematics. While Africa lags behind in this respect, efforts have been made in several countries where parasitologists have worked to identify and classify parasites from African freshwater fishes (Table 1.1). Paperna (1996) and Khalil and Polling (1997) published the most recent updates on the biology, distribution and classification of the parasites of African fishes. Khalil and Polling (1997) recorded a total of 568 species of adult helminths in African fishes representing 342 monogeneans, 62 digeneans, 61 cestodes, 80 nematodes, 21 acanthocephalans and two aspidogastreans.

Research on fish parasites is still lacking in many African countries with some having no records of fish parasites (Paperna, 1996). In southern Africa, only South Africa has a well-established database of parasites of fish and related aquatic vertebrates (van As and Basson, 1984; Khalil and Polling, 1997). A number of South African universities have trained fish parasitologists over the last three decades and they subsequently established and mobilised extensive research in their country's drainage systems (Mashego, personal communication). These scientists form the core of the Parasitological Society of Southern Africa (PARSA) which convenes an annual conference where research findings are presented (Avenant-Oldewage, personal communication). Some

research has been done at masters and doctoral levels on helminths and pentastomids of fish, birds and crocodiles (Mashego, 1977, 1982; Mokgalong, 1996).

Fish parasites have not been studied much in Zimbabwe. Apart from platyhelminths recorded by Mettrick (1960) and Beverly-Burton (1962) from near Harare, Mazowe and Kadoma, the only other study of fish parasites was done on fish from Lake Kariba (Chishawa, 1991; Douëllou, 1992a, 1992b; Douëllou and Erlwanger, 1993; Douëllou and Chishawa, 1995).

Two phyla of parasitic helminths, the Platyhelminthes (flatworms) and the Nemathelminthes (roundworms) occur in fish. Flatworms of the class Monogenea are ectoparasites which infest the gills and skin of the host, while the Digenea (flukes) and Cestoda (tapeworms) are endoparasites of the body cavity and organs of the host, although their larval stages may encyst on the gills and skin (Roberts and Janovy, 2000). Nematodes and acanthocephalans are the most common roundworm parasites of fish and are always found in the internal organs. Save for the Monogenea, most helminth parasites have indirect life cycles involving one or more intermediate hosts, which may be fish or aquatic invertebrates. Definitive or final hosts of most digenean, nematode and even some cestode parasites are fish-eating birds or mammals. A complete parasitological study of aquatic ecosystems should therefore encompass all hosts in order to follow the life stages.

Fish-eating birds are ubiquitous in their distribution in all freshwater and marine habitats of Africa (Tuck and Heinzel, 1979). In Zimbabwe, birds belonging to the orders Pelecaniformes (cormorants (Phalacrocoracidae) and darters (Anhingidae)), Ciconiiformes (storks (Ciconiidae),

Table 1.1. Some publications on freshwater fish parasites from African countries.

Country	Parasites	Fish	Source
Burkina Faso	Nematodes	Centrarchids	Kabre and Petter, 1996
Democratic Republic of Congo	Nematodes, cestodes, trematodes	Cichlids, mormyrids	Khalil, 1973
Egypt	Nematode, cestodes	Cichlids, Clariids	Amin, 1978
Ethiopia	Nematodes, cestodes	Cichlids, Cyprinids	Yimer, 2000; Dejen, 2003
Ghana	Trematodes	Cichlids, Clariids	Ukoli, , 1992
Kenya	Nematodes	Cichlids	Malvestuto and Ogambo-Ongoma, 1978; Aloo, 1999, 2001
South Africa	Protozoa, trematodes, cestodes nematodes and crustaceans	Clariids, cichlids, characids and cyprinids	Mashego, 1977, 1982, 1989, 2001; Prudhoe and Hussey, 1977; Whitfield and Heeg, 1977; Mashego and Saayman, 1981; van As and Basson, 1984; Britz *et al.*, 1985, Saayman *et al.*, 1991; Boomker, 1982, 1994a, b; Avenant-Oldewage, 2001.
Sudan	Nematodes, trematodes	Clariids, cichlids, mormyrids	Khalil, 1963
Swaziland	Nematodes	Clariids	Prudhoe and Hussey, 1977
Tanzania	Cestodes	Cyprinids	Wanink, 1992; Marshall and Cowx, 2003
Uganda	Acanthocephalans, nematodes, cestodes, trematodes		Khalil and Thurston, 1973; Paperna, 1974; Marshall and Cowx, 2003
Zambia	Trematodes, cestodes and acanthocephalans	Cichlids	Beverly-Burton, 1962; Batra, 1984

hamerkops (Scopidae) and herons (Ardeidae)) and Halcyoniformes (kingfishers (Alcedinidae)), are by far the most abundant on aquatic habitats (Irwin, 1981). The Reed Cormorant, *Phalacrocorax africanus* (Gmelin, 1789), for example, has adapted well to dams becoming the most abundant piscivorous species on Lakes Kariba and Mutirikwi (Junor and Marshall, 1987; Hustler and Marshall, 1996). The White-breasted Cormorant, *P. carbo* (L.), has always been outnumbered by *P. africanus* as a breeding species on most impoundments in Zimbabwe, the only exception being Lake Chivero where it is more numerous (Irwin, 1981).

The Darter, *Anhinga melanogaster* Pennant, 1769, like the cormorants, responded in a similar manner to the increase in artificial impoundments (Irwin, 1981). Piscivorous birds, which were relatively rare in the past, have now increased in Zimbabwe because of man-made reservoirs (Irwin, 1981), and the following species are found in the Manyame catchment: Grey Heron (*Ardea cinerea*), Goliath Heron (*A. goliath*), Purple Heron (*A. purpurea*), Little Egret (*Egretta garzetta*), Great Egret (*E. alba*), Cattle Egret (*Bubulcus ibis*), Pied Kingfisher (*Ceryle rudis*), Fish Eagle (*Haeliatus vocifer*) and Night Heron (*Nycticorax nycticorax*), as well as cormorants and darters (Couto, personal communication).

The birds mentioned above, as well as pelicans, are also found throughout South Africa and Mozambique (Tuck and Heinzel, 1979; Maclean, 1985; Olivier *et al.*, 1991). Most of them have been found to harbour gastrointestinal helminths such as clinostomid species, diplostomid species and nematodes of the genus *Contracaeum* (Saayman *et al.*, 1991; Mokgalong, 1996). The incidence of parasites in African aquatic birds has also been determined in Ghana (Ukoli, 1968), Swaziland (Prudhoe and Hussey, 1977) and South Africa (Whitfield and Heeg, 1977). In

Zimbabwe there are no published records of avian parasites in the past forty years, which make this study the first in that respect.

Study Area

The upper Manyame catchment extends over an area of 2136 km^2, and is about 10% urban and 90% rural, the latter being commercial farmland and communal land (Magadza, 1997). This study was carried out at three localities within the catchment: (1) Lake Chivero, (2) Munwahuku Dam, and (3) three sites along the Marimba River (Figure 1.1).

Lake Chivero (formerly Lake McIlwaine) was formed in 1952 by damming the Manyame River and is situated about 37km southwest of Harare, the capital city of Zimbabwe. The lake is the fifth largest impoundment in Zimbabwe (Sugunan, 2000) with an area of 2630ha and a mean depth of 9.5m (Table 1.1). It is a popular recreational site, forming part of the Robert McIlwaine Recreational Park and is the primary water supply for the city of Harare and its dormitory town of Chitungwiza. Farms downstream use the lake as an irrigation source and it has also become one of the most important fisheries in the country. Lake Chivero is eutrophic because of sewage effluent that is discharged into its feeder streams from Harare and Chitungwiza (Marshall, 1997).

The upper Munwahuku Dam is situated on Ingwerati Farm, about 10km northwest of Lake Chivero. It is a small reservoir on the upper reaches of the Munwahuku stream (Area approximately 5.7ha) , which drains into Lake Manyame, a few kilometres downstream of Lake Chivero (Fig. 1.1). It is oligotrophic (Table 1.1) and during the rainy season, *Nymphaea* spp. and *Polygonum senegalense* Meisn are the dominant macrophytes in the shallow areas of the dam.

The Marimba River flows through the northern and western parts of Harare, originating on the University of Zimbabwe campus as a small stream, and eventually draining into Lake Chivero (Fig 1.1). Along its length, the river gradually becomes polluted by sewage effluent, especially after the Crowborough Sewage Works, Harare's second largest sewage treatment plant, located a few kilometres upstream from Lake Chivero that discharges partially treated effluent into the river (Moyo, 1997).

Table 1.2. Some characteristics of Lake Chivero (from Elenbaas & Grundel, 1994), Munwahuku Dam and Marimba River.

	L. Chivero	Munwahuku Dam	Marimba R. Site 1	Marimba R. Site 2	Marimba R. Site 3
Altitude (m)	1350	-	-	-	-
Area (ha)	2630	5.7	N/A	N/A	N/A
Volume ($x10^6$ m^3)	250.04	0.007	N/A	N/A	N/A
Maximum depth (m)	27.4	5.0	1.0	1.5	1.2
Mean Depth (m)	9.5	2.5	0.6	0.8	0.7
Conductivity (μS cm^{-1})	47-338	9-132	390-403	475-536	534-585
Temperature (°C)	18-26.2	18-27.8	19.2-21.3	17.1-20.3	18.2-20.7
pH	6.6-9.2	6.5-8.2	7.9-8.7	7.6-8.0	6.8-7.7
Transparency (Secchi disc) (m)	0.6-2.2	0.1-1.6	0.4-0.5	0.1-0.2	0-0.2
Dissolved O_2 (surface)	5.6-7.7	3.6-7.6	9.7-10.5	4.3-6.4	0.3-0.9

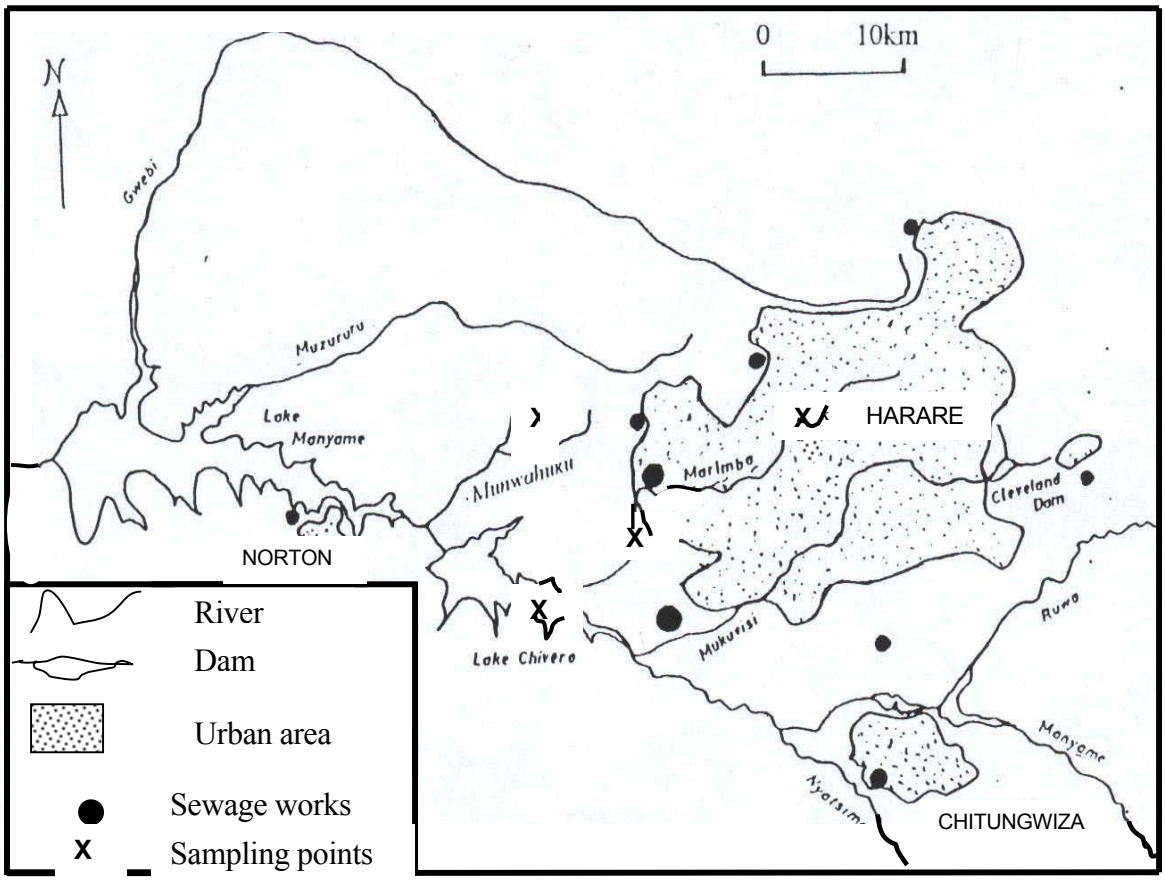


Fig. 1.1. The western portion of the upper Manyame catchment showing the location of Lake Chivero, the Munwahuku Dam and the Marimba River in relation to the city of Harare and the town of Chitungwiza.

Study Purpose

Little work has been done on fish and avian parasites in Zimbabwe, so this study sought to add to knowledge in this particular subject. The few previous studies of Zimbabwean fish parasites were mainly taxonomic, so the aim was to include ecological aspects by determining the prevalence and intensities of infection and relating them to the condition of the fish, while attempting to identify the parasites as accurately as possible. The work on avian parasites was done to determine the life cycle of the parasitic helminths and also to highlight the importance of piscivorous birds in parasite transmission.

CHAPTER 2

THE OCCURRENCE OF *CONTRACAECUM* SPECIES (NEMATODA: ANISAKIDAE) IN CATFISH, *CLARIAS GARIEPINUS*, AND FISH-EATING BIRDS FROM LAKE CHIVERO, ZIMBABWE.

2.1. INTRODUCTION

Nematodes are considered to be amongst the most economically important parasites of fishes (Dick and Choudhury, 1995). According to Paperna (1974, 1996), larval *Contracaecum* and *Eustrongylides*, and adult *Anguillicola* are the most important fish parasites in the tropics. The fact that larval nematodes can form cysts in the flesh and musculature of fish reduces its quality and results in economic losses to the food industry because the displeasing appearance of larval parasites in fish may lead to widespread rejection by consumers (Paperna, 1974). Where raw or improperly cooked infected fish are eaten, these nematodes may infect humans, raising public health concerns. In fish, high nematode loads have been shown to cause anaemia and emaciation, eventually killing the fish (Ferguson, 1989; Branson and Southgate, 1992; Roberts, 2001).

In Africa, more and more parasitologists are becoming interested in the helminth fauna of fish (Table 1.1). From Zimbabwe, Khalil and Polling (1997) recorded only *Contracaecum* species on the basis of the work done at Lake Kariba by Chishawa (1991) and Douëllou (1992a, b). In all of these publications, the larvae of the genus *Contracaecum* were reported from the body cavities and intestines of siluriform and cichlid fishes as well as the tigerfish, *Hydrocynus vittatus* (Castelnau) and the Cornish Jack *Mormyrops anguilloides* (Peters).

Contracaecum is an ascaridoid nematode genus belonging to the family Anisakidae and they are parasites of aquatic birds and mammals (Anderson, 1992). Its larval stages usually occur in the body cavity and mesenteries of fish while the adults occur in the gut of piscivorous birds, notably pelicans, cormorants, herons and darters (Mokgalong, 1996; Suter, 1998) as well as mammals such as otters, whales and seals. The occurrence of the parasite in cichlids and catfish has been widely reported from several African countries such as Egypt (Amin, 1978), East Africa (Malvestuto and Ogambo-Ongoma, 1978; Aloo, 2001), and South Africa (Prudhoe and Hussey, 1977; Mashego and Saayman, 1981; Boomker, 1982, 1994a, b; van As and Basson, 1984).

Huizinga (1967) successfully demonstrated the life cycle of *C. multipapillatum* (Von Drasche, 1882) Lucker, 1941. Eggs from adult female worms are shed into the water via the faeces of a definitive host, and they develop into second-stage larvae, which subsequently infect freshwater cyclopoid copepods. When the copepods are eaten by fish, the worms develop into third-stage larvae (L3). Adult nematodes will only develop in the gut of a definitive host after it eats an infected fish, thus completing the life cycle.

Although the ecology of fish-eating birds has been studied in Zimbabwe, no published work on their parasites is available, the only exception being two dead cormorants found on Lake Chivero in 1995, which were examined at the Government Veterinary Laboratory and found to be infected with nematodes of the genus *Spiroptera* (renamed *Acuaria*) with a mean intensity of 32 per bird (range = 26-38) (Couto, personal communication). While there are 26 species of aquatic birds representing nine families on the Middle Letaba Dam in South Africa (Olivier *et al.*, 1991), when 16 of them were examined for parasites, only five species (2 cormorants, the Darter, and 2

herons) were found to be infected by *Contracaecum* (Saayman *et al.*, 1991; Mokgalong, 1996). Six species of *Contracaecum* have so far been identified from South African birds while one is yet to be classified (Mokgalong, 1996).

The sharptooth catfish, *Clarias gariepinus* Burchell, 1822, was selected for this investigation because of its wide distribution in Zimbabwe and throughout sub-Saharan Africa (Roberts, 1975; Skelton, 1993). The fish is known to survive adverse conditions such as muddy water and extremely low oxygen concentrations (Roberts, 1975). Because it is omnivorous, *C. gariepinus* is usually more heavily parasitized than most fish species (Chishawa, 1991); it also used to be one of the dominant species in Lake Chivero (Munro, 1967). Four bird species, representing three families, were also selected for this investigation, namely, the Reed Cormorant *Phalacrocorax africanus*, the White-breasted Cormorant *P. carbo*, the Darter *Anhinga melanogaster* and the Grey Heron *Ardea cinerea* because they were abundant on the lake.

The aims of this investigation were to determine the occurrence of larval *Contracaecum* spp. in *C. gariepinus,* and of adults in fish-eating birds from Lake Chivero, Zimbabwe, and to note its seasonal distribution, infection rate, variation with host size and sex, and the effect of the parasite on the health of the host and, if possible, to confirm the life cycle of the parasite.

2.2. MATERIALS AND METHODS

2.2.1. Collection of fish and their parasites

Fish were collected once a month for 18 months (November 2000 – April 2002) using a fleet of 8-10 cotton gill nets. The standard length, weight and sex of each fish were recorded and every

fish was then either examined immediately for parasites or frozen for later examination in the laboratory.

In the laboratory the entire digestive tract, together with the liver, spleen and gills of each fish was removed and preserved in 10% formalin. The body cavity was examined macroscopically for nematode larvae and cysts, which were then excised and fixed in glacial acetic acid before being preserved in 70% ethyl alcohol. The larvae were then stained with Horein's trichome stain, cleared in lactophenol and permanently mounted in lactophenol or Canada balsam. The preserved organs were examined for nematode larvae or cysts with the aid of an Olympus SZ 40 stereomicroscope. The worms were identified to generic level using the keys of Bykhovskaya-Pavlovskaya *et al.* (1964), Hartwich (1974) and Yamaguti (1960) based on head profile and oesophageal morphology under an Olympus CK 40 Culture Microscope.

The rate of infection was determined as the prevalence (% infected) and the mean intensity (average number of parasites per fish) for each month (Margolis *et al.,* 1982; Gregory and Blackburn, 1991). The effect of the larval nematode parasites on the health of their host was determined by calculating the condition factor (K) of the host, in which

$$K = \frac{(W \times 100)}{SL^3}$$

where W = weight (g), and SL = standard length (cm) (Bagenal and Tesch, 1978)

2.2.2 Collection of birds and their parasites

Four Reed Cormorants, four White-breasted Cormorants, four Darters and four Grey Herons were collected in Lake Chivero by shooting them with a 0.22 rifle or a 12-bore shotgun firing buckshot. Their beaks were immediately sealed with rubber bands to prevent the escape of clinostomes that are usually lodged in the buccal cavity (Mokgalong, 1996) and taken to the laboratory where they were weighed and dissected. The alimentary canal was removed and opened from the pharynx to the rectum. Undigested and partly digested fish encountered in the oesophagus and stomach were removed, identified to species level where possible, and weighed.

Nematodes found either in the oesophagus or attached to the stomach mucosa were removed, fixed in glacial acetic acid and stored in 70% ethyl alcohol. Sub-samples of nematodes from each infected bird were set aside for electron microscopy. The remainder were cleared and mounted in lactophenol and the cover slides were sealed with clear nail varnish. The structures of the nematodes were measured under a compound microscope using a graticule eyepiece.

Specimens selected for scanning electron microscopy (SEM) were examined in the Microscopy and Imaging Centre at the University of the North, South Africa. The worms, preserved in 70% alcohol, were first cleaned three times with a Branson 3200 ultrasonic cleaner for about 4 seconds to remove debris from the cuticle. They were then dehydrated in an ascending alcohol series (80%, 90%, industrial methanol and absolute ethanol) after which they were dried in a critical point drier. They were then mounted on stubs and coated for 40 minutes with gold particles, after which they were mounted and examined under a Jeol 6100 Scanning Microscope.

2.3. RESULTS

2.3.1. Nematode parasites of fish

Third-stage larvae of *Contracaecum* sp. were found usually encapsulated in the mesenteries and visceral cavities of *C. gariepinus* and there was no evidence that they had infected any other internal organs. Of the 202 specimens of *C. gariepinus* collected over a period of 19 months, 86 (42.6%) were infected with L3 larvae of *Contracaeum* with mean and maximum intensities of 2.2 and 7 worms per fish, respectively. There was no substantive evidence of seasonal variation in the prevalence of *Contracaecum* in *Clarias* (Table 2.1) nor was the condition of the fish affected by the presence of this parasite (Fig. 2.1). Although more worms were recovered from female fish there was no significant difference in the prevalence of infection between the sexes ($\chi^2 = 2.228$; $p > 0.05$, $df = 0$) (Table 2.2) and there was no relation between the size of the host (standard length) and the rate of infection in males and females (Fig. 2.2).

1.3.2 Nematode parasites of birds

All of the cormorants and darters were infected with at least two nematodes in their alimentary canal giving a parasite prevalence of 100% (Table 2.3). The intensity was greatest in the Darter with a mean intensity (MI) of 30 worms per bird, compared to 18.75 in the Reed Cormorants and 6.5 in the White-breasted Cormorant. The infection rate was low in the Grey Heron, with one bird being infected. The nematodes that could be identified were *Contracaecum microcephalum* (Rudolphi, 1809), *C. rudolphii* (Hartwich, 1964) and *C. carlislei* (Ortlepp, 1938) which were all found in the two cormorant species. *Contracaecum tricuspe* (Gedolst, 1916) was taken from the darters but the species taken from the Grey Heron

could not be identified. The Zambezi bream, *Pharyngochromis acuticeps* (Staindachner), and the Nile bream, *Oreochromis niloticus* (L.), were the most abundant species in the diet of the birds (Table 2.5). Darters and herons preferred the latter while cormorants were not very specific.

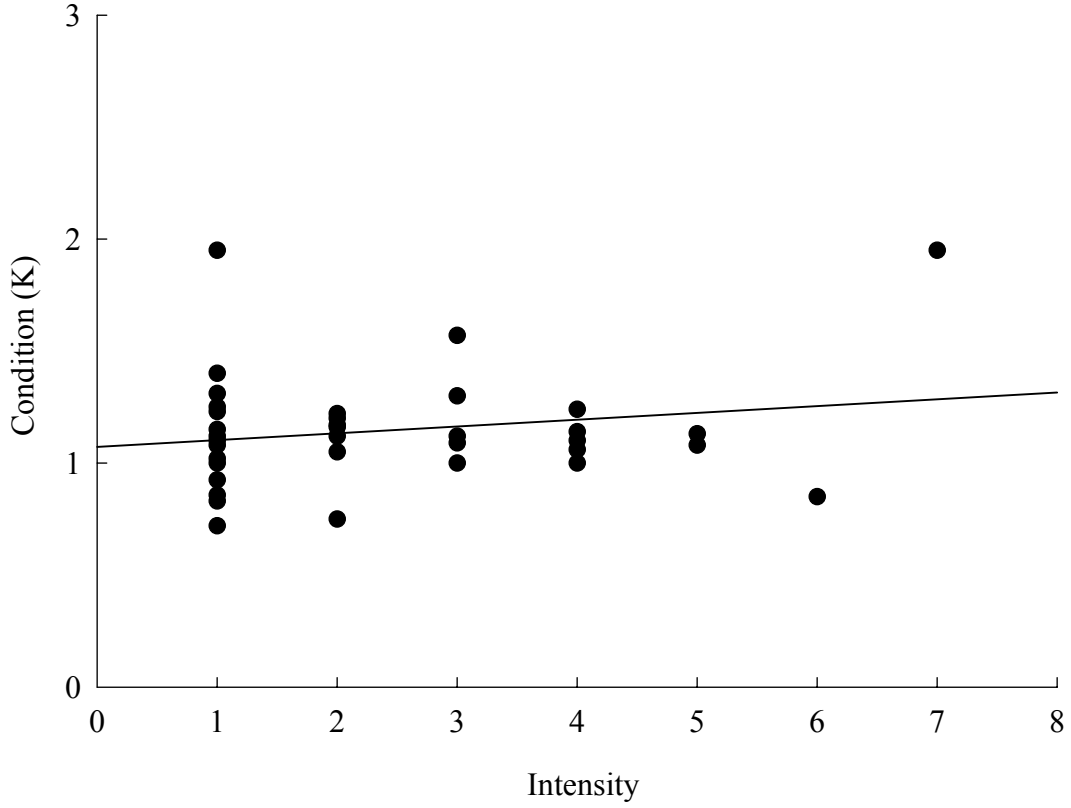
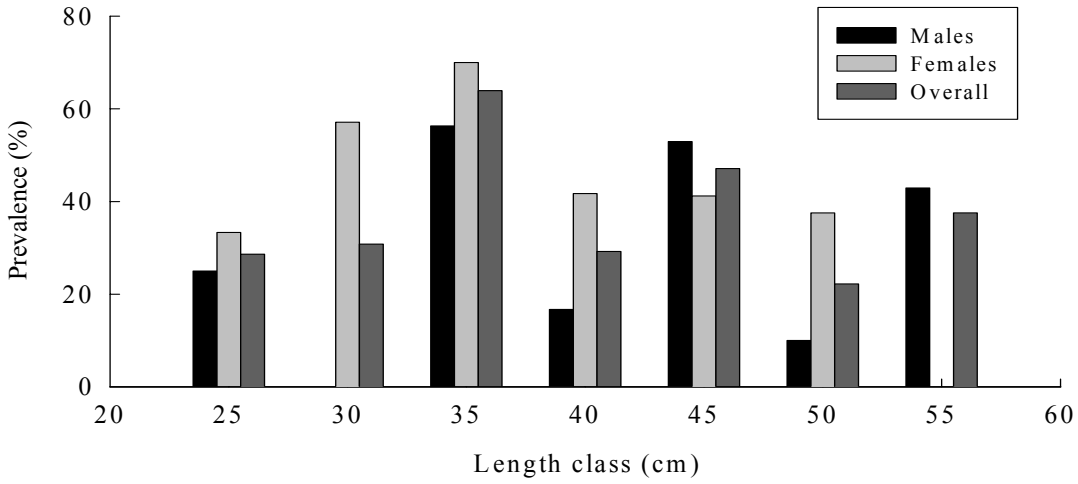


Fig. 2.1. The relationship between intensity and the condition factor (K) of *C. gariepinus* in Lake Chivero ($y = 1.0722 + 0.0303\,x$, $r = 0.19$, $p > 0.05$)

Table 2.1. The prevalence (% infected) of *Contracaecum* larvae in *C. gariepinus* from Lake Chivero (November 2000 – May 2002). N = number of fish sampled.

	Female		Males		Total	
	N	%	N	%	N	%
Nov 2000	8	12.5	4	0.0	12	8.3
Dec 200	7	0.0	3	33.3	10	10.0
Jan2001	6	33.3	4	0.0	10	40.0
Feb 2001	6	0.0	4	0.0	10	0.0
Mar 2001	5	0.0	3	0.0	8	0.0
Apr 2001	8	50.0	2	0.0	10	40.0
May 2001	8	62.5	3	66.7	11	63.6
Jun 2001	3	0.0	4	0.0	7	0.0
Jul 2001	1	0.0	2	0.0	3	0.0
Aug 2001	6	0.0	4	0.0	10	0.0
Sep 2001	6	0.0	4	0.0	10	0.0
Oct 2001	3	0.0	4	25.0	7	28.6
Nov 2001	6	33.3	10	20.0	16	31.3
Dec 2001	4	50.0	9	30.0	13	38.5
Jan 2002	5	60.0	4	75.0	9	66.7
Feb 2002	-	-	-	-	-	-
Mar 2002	10	60.0	10	30.0	20	45.0
Apr 2002	3	66.7	13	53.8	16	56.3
May 2002	10	70.0	10	30.0	20	50.0
Total (N)/ Mean (%)	105	43.8	97	30.9	202	42.6

(a)



(b)

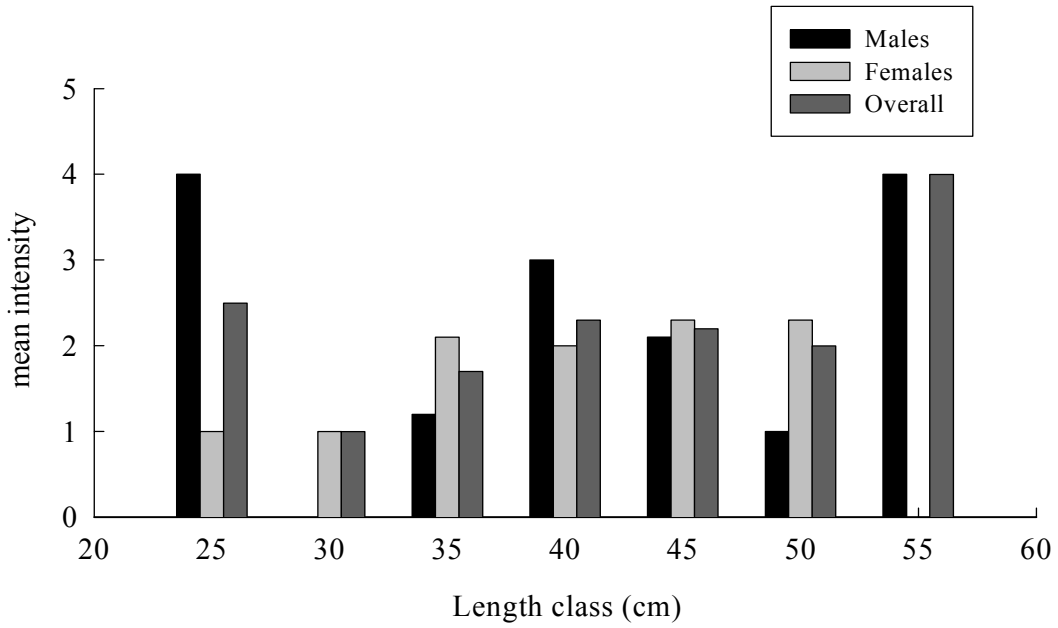


Fig. 2.2. The variation in (a) prevalence and (b) mean intensity of *Contracaecum* infection of *C. gariepinus* of different size classes and sexes.

Table 2.2. The infection statistics of *Contracaecum* species found in the alimentary canal of four species of piscivorous birds at Lake Chivero. MI = mean intensity, P = prevalence.

Bird species	Sex	Weight (g)	Parasites in oesophagus	Parasites in stomach	Intensity	MI (per species)	P (% - per species)
Phalacrocorax africanus	F	518	8	16	24	18.75	100
	F	551	0	22	22		
	M	552	0	11	11		
	M	619	2	16	18		
P. carbo	F	2065	3	1	4	6.50	100
	M	2525	3	7	10		
	M	2200	4	0	4		
	M	2225	0	8	8		
Anhinga melanogaster	F	1265	0	4	4	30.00	100
	F	1375	11	31	42		
	F	1590	3	53	56		
	M	1205	1	17	18		
Ardea cinerea	F	1775	0	0	0	0.50	25
	F	1635	0	0	0		
	F	1260	1	1	2		
	M	1430	0	0	0		

Table 2.3. The diet of four bird species collected from Lake Chivero. *Pa = Pharyngochromis acuticeps, On = Oreochromis niloticus, Cc = Cyprinus carpio*, uc = unidentified cichlids, ND = not determined.

Bird species	Sex	Weight (g)	Mass of gut contents (g)	Fish sp. in diet	No. of fish of each sp.	Mass of fish in diet (g)	Mean mass of fish in diet (g)
P. africanus	F	518	ND	*Pa*	2	ND	ND
	F	551	41	*On*	7	29	4.14
				uc	1	3	3.00
	M	552	ND	uc	16	ND	ND
	M	619	ND	*On*	5	ND	ND
P. carbo	F	2065	85	*On*	5	37	7.40
				uc	2	5	2.50
				Pa	1	7	7.00
	M	2525	ND	*Pa*	4	ND	ND
	M	2200	56	*On*	3	15	5.00
				Cc	1	5	5.00
				Pa	2	15	7.50
				uc	1	4	4.00
	M	2220	119	*On*	1	48	48.00
				Pa	2	8	4.00
				uc	2	10	5.00
A. melanogaster	F	1265	147	*On*	5	ND	ND
	F	1375	ND	*On*	2	ND	ND
	F	1590	ND	*On*	7	127	18.14
				uc	1	5	5.00
	M	1205	71	*On*	6	56	9.33
A. cinerea	F	1775	ND	uc	16	ND	ND
	F	1635	114	*On*	2	109	54.5
	F	1260	ND	*On*	1	8	8.00
	M	1430	ND	-	0	ND	ND

2.4. DISCUSSION

Contracaecum larvae could not be identified to species level because the reproductive organs of the worms, which are used to separate the species, were not developed. The lack of any seasonal pattern in the infection rate of *Contracaecum* in *C. gariepinus* is consistent with the findings of other workers (Mashego & Saayman, 1981; Aloo 2001). The monthly host sample sizes were usually small (mean = 11.2), and during some months, prevalence values of zero were recorded which, as Gregory & Blackburn (1991) point out, could indicate a low prevalence that can only be detected in larger samples.

The low prevalence of *Contracaecum* during the winter months may reflect lower feeding activity of the fish because of the lower temperatures, thus reducing the chances of infection via copepods. *Contracaecum* eggs hatch in water with an optimum temperature of 21°C (Huizinga 1967), and the fact that the winter temperatures of the lake fall to <16ºC in winter (Marshall and Falconer, 1973) might also account for the low parasite prevalence at this time.

The highest intensity of *Contracaecum* in *C. gariepinus* was only seven worms per and the fish from Lake Chivero were not heavily infested compared to catfish from other dams in southern Africa (Table 2.4). Their condition was not affected by the presence of these parasites, which suggests that they have little effect on the health at low intensities. In natural environments, this is expected since parasites are normally in equilibrium with their hosts (Paperna 1996), which ensures that the parasite reaches its final host and completes its life cycle. Even very heavy infestations of *Contracaecum* have not affected the condition of the host (Mashego & Saayman, 1981; Aloo 1999, 2001). In Lake Naivasha, Kenya, the prevalence was higher in females than

males (Aloo 1999), but this was not the case in Lake Chivero (this study) or elsewhere (Aloo 2001; Mashego 1989).

Hartbeespoort Dam in South Africa is hypertrophic (National Institute of Water Research 1985), and high parasitic loads have been recorded from catfish there (Boomker 1982 but although Lake Chivero is also hypertrophic the parasite prevalence and intensity was low. This suggests that trophic state alone is not important in parasite infections and the reasons why the fish in Lake Chivero were not heavily parasitized should be sought elsewhere. It is possible that the infection cycle might be affected at some stage if piscivorous birds are unable to catch fish. In Lake Chivero extensive mats of *Eichhornia crassipes* (water hyacinth) and *Hydrocotyle ranunculoides* (spaghetti weed) extend from the shore and form a barrier to wading species like herons. They probably also provide shelter for smaller fish and make it harder for diving species like cormorants and darters to catch them.

The low level of parasitism may also reflect sampling bias. The gill nets used for sampling selected large fish (>200g) but most piscivorous birds, with the exception of Fish Eagles, prefer small-sized prey (<100g) (Hustler 1995) and the fish taken from the stomachs of the birds were small (2.5g - 48g) (Table 2.5). Furthermore, Reed Cormorants and Darters, which are abundant on Lake Chivero mainly prey on cichlids (Donnelly & Hustler, 1986) and rarely on *Clarias*.

Cormorants and darters from Lake Chivero were all infected by adult *Contracaecum* species but only one Grey Heron had a larval *Contracaecum* species in its gut. Cormorants seem to be

the most important definitive hosts of the nematode and a 91-100% prevalence of *C. rudolphii* in the European Cormorant, *Phalacrocorax carbo,* has been recorded (Suter, 1998). In southern Africa, the prevalence of different *Contracaecum* species in *P. carbo, P. africanus* and *A. melanogaster* ranged from 45-100% (Mokgalong, 1996; Saayman *et al.,* 1991).

Table 2.4. The prevalence and intensity of *Contracaecum* larvae in *C. gariepinus* from Lake Chivero in comparison with other dams and in southern Africa.

Locality	Prevalence (%)	Intensity	Source
Bufeldoon Dam, S. Africa	100.0	1-2860	Mashego and Saayman, 1981
Hartbeespoort Dam, S. Africa	95.3	53-775	Boomker, 1982
Seshego Dam, S. Africa	93.0	1-698	Mashego and Saayman, 1981
Piet Gouws Dam, S. Africa	78.0	1-69	"
Coetzeesdraai Dam, S. Africa	72.0	1-336	"
Krokodilsheuwel Dam, S. Africa	67.0	1-61	"
Lake St. Lucia, S. Africa	46.0	-	Whitfield and Heeg, 1977
Lake Chivero, Zimbabwe	**42.6**	**1-7**	**Present study**
Lepellane Dam, S. Africa	31.0	1-96	Mashego and Saayman, 1981
Namakgale Dam, S. Africa	25.0	1-2	"
Lake Kariba, Zimbabwe	22.0	1-3	Chishawa, 1991

Some species of *Contracaecum* seem to be host-specific. For example, *C. tricuspis* was found only in Darters from Lake Chivero with all specimens being infected which was consistent with the findings of Saayman *et al.* (1991) and Mokgalong (1996) in South Africa. Data from Zimbabwe and South Africa indicate a possible regional distribution of the species. *Contracaecum carlislei* was first described by Ortlepp in 1938 from the stomach and oesophagus of *Phalacrocorax africanus* (Saayman *et al.*, 1991; Mokgalong, 1996) and was thought to be indigenous to South Africa only, but it was taken in both species of cormorants in Lake Chivero together with the cosmopolitan *C. rudolphii*. As it has not been found in other parts of the continent, it is possible that *C. carlislei* is restricted to the southern African region, although this assumption can only be validated by a greater sampling effort from many African countries.

In contrast, *C. rudolphii* is one of the most widely distributed species in piscivorous birds worldwide. In Africa, it has been reported from cormorants and pelicans in Lake Naivasha, Kenya (Malvestuto and Ogambo-Ongoma, 1978) and Lake St Lucia, South Africa (Whitfield and Heeg, 1977). In Europe, it has been reported in cormorants from in Switzerland and Italy (Suter, 1998; Volponi, 1999). Although Saayman *et al.* (1991) and Mokgalong (1996) also found *C. rudolphii* in darters; those from Lake Chivero were not infected by this parasite.

Contracaecum microcephalum, found in the White-breasted Cormorant and the Darter has a worldwide distribution and a wide range of hosts among most families of piscivorous birds (Yamaguti, 1961). It was interesting to note that, while the Grey Heron is one of the major hosts of *Contracaecum* species (Prudhoe and Hussey, 1977; Mokgalong, 1996), it was the least

infected of the species examined during this study, with only one immature nematode recovered from one bird.

The stomach contents of birds from Lake Chivero suggest that cichlid fishes, mainly *O. niloticus* and *Pharyngochromis acuticeps*, are intermediate hosts of *Contracaecum*. It was unfortunate that these fish species were not examined during this study, but some specimens recovered from the guts of the birds had nematodes coming out from their flesh. These juvenile worms in fish tissues are usually encysted and only emerge in response to enzymatic activation in the alimentary canal of the bird (Paperna, 1996). Douëllou (1992a, b) found *Contracaecum* larvae in the body cavity of *Tilapia rendalli* (Boulenger), *Sargochromis codringtonii* (Boulenger) and *Serranochromis macrocephalus* (Boulenger)

Clarias gariepinus were not found in any of the birds, although they will eat these fish if the opportunity arises. The only bird species around Lake Chivero which might feed regularly on large fish such as *Clarias* is the African Fish Eagle, *Haliaetus vocifer* (Daudin), (Whitfield and Heeg, 1977). Unfortunately, this bird could not be sampled from Lake Chivero because its numbers are low and it is a protected species (Couto, personal communication).

The case of two cormorants found dead on Lake Chivero in 1995 is the only instance in Zimbabwe where the pathology of parasitic infestation in birds is discussed. According to the Government Veterinary Laboratory Report (18 October 1995; supplied by T. Couto, personal communication), the cormorants were subjected to pathological examination and found to be in "… moderate condition; stomachs empty; generalized inflammation; nematodes in intestine; A+B

focal necrosis of intestines; and granulomas in livers". The proventriculus of the first bird had 38 *Spiroptera* adults and the other had 26 *Spiroptera* and an egg count constituting 280 *Capillaria carbonis* (Nematoda) eggs per gram (epg) of faeces, and 780 *Spiroptera* epg. The nematode genus *Spiroptera* has been renamed *Acuaria* (Spiruridae: Acuarioidea), is normally a parasite of terrestrial birds and is usually found in the gizzard (Chabaud, 1974; Anderson, 1992). This parasite has not previously been documented from fish or any other fish-eating bird species and it would be worthwhile to re-examine the specimens, if available, just to dispel any doubts of misidentification.

CHAPTER 3

TAPEWORMS OF FISH: THE OCCURRENCE OF *LIGULA INTESTINALIS* IN *BARBUS PALUDINOSUS* AND PROTEOCEPHALIDS IN *CLARIAS GARIEPINUS*

3.1. INTRODUCTION

Together with the nematodes and flukes (Trematoda), tapeworms (Class: Cestoda) are some of the most important helminth parasites of fish (Roberts & Janovy, 2000). In Zimbabwe, very little is known about tapeworms infecting fish. Most of the available data were collected by Douëllou (1992a, b) from Lake Kariba, where five species of cestodes were found in the intestines and body cavities of nine fish species. The tapeworms included *Ligula intestinalis* (L.) (Cestoda: Pseudophyllidea) which mostly infects cyprinid fish and *Proteocephalus* sp. Weinland, 1858, which mostly infects bass and catfish (Mashego, 1977).

The only records of *L. intestinalis* larvae in Zimbabwean fish are of some taken from the body cavity of *Barbus fasciolatus* Günther from Lake Kariba (Douëllou, 1992a), and from the body cavity of *B. paludinosus* and *B. lineomaculatus* caught near Harare (Mettrick, 1960). It has also been found in *Mesobola brevianalis* (Boulenger) from the Runde River in south-eastern Zimbabwe and in *B. paludinosus* from Udu Dam, Nyanga (B.E. Marshall, personal communication). Chishawa (1991) recorded three individual ligulids in the body cavity of one catfish, *Clarias gariepinus* from Lake Kariba. In Zimbabwe, proteocephalid tapeworms have only been recorded from the intestines of *C. gariepinus* from Lake Kariba (Chishawa, 1991; Douëllou, 1992a), and even these were not identified to generic level. Van As and Basson (1984), Mashego

and Saayman (1989), and Khalil and Polling (1997) all recorded only one species, *Proteocephalus glanduliger* among specimens collected and described by Mashego (1977, 2001). No other record of proteocephalids in southern African fishes is available. Elsewhere in Africa, 12 species of *Proteocephalus* found in eight fish species have been described and recorded by Khalil (1963, 1973), Khalil and Thurston (1973), Paperna (1996), and Khalil and Polling (1997).

Ligula intestinalis is a cosmopolitan tapeworm that infects fish-eating birds, notably cormorants, darters and herons (Prudhoe and Hussey, 1977; Mokgalong, 1996). The eggs of the parasite are shed in the faeces of the birds and hatch in water to form infective coracidia which are ingested by cyclopoid and calanoid copepods. The coracidia escape the gut of the copepod and develop into procercoids in the haemocoel. Upon feeding on infected copepods cyprinid fishes become the second intermediate hosts of the tapeworm where it transforms into the second-stage larva or plerocercoid. Plerocercoids of *Ligula* have been studied extensively and cause a number of pathological conditions in fish (Prudhoe and Hussey, 1977; Sweeting, 1977; Bean and Winfield, 1989; Mashego and Saayman, 1989; Mokgalong, 1996; Paperna, 1996; Barber, 2003).

In Africa, *L. intestinalis* infections have been recorded in fish from the following countries: Democratic Republic of Congo (Khalil & Polling, 1997), Ethiopia (Dejen, 2003), Tanzania (Wanink, 1992; Marshall and Cowx, 2003), South Africa (Mashego, 1982; Mashego and Saayman, 1989) and Uganda (Marshall and Cowx, 2003). Aspects of the epidemiology, pathology and behaviour of *Ligula* have been studied in developed countries such as Canada (Szalai *et al.*, 1989), France (Loot *et al.*, 2001), Northern Ireland (Bean and Winfield, 1989), and the United Kingdom (Sweeting, 1977; Wyatt and Kennedy, 1989). Barber (2003) reviewed literature and case studies of the role of *Ligula* in fish-bird interactions.

The main objective of this study was to find out which cestodes parasitize *B. paludinosus* and *C. gariepinus* in some water bodies in the Manyame Catchment, Zimbabwe. These two fish species are ubiquitous in the country and in the southern African region (Skelton, 1993) and are relatively tolerant of pollution extreme conditions such as muddy waters and anoxic environments.

3.2. MATERIALS AND METHODS

3.2.1. Fish Sampling

Specimens of *C. gariepinus* and *B. paludinosus* were collected on monthly basis using 8mm, 10mm and 12mm nylon monofilament nets, multifilament cotton nets (14mm – 58mm mesh size), and fyke nets in the upper Munwahuku Reservoir from July 2000 to July 2001. During the dry season (October 2000 to January 2001), only fyke nets were used for fishing because the water level was too low and the dam too shallow for gill nets. *Clarias gariepinus and B. paludinosus* were also collected from Lake Chivero using cotton gill nets (November 2000 – March 2002), and from the Marimba River by electrofishing (November 2001 – May 2002) using a Smith-Root 5kW Type VI-A Electrofisher.

Zooplankton samples were collected monthly from the Munwahuku Dam (January – July 2001) and the Marimba River (March – May 2002) using 64μm-mesh plankton nets, and were fixed in 10% formalin solution. Each sample was condensed to 1cm^3 prior to enumeration and identification. In the laboratory, the zooplankton were examined under an Olympus CK 40 microscope and identified using the taxonomic keys of Harding and Smith (1974) and Rayner (2001). Their population densities were calculated and then compared with those of zooplankton from Lake Chivero (Elenbaas and Grundel, 1994).

3.2.2. Collection of Parasites

Barbus paludinosus were killed immediately after collection and examined macroscopically for ectoparasites, after which the standard lengths, weight and sex of each fish were recorded. Each fish was opened ventrally and the body cavity was searched for plerocercoids of *Ligula intestinalis* and the weight of each plerocercoid was recorded. The worms were then killed in warm water (60°C) and fixed in 10% formalin for 24h before being transferred to 70% ethyl alcohol for permanent preservation.

The parasite index (PI) for each parasitized fish was calculated using the formula:

$$PI \equiv \frac{W_p \times 100}{W_h - W_p}$$

where W_p = weight of parasite (g) and W_h = weight of host (g) (Kennedy and Burrough, 1981; Bean and Winfield, 1989). The condition of the fish was determined by Fulton's condition factor (K), which was calculated using the formula:

$$K = \frac{(W \times 100)}{SL^3}$$

where W = weight (g), and SL = standard length (cm) (Bagenal and Tesch, 1978)

Samples of *C. gariepinus* were examined in the same way, but the stomachs and intestines were examined immediately for proteocephalid tapeworms. After removal from the fish, the worms were swirled in 10% sodium chloride solution to relax the scolices, and then fixed in hot alcohol-formal-acetate (AFA) for 10 minutes, after which they were stored in 70% ethyl alcohol. Before preparing whole mounts of *Proteocephalus*, the specimens were brought down to water through

descending grades of alcohol (70%, 50%, and 30%) and finally washed in distilled water. The worms were then stained for 20 minutes in aceto-alum-carmine solution. An aqueous solution of hydrochloric acid (2% HCl) was used for de-staining over-stained specimens, while an aqueous alkaline solution (1% KOH) was used for differentiation. After staining, the specimens were dehydrated in an ascending series of alcohol (30% up to two changes of 100%). Clearing was done in xylene and the specimens were finally mounted in Canada balsam (Schmidt, 1986). Keys by Yamaguti (1959), Schmidt (1986) and Rego (1994) were used to identify the parasites.

3.3. RESULTS

3.3.1. *Ligula intestinalis*

More than 2,000 specimens of *Barbus paludinosus* were collected over a period of 13 months from the upper Munwahuku Dam, while only 96 and 108 specimens were collected from Lake Chivero and the Marimba River respectively (Table 3.1). A sub-sample of 885 barbs caught with fyke nets in the Munwahuku Dam was examined for tapeworms, of which 64 (or 7.23%) were infected with *L. intestinalis* in the body cavity (Table 3.1). Only one of these fish (1.6%) harboured three tapeworms, two (3.1%) had two tapeworms each, and the remainder had only one parasite in the body cavity. There were no cestode parasites in any of the *C. gariepinus* from the dam. No tapeworms were found in any of the fish sampled from the Marimba River (Table 3.1).

There was no obvious pattern of seasonal variation in the prevalence of *L. intestinalis* during the sampling period (Figs 3.1). The prevalence was high (21.3 – 46%) between July and September

2000, after which it was low (<6%) throughout the remainder of the year. A prevalence of zero was recorded in October 2000, December 2000 and July 2001.

The abundance of copepods in Munwahuku Dam increased gradually from 1.1 x 10^3 m^{-3} in July 2000 to a peak of 84.9x 10^3 m^{-3} in January 2001, after which it decreased to about 2.5 x 10^3 m^{-3} in June 2001 (Fig. 3.1). In comparison, the numbers of copepods in the Marimba River was very low while the population in the Munwahuku Dam was only slightly lower than published estimates for Lake Chivero (Table 3.2).

Table 3.1. The occurrence and prevalence of cestode parasites in *Barbus paludinosus* and *Clarias gariepinus* from Munwahuku Dam, Lake Chivero and the Marimba River. P = prevalence (%), I = intensity, MI = mean intensity.

Sampling site	Host	N	Tapeworm species	No. parasitized	P (%)	I	MI
Munwahuku Dam	*B. paludinosus*	885	*Ligula intestinalis*	64	7.2	1-3	1.06
	C. gariepinus	130	-	0	0	0	0
Lake Chivero	*B. paludinosus*	96	-	0	0	0	0
	C. gariepinus	99	*Proteocephalus* sp.	7	7.1	1-14	5.57
Marimba River	*B. paludinosus*	108	-	0	0	0	0
	C. gariepinus	42	-	0	0	0	0

Table 3.2. The mean abundance (numbers x 10^3 m^{-3}) of copepods in Lake Chivero (from Elenbaas and Grundel, 1994), the Marimba River and the Munwahuku Dam.

Location	Range	Mean	Standard deviation
Marimba River	0.002-0.069	0.025	± 0.015
Munwahuku Dam	1.12-84.93	28.367	± 27.408
Lake Chivero	-	33.4	-

There was a positive correlation between the weight of *Ligula* and the weight of the host (Fig. 3.3), but there was no significant relationship between the condition factor of the fish and the weight of the parasite (Fig. 3.4)

3.3.2. *Proteocephalus* species

Of the 99 catfish collected from the lake, only seven (or 7.1%) contained adult *Proteocephalus* in their anterior intestine (Table 3.1). The largest parasite was 7.8cm long and 4.2mm wide while the smallest was 1.8cm and 2.2mm. Fourteen proteocephalids were found in the most heavily infested fish and the mean intensity of *Proteocephalus* infection was 5.57 per fish (Table 3.1).

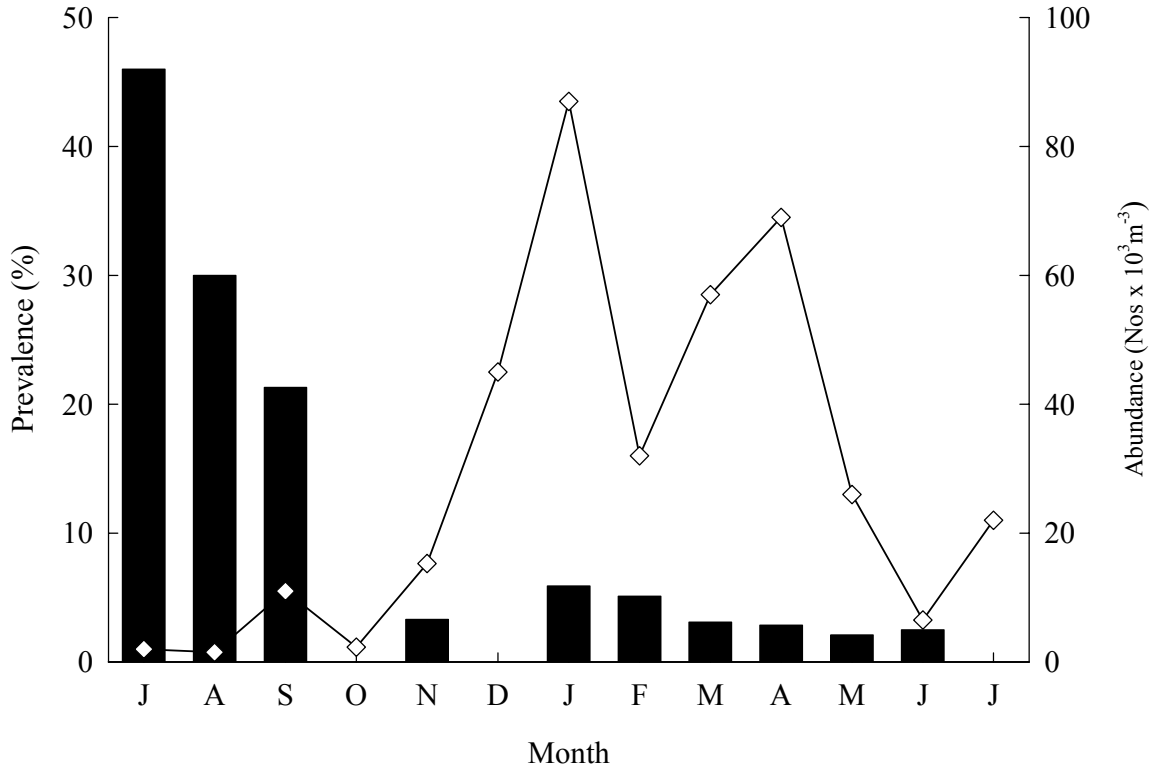


Fig. 3.1. The monthly prevalence of *Ligula intestinalis* in *Barbus paludinosus* from Munwahuku Dam (vertical bars), and the abundance of copepods (calanoids + cyclopoids) collected from the dam between July 2000 and July 2001 (line plot).

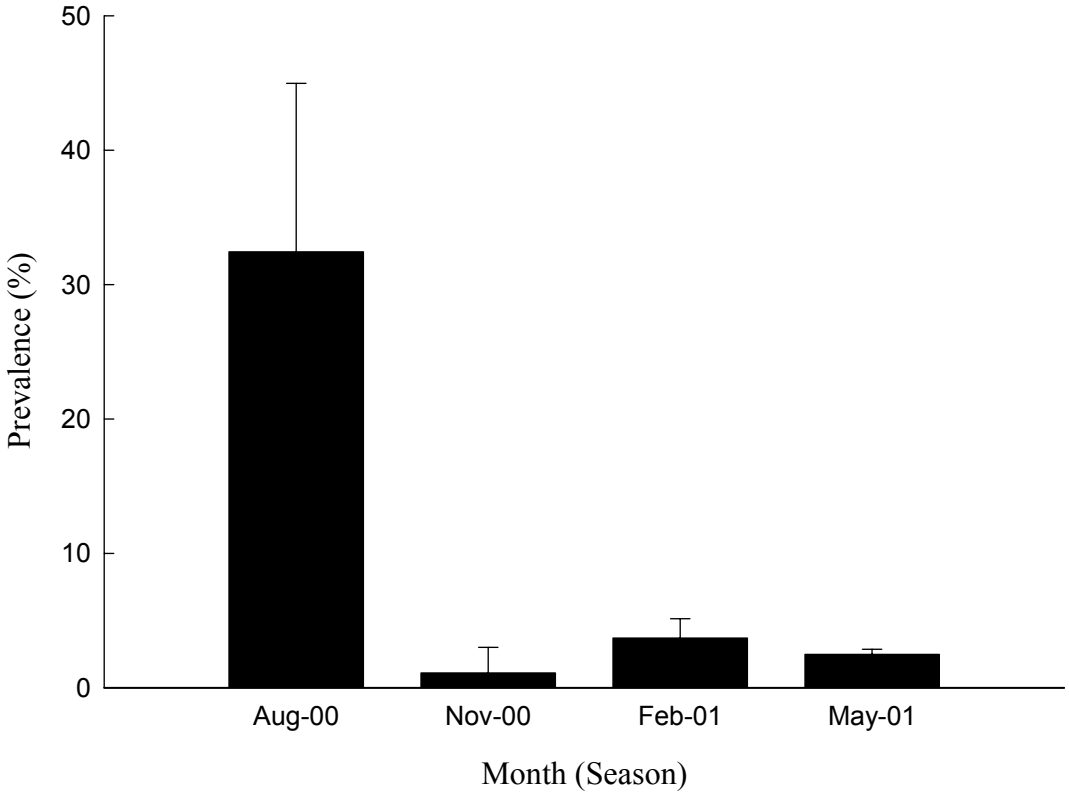


Fig. 3.2. The seasonal prevalence of *Ligula intestinalis* in *Barbus paludinosus* from Munwahuku Dam. Each Season is represented by the median month.

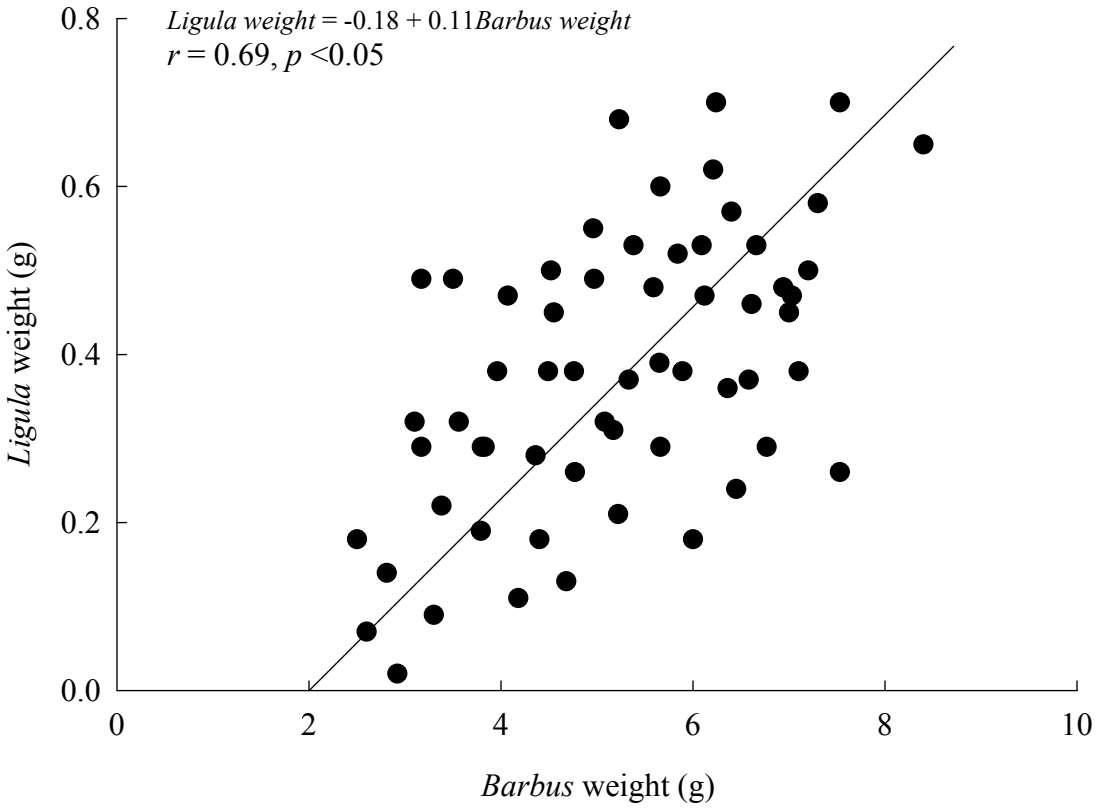


Fig. 3.3. The relationship between the weight of *Barbus paludinosus* and the weight of *Ligula intestinalis* from the Munwahuku Dam. Dotted lines indicate the 95% confidence limits

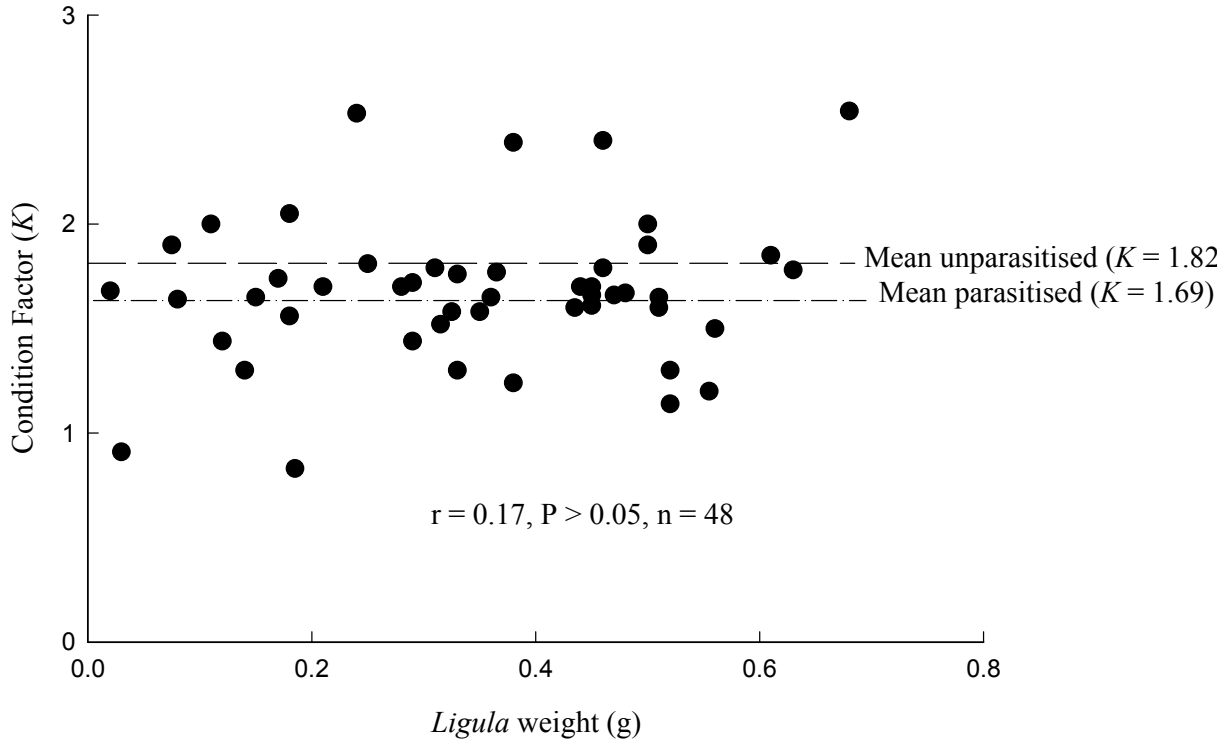


Fig. 3.4. The relationship between the weight of *Ligula intestinalis* and the condition factor (*K*). of *Barbus paludinosus* from the Munwahuku Dam.

3.4. DISCUSSION

Ligula intestinalis was the only species of tapeworm found in *Barbus paludinosus* and was recorded only in fish from Munwahuku Dam, being absent in those from Lake Chivero and the Marimba River. Fish are infected by tapeworms via copepods which are the intermediate hosts and since copepods are generally more numerous in standing rather than running waters (Wetzel, 2001) it is to be expected that fish from dams will be more heavily parasitized than those from rivers. The absence of cestode parasites in fish from Marimba River can probably, therefore, be attributed to the low density of copepods in the river. Using this argument, a higher rate of *Ligula* infection would be expected in *Barbus* from Lake Chivero, than in the Munwahuku Dam This was not the case, however, and the reasons why are not immediately obvious. The low intensity of parasitism may result from the fact that the lake is not an optimal habitat for *Barbus* since fishes of this genus do not adapt well to large impoundments (Marshall, 1982; Skelton, 1993). Consequently, the sample was very small with an average of only seven fish per month being collected. It is also possible that *Barbus* in the larger lake do not feed on copepods to the same extent as in the smaller ones, although this assumption has not been tested.

The prevalence of *L. intestinalis* in *B. paludinosus* in the Munwahuku Dam was lower than that of *B. paludinosus, B. unitaeniatus, B. toppini* and *B. bifrenatus* in South Africa, while it was higher than that of *B. trimaculatus, B. radiatus* and *Labeobarbus marequensis* from the same country (Table 3.3).

Table 3.3. The prevalence and intensity of *Ligula* parasitism in *B. paludinosus* from Munwahuku Dam compared with some *Barbus* and *Labeobarbus* species from South African impoundments. N = sample size, P = prevalence, I = intensity, MI = mean intensity. Sources are (1) Saayman *et al*. (1991): (2) Mashego (1982); (3) this study.

Species	Locality	N	P (%)	I	MI	Source
B. toppini	Middle Letaba Dam	178	95.51	1-5	1.57	1
B. unitaeniatus	Middle Letaba Dam	98	77.55	1-9	2.04	1
B. bifrenatus	Middle Letaba Dam	33	33.33	1-2	1.36	1
B. unitaeniatus	Luphephe and Seshego Dams	-	19.00	1-2	1.00	2
B. paludinosus	Luphephe and Seshego Dams, Nwanedzi River	-	13.00	1-3	1.00	2
B. paludinosus	**Munwahuku Dam**	**885**	**7.23**	**1-3**	**1.06**	**3**
B. radiatus	Luphephe Dam	-	5.00	1-1	1.00	2
B. trimaculatus	Middle Letaba Dam	72	2.78	1-1	1.00	1
L. marequensis	Piet Gouws Dam	-	1.00	1-1	1.00	2

The intensity of *Ligula* infection in *B. paludinosus* from Munwahuku Dam was similar to that recorded by Mashego (1982) from two dams and a river system in South Africa (Table 3.3). However, high intensities of up to 30 (mean = 4.2 have been observed (Sweeting, 1977).

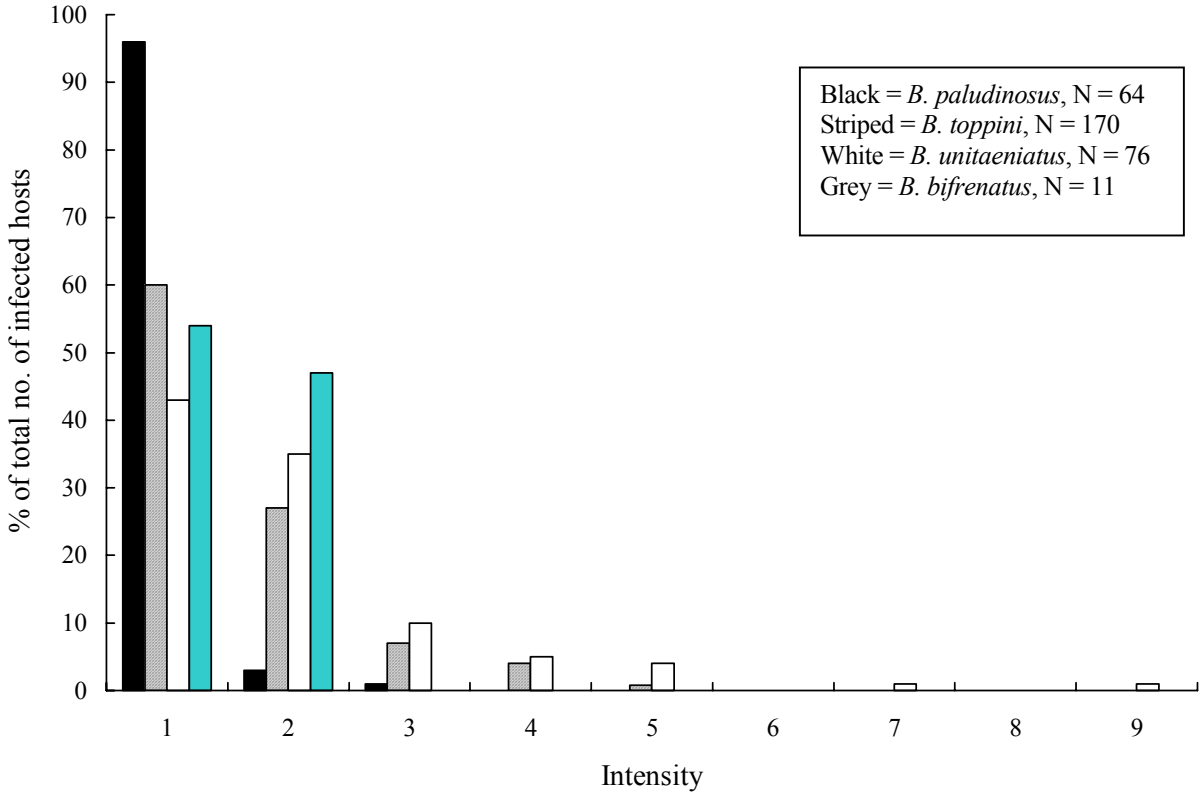


Fig. 3.5. The frequency of different levels of intensity of *Ligula intestinalis* infestation in *B. paludinosus* from the Munwahuku Dam and four other *Barbus* species from the Middle Letaba Dam (Saayman *et al.,* 1991).

Small *Barbus* species are apparently able to survive infestations of more than ten parasites without drastically affecting the size of the parasite or killing the host and Mashego (1982) suggested that the larger cyprinid species such as *L. marequensis* could perhaps carry a heavier parasite load although they seem not to be regular hosts of the parasite (Table 3.3). According to Kennedy and Burrough (1981) *Ligula* infestations are scarcer in large fish partly because of selective mortality of infected fish and partly because of a change in the host's diet.

There was no obvious seasonal variation in the prevalence of *L. intestinalis* in *B. paludinosus* from Munwahuku Dam which is similar to the observations of Mashego (1982) who also noted the lack of seasonality in the infection of *Barbus* species by *L. intestinalis*. Gregory and Blackburn (1999) argue that parasite prevalence is not a reliable measure when the sample size is small (<30) which may explain the situation in Lake Chivero and in the Munwahuku Dam from October 2000 onwards. Large numbers of *B. paludinosus* (mean = 53.7 per month) were collected in July-September 2000 when the prevalence of parasites was high but fewer were collected (22.3 per month) during the period from October-December 2000, which was the later part of the dry season and the Munwahuku Dam almost dried up, leaving only a number of shallow ponds thinly connected by a trickling stream. The parasite prevalence was low, with values of zero being recorded in October and December.

As piscivorous birds could more easily capture the fish at this time, the possibility that parasitized fish were more readily captured than healthy ones might explain the low prevalence of infected fish. In Lake Tanganyika individuals of the cyprinid *Rastrineobola argentea* (Pellegrin) that were infected with *L. intestinalis* were more likely to be captured by the Pied Kingfisher, *Ceryle rudis* (L.) because they cannot swim as efficiently as healthy ones (Wanink, 1992). The behaviour of fish also changed with infected individuals staying close to the surface during the day, when they were most likely to be captured by the kingfishers. The prevalence of parasites remained low after the Munwahuku Dam filled up again in January-February 2001 (Fig. 3.1) possibly because the sample size remained low (mean = 31.2) as the *B. paludinosus* population started to re-establish itself again in the dam.

The increase in the size of *Ligula* plerocercoids as the host size increased may explain why the parasite intensity is low. As tapeworms grow in the body cavity of the host, they induce retardation of its gonadal development (Paperna, 1996), thus creating more space for themselves to grow. Thus larger hosts provide a larger space for the plerocercoid to occupy resulting in a larger tapeworm (Kennedy and Burrough, 1981). Pathological effects of *L. intestinalis* on its host include the suppression of gonad development and gonadal atrophy, a reduction in liver size, retardation of growth, reduced buoyancy, distension of the body wall, and muscular degeneration (Sweeting, 1977; Bean and Winfield, 1989; Szalai *et al.,* 1989; Paperna, 1996; Mokgalong, 1996). Infected specimens examined during the present study showed obvious distension of the body wall and the gonads, where present, were atrophied. These factors probably make parasitized fish more susceptible to predation ensuring a speedy transmission to the final host.

A high parasite index (PI) is generally associated with a higher level of damage to the host's body wall and musculature degenerates uniformly as the PI increases (Sweeting, 1977). In the roach (*Rutilus rutilus*) with a PI greater than 30%, it was observed that the muscles degenerated completely causing ruptures of the body wall. The parasite index in *B. paludinosus* recorded in this study was generally very low (Table 3.4) and did not affect their condition and there was no evidence that their musculature had been affected.

Adult *Proteocephalus* were found only in the intestines of *C. gariepinus* from Lake Chivero but larvae, which could not be identified to generic level have also been found in *Clarias* from Lake Kariba (Chishawa, 1991; Douëllou, 1992a). *Proteocephalus glanduliger* (Janicki), which is very much smaller than the specimens collected in this study (maximum length = 1.9mm, max. width =

0.3mm) have been recorded from *Clarias* in four South African impoundments (Mashego, 1977, 2001, Mashego and Saayman, 1989) and in the Middle Letaba Dam (Saayman *et al.,* 1991). *Proteocephalus*, like most intestinal helminths of fish, does not have serious pathological effects on its host (Ferguson, 1989; Roberts, 2001) although, because it is usually found embedded in the intestinal mucosa with its scolex attached to epithelial cells, it may cause haemorrhagic lesions in the gut especially in heavy infestations when it might obstruct the intestinal lumen (Saayman *et al*., 1991). The data available so far from southern Africa (Mashego, 1977; Mashego, personal communication; Saayman *et al.*, 1991, this study) suggest that infection rates are generally low and the effect of these parasites is probably insignificant effect. Proteocephalid cestodes show some degree of host specificity, at least in Africa, where the majority parasitize siluroid fishes (Khalil & Polling, 1997).

It was not clear why these tapeworms were recorded only in *Clarias* from Lake Chivero but not at the other sampling sites. The life cycle of the parasite gives a possible explanation; the procercoids develop only in cyclopoids (Saayman *et al.*, 1991) and the density of cyclopoids was much greater in Lake Chivero than at the other two stations. Zooplankton (Cladocera and Copepoda) dominates the diet of *C. gariepinus* in the lake, especially in large fish and when the availability of benthic invertebrates is low (Munro, 1967).

Table 3.4. A comparison of the parasite index (PI) values of *B. paludinosus* collected from Munwahuku Dam with those of other *Barbus* species from Middle Letaba Impoundment, South Africa (from Saayman *et al.*, 1991).

Species	N	Mean PI (± SD)	Range (PI)
B. paludinosus	64	7.97 ± 3.38	0.69 – 16.28
B. unitaeniatus	54	16.26	5.57-34.40
B. toppini	128	24.31	8.18-49.33
B. bifrenatus	4	19.02	11.33-33.49

Table 3.5. The prevalence and intensity of *Proteocephalus* infection in *C. gariepinus* from some dams in South Africa compared with Lake Chivero. N = sample size, P = prevalence, I = intensity, MI = mean intensity.

Locality	Source	Parasite	N	P (%)	I	MI
Buffeldoorn, Coetzeesdraai, Krokodilsheuwel and Piet Gouws Dams	Mashego (1977); Mashego and Saayman (1989)	*Proteocephalus glanduliger*	149	3.00	1-17	7.00
Middle Letaba Dam	Saayman *et al.*, 1991	*P. glanduliger*	28	17.86	2-9	5.60
Lake Chivero	Present study	*Proteocephalus* sp.	99	7.07	1-14	5.57

CHAPTER 4

TREMATODE PARASITES OF FISH: THE OCCURRENCE OF *HARVARDIA SANDGROUNDI* (DIPLOSTOMIDAE) IN *BARBUS PALUDINOSUS* FROM THE MARIMBA RIVER AND *CLINOSTOMUM COMPLANATUM* (CLINOSTOMIDAE) IN PISCIVOROUS BIRDS FROM LAKE CHIVERO.

4.1. INTRODUCTION

The digenetic trematodes, or flukes, are the second largest group of parasitic worms after the nematodes (Roberts & Janovy, 2000). They belong to the phylum Platyhelminthes (flatworms) and they have a heteroxenous life cycle, i.e. one that requires at least two hosts of which molluscans are the most common first intermediate hosts (Paperna, 1996). Digeneans parasitize all groups of vertebrates and, in man, they cause severe infections such as schistosomiasis (Roberts and Janovy, 2000).

The embryonated eggs of digeneans are shed into the water through the faeces of the final host, upon which they immediately hatch into free-swimming ciliated miracidia (Meyer, 1954). These either actively penetrate the skin or are accidentally ingested by a molluscan host where they develop through redia and sporocyst stages, finally becoming cercariae. The tailed cercariae are shed from the snail into the water where they penetrate the skin of fish and encyst to form metacercariae (Roberts and Janovy, 2000). Because of taxonomic difficulties, all metacercariae that formed “black spots” on the skin of fish used to be assigned to the genus *Neascus* until they were identified, while whitish or yellowish cysts (“yellow grubs”) in the musculature were assigned to the Clinostomidae (Khalil, 1963; Paperna, 1996; Saayman *et al.*, 1991). Most of the “black spots” have

now been identified as larvae of the Diplostomidae (genus *Posthodiplostomum* and larval genus *Diplostomulum*) whereas the "yellow grubs" were either *Clinostomum* or *Euclinostomum* species (Britz *et al.*, 1985; Douëllou and Erlwanger, 1993). The metacerariae excyst in the gut of a piscivorous bird when an infected fish is ingested, and develop into adult flukes which find their way to the buccal cavity of the bird.

Clinostomum complanatum has been found in North America (Meyer, 1954), Australia (Matthews and Cribb, 1998) and South Africa (Mashego, 1982; Saayman *et al.*, 1991; Mokgalong, 1996) in fish and fish-eating birds, while metacercarial infections of fish have been reported from Angola, Sudan and South Africa (Khalil and Polling, 1997) as well as Zambia (Batra, 1984) and Zimbabwe (Douëllou and Erlwanger, 1993). Human infections by *C. complanatum* have been reported from Israel, India and Japan (Douëllou and Erlwanger, 1993; Matthews and Cribb, 1998), as well as Korea (Chung *et al.*, 1995) where it was implicated in cases of human laryngopharyngitis. An increase in the infection rate by this parasite in birds and fish should raise public health concerns especially in communities were fish are not properly cooked.

Beverley-Burton (1962), Douëllou (1992a), and Douëllou and Erlwanger (1993) have recorded Digenea infecting Zimbabwean fish. Of the 27 metacercariae recorded by Douëllou, none were found infecting *Barbus* species, the only exception being *Centrocestus formosanus* (Nigishori) on the gills of *B. fasciolatus*. Only one adult trematode genus, *Orientocreadium*, has been found in Zimbabwean fish (Beverley-Burton, 1962; Douëllou, 1992a), while five genera of metacercariae are known (*Centrocestus, Clinostomoides, Clinostomum, Diplostomum (Diplostomulum)* and *Euclinostomum*) (Douëllou, 1992a; Douëllou and Erlwanger, 1993).

There is only one record from Zimbabwe of trematodes from a fish-eating bird, in which a new strigeid, *Diplostomum* (*Tylodelphys*) *mashonense*, was found in the Grey Heron, and its life cycle experimentally demonstrated (Beverley-Burton, 1963). Ukoli (1968) described three strigeid digenean species in the gut of *Anhinga melanogaster* in Ghana, while in South Africa, 18 digenean species have been described from bird species, mostly cormorants and darters (Saayman *et al.*, 1991).

This study aimed to recover and identify digenean metacercariae collected from the cyprinid, *B. paludinosus*, and adult digeneans from birds in the Manyame catchment. The latter were only sampled from Lake Chivero while fish were sampled from all three water bodies (Lake Chivero, the Munwahuku Dam and the Marimba River).

4.2. MATERIALS AND METHODS

Fish were collected from Lake Chivero and the Munwahuku Dam as described in Sections 2.2.1 and 3.2.1. In April 2002, they were collected from three further sites along the Marimba River using a Smith-Root 5kW Type VI-A Electrofisher. The first site was on the upper reaches of the river near the University campus, where the water was generally clear and unpolluted. The second site was less than a kilometre upstream of the sewage works where the water was quite turbid and smelly. The last site was just about 150 m downstream of the point of sewage discharge and about six kilometres before the confluence of the river and Lake Chivero (Fig. 1.1). The water was black and thick with suspended solids, with a very unpleasant odour.

The samples were transported in fresh condition to the laboratory where their standard lengths and weights were recorded. Each fish was then examined for blackspot skin cysts containing metacercariae, and these were counted and recorded. The cysts were then excised and the metacercariae removed by manually teasing with a needle, after which they were immediately fixed in alcohol-formol-acetate (AFA).

The specimens were brought down to water through descending grades of ethyl alcohol (70%, 50%, and 30%) and finally washed in distilled water. They were then stained for 20 minutes in acetocarmine solution, using 2% acid alcohol for differentiation. The metacercariae were dehydrated in ascending series of alcohol (30%, 50%, 70%, 90%, 96% two changes of absolute alcohol). Clearing was done in xylene and the specimens were permanently mounted in Canada balsam (McLaughlin, 2000).

The metacercariae were identified to species level using the keys of Yamaguti (1958) and Niewiadomska (2002). The prevalence, intensity and mean intensity of infection were calculated. Birds from Lake Chivero were shot as described earlier (Section 2.2.2) and clinostomes removed from their buccal cavities. These were fixed in hot AFA solution and stored in 70% ethyl alcohol then stained in Delafield's haematoxylin, counterstained in eosin, cleared in xylene and mounted whole in Canada balsam (McLaughlin, 2000). They were then examined under the light microscope and identified with the aid of keys in Yamaguti (1958) and Kanev *et al.* (2002).

4.3. RESULTS

Ninety six *B. paludinosus* were collected from Lake Chivero and 885 from the Munwahuku Dam, but none of them were infected by trematodes. A total of 108 fish was collected from sampling sites 1 and 2 on the Marimba River, upstream of Crowborough sewage plant (Fig. 1.2 but none was caught at site 3 which was heavily polluted by raw sewage. Only 12.2% of the fish from site 1 were infected with "blackspot cysts" on the skin, which proved to be diplostomid metacercariae identified as *Harvardia* sp., with an intensity of up to six cysts per host and 22% of those from site 2 were infected with an intensity of up to 11 cysts per fish (Table 4.1) and the mean intensity was similar at both stations where fish occurred. The metacercaria were tentatively identified as *Harvardia sandgroundi* (Baer), the only species which parasitizes piscivorous birds in southern Africa. Two adult clinostomes were recovered from the buccal cavities of one White-breasted Cormorant and one Darter and identified as *Clinostomum complanatum* (Rudolphi, 1819). None of the other birds examined had any digenean parasites.

Table. 4.1. Diplostomid metacercariae on the skin of *Barbus paludinosus* from the Marimba River. N = sample size, P = prevalence (%), I = intensity, MI = mean intensity.

Sampling Site	N	No. infected	P	I	MI
1	49	6	12.2	1-6	2.7
2	59	13	22.0	1-11	3.8
3	0	-	-	-	-

4.4. DISCUSSION

Like larval nematodes, metacercariae of trematodes are difficult to diagnose to species level because they lack reproductive structures (Roberts and Janovy, 2000). Diagnostic keys mainly describe adult features which do not appear in the larval forms and were therefore of limited value. Black strigeoid cysts in southern African fish produce metacercariae most closely related to adults of *H. sandgroundi*, while green-pigmented cysts are considered to be metacercarial forms of *Hysteromorpha triloba* (Rudolphi, 1819) (Saayman *et al*., 1991).

The absence of parasites from the Munwahuku Dam suggests that fish from polluted systems (e.g. the Marimba River) are more likely to be parasitized than those in less polluted water. There is some evidence that fish stressed by environmental factors such as pollution are more susceptible to parasitism than those in an unpolluted environment (Avenant-Oldewage, 2001).

The fact that the darters and cormorants from Lake Chivero were not infected by *Harvardia* or any other diplostome might suggest that either they do not feed on *Barbus* in the lake or, if they do, that the fish were healthy and there was no parasite transmission This is supported by the analyses of stomach contents of birds collected from Lake Chivero, as well as others from Lake Kariba (Birkhead, 1978; Donnelly and Hustler, 1986), which indicate that cichlids are preferred by these birds.

The prevalence of *Clinostomum complanatum* infection in *P. carbo* (25%) and *A. melanogaster* (25%) on Lake Chivero was quite low compared to the prevalence in these birds on the Middle

Letaba Dam, South Africa (100% and 96% respectively; Saayman *et al.*, 1991). Although herons are the principal hosts of *Clinostomum* (Meyer, 1954), none of the Grey Herons from Lake Chivero were infected; the absence of clinostomes in these birds may be an artifact reflecting the small size of the sample. In South Africa, though, a small sample of *Ardea cinerea* (n = 3) was moderately infected by *C. complanatum* (prevalence = 66%, mean intensity = 2) and *Euclinostomum heterostomum* (P = 100%. MI = 5) (Saayman *et al.*, 1991).

CHAPTER 5

TAXONOMIC NOTES ON THE MAJOR HELMINTH SPECIES PARASITIZING *BARBUS PALUDINOSUS, CLARIAS GARIEPINUS* AND PISCIVOROUS BIRDS FROM THE MANYAME CATCHMENT AREA

This chapter outlines the classification of the parasitic helminths of fish and birds collected during this study. Since taxonomy is a dynamic subject, the generic and species names given here may not agree with some authors, in which case synonyms are given. A number of keys were consulted, and so were experienced parasitologists such as Prof. S.N. Mashego (University of the North-West, South Africa) and Prof. N.M. Mokgalong (University of the North, South Africa) to confirm these identifications. Drawings and photomicrographs were made to illustrate some of the characteristic features, and for some species, various structures were measured and compared with measurements taken by other authors. Voucher specimens were deposited in the collection of the Southern African Institute of Aquatic Biodiversity (Grahamstown), Parasitology Section at Rand Afrikaans University, South Africa.

5.1. Trematodes

PHYLUM : Platyhelminthes

CLASS : Trematoda

ORDER : Digenea

FAMILY : Diplostomatidae

SUBFAMILY : Diplostominae

GENUS : *Harvardia* (Baer, 1932) (Larval genus = *Diplostomulum*)

***Harvardia sandgroundi* (Baer, 1932) (Fig. 5.1)**

Distribution

Africa. Known from South Africa where it infects cormorants; metacercariae have been found in the integument of *Oreochromis mossambicus* (Peters, 1852) (Mokgalong, personal communication, Saayman *et al.*, 1991). In Zimbabwe, it has been recorded in the skin of *B. paludinosus* collected from the Marimba River (this study).

Description

The metacercaria of *Harvardia* have a characteristic S-shaped body which is bipartite, i.e., a distinct concave forebody and a retroflexed, cylindrical or bluntly conical hindbody (Yamaguti, 1958; Kanev *et al.*, 2002). It ranges from 1.5-2.3mm in length and is about 0.7-1.1mm in width (Table 5.1). Reproductive organs were apparently not yet developed.

SUBORDER : Prosostomata

FAMILY : Clinostomatidae

SUBFAMILY : Clinostominae

GENUS : *Clinostomum* Leidy, 1856

***Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 metacercaria (Fig. 5.2)**

Synonyms: *C. marginatum* (Rudolphi, 1819); *C. hornum* Nicoll, 1914; *C. chrysichthys* Dubois, 1930; *C. van der hostii* Ortlepp, 1935.

Distribution

Cosmopolitan (Mashego, 1982), although Kanev *et al.* (2002) say *Clinostomum* species are most

prevalent in the Neotropical region. Only two specimens were recovered in this study, one from the White-breasted Cormorant and one from the Darter from Lake Chivero.

Description

The oral sucker was surrounded by a collar-like fold (Fig. 5.2). Vitelline folds extended from the posterior end of the body into the forebody; the uterus was intercaecal, almost reaching the level of the ventral sucker. Measurements of the specimens were compared to those from Asia (Yamaguti, 1933 in Chung *et al.* (1995), Kagei *et al.*, 1988 in Chung *et al.* (1995)) and Australia (Matthews and Cribb, 1998) and no significant differences were found in the size of the organs of the present specimens in comparisons to specimens of the above-mentioned workers (ANOVA , P = 0.974)

Table 5.1. Measurements (millimetres) of *Harvardia sandgroundi* metacercariae from *Barbus paludinosus* collected in the Marimba River

Characteristic	Range	Mean (N = 6)
Length	1.537-2.297	1.928
Length of forebody	1.007-1.482	1.260
Length of hindbody	0.531-0.814	0.668
Hind: forebody ratio	-	≈1:2
Maximum width	0.750-1.116	0.860
Diameter of oral sucker	0.082-0.183	0.126
Length of pseudosucker	0.424-0.659	0.531
Ventral sucker (length*width)	-	0.183*0.138
Distance of ventral sucker from anterior end	0.860-1.226	1.027
Tribocytic organ (L*W)	-	0.320*0.096
Pharynx (L*W)	-	0.094*0.084

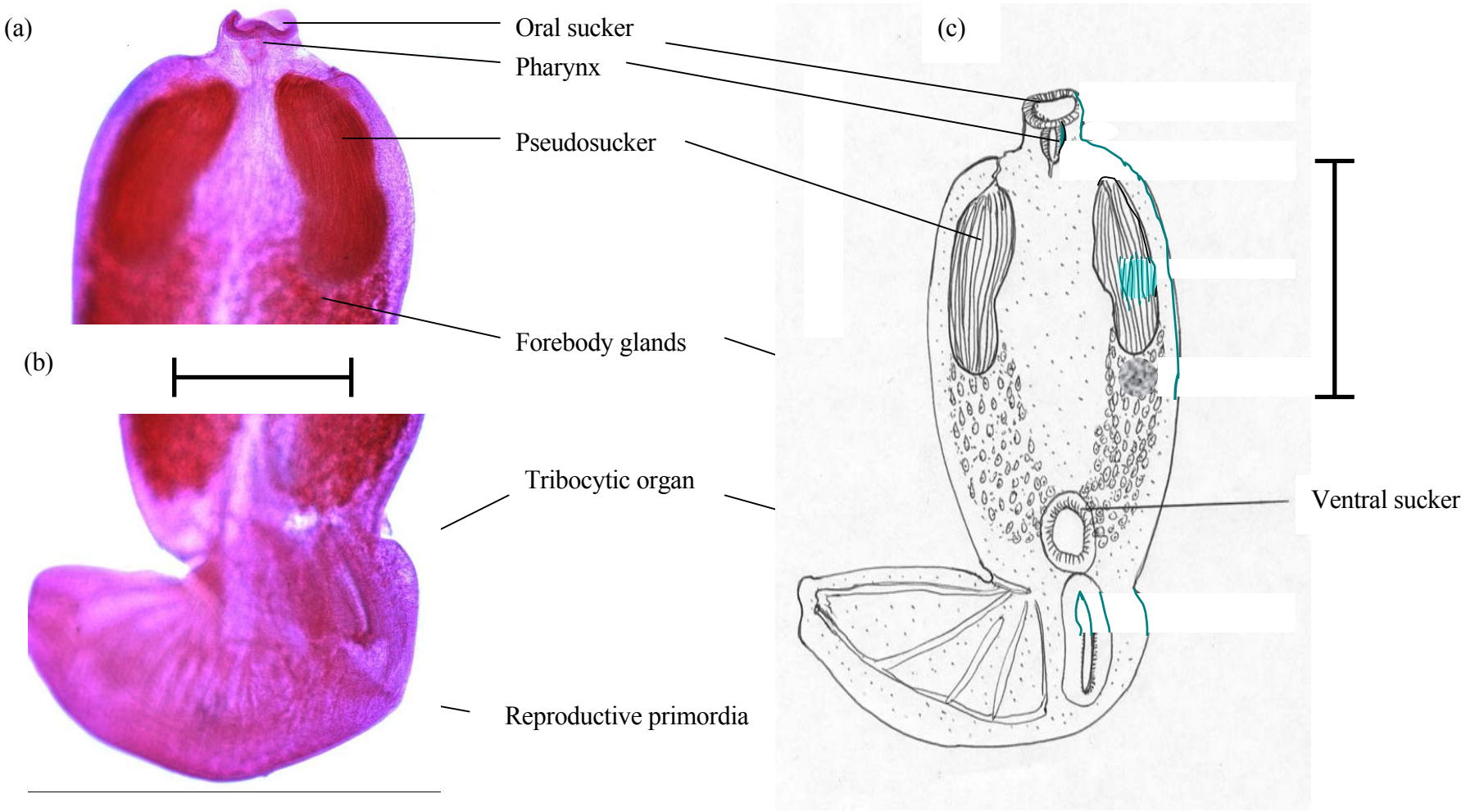


Fig 5.1. *Harvardia sandgroundi* – photomicrographs of (a) forebody and (b) hindbody (scale bar = 0.8mm). (c) Drawing to illustrate features in (a) and (b) (Scale bar = 1.0mm).

Table 5.2. Measurements (micrometers) of Clinostomum complanatum from birds on Lake Chivero compared with those recorded by other workers. Mean values in parentheses.

	Lake Chivero (2002) N=2	Matthews & Cribb (1998)	Chung *et al.* (1995) N=1	Kagei *et al.* (1988) N=10	Yamaguti (1933)
Body length	5552-8064 (6808)	2384-6320 (4040)	4740	3210-5580 (4395)	4600
Body width	1466-1558 (1512)	992-1984 (1411)	1050	1940-2360 (2150)	1680
Oral sucker length	73-110 (912)	123-308 (205)	290	240-400 (320)	280
Oral sucker width	202-257 (229)	164-308 (236)	430	340-460 (400)	330
Ventral sucker length	367-422 (394)	320-720 (489)	720	450-830 (640)	700
Ventral sucker width	183-312 (247)	352-688 (508)	640	610-800 (705)	-
Uterine sac length	3666 (N=1)	398-2048 (961)	-	-	-
Uterine sac width	623 (N=1)	77-1120 (407)	-	-	-
Anterior testis length	440-715 (578)	212-520 (336)	530	310-760 (535)	400
Anterior testis width	293-513 (403)	321-931 (530)	510	610-1040 (825)	710
Posterior testis length	403 (N=1)	141-514 (322)	350	200-440 (320)	250
Posterior testis width	586 (N=1)	353-899 (589)	550	260-1210 (735)	790
Ovary length	421 (N=1)	128-334 (203)	150	190-380 (285)	250
Ovary width	495 (N=1)	71-462 (186)	120	210-870 (540)	160
Cirrus sac length	733 (N=1)	161-545 (320)	290	280 (N=1)	-
Cirrus sac width	586 (N=1)	84-513 (187)	160	180 (N=1)	-

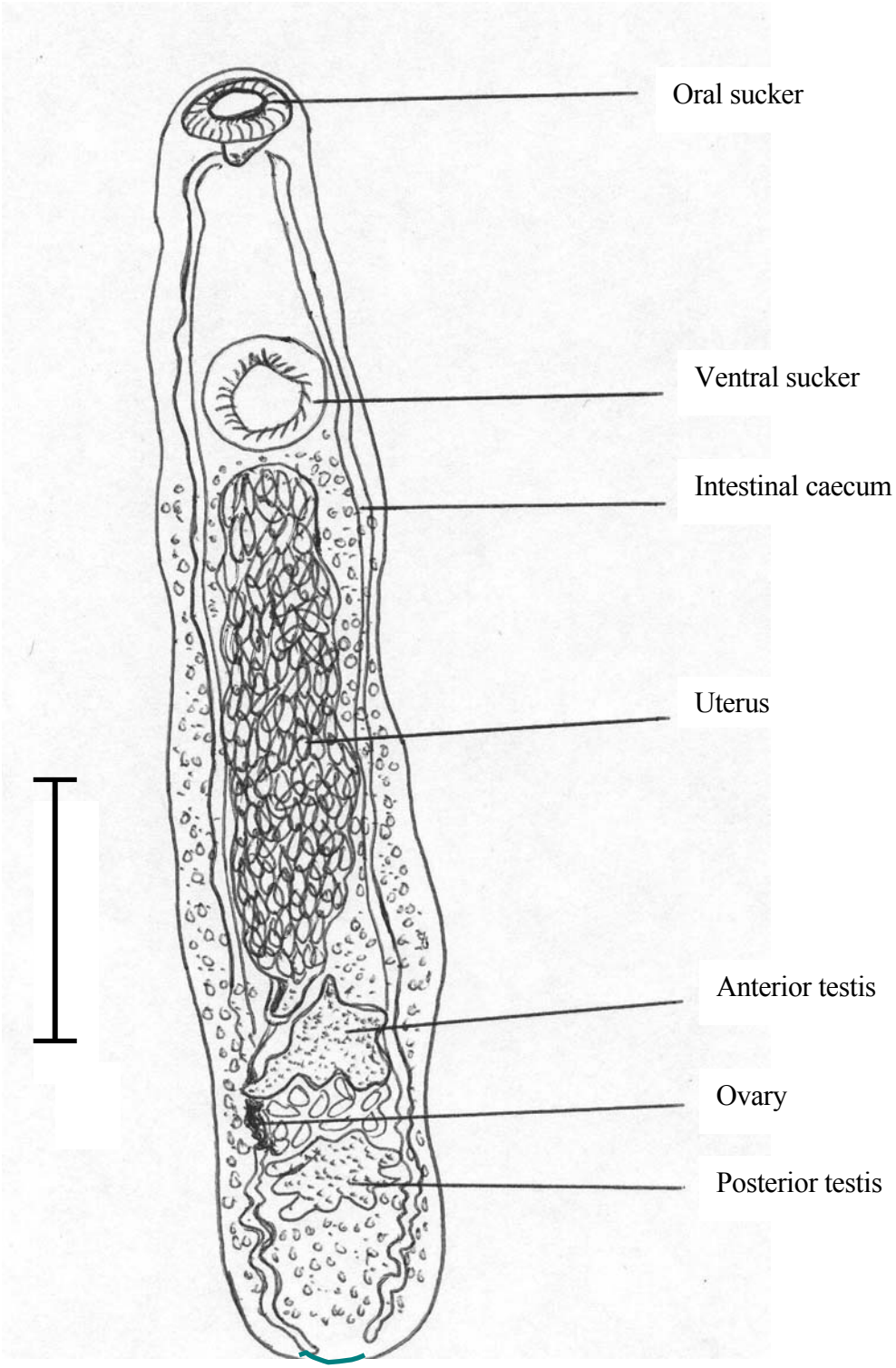


Fig. 5.2. Clinostomum complanatum from the buccal cavity of Phalacrocorax *carbo* (Lake Chivero). Scale bar = 1.0mm

CLASS : Cestoda

SUBCLASS : Eucestoda

ORDER : Pseudophyllidea

FAMILY : Diphyllobothriidae

GENUS : *Ligula* (Bloch, 1782)

***Ligula intestinalis* (L.)**

Distribution

According to Prudoe and Hussey (1977), *L. intestinalis* in Africa was first recognized by Baer in 1933 from reed cormorants in Mozambique and, based on a number of morphological differences, it was renamed *L. intestinalis* var. *Africana*. Ligulae recovered by Fuhrmann in 1943 from *P. africanus* in Angola, and Mahon in 1954 from the Green Pigeon *Treron calva* in the Congo, were subsequently named after this new variety (Prudoe and Hussey, 1977). In spite of this proposal some authors who described the adult worm from bird hosts referred to it simply as *L. intestinalis* (Saayman *et al.*, 1991; Mashego and Saayman, 1989; Mokgalong, 1996). Wanink (1992) recorded the occurrence of *L. intestinalis* in the pelagic cyprinid, *Rastrineobola argentea* (Pellegrin), and the final host *Ceryl rudis* (Pied Kingfisher). It has also been found infecting *Barbus tanapelagius* de Graaf, Dejen, Sibbing & Osse and *B. humilis* Boulenger from Lake Tana, Ethiopia (Dejen, 2003). No adult ligulids were recovered from piscivorous birds on Lake Chivero; only plerocercoid larvae were collected from *B. paludinosus* in the Munwahuku Dam.

Description

Positive identification of plerocercoids from the body cavity of *B. paludinosus* (Munwahuku Dam) was confirmed by Mashego (personal communication). Using the keys of Yamaguti (1959) and Bykhovskaya-Pavlovskaya *et al.* (1964), the following features were diagnostic:

Belt-like, extremely muscular worms, with central longitudinal groove extending along ventral surface. Neck absent. External segmentation of strobila absent.

ORDER : Proteocephalidea

FAMILY : Proteocephalidae

GENUS : *Proteocephalus* Weinland, 1858

***Proteocephalus* sp. (Fig. 5.3)**

The adult tapeworms recovered from the intestine of *C. gariepinus* from Lake Chivero belonged to the genus *Proteocephalus* as confirmed by Mashego and Mokgalong (personal communications). Based on the keys of Schmidt (1986) and Rego (1994), the following diagnostic features were described from the specimens:

Four typical lateral suckers near the anterior end of the head (Fig. 5.3a, d); neck region not differentiated into well-defined proglottides; proglottides increasingly longer than broad towards the posterior end of strobila. Gravid proglottides at the posterior end of the strobila. Testes located in medullary tissue dorsal to uterus and flanked laterally by vitellaria (Fig. 5.3b). Ovaries located posterior to testes in mature proglotides (Fig. 5.3c).

PHYLUM : Nemathelminthes

CLASS : Nematoda

ORDER : Ascaridida

FAMILY : Anisakidae (= Heterocheilidae)

SUBFAMILY : Anisakinae

GENUS : *Contracaecum* Raillet and Henry, 1912

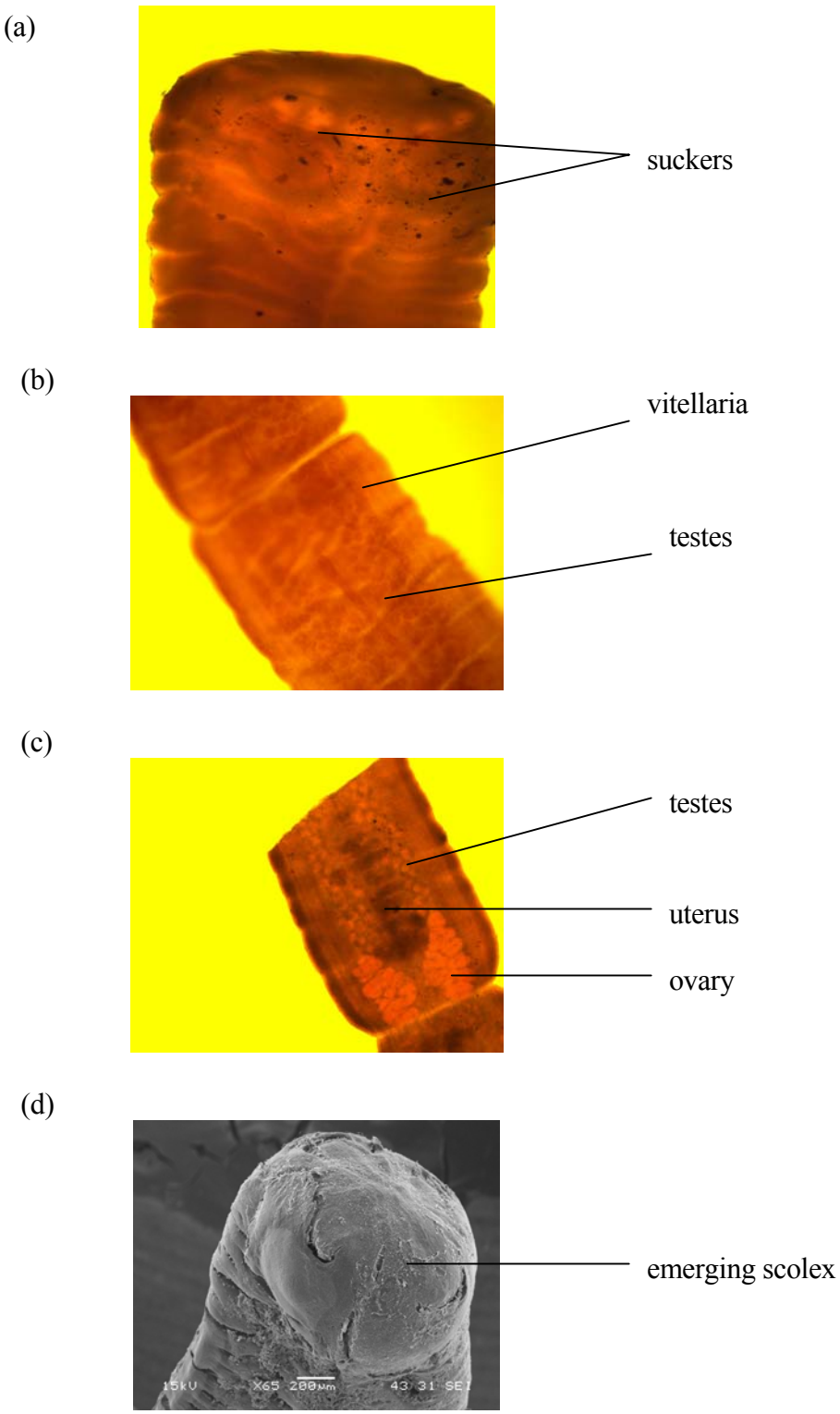


Fig. 5.3. Photomicrographs of *Proteocephalus* sp. (a) scolex, (b) mature proglottide showing arrangement of testes and vitellaria, (c) mature proglottide showing position of ovaries and uterus, (d) scanning electron micrograph of scolex of an immature adult. Note that the suckers are not yet fully developed.

***Contracaecum* sp.**

Third-stage larvae (L3) of the genus *Contracaecum* are characterized by two blind caeca branching off from intestinal canal at the boundary between oesophagus and midgut; ventricular appendix longer and pointing posteriorly; intestinal caecum shorter and pointing anteriorly (Fig. 5.). Rudimentary labia lacking interlabia present; tail curved ventrally with a terminal spine; genital organs not developed. Measurements taken were recorded in Table 5.3. Because of the morphological similarities between L3 larvae of different species of this genus and the related genus, *Phocascaris*, it is not possible to differentiate between species at this stage of development in fish (Mokgalong, 1996).

Four species of adult *Contracaecum* were recovered from birds on Lake Chivero. The nematodes were identified on the basis of the dimensions of the ventricular appendix and intestinal caecum using light microscopy, while scanning electron microscopy (SEM) revealed details of head and tail structures, as well as the size and number papillae and spicules in males, which were very useful in diagnosis (Table 5.4).

Table 5.3. Morphological features used in identification of L3 larvae of *Contracaecum* sp. in *Clarias gariepinus* from Lake Chivero. All measurements are in mm.

Morphological characteristic	Range	Mean (N = 17)
Body length	15.00-30.00	24.50
Body width	0.62-0.98	0.83
Head diameter	0.11-0.18	0.14
Oesophagus length	1.74-5.47	3.05
Oesophagus width	0.05-0.07	0.06
Ventriculus length	0.10-0.21	0.17
Ventriculus width	0.12-0.51	0.37
Ventricular appendix length	-	0.29 (N=1)
Ventricular appendix width	-	0.08 (N=1)
Intestinal caecum length	1.33-5.57	2.80
Intestinal caecum width	0.14-0.48	0.29
Intestine length	13.02-25.62	21.17
Intestine width	0.29-0.61	0.47
Tail length (from anus to tip of tail)	0.12-0.17	0.16

***Contracaecum rudolphii* (Hartwich, 1964)** (Figs. 5.3-5.6)

Lips slightly wider than long, with marked medial deepening on upper lip. Lip palp forms two lobes in interior margin, each divided into a rounded lateral and medial lobulus. Interlabia reach four-fifths of length of lips with tips distinctly bifurcated. Male body length 12.1-33.0mm. Cloaca 0.14-0.24mm from tip of tail. 27-43 pairs of precloacal papillae arranged irregularly in two longitudinal rows; seven pairs of simple postcloacal papillae. Spicules similar, subequal (left 4.05-9.98mm; right 4.46-9.19mm long), with longitudinal alae.

C. rudolphii is the most widely studied species of *Contracaecum* with a cosmopolitan distribution (Yamaguti, 1959; Mokgalong, 1996). It utilizes both marine and freshwater bird species as final hosts with little host specificity (Anderson, 1992). During this study, however, this parasite did not occur in the Grey Heron, infecting only the darters and cormorants. A similar trend was also observed in South Africa (Whitfield and Heeg, 1977; Mokgalong, 1996) but in both cases the host samples were small, so definite conclusions could not be made.

***Contracaecum carlislei* (Ortlepp, 1938)** (Figs. 5.7-5.9)

> Anisakidae reaching a length of 15mm for male and 27mm for females. Three somewhat rectangular lips, anterior surfaces bilobed; interlabia large and curved; dorsal lip with two single papillae and subventral lips with a double and a single papilla. Intestinal caecum long. Tail in both sexes about 0.25mm long; gubernuculum absent. 30 or more pairs of precloacal papillae and six pairs of postcloacal papillae of which the first and second pairs are close together but not forming twin papillae. Vulva divides body into ratio 2:3. Eggs round with thin shells.

This species was first described in 1938 by Ortlepp from South Africa (Mokgalong, 1996) where it seems to be well established. This study records it for the first time in Zimbabwe.

***Contracaecum microcephalum* (Rudolphii, 1809)** (Figs. 5.10-5.12)

> Lips hexagonal, rounded, distinctly longer than wide. Lip pulp forms two lobes anteriorly, each further divided into a markedly rounded lateral lobe and a finger-like medial lobe. Interlabia reach the length of four-fifths of

lips with tips not bifurcated but distinctly rounded. Male length 13.10-36.92 mm. Cloaca 0.17-0.30mm from tip of tail. 22-30 pairs of precloacal papillae arranged in two longitudinal rows; seven pairs of simple postcloacal papillae. Spicules with narrow longitudinal alae, similar, subequal (left 1.41-3.65mm; right 1.40-3.50mm long).

C. microcephalum, according to Yamaguti (1959) and Anderson (1992), has a worldwide distribution, occurring in Europe, Africa, Asia and South America. Although Saayman *et al.* (1991) and Mokgalong (1996) found it in the herons, *Ardea cinerea* and *A. melanocephala*, as well as the cormorants and darters from South Africa, none of the specimens of the Grey Heron examined during this study harboured this parasite.

***Contracaecum tricuspis* (Gedoelst, 1916) Baylis, 1920** (Figs. 5.13-5.15)

Three lips and three interlabia of elaborate structure; lateral surfaces of lips notched with a point where interlabia fit into notches; interlabia with large base and slender corpus ending in three tips – one internal and two lateral; dorsal lip with two simple papillae; latero-ventral lips each with one double papilla. No lateral alae. Male 13.8mm long with at least 56 pairs of caudal papillae arranged as follows: 4 pairs near caudal end, two pair each which are situated laterally, ventrally and distally to cloacal aperture; one pair of ad anal papillae and a series of about 50 pairs of preanal papillae arranged on two regular rows. Spicules subequal, 4.6mm long, of similar construction to those of *C. microcephalum*.

Originally described from a heron from the Democratic Republic of Congo (Mokgalong, 1996), *C. tricuspis* obtained from Lake Chivero was host-specific, occurring only in the darters, an observation also shared by Saayman *et al*. (1991) and Mokgalong (1996) from South Africa.

Table 5.4. Numbers of pre- and postanal papillae in male *Contracaecum* species in birds from Lake Chivero

Parasite	Host	No. of preanal papillae	No. of postanal papillae
C. rudolphii	*Phalacrocorax carbo*	25	6
	P. carbo	44	7
	P. africanus	25	7
C. carlislei	*P. carbo*	34	0
	P. africanus	26	5
C. microcephalum	*Anhinga melanogaster*	32	5 + 1 double
C. tricuspis	*A. melanogaster*	55	3
	A. melanogaster	76	5 + 1 double
	A. melanogaster	78	5 + 1 double

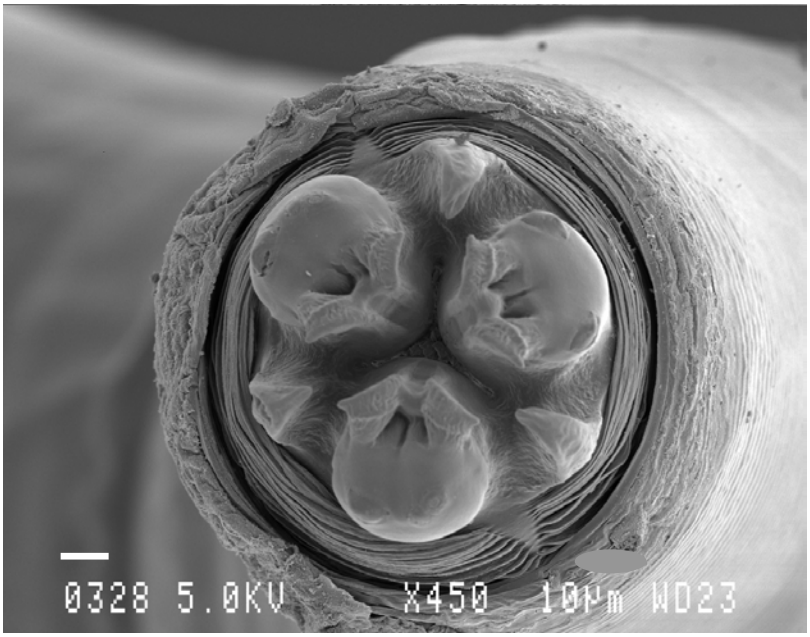


Fig. 5.4. The head of a female *Contracaecum rudolphii* from the Reed Cormorant showing two lobes in interior margin of each lip. Scale bar = 10µm

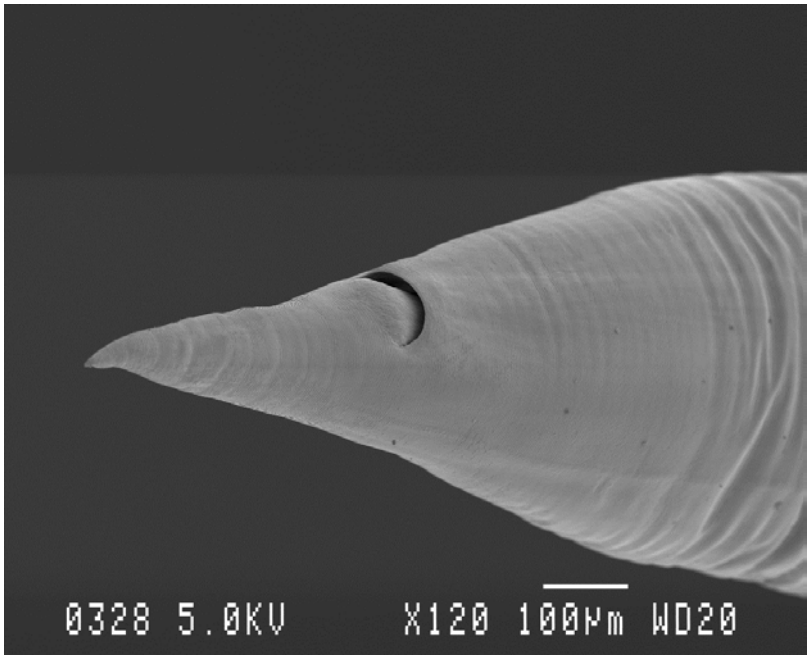


Fig. 5.5. The tail of a female *C. rudolphii* from the White-breasted Cormorant. Note the absence of pre- and postcloacal papillae. Scale bar = 100µm

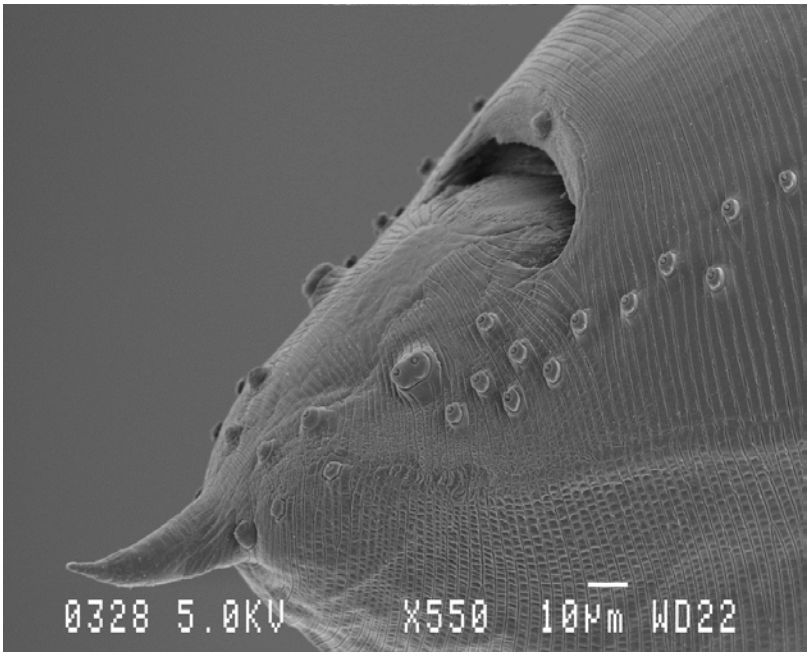


Fig. 5.6. The tail of a male *C. rudolphii* from the White-breasted Cormorant showing the arrangement of postcloacal papillae. Note the two double papillae, lateroventral and midway between tip of tail and cloacal opening. Scale bar = 10µm

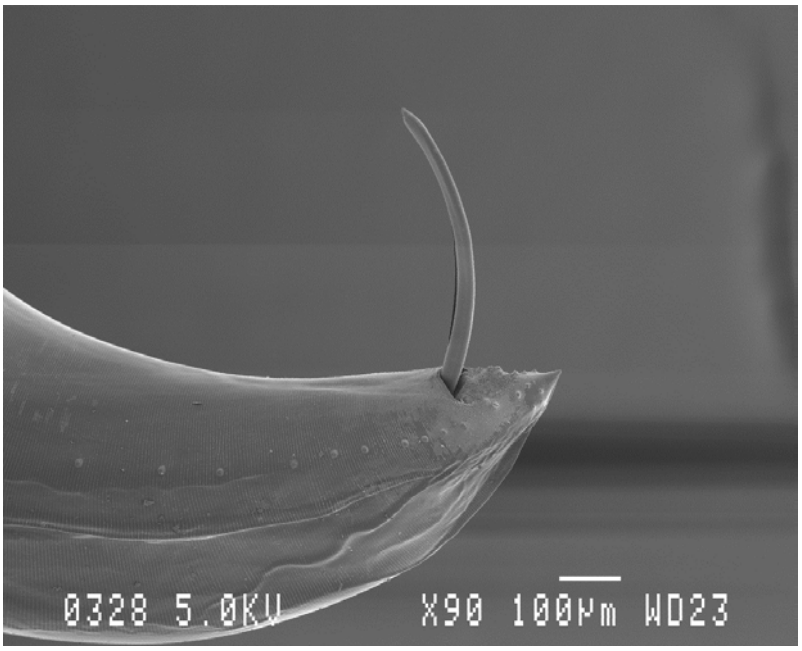


Fig. 5.7. The tail of a male *C. rudolphii* from the Reed Cormorant showing the spicules (fused) and papillae. Scale bar = 100µm

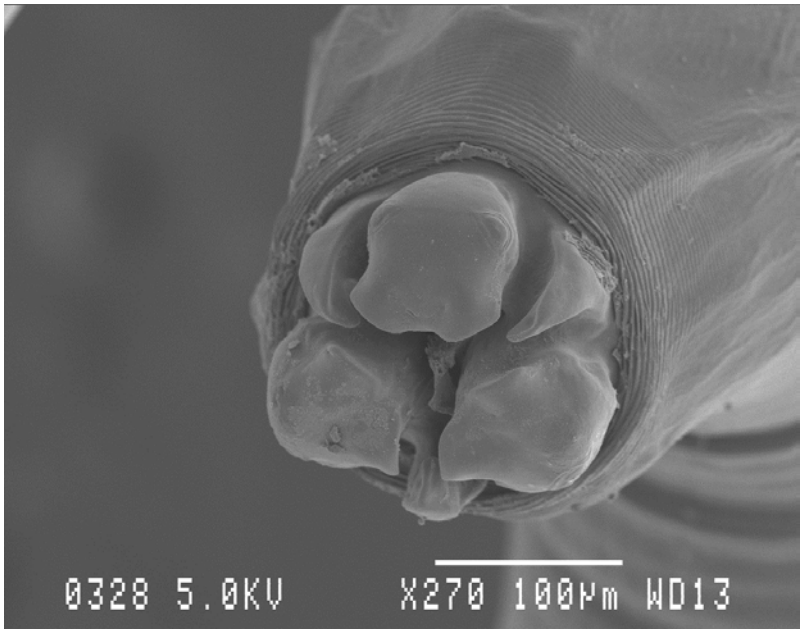


Fig. 5.8. The head of *C. carlislei* from the White-breasted Cormorant characterized by somewhat rectangular lips and two single papillae on dorsal lip. Scale bar = 100µm

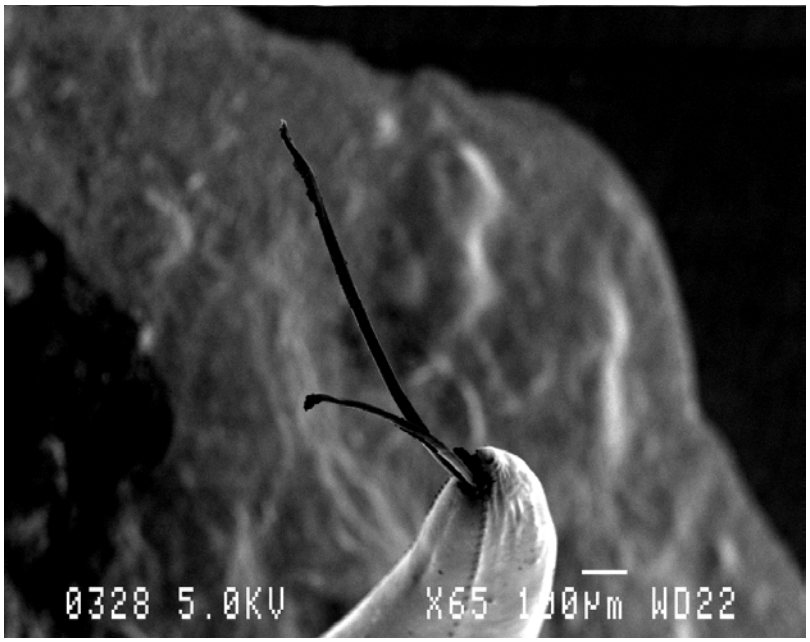


Fig. 5.9. The tail of a male *C. carlislei* from the White-breasted Cormorant. Note the subequal spicules. Scale bar = 100µm

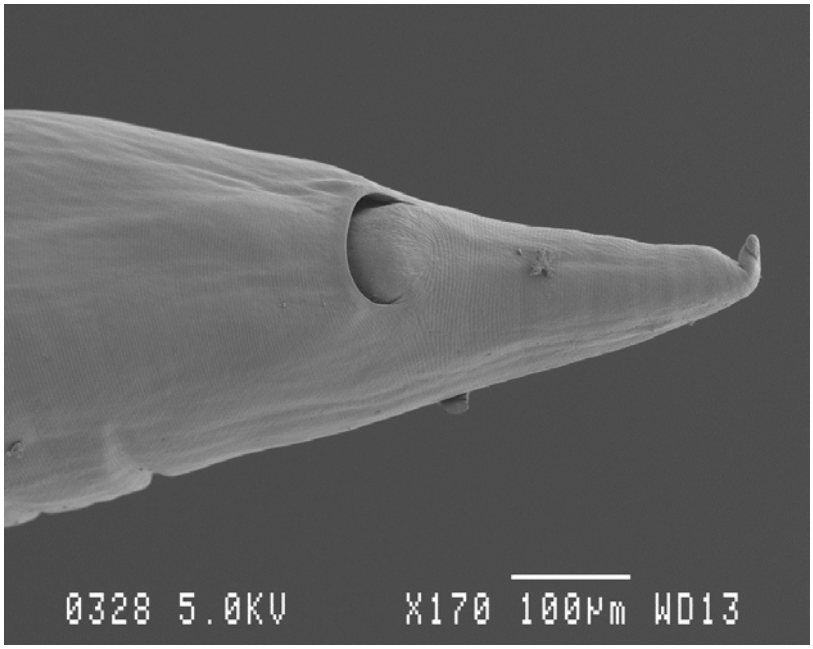


Fig. 5.10. The tail of a female *C. carlislei* from the White-breasted Cormorant. Scale bar = 100µm

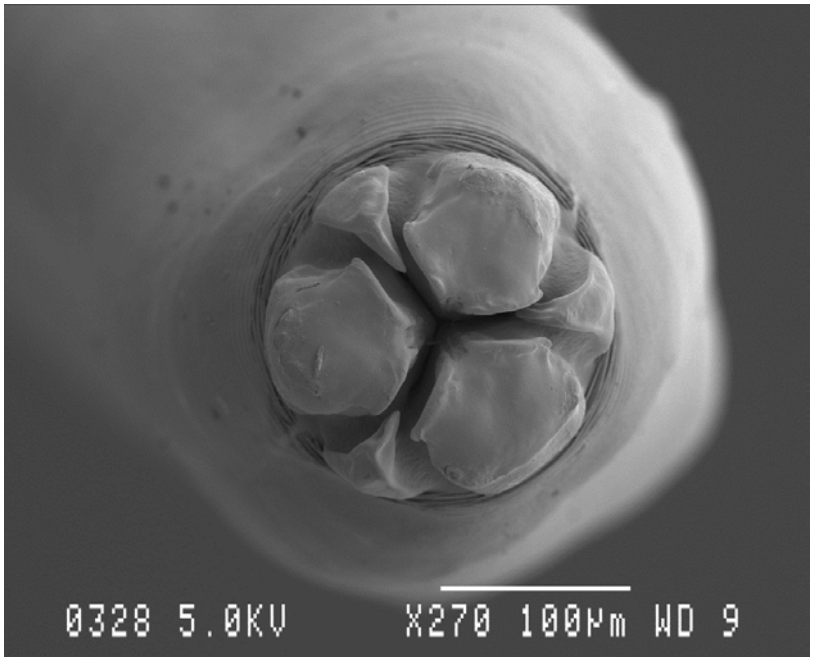


Fig. 5.11. The head of *C. microcephalum* from the Darter. Note the hexagonal lips. Scale bar = 100µm

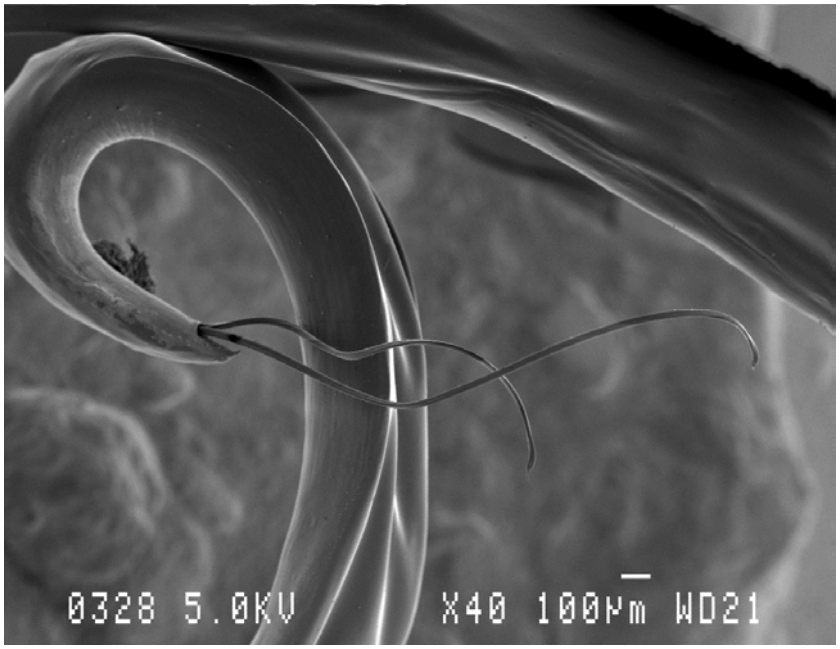


Fig. 5.12. The tail of a male *C. microcephalum* from the Darter with subequal spicules. Note the longitudinal, wrinkle-like alae. Scale bar = 100µm

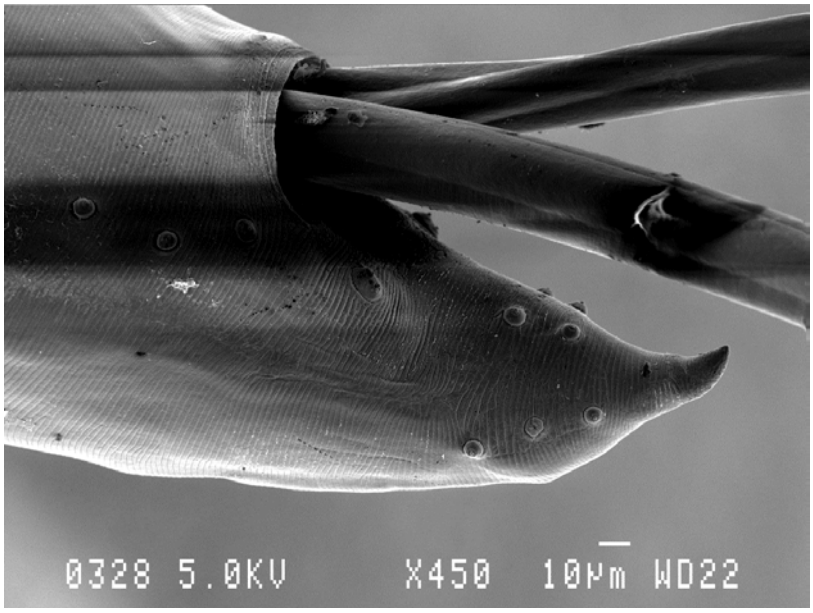


Fig. 5.13. The tail of a male *C. microcephalum* from the Darter showing the arrangement of papillae. Scale bar = 10µm

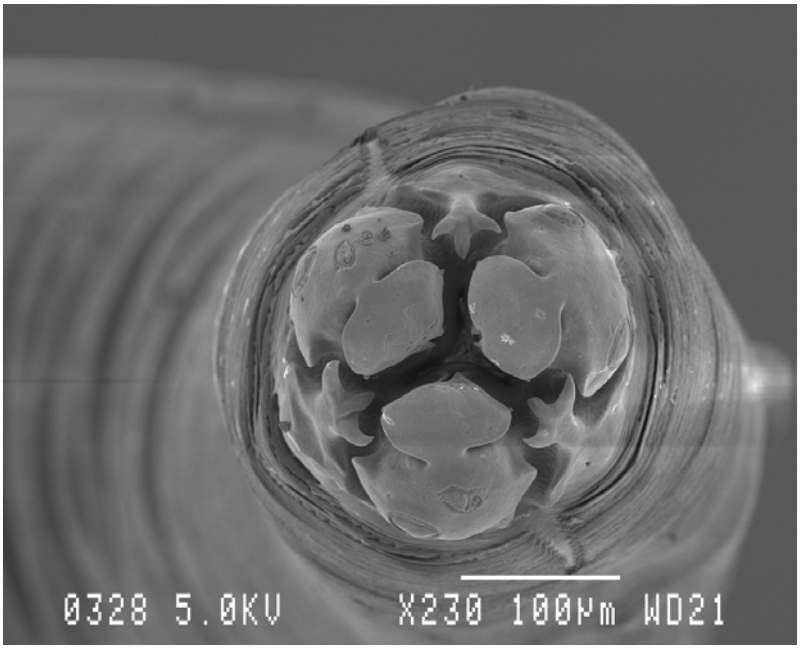


Fig. 5.14. The head of *C. tricuspis* from the Darter showing the characteristic tri-forked interlabia. Scale bar = 100µm

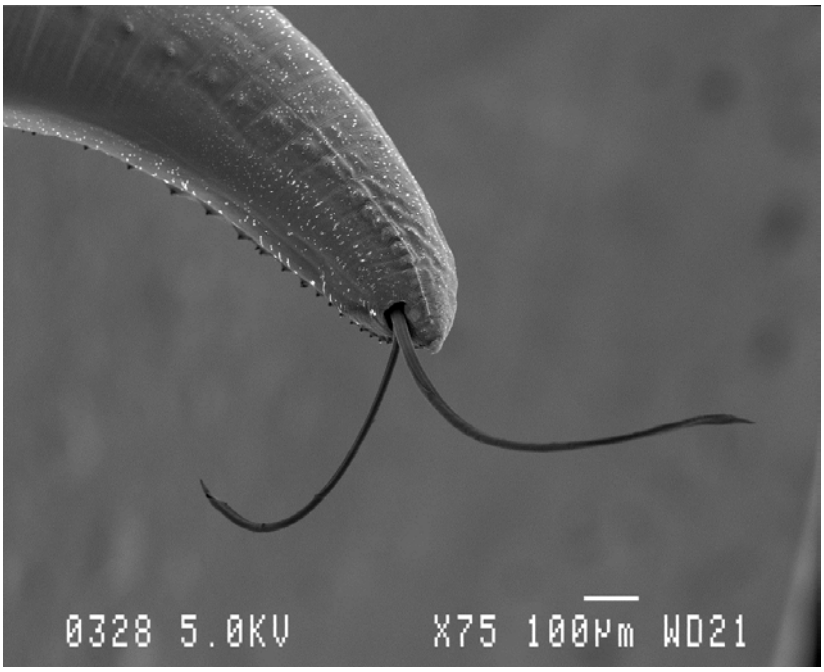


Fig. 5.15. The tail of a male *C. tricuspis* from the Darter showing papillae and subequal spicules. Note the absence of lateral alae. Scale bar = 100µm

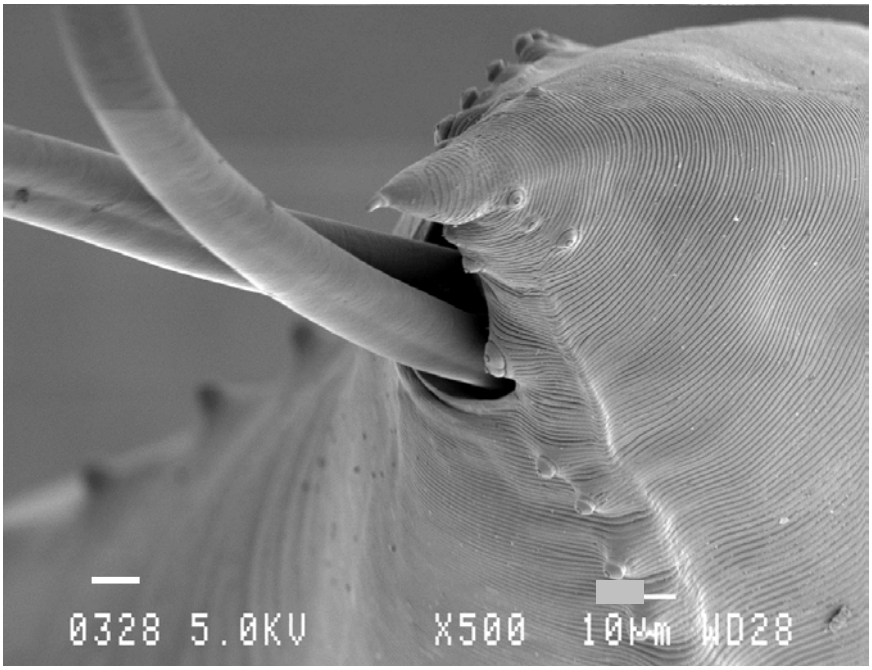


Fig. 5.16. The tail of *C. tricuspis* from the Darter showing the arrangement of papillae. Scale bar = 100µm

CHAPTER 6

CONCLUSIONS

The findings of this study show that helminth parasites were prevalent in *C. gariepinus* and *B. paludinosus*, and the birds *P. africanus*, *P. carbo*, *A. melanogaster* and A. *cinerea* in the Manyame catchment area. These parasites include the nematodes *Contracaecum rudolphii*, *C. microcephalum*, *C. carlislei* and *C. tricuspis*, the cestodes *Ligula intestinalis* and *Proteocephalus* sp., and the digenean trematodes *Harvardia sandgroundi* and *Clinostomum complanatum*. The presence of cichlid species in the guts of birds infected with *Contracaecum* species suggests that they are possible hosts to these parasites, which has been shown to infect cichlids in some other African countries (Douëllou, 1992; Boomker, 1994b; Aloo, 2001). The parasite prevalence and intensity in most hosts sampled were not related to host size or sex but no substantive conclusions could me made since sample sizes were usually low.

The metacercariae of *Harvardia sandgroundi* infecting *B. paludinosus* are recorded for the first time in Zimbabwe, with moderate infection rates. Since this fish prefers riverine habitats and is of little commercial importance in the country, the parasite is most likely transmitted to fish-eating birds and no extreme pathological effects on them are known. In small reservoirs such as the Munwahuku Dam, where *B. paludinosus* thrives because of high plankton availability, "blackspot" infections of *H. sandgroundi* are potentially risky to human populations that fish for subsistence especially if they do not cook the fish thoroughly to kill the cysts. Even though larval clinostomes infecting fish in Zimbabwe were previously described (Douëllou and Erlwanger, 1993), adult *C. complanatum* was described for the first time from fish-eating birds in the country. This parasite also has the potential of infecting humans if improperly cooked fish are ingested.

The infection of cyprinid fishes by *L. intestinalis* is a world-wide problem. Although a high prevalence of the tapeworm in *B. paludinosus* was recorded, the low parasite index dispels fears of any serious threat to the fish population in the Munwahuku Dam. Since the eggs of *Ligula* are transmitted via infected birds, this worm is difficult to control, although fish farmers effectively keep birds away by covering their ponds with fine mesh nets (Paperna, 1996). Further studies on the life cycles and taxonomy of the helminths found in this study are recommended, and should take into account new technologies such as molecular systematics. Experimental infection studies, especially on trematode metacercariae, will also go a long way in determining the final host species (Beverly-Burton, 1963; Saayman *et al.*, 1991).

There were a number of limitations to this study that resulted in some of the objectives not being fully achieved. The study sites selected each represented a lotic system (Marimba River), a small seasonal lentic system (Munwahuku Dam) and a large impoundment (Lake Chivero), the physico-chemical and environmental conditions of which were so different and highly variable that a controlled comparative study would have been difficult to do. However, fish species common to all the three systems (*C. gariepinus* and *B. paludinosus*) were chosen in the hope that they would contain similar parasitic fauna that could then be compared in relation to the respective environmental factors. The results of the study showed that this was not the case, fish from each species having different helminth parasites, suggesting that habitat preferences and feeding habits of the fish in the different systems probably have an effect of the parasite species found in them.

Studies on water bird parasites were only conducted at Lake Chivero where permission by the authorities had been granted, thus gaps still exist for the other sampling sites that need to be investigated. Sample sizes for the birds were quite small to make concrete conclusions on their

infection statistics by helminths. In some cases where whole guts were preserved in formalin for laboratory examination of parasites, cestode parasites were sometimes contracted, brittle and hardened, making them difficult to identify using the normal staining techniques. It was also noted that zooplankton samples from the Marimba River and bird samples from Lake Chivero were only collected during the final months of sampling; therefore no seasonal trends could be observed.

In Zimbabwe, a lot of work needs to be done on fish parasites since little is known on the subject. This entails the need to train more fish parasitologists or put more emphasis on the subject in veterinary and aquatic ecology programmes. For Lake Chivero, an exhaustive taxonomic study similar to Douëllou's (1992a) work on Lake Kariba, encompassing all parasites encountered in all the fish species, is recommended. Aspects of the ecology of the parasites, as well as some applied aspects such as the use of fish parasites as bioindicators of pollution (Avenant-Oldewage, 2001) will be beneficial, especially in the Upper Manyame catchment where heavily pollutes stream and rivers drain into Lake Chivero. Work on the parasites of aquatic birds is quite promising, and the relevant authorities need to be persuaded to authorise the sampling of more birds for such work.

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