

HELMINTHOLOGIA, 52, 3: 229 - 235, 2015

## Seasonal occurrence of some larval stages of endoparasites in three cyprinids from the Nwanedi-Luphephe dams, the Limpopo River System, South Africa

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### Article info

Received October 17, 2014  
Accepted January 28, 2015

### Summary

This study provides information on seasonal occurrence of developmental stages of endoparasites infecting three cyprinids in the Nwanedi-Luphephe dams, Limpopo River System. *Labeobarbus marequensis* (Smith, 1841), *Barbus trimaculatus* Peters, 1852 and *Barbus radiatus* Peters, 1853 were investigated seasonally from January 2008 to October 2008. The following larvae of metazoan parasites were collected: *Diplostomum* sp. from the eyes of *L. marequensis* and *B. trimaculatus*; *Ornithodiplostomum* sp. from the gills of *B. trimaculatus*; *Posthodiplostomum* sp. from muscle, skin and fins of *B. trimaculatus* and *B. radiatus*; third-stage *Contracaecum* larvae (L3) from the mesentery fats and on the liver lobes of *L. marequensis* and *B. trimaculatus* and gryporynchid cestode larvae from the outer intestinal wall of *B. radiatus*. All the flukes encountered were metacercariae. *Diplostomum* sp. and *Contracaecum* sp. dominated the parasite communities. Their prevalence exhibited seasonal fluctuations with maxima in summer. Factors likely to influence fish infection such as the body size of fish and their condition factors were also briefly considered in this study.

**Keywords:** Limpopo River Systems; *Labeobarbus marequensis*; *Barbus trimaculatus*; *Barbus radiatus*; *Diplostomum* sp.; *Ornithodiplostomum* sp.; *Posthodiplostomum* sp.; *Contracaecum* sp.; gryporynchid cestode

### Introduction

Larval forms of many helminth digeneans (flukes), cestodes (tapeworms) and nematodes (roundworms) use a variety of freshwater invertebrates such as snails and copepods as first intermediate hosts and fish species as second intermediate hosts, with the life cycle being completed in the intestinal tract of piscivorous birds (Barber *et al.*, 2000). The migratory nature of piscivorous birds is crucial in the dispersal of endoparasites over long distances, hence making it difficult to control the spread of infections between water bodies in different catchments (Barson & Avenant-Oldewage, 2006). Digeneans, cestodes and nematodes usually display a low specificity to both intermediate and definitive hosts that ensure their wide geographical distribution. Parasites could affect the health of fish, growth, behavior, fecundity and mortality and also regulate host population dynamics and community structure (Marcogliese, 2004).

The composition of fish endohelminth communities is influenced by numerous environmental and ecological factors such as the seasonal changes, geographical range, water characteristics, size and gender of the fish host, diet, abundance, mobility and life span of the fish, interdependence of parasites in the fish, as well as composition of fauna in the habitat (Lyholt & Buchmann, 1996; Dörücü & İspir, 2001; Dörücü *et al.*, 2002; Campos *et al.*, 2009; Alam *et al.*, 2010). Ecological studies on parasites provide information about interrelationships between parasites, and their hosts about biodiversity of parasites and their distribution in a particular water system (Marcogliese, 2004; Vankara *et al.*, 2011). The epidemiological data are particularly important when fish parasites are considered as the potential risks for aquaculture candidate species.

Therefore, the present study was conducted to obtain epidemiological data on larval helminth parasites of three cyprinid fish, namely *Barbus trimaculatus* Peters, 1852, *Barbus radiatus* Peters,

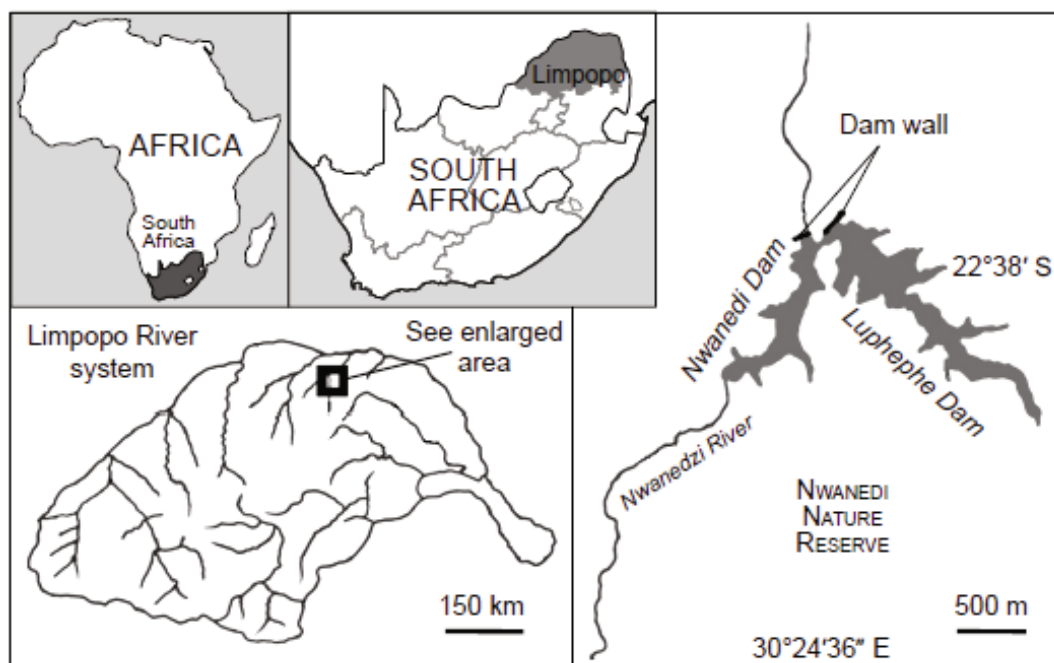


Fig. 1. The Nwanedi-Luphephe dams forms a part of the Limpopo River System, South Africa

1853 and *Labeobarbus marequensis* (Smith, 1841) in the Nwanedi-Luphephe dams from the Limpopo River System (South Africa). This work represents a part of a comprehensive research programme involving investigations of the parasite fauna of fish from the Nwanedi-Luphephe dams.

## Material and Methods

### Study area

The study was conducted at the Nwanedi-Luphephe dams (22°39'S, 30°25'E, altitude 600 m above sea level) situated within

Table 1. Summary of water quality variables measured seasonally at the Nwanedi-Luphephe dams in 2008. Units are in mg/l unless otherwise indicated. Dashes denote unavailability of data.

Water quality Variable	Season				TWQR
	Autumn	Winter	Spring	Summer	
Temperature (°C)	23.1	17.4	27.7	27.2	-
Dissolved oxygen	8.6	8.4	6.1	6.6	6-9
pH	7.6	7.2	7.7	8.0	-
Conductivity (µS/cm)	0.06	0.06	0.07	0.08	-
Turbidity (NTU)	2.2	1.4	2.6	4.2	-
Salinity (no unit)	- 0.03	- 0.03	- 0.03	- 0.04	-
Total alkalinity (as CaCO <sub>3</sub> )	20	20	24	24	-
Nitrate	<0.2	<0.2	<0.2	0.5	0.5
Nitrite	<0.1	<0.1	<0.1	<0.1	0.2
Ammonium	<0.2	<0.2	<0.2	<0.2	0.2
Ortho-phosphate	< 0.2	< 0.2	<0.2	<0.2	0.10
Chloride (Cl)	12	14	14	14	600
Sulphate (SO <sub>4</sub> )	5	6	6	5	100.0
Sodium (Na)	7	11	11	13	100
Potassium (K)	<0.1	<0.1	<0.1	<0.1	200
Calcium (Ca)	5	4	5	5	-
Magnesium (Mg)	2	2	3	3	70.0
Aluminium (Al)	<0.100	<0.100	<0.100	<0.100	0.01
Arsenic (As)	<0.010	<0.010	<0.010	<0.010	100
Copper (Cu)	<0.025	<0.025	<0.025	<0.025	0.500
Iron (Fe)	<0.025	<0.025	<0.025	<0.025	0.020
Lead (Pb)	<0.020	<0.020	<0.020	<0.020	0.012
Manganese (Mn)	<0.025	<0.025	<0.025	<0.025	0.180
Zinc (Zn)	<0.025	<0.025	<0.025	<0.025	1.000

Key: TWQR=Target Water Quality range for South African aquatic ecosystems

Table 2. Mean total length (mm) and condition factors of three cyprinids sampled at Nwanedi-Luphephe dams during the four seasons (January-October 2008)

Species	Season	Fish length		Weight		Condition factor	
		Mean	±SD	Mean	±SD	Mean	±SD
<i>Labeobarbus marequensis</i>	Autumn	360.0	41.3	981.6	493.2	1.9	0.7
	Winter	187.1	53.2	176.9	109.9	2.3	0.3
	Spring	309.3	105.1	711.5	629.1	2.2	0.2
	Summer	392.0	53.1	1318.5	345.9	2.2	0.6
<i>Barbus radiatus</i>	Autumn	30.7	2.2	0.5	0.1	1.8	0.4
	Winter	31.7	5.3	0.7	0.3	2.1	0.5
	Spring	59.1	9.6	5.3	2.3	2.4	0.3
	Summer	41.2	7.9	1.7	1.6	2.1	0.6
<i>Barbus trimaculatus</i>	Autumn	41.8	25.5	3.4	6.4	1.9	0.2
	Winter	90.8	14.8	19.2	5.8	2.4	0.3
	Spring	76.9	25.9	14.0	10.8	2.5	0.2
	Summer	90.5	20.6	17.1	6.6	2.1	0.4

±SD = Standard deviation

the Nwanedi Nature Reserve in the Limpopo Province of South Africa (Fig. 1). The dams form twin impounds connected by a 2.5 m canal with a carrying capacity of 19.1 million m<sup>3</sup>. The drainage system of the dams forms part of the Limpopo River System. There are no agricultural, industrial and mining activities in close proximity to these dams (Oberholster *et al.*, 2009).

A handheld YSI (556 MPS) measuring instrument was used to determine the water parameters and additional subsurface water parameters *in situ* during each survey (Table 1). The water samples were frozen and subsequently analyzed in accredited laboratory (ISO 17025, SANAS, Laboratory no. T0391). All water values did not exceed the limits specified by the South African target water quality range (TWQR) as indicated by the then Department of Water Affairs and Forestry (1996).

#### Collection of hosts

A total of 162 fish, *L. marequensis* (n = 53), *B. trimaculatus* (n = 63), and *B. radiatus* (n = 46) were caught using gill nets of stretched mesh sizes 30 – 110 mm, cast nets, electro-fishing and seine nets over four seasonal periods in 2008: January (summer), April (autumn), July (winter), and October (spring). *B. trimaculatus* and *B. radiatus* were also collected in the streams feeding the dams. Live fishes were transported to the field laboratory and kept alive in aerated holding tanks until examination. Total length, weight and sex of each fish were recorded. Fish were sacrificed by severing the spinal cord, dissected and examined for parasites. Biometrical data on the three fish hosts and their condition factors are summarized in Table 2.

#### Collection of parasites and parasitological analysis

The eyes, brain, heart, stomach, intestine, liver, spleen, swim bladder and urinary bladder, body cavity, surface of the visceral organs and serous membrane were placed separately in Petri dishes containing a saline solution and examined microscopically for the presence of larval stages of endoparasites. Fixation, preservation, whole mount preparations as well as identification of parasites followed standard procedures by Pritchard and Kruse (1982). Due to difficulties in the identification of the larval forms to the species level, only the genus names of parasites were employed in the present paper.

#### Data analysis

The prevalence, mean abundance and mean intensity of each parasite were calculated according to Bush *et al.* (1997). A general linear model (GLM) was performed to ascertain which of the predictor variables (season, size, and sex) was associated with the infection level of the two predominant parasites, *Diplostomum* sp. and *Contracaecum* sp. Data were tested for normality and homogeneity of variance using the Shapiro-Normality test and Levene's test, respectively. The LSD (Least Significant Difference) post-hoc test was performed using SPSS 17.0 software package (SPSS Inc., Chicago, Illinois, USA) to determine the seasonal differences in the occurrence of the predominant parasites. A Pearson's correlation was performed to determine the relationship between the parasite burden and the fish length as well as between parasite burden and the condition factors of the fish. All statistical analyses were performed at the significance level of 5 %.

Table 3. Seasonal occurrence of *Diplostomum* sp. and *Contracaecum* sp. from *Labeobarbus marequensis* at the Nwanedi-Luphephe dams

Taxon	Season	No. of examined fishes (n)	No. of fish infected	MA	MI	P (%)	Total No. of parasites
<i>Diplostomum</i> sp.	Autumn	15	13	8.8	10.2	86.7	132
	Winter	10	8	7.2	9.0	80.0	72
	Spring	10	10	9.7	9.7	100.0	97
	Summer	18	17	19.3	20.4	94.4	347
<i>Contracaecum</i> sp.	Autumn	15	4	4.5	16.8	28.0	67
	Winter	10	0	0.0	0.0	0.0	0
	Spring	10	1	0.3	3.0	10.0	3
	Summer	18	16	68.2	76.8	89.0	1228

MA = mean abundance, MI = mean intensity, P = prevalence

The condition factor of fish was calculated as follows:

$CF = W \times 10^5 / L^3$ , where CF = condition factor, W = weight in grams and L = total length in millimetres (Klemm *et al.*, 1992).

## Results

In total, three types of metacercariae larvae, gryporhynchid cestode larvae and nematode-third-stage *Contracaecum* larvae (L3) were recorded in this study. *Diplostomum* sp. were collected from the eyes of *L. marequensis* (648) and *B. trimaculatus* (20) while a total of 177.0 *Ornithodiplostomum* sp. (encysted) from gills of *B. trimaculatus* (Table 4). *Posthodiplostomum* sp. encysted in the muscles, skin and fins of six *B. trimaculatus* (20) and five *B. radiatus* (17) during the winter survey (Table 4). *Contracaecum* sp. were collected from the mesentery fats in the body cavity and liver lobes of *L. marequensis* (1298) and *B. trimaculatus* (23) whereas the gryporhynchid cestode larvae (Cyclophyllidae) was found from the external surface of the intestine of *B. radiatus*. The gryporhynchid cestode larvae (10 specimens) were isolated exclusively during the winter survey; with a mean intensity of 1.6 and a prevalence of 13.0 %.

The most abundant parasites were *Diplostomum* and *Contracaecum* sp. and their seasonal variations and infection statistics are summarised in Table 3. The overall occurrence (present or absent) of endoparasites recorded in this study is presented in Table 4

while Table 5 shows the overall level of infection calculated for each fish per parasite.

*Diplostomum* sp. were the most abundant larvae throughout the sampling period. In *B. trimaculatus*, the prevalence of this parasite reached 10.0 % and 12.2 % in spring and in summer, respectively. The GLM model indicated that season significantly influenced the intensity of *Diplostomum* sp. ( $F=5.20$ ,  $P<0.05$ ). In the other host, *L. marequensis*, significant differences in the intensity of infection were found between summer and the remaining three periods of the year, but not between autumn and winter, autumn and spring as well as between winter and spring (LSD post-hoc test). The mean intensity of the *Diplostomum* sp. was 19.1 in summer compared to 10.2, 9.0 and 9.7 in autumn, winter and spring, respectively (Table 3). On the other hand, no significant effect of gender and size of the fish host on the intensity of *Diplostomum* sp. ( $F=1.01$ ,  $P>0.05$ ;  $F=0.12$ ,  $P>0.05$ , respectively) was observed. The Pearson's correlation results indicated only a weak, positive correlation between the mean intensity of *Diplostomum* sp. and the condition factor of *L. marequensis* ( $r=0.09$ ,  $P>0.05$ ).

A prevalence of 20.0 % for *Contracaecum* sp. (L3) in *B. trimaculatus* was recorded in the winter and summer surveys. The mean intensity was 2.7 and 5.0 in these samplings, respectively. No *Contracaecum* larvae were found in this host during autumn and spring. The GLM and Pearson correlation analyses indicated no

Table 4. Presence of larval stages of endoparasites in three cyprinids during different seasons in 2008 at the Nwanedi-Luphephe dams

Parasite groups	Taxon	Sampling periods			
		April	July	October	January
Digenea	<i>Diplostomum</i> sp.	+	+	+	+
	<i>Posthodiplostomum</i> sp.	-	+	-	-
	<i>Ornithodiplostomum</i> sp.	+	+	+	+
Cestoda	Gryporhynchid larvae	-	+	-	-
Nematoda	<i>Contracaecum</i> sp.	+	-	+	+

+ = Presence of parasite, - = absence of parasite

Table 5. Overall level of infection of parasites for three fish species from Nwanedi-Luphephe dams (January-October 2008)

Host species	Parasite species	MA	MI	P (%)
<i>Labeobarbus marequensis</i>	<i>Diplostomum</i> sp.	12.2	13.5	90.6
	<i>Contracaecum</i> sp.	24.5	61.8	39.6
<i>Barbus trimaculatus</i>	<i>Diplostomum</i> sp.	0.3	2.8	11.1
	<i>Ornithodiplostomum</i> sp.	2.8	4.0	69.8
	<i>Posthodiplostomum</i> sp.	0.3	3.3	9.5
	<i>Contracaecum</i> sp.	0.3	3.8	9.5
<i>Barbus radiatus</i>	<i>Posthodiplostomum</i> sp.	0.4	4.3	10.9
	Gyroporynchid larvae	0.2	1.6	13.0

MA = mean abundance, MI = mean intensity, P = prevalence

effect of season and condition factor of the fish host on the intensity of infection of *Contracaecum* sp. in *L. marequensis*.

## Discussion

*Diplostomum* sp. and *Contracaecum* larvae were previously recorded from *L. marequensis* as well as from other fish species in South Africa (Olivier *et al.*, 2009; Smit & Luus-Powell, 2011; Madanire-Moyo *et al.*, 2012). A high prevalence and intensity of infection of the two parasites were also recorded in *B. trimaculatus* in this study. In the present study, the maximum prevalence and intensity of *Diplostomum* sp. occurred in summer, most probably due to higher water temperature in this season. More intense cercarial production associated with the increasing water temperature was formerly reported by Stables and Chappell (1986), Taskinen *et al.* (1994) and Lyholt and Buchmann (1996). Wang *et al.* (2001) reported that the release of the free-swimming cercariae of *Dollfusotrema vaneyi* (Echmann, 1934) in warm seasons contributed to higher infection levels in the bullhead catfish *Pseudobagrus fulvidraco* (Richardson, 1846) in China.

Life cycles of endoparasites are complex with multiple intermediate and often migratory definitive hosts and, thus, consequent infection values may be assigned to the richness and availability of appropriate hosts. Different feeding patterns of the fish could also play a significant role in the diverse abundance of their parasites. *Labeobarbus marequensis* is an omnivorous fish species (Skelton, 2001), a diet of which is mostly composed of snails and crustaceans (intermediate hosts of *Contracaecum* sp.). Contrary to that, *B. trimaculatus* feeds mostly on phytoplankton that limits its possibility to be infected.

The abundance of *Diplostomum* sp. and *Contracaecum* sp. may also be influenced by the type of habitat. Lentic ecosystems provide specific conditions, such as the higher water temperature and lower water currents (Ondračková *et al.*, 2004), which facilitates concentration of the first and second intermediate hosts, thus increasing the probability of the cercariae locating its host. In addition, these conditions make the second intermediate host more vulnerable to predation by piscivorous birds. The high intensities of *Diplostomum* sp. and *Contracaecum* sp. recorded in *L. marequensis* in the present study coincided with the low water levels in the dams, in contrast to *B. trimaculatus* collected in the streams, which was free of parasites.

The present study evidenced no relationship between parasite burden and the condition factor of the fish host. In natural conditions, many parasites exist in benign relationships with their hosts, without causing any obvious distress. Nevertheless, high numbers of *Diplostomum* sp. are associated with several fish diseases such as exophthalmia, local haemorrhage, lens cataract, which may yet lead to fish mortality. None of these symptoms were observed in the present study.

Results of this study also showed that the prevalence and intensity of *Contracaecum* sp. and *Diplostomum* sp. were not influenced by the size of the host. It is generally known that the size of fish may have an impact on the occurrence of parasites. Larger fish usually have higher numbers of parasites, which can be attributed to the larger surface area providing more space and food resources to parasites. Fish investigated herein have different body size: The maximum length of *L. marequensis* is 470 mm and that of *B. trimaculatus* and *B. radiatus* only 120 mm (Skelton, 2001). The number of parasites recorded in this study was also higher in larger fish.

The metacercariae embedded in gills of *B. trimaculatus* closely resembled the *Ornithodiplostomum* sp. collected by Barson and Avenant-Oldewage (2006) from the muscles of catfish *Clarias gariepinus* (Burchell, 1822). Although the life cycle of these parasites is similar to other diplostomoids and a number of related genera such as *Diplostomum*, *Neodiplostomum* and *Posthodiplostomum* are quite common in the Limpopo Province (Khalil & Polling, 1997), *Ornithodiplostomum* sp. has not been registered in this area before. Our finding of *Ornithodiplostomum* sp. in *B. trimaculatus* represents a new host and geographical record.

*Posthodiplostomum* sp. is referred as a causative agent of black grub or black spot disease and are located in spherical or oval bead-like cysts embedded in the fish muscle, or under the skin and on the fins. According to Lane and Morris (2000), the outer black cyst wall is of host origin (cells of the host) and inner transparent cyst are formed by secretions of the parasite, which leads to clearly recognizable black spots in the muscle, skin and fins of the host. The low number of fish infected with *Posthodiplostomum* sp. may



suggest a scarcity of suitable intermediate hosts in the Nwanedi-Luphephe dams and thus, *Posthodiplostomum* sp. metacercariae do not represent a serious threat to fish health in this locality. Larval stages of a gryporhynchid cestode were found in the outer intestinal wall of *B. radiatus* during the winter survey. Generally, low infection levels of these metacercariae might be related to the fact that gryporhynchid larvae are often overlooked due to their minuscule size and specific site. Mashego (2001) recorded a prevalence of only 4.0 % of these metacercariae in *B. radiatus* and mean intensities of 1.0 – 6.0 specimens with prevalence ranging from 4.0 – 13.0 % in other five cyprinids from the Limpopo Province. Water quality variables measured in this study show that the Nwanedi-Luphephe dams are relatively un-polluted. During the entire course of the survey, levels of the selected physico-chemical parameters measured were relatively low and within permissible TWQR range. This agrees with previous studies from the Nwanedi-Luphephe dams, characterizing them as essentially unpolluted due to little urban development, absence of intensive agricultural, industrial and mining activities in the area (Oberholster *et al.*, 2009; Madanire-Moyo *et al.*, 2012). The infective stages are considered highly sensitive to adverse water conditions, therefore, their presence or absence may serve as an indication of the status of the water body (Tieri *et al.*, 2006). Our results demonstrated that the quality of the environment favored development of larval stages of various parasites in their intermediate hosts. Although all the above-mentioned larval stages have previously been recorded from other fish species in South Africa, nevertheless, they represent new ecological records for the Nwanedi-Luphephe dams.

## Acknowledgements

The authors thank students from the Department of Biodiversity and the Aquaculture Research Unit, UL, for technical assistance in fish necropsy and field work. We also wish to thank Prof. PAS Olivier, Dr. MM Matla, Dr. GN Madanire-Moyo, Mr. WJ Smit and Mr. HE Hattingh who assisted with field work. The funding for this study was provided by the National Research Foundation (NRF); and the Research Development and Administration of the University of Limpopo.

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