OCCURRENCE OF METACERCARIAE OF *DIPLOSTOMUM* AND *TYLODELPHYS* SPECIES (DIPLOSTOMIDAE) IN *CLARIAS* SPECIES (CLARIIDAE) FROM LAKE VICTORIA

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ABSTRACT

The localisation and abundance of the metacercariae of Diplostomum *and* Tylodelphys *species among the clariids of Lake Victoria were investigated between April and December 2001. Four species viz.* Clarias gariepinus (*271*), C. alluaudi (*65*), C. werneri (*50*) *and* C. liocephalus (*47*) *were examined, but only* C. gariepinus *was infected in the brain case and along the nasal cavity. Prevalence of metacercariae was high (>70%). Prevalence and mean intensity were similar between male and female fish. Mean intensity increased with fish size and declined in larger fish for* D. mashonense; *but declined with the increase in fish size for* Tylodelphys *species. The metacercariae were over-dispersed and the variance to mean ratio curves behaved similarly to mean intensity. Possible factors leading to the observed infection patterns are discussed.*

INTRODUCTION

Parallel with the development of fish culture, the importance of fish diseases and parasites in the fishery industry has been underlined. Parasites can affect fish health in intensive culture systems causing considerable losses to the fishery industry (Parperna 1980, Field and Irwin 1994).

In the northern hemisphere, *Diplostomum* species have attracted attention because of their pathogenicity on fingerlings on fish farms (Field and Irwin 1994, Chappell 1995). Hence their biology has been studied intensively (Stables and Chappell 1986, Field and Irwin 1994, Chappell 1995). The metacercariae of *Diplostomum* species have been recorded in over 150 fresh and brackish water fish (Sweeting 1974, Niewiadomska 1996). The genus *Tylodelphys* is closely related to *Diplostomum*. The adults of both species infect closely related birds as final hosts (Niewiadomska 1996). Similarly, the metacercarial stages often co-exist in the same fish (Burrough 1978, Mwita 2002, Musiba 2004). The abundance of metacercariae of both genera varies among fish host species and with the size of the host (Betterton 1974, Nkwengulila 1995).

Studies on *Diplostomum* species in Africa are few and only one is known on *Tylodelphys* species (Mwita 2002). Similarly, in Tanzania few studies are known on *Diplostomum* species, and all refer to one fish species, *C. gariepinus* (Nkwengulila 1995, Mwita 2002, Musiba 2004). Nkwengulila (1995) also reported *Diplostomum* species from *Oreochromis* species (Cichlidae). The aim of the present study was to establish if other *Clarias* species that co-exist with *C. gariepinus* are also infected with *Diplostomum* species. Thus, the occurrence, localisation, and abundance of *Diplostomum* species among four *Clarias* species (*C. gariepinus*, *C. alluaudi*, *C. werneri*, and *C. liocephalus*) in the Mwanza gulf were investigated.

MATERIALS AND METHODS

Collection and handling of fish and metacercariae

Fish were caught using gill nets, traps and baited long lines and handlines. Dead fish were transported, to the laboratory, in an ice-cooled box and live ones in buckets containing lake water, at the Tanzania Fisheries Research Institute (TAFIRI), Mwanza Centre. Fish were examined soon on arrival, after the weight, standard length, sex and maturity stage of each fish have been recorded. Fish were then decapitated and the cranium opened using a strong and sharp scalpel. Metacercariae were flushed from their localisation by squirting tape water using a pipette. Metacercariae were pipetted into a watch glass, washed, sorted and counted.

Data analysis

Data for prevalence and mean intensity with respect to sex and size of fish were analyzed by χ^2 and Kruskal-Wallis tests, respectively (Zar 1996).

RESULTS

Occurrence, prevalence and mean intensity of metacercariae

Of the four *Clarias* species examined, only *C. gariepinus* was infected. Three metacercariae, *Diplostomum mashonense* and two species of *Tylodelphys* (designated 1 and 2) co-existed in front of the brain along the nasal cavity and the fat tissue covering the olfactory nerve. About 97% of *C. gariepinus* examined were infected by at least one of the three types of metacercariae. The prevalence of *D. mashonense*, *Tylodelphys* 1 and 2 was generally high, 97%, 74% and 94%, respectively. The intensity of infection among individual fish was extremely variable (Table 1). The mean intensity of both *D. mashonense* and *Tylodelphys* 2 were higher than that of *Tylodelphys* 1.

Table 1; Occurrence of the metacercariae of *Diplostomum mashonense* and *Tylodelphys* species in 271 *Clarias gariepinus*

Parasite	No. infected	Prevalence (%)	Mean intensity	std	Range	Abundance	Proportion (%)
Diplostomum mashonense	262	96.7	619.5	780.5	1 - 5024	598.9	58.35
Tylodelphys sp. 1	202	74.5	76.7	260.5	1 - 1800	57.2	5.58
Tylodelphys sp. 2	255	94.1	393.5	593	1 - 3872	370.2	36.07
Overall	264	97.4	1053.5	1099	1 - 5024	1026.3	100

In general, *Tylodelphys* 1 occurred in lower proportions than *Tylodelphys* 2 and *D. mashonense* (Table 1). The interactions between the pairs of these parasites are shown on Fig. 1. Heavy infections of any two species seldom occurred in the same fish, but light infections of one species could co-exist with light or heavy infections of the other. *Tylodelphys* 1 always occurred in lighter infections whereby more than 250 metacercariae could hardly be found together with ≥1000 *Tylodelphys* 2 and / or *D. mashonense*. However, in some instances, heavy infections by *D. mashonense* and *Tylodelphys* 2 (≥ 2500 metacercariae) were found in the same fish.

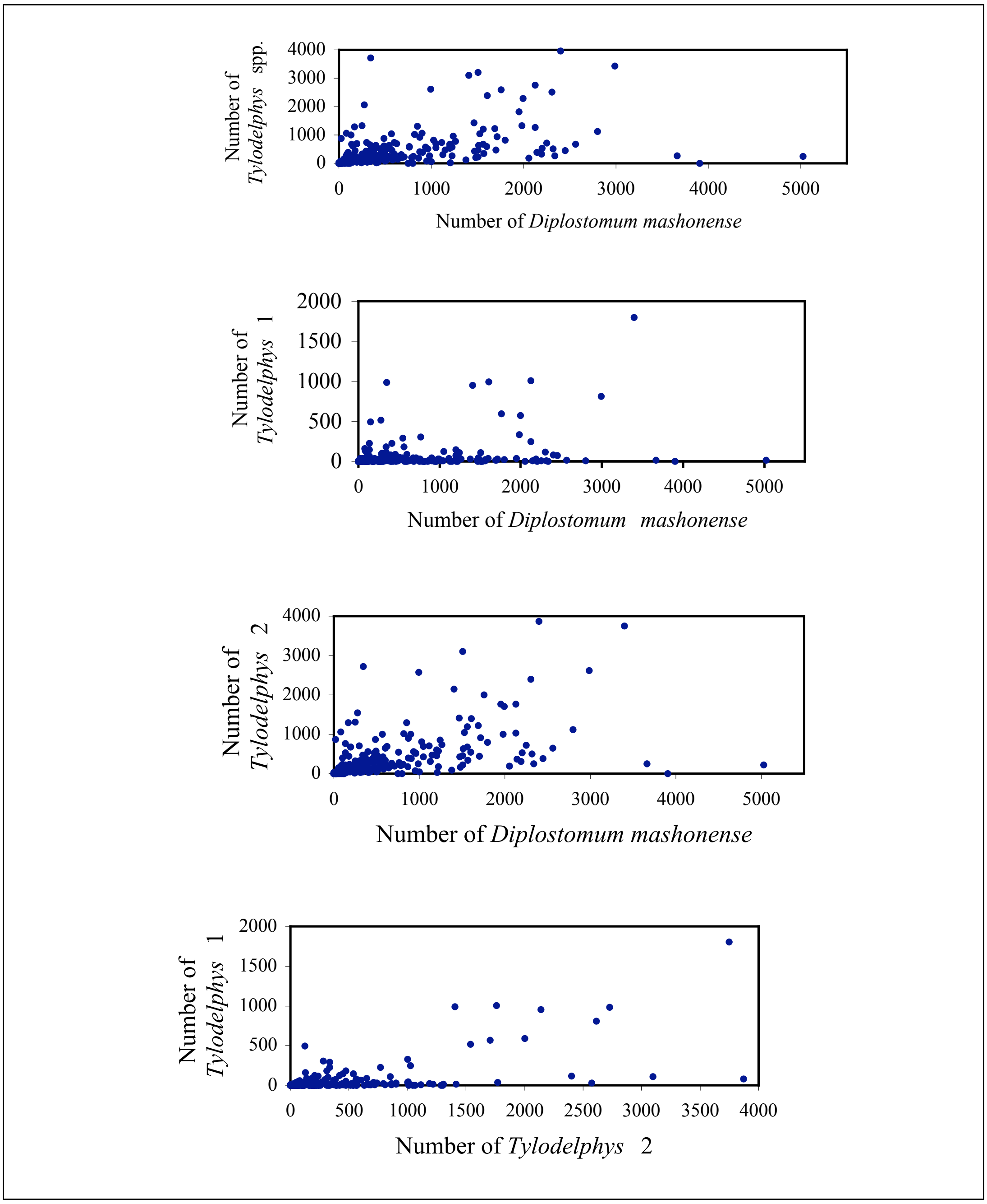

Figure 1: The relationship between the number of *Diplostomum mashonense* and *Tylodelphys* species 1 and 2 (pair wise comparisons) in the cranium of *Clarias gariepinus* (each dot represents one fish).

Abundance of metacercariae in relation to sex and size of *C. gariepinus*

The prevalence and mean intensity of all the three metacercariae were not significantly different between female and male *C. gariepinus* (Table 2).

Table 2: Statistical test results for differences in the prevalence and intensity of *Diplostomum* and *Tylodelphys* metacercariae between male and female *Clarias gariepinus*

Parameter	Test	*D. mashonense*	*Tylodelphys* sp 1	*Tylodelphys* sp 2
Prevalence	χ^2	1.77	3.09	1.85
	p	0.1833	0.0786	0.1732
	Significance	NS	NS	NS
Mean	**KW (H)**	10.63	11.29	10.07
Intensity	p	0.2235	0.1854	0.1732
	Significance	NS	NS	NS

The prevalence of *D. mashonense* and *Tylodelphys* 2 was similar in all size classes with a slight decline in the 45 – 50 cm class: *D. mashonense* (χ^2= 1.959, p = 0.9921) and *Tylodelphys* 2 (χ^2 = 1.860, p=0.9935) (Fig. 2). The prevalence of *Tylodelphys* 1 was slightly low but reached 100% in the smallest and largest size classes, the variations were not statistically significant (χ^2 = 15.788, p = 0.0714) (Fig. 2). The mean intensity of *D. mashonense* rose steadily with fish size but declined from fish of 20 - 25 cm size class; these variations too were not statistically different (H = 7.038, p = 0.6332). The mean intensity of *Tylodelphys* 1 and *Tylodelphys* 2 decreased with fish size (Fig. 2), but again the variations were statistically not significant: *Tylodelphys* 1 (H = 10.948, p = 0.2793) and *Tylodelphys* 2 (H = 6.634, p = 0.6751).

Frequency distribution of the intensity of metacercariae

Variance to mean ratio showed that both *D. mashonense* and *Tylodelphys* species were over dispersed (Fig. 3). The variance to mean ratio curves for all three species were similar to the mean intensity curves (Fig. 3 c.f. Fig. 2).

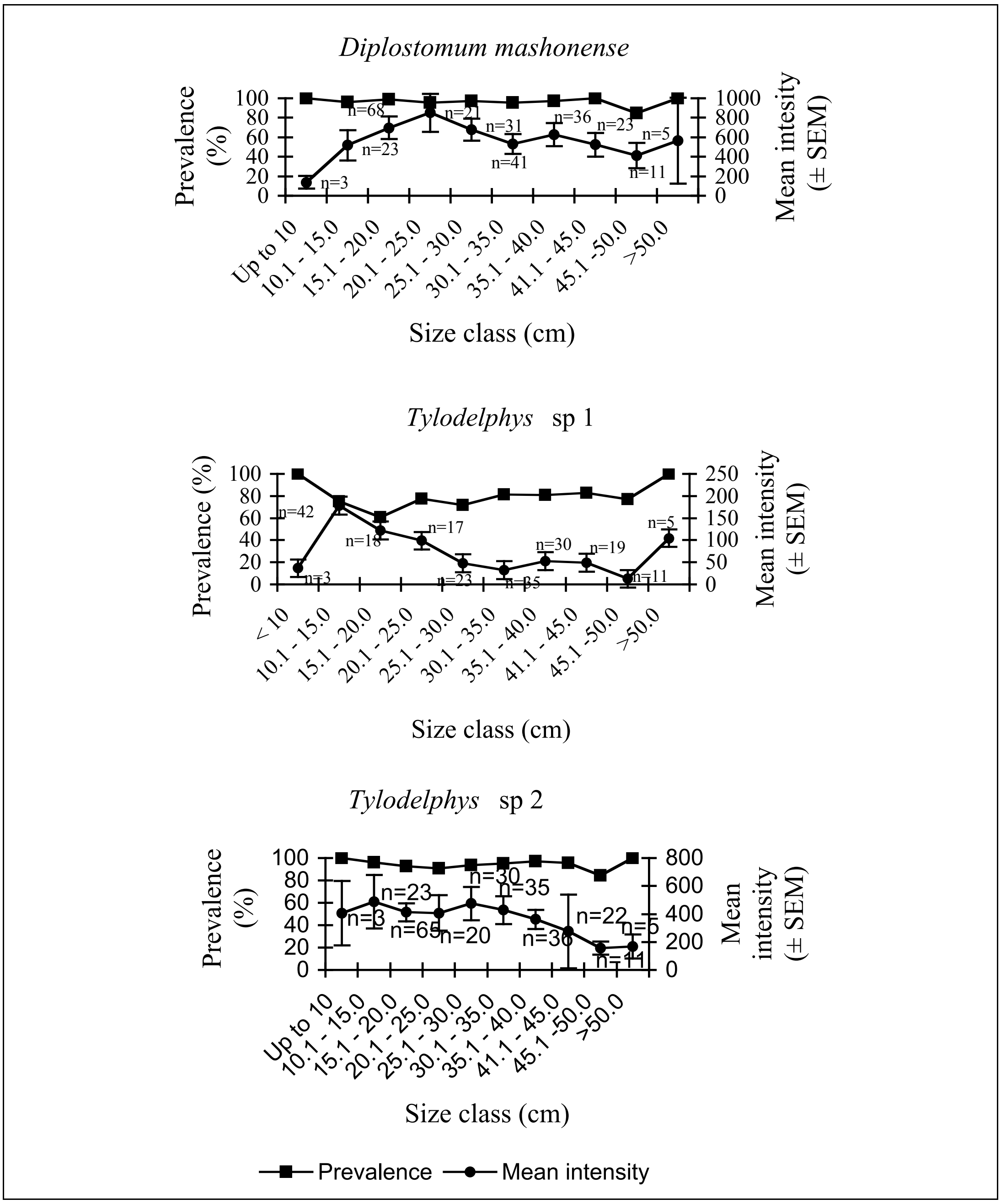

Figure 2: Prevalence and mean intensity of the three metacercariae in relation to the length of *Clarias gariepinus*.

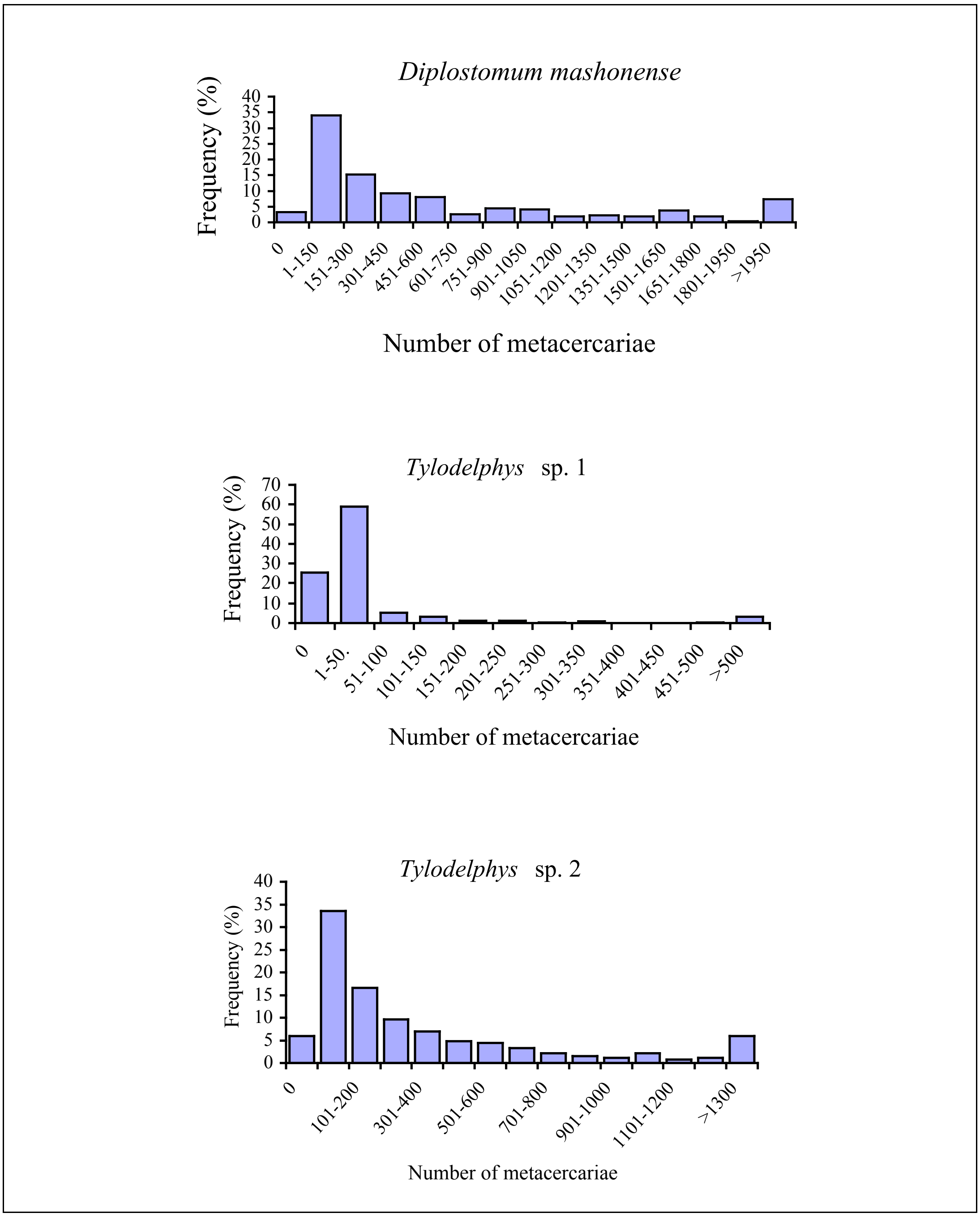

Figure 3: Frequency distribution of *Diplostomum mashonense* and *Tylodelphys* species in *Clarias gariepinus*.

DISCUSSION

Occurrence, prevalence and mean intensity of metacercariae

Metacercariae of *D. mashonense* were first reported by Beverley-Burton (1963) from the cranial cavity of *C. gariepinus* and *C. ngamensis* in Zimbabwe and Zambia, respectively. Prudhoe and Hussey (1977), and Mashego and Saayman (1989) recovered metacercariae of *D. mashonense* from the cranial cavity of *C. gariepinus* in South Africa. In Tanzania, metacercariae of *D. mashonense* have been reported in *C. gariepinus* from the Ruvu Basin (Nkwengulila 1995) and Lake Victoria (Mwita 2002). The earliest record of *Tylodelphys* species in Africa is that of adult worms from a kite, *Buteo refofuscus augur* Rüpp (Dubois and Fain 1956) from the Democratic Republic of Congo. The latest is by Mwita (2002) who recorded metacercaria of *Tylodelphys* species in *C. gariepinus* from the Mwanza Gulf, Lake Victoria. Nkwengulila (unpub. data) also reported *Tylodelphys* species in eyes of *Oreochromis* species and the cranial cavity of *C. gariepinus* from the Ruvu basin. The findings of the present study confirm the localisation and specificity of metacercariae of *D. mashonense* to *C. gariepinus*.

Doplostomum mashonense and *Tylodelphys* species were recorded from *C. gariepinus* but none in the other three clariids. Differential susceptibility of closely related fish species to metacercariae of *Diplostomum* species is well documented (Betterton 1974, Sweeting 1974, Burrough 1978, Nkwengulila 1995). Since the four *Clarias* species are closely related and inhabit similar habitats, the absence of infection in the other three *Clarias* species may most likely be due to failure of migrating cercariae to penetrate the tissues of the other three clariids. Other physiological factors, such as absence of stimuli or presence of conflicting stimuli, which disorient migrating cercariae, and a strong immune response may retard or hinder establishment within the host (Betterton 1974, Sweeting 1974).

The high prevalence (> 70%) of *D. mashonense* and *Tylodelphys* species observed is supported by reports of earlier workers (Nkwengulila 1995, Mwita 2002) in Tanzania, and elsewhere in Africa (Beverley-Burton 1963, Mashego and Saayman 1989). The high infection statistics suggests that, *C. gariepinus* is highly susceptible to *D. mashonense* and *Tylodelphys* species.

There were always light infections of *Tylodelphys* 1 with heavy infections of *D. mashonense* and / or *Tylodelphys* 2 and that these two parasites (*D. mashonense* and *Tylodelphys* 2), were in some instances, found heavily infecting the same fish. There could be two explanations for this; either that heavy infection of *D. mashonense* and / or *Tylodelphys* 2 on the one hand, and those of *Tylodelphys* 1, is mutually exclusive. Alternatively, factors governing the infection processes prevent acquisition of large numbers of the two groups (i.e. *D. mashonense* and *Tylodelphys* 2, group 1 and *Tylodelphys* 1, group 2) of metacercariae (Kennedy and Burrough 1977, Burrough 1978). Heavy infections of *D. mashonense* and *Tylodelphys* species in the same fish suggest that these parasites share the same snail population as their first intermediate host (Machado *et al*. 1996). The snail intermediate hosts for *Diplostomum* and *Tylodelphys* species in Africa are not known. Elsewhere, lymnaeid species act as first intermediate hosts (Niewiadomska 1996).

Abundance of metacercariae in relation to sex and size of **Clarias gariepinus**

There were minor variations in prevalence and mean intensity between male and female fish. Other workers have reported similar observations (Shotter 1980, Nkwengulila 1995, Mwita 2002, Musiba, 2004), and this has been attributed to similar ecological relationships between female and male fish.

Mean intensity of *D. mashonense* increased with size but declined in larger fish, whereas that of *Tylodelphys* 1 and 2 decreased with size. Furthermore, few infected fish were ≤ 10 cm long, reflecting perhaps death of these smaller fish due either to massive cercarial penetration or by predation (Brassard *et al.* 1982). Fish size and age are known to be positively associated with abundance of metacercariae of diplostomids (Sweeting 1974, Nkwengulila 1995, Mwita 2002, Musiba, 2004). This has been attributed to; the long life span of metacercariae, continuous exposure of the fish to re-infection throughout their life and their inability to develop age related immunity.

The decline of intensity of infection in older fish might be due to low establishment rate caused by either development of immunity or behavioural changes in old fish, which result in low recruitment rate. Although evidence is lacking, it is possible that the parasites were dying within the fish. This would infer that the parasites' life span is short and that mortality rate exceeded recruitment rate in older fish. However, most *Diplostomum* species are known to have long life spans in their fish hosts, hence their accumulation with host size (Kennedy & Burrough 1977, Chappell 1995, Nkwengulila 1995). No dead or moribund metacercariae were recorded during the present study, neither did Nkwengulila (1995), Mwita (2002) and Musiba (2004). Many workers have reported similar results (Chappell 1969, Kennedy and Burrough 1977); some however, have reported dead metacercariae in fish in the Palaearctic Region (Pennycuick 1971, Sweeting 1974, Nkwengulila 1995).

Frequency distribution of metacercariae

Over dispersion of the metacercariae of *Diplostomum* species in their fish hosts is a common phenomenon (Sweeting 1974, Kennedy 1984, Nkwengulila 1995, Mwita 2002, Musiba 2004). The decline in the degree of over dispersion coupled by a decline in mean intensity may be indicative of loss of the parasite population within the hosts, to which the explanation given above for the decline in mean intensity with age should apply.

The over dispersion of metacercariae in fish host populations has been attributed to heterogeneity in susceptibility, and uneven exposure of fish to infection that is a result of non-random distribution of cercariae in the habitat caused by aggregated distribution of snail intermediate hosts and chance effects (Anderson and Gordon 1982, Esch and Fernandez 1993, Bush *et al.* 2001).

CONCLUSION

The metacercariae of *D. mashonense* and *Tylodelphys* species were specific to *C. gariepinus*. Physiological differences may account for the absence of infection in the other clariids. Mutual exclusion or some factors governing the infection processes prevented the acquisition of large numbers of *Tylodelphys* 1 concurrent with large numbers of *D. mashonense* and / or *Tylodelphys* 2. Development of immunity, a short life span of the parasites and changes in recruitment rates with age of fish may explain the observed infection patterns. The over dispersed distribution of metacercariae in *C. gariepinus* is a result of inherent differences in susceptibility and uneven exposure of fish to infection.

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