Co-infection and Morphometrics of Three Clinostomatids (Digenea: Clinostomatidae) in *Tilapia guinensis* Bleeker, 1862 from Opi lake, Nigeria

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Abstract

Co-infection and morphometrics of helminth parasites of Tilapia guinensis in Opi Lake (GPS N06.75275*, E007.49104*), were studied from (Nov 2007– Oct 2008) using multiple fishing gear techniques; cast nets, hook and line, and seine nets (150 mm – 200 mm). The parasites recovered were C. tilapiae, C. complanatum and E. heterostomum. The prevalence was low, indicative of parasitic infection in the wild. Prevalence of (33.9 %) was recorded in C. complanatum, (7.2 %) in E. heterostomum and (12.6 %) in C. tilapiae. But high mean intensity was suggestive of heavy parasite burden; C. complanatum (4.8), C. tilapiae (2.8), and E. heterostomum (5.1). Morphometrics of the parasites showed no significance difference in the distance between oral and ventral suckers. However, they differed in all other parts significantly (p < 0.05). In the rank-abundance curve for parasite communities, C. complanatum was most abundant (pi 0.63). Differential parasitic effects were due to selection for relatively better adaptiveness to host's microhabitats, more population size, better host location, and larger body size. Consequently, this resulted in a trade-off between larger morphometric parts and population size among the parasites.

Keywords: Clinostomatids, Co-infection, Morphometrics, *Tilapia guinensis,* Opi Lake, Selection

Introduction

Species of *Clinostomum* have been described from freshwater fish in Nigeria and many parts of the world. Ukoli (1966) described *Clinostomum tilapiae* in the intestine of *Oreochromis niloticus* and *Sarotherodon galilaeus* in the River Niger. Other species of *Clinostomum,* e.g. *Clinostomum complanatum* has been recorded in the fish of River Niger (Ukoli, 1969), *Clinostomum* sp. were found to be common in *Tilapia* sp. While those of *Euclinostomum* sp. were recovered mainly from the osteoglossid *Heterotis niloticus.* However, in the species of *Tilapia*, the metacercariae occurred in the sub mucosa of the mouth cavity, gill chamber, below the operculum and pharyngeal bone, orbit, muscles of the body, around the heart, abdominal cavity, mesentery, viscera and the swim bladder (Awachie, 1965). *Clinostomum tilapiae* n. sp. and *Clinostomum phalacrocoracis* Dubois, 1931 from Ghana (Ukoli, 1966). The adult trematode of *Clinostomum* are attached to the upper and lower jaws of cattle egrets, herons etc. Family *Clinostomatidae* has a widespread distribution. Members of this family have been recorded from the area where mean temperature is about 10^0C (Grabda-Kazubska, 1974). Infected cases of fish have been reported from Japan and Korea (Chung *et al*., 1995; Hiral *et al*., 1987; Isobe *et al*., 1994). *Clinostomum complanatum* and *Clinostomum tilapiae* have been recovered from African continent with *Euclinostomum heterostomum* being cosmopolitan. The metacercariae of *Clinostomum complanatum* have been recorded from *Perca fluviatilis* (Grabda-Kazubska, 1974), *Rutilus rutilus* (Grabda-Kazubska, 1974), *Plecoglossus altivelis* (Lo *et al*., 1987), *Misgurnus anguillicaudatus* (Lo *et al*., 1992), *Carassius carassius, C. gibelio langsdrfi, C. cuvieri, Cobitis anguillicaudatus, Cyprinus carpio, Pseudogobio esocinus, Pseudorasbora parva, Rhodeus lanceolatus, R. ocellatus* and *Hypomesus transpacificus* (Aohagi *et al*., 1993), *Ccarassius* sp. (Aohagi and Shibaharam 1994), *Zacco temminki, Acheilognathus rhombea, Microphysogobio yaluensis, Carassius auratus* (Chung, 1995), *Lateolabrax japonicus, Leuciscus hakonensis* (Aohagi *et al.,* 1995) and *Eutycea neotenes* (Mitchell, 1995). Adult stage of parasite has been reported from *Nycticorax nycticorax* (Aohagi *et al*., 1992), *Ardea cinnerea* (Aohagi *et al.,* 1992; Lo *et al*., 1987). Metacercariae may affect growth and survival, or disfigure fish so that they lose their market value as a food or ornamental product (Paperna, 1991). Some metacercariae in fisheries and aquaculture products (fish and shellfish) are a source for infections in humans and domestic animals (Deardoff and Overstreet, 1991). The objectives of the present study were to study co – infection and morphometrics in relation to better adaptiveness and population size.

Material and Methods

Study area: Opi lakes are located between 6^0 45' 0"- 6^0 45' 28" N and 7^0 29'28" - 7^0 29' 35" E in the valley of River Uhere Northeast of Nsukka, Enugu State, Nigeria. Each lake is not more than 300 meters from Uhere river. The soil is porous and subject to sever erosion. The vegetation and climate of the area are described by Hare and Carter (1984). The lake has no permanent inlet, but during the flood period the lake overflows through a small channel at the southern end. The lake has a gentle sloppy shoreline with thick marginal vegetation (Inyang, 1995). The western side has a wide beach overgrown with saprophytes dominated by *Cyrtosperma senegalenses* (Schott), *Jussiaea repens* var *diffusa* (Forsk) and *Rynchospora* sp. Its surface area and maximum depth (Zmax) fluctuate

Table 1: Clinostomatid composition and prevalence in relation to micro-habitats in *Tilapia guinensis* from Opi Lake

Parasite species	Host fish & No of infected hosts	Sex of hosts	No of infected host	Microha-bitats in host fish	Total No of Parasites	*Prevalence (%)	¶Mean intensity	#Abundance
Clinostomum complanatum	*Tilapia guinensis* (n = 206) infected hosts = 70	Male	24	B. cavity	192	11.65	2.74	0.93
		,,	18	Skin	78	8.74	1.11	0.38
		,,	22	Eye	50	10.68	0.71	0.24
		Female	3	B. cavity	7	1.46	0.10	0.03
		,,	2	Skin	9	0.97	0.13	0.04
		,,	1	Eye	2	0.49	0.03	0.01
						33.99		
Euclinostomum heterostomum	*Tilapia guinensis* (n = 206) infected hosts = 15	Male	2	B. cavity	14	0.97	0.93	0.07
		,,	3	Skin	8	1.46	0.53	0.04
		,,	1	Eye	5	0.49	0.33	0.02
		Female	4	B. cavity	22	1.94	1.47	0.11
		,,	2	Skin	18	0.97	1.20	0.09
		,,	3	Eye	10	1.46	0.67	0.05
						7.29		
Clinostomum tilapiae	*Tilapia guinensis* (n = 206) infected hosts = 26	Male	6	B. cavity	43	2.91	1.65	0.21
		,,	10	Skin	20	4.85	0.77	0.10
		,,	7	Eye	8	3.40	0.31	0.04
		Female	1	B. cavity	2	0.49	0.08	0.01
		,,	1	Skin	1	0.49	0.04	++
		,,	1	Eye	1	0.49	0.04	++
						12.63		

Key: *B. cavity = Buccal cavity, *Prevalence: number of host infected divided by the number examined expressed as a percentage; ¶Mean intensity: Mean number of parasites per infected host; Abundance: Mean number of parasites per host examined; + + is for values less than 0.01*

seasonally and range between 1.3 and 2.0 ha and 2.0 3.9 m respectively (Inyang, 1995). The mid lake deposit is mud mixed with coarse organic materials from the marginal vegetation on the other parts of the shoreline. The ichthyofauna of the lake includes *Tilapia zilli, Hemichromis fasciatus, Parachana obsura, Malapterus electricus, Chrysicthys auratus, Epiplatys sexfasciatus, Heterobranchus longifilis, Clarias angullaris, C. gariepinus, Barbus aboinensis, Nannaethiops unitaeniatus, Mormyrops engystoma* and *M. hasselguistii* (Inyang, 1995).

Sampling: Two hundred and six species of *T. guinensis* (Olasebikan and Raji, 1998) were caught using multiple fishing gear techniques - cast nets, hook and line, and seine nets (150 mm – 200 mm) monthly (Nov 2007 – Oct 2008).

Parasite prevalence: Freshly caught fish were examined for parasites using procedure in Arthur and Albert (1994). Prevalence was calculated as the number of host infected divided by the number of host examined expressed in percentage. Treatment, fixation and preservation of parasites were according to Ash and Orihel (1987).

Parasite morphometrics: Eye piece and stage micrometers were used to measure the diameters of oral sucker (OS), ventral sucker (VS) and the pharynx (PHA) to the nearest 0.1 micrometers. Other measurements taken were body length (BL) (nm) and the distance between oral and ventral suckers (DOVS) (nm).

Data analysis: Data generated on the prevalence of parasites were analyzed using the infection statistics of Bush *et al.* (1997), Rank–abundance, species diversity and quantitative index of Shannon-Wienner (Molles, 2002). Differences in the various morphometric characters were established using analysis of variance with Duncan's post hoc. All statistical analysis were done using SPSS version 15 statistical package.

Results

Three clinostomatid metacercariae co-infected *T. guinensis.* They include *Clinostomum tilapiae* Ukoli, 1966, *Clinostomum complanatum* Rud, 1819, and *Euclinostomum heterostomum* Rudolphi, 1809. The prevalence of the parasites was characteristically low, indicative of parasitic infection in the wild – *C. complanatum* (33.9%), *E. heterostomum* (7.2 %), and *C. tilapiae* (12.6 %). But high mean intensity was suggestive of heavy parasite burden. For instance, *C. complanatum* (4.8), *C. tilapiae* (2.7) and *E. heterostomum* (5.1). Out of 206 *T. guinensis* examined, 111 were infected. 26 hosts were infected by *C. tilapiae,* whereas 15 and 70 hosts were infected by *E. heterostomum* and *C. complanatum* respectively. 338 *C. complanatum,* 77 *E. heterostomum,* and 75 *C. tilapiae* were recovered from the infected hosts. *C. complanatum* (pi 0.63) was more abundant than the other two species (Table 1). Duncan's one way analysis of variance for comparison of means of external morphometric parts showed that except for the oral sucker, the parasites differed significantly only in their pharynx and oral sucker ($p<0.05$) (Table 2). The rank-abundance curve for parasite communities showed that *C. complanatum was* more abundant than the other two species (Fig. 1). The three major microhabitats viz: buccal cavity, skin and eye were all harboured by the parasites.

Table 2: Morphometric parts of clinostomatids from *Tilapia guinensis*

Morphometric parts(Micro-meter)	*Clinostomum complanatum*	*E. heterostomum*	*Clinostomum tilapiae*
OS (mm)	2.56 ± 0.94^{c}	1.62 ± 0.49^{a}	1.96 ± 0.84^{b}
VS (mm)	4.23 ± 1.20^{b}	4.00 ± 1.25^{a}	4.31 ± 1.33^{a}
DOVS (mm)	10.78 ± 2.65^{a}	9.71 ± 2.08^{a}	11.14 ± 2.76^{a}
BL (mm)	66.88 ± 12.28^{b}	63.60 ± 11.47^{a}	67.34 ± 11.70^{ab}
Pha (mm)	1.60 ± 0.46^{c}	1.26 ± 0.34^{a}	1.58 ± 0.40^{b}

Key: *Letters a, b, and c show significance difference at 95 % confidence interval ($p < 0.05$ %), the figures with similar letters indicate no significance difference, while those with different letters show significance difference.*

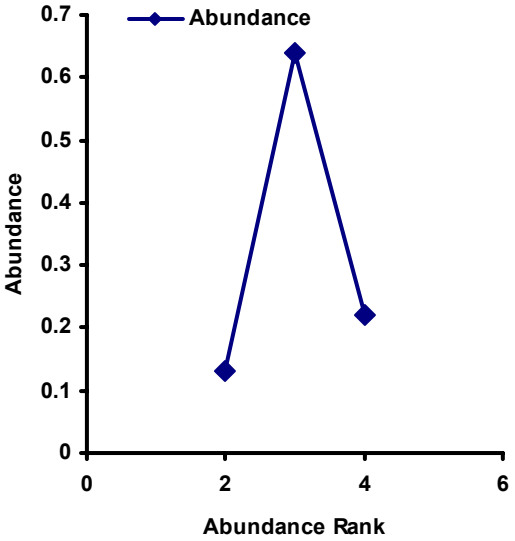

Fig. 1: Community of parasites in Tilapia gunenisi from Opi lake

Also, the infection of the buccal cavity had the highest infection than the other two microhabitats of eye and skin. This is indicative of high predilection for nutrients in the blood of this vascularized area, which these metacercariae depended on. Implications of their presence stem from obstruction of normal blood circulation, especially to the gills, cardiac pathologies, blood flow obstructions, possible mortalities etc. They caused pronounced inflammatory responses due to haemorrhages accompanied by the penetration, early migration, and damage of important organs especially sense organs like eye and skin. Eye damage due to corneal infection resulted in total blindness and other degrees of eye infection; exophtalamus, necrotic cells, and ulceration of the lining membranes. Roughening of the skin by bumps/ yellow grubs caused by encysting metacercariae was observed.

Discussion

The high prevalence and abundance of parasites in the buccal cavity, under the mouth, to behind the operculum (Table 1) is probably due to the presence of blood capillaries because of high penchant for feeding on nutrients in these vascularised areas than other microhabitats of eye and skin. They take-up nutrients (glucose, amino acids, vitamins, fatty acids etc) by both facilitated diffusion and active transport. Due to dearth number of snail intermediate hosts (*Bulinus truncatus*), the few available ones suffer much problem of heavy parasite burden with its own appreciable consequences. Metacercarial development of clinostomatids was correlated with the longevity of the metacercariae due to low rate of infections of bird intermediate hosts. Also, because they do not depend on stored food and can obtain some nutrients from their intermediate hosts thereby remaining viable for the longest periods in the hosts. It might last throughout the lives of the host fish due to difficulty in locating their definitive hosts. Although, sexual maturity was absent every other attribute of adult was well developed. Metacercariae of some flukes can stay up to seven years. After the required development, metacercariae go into a quiescent stage and remain in readiness to excyst on reaching a definitive host. Two forms of occurrence of metacercariae in fish (encysted and excysted) possibly indicate some fish developed resistance to the metacercarial stage of *Clinostomum*. The later form; excysted metacercariae in the various localized sites could be more harmful to human health when they are consumed in semi cooked form. This is because attachment to the mucus membrane of pharynx of the definitive hosts (Egret, Herons etc) causes laryngopharyngitis. There were more excysted forms of *E. heterostomum* than the other two parasite species. Only 28 *E. heterostomum* were not excysted, 35 *C. tilapiae* were all encysted whereas 40 of *C. complanatum* were encysted. These excysted forms which recorded more figure of 298 of *C. complanatum* than the other two species caused serious damages to the infected fish than the excysted forms. These effects include blindness, myositis, muscle bumps (yellow grubs) etc. This will affect the palatability and marketability of the infected fish as well as the acceptance of fish as the primary source of animal protein.

The most general postulate of the theory of natural selection is that the environment determines the evolution of the anatomy, physiology and behaviour of organisms as surmised by Darwin. This great diversity of clinostomatids; differences in the anatomy, especially the length of their body, the diameter of oral sucker, ventral sucker, distance between the oral and ventral suckers and pharynx, reflected selection for firm attachment on host mesenteries for effective use of nutrients. The attribute of nutrition that appears to be most significant is the larger surface area per unit volume of *C. complanatum.* This relative larger surface area of the body length and the diameter of other parts in the parasite populations resulted in a trade – off between selections for maximum nutrient absorption and reproductive success / population. Adaptation is a heritable trait that either spread because of natural selection or has been maintained by selection to the present or currently spreading relative to alternative traits because of

natural selection. In all such cases, the said trait has conferred and continues to confer or is just beginning to confer higher genetic or reproductive success on the species of *C. complanatum* in *T. guinensis*. Similar studies have been documented for two genetically similar strains of laboratory mice, *Cenorhabditis elegans*, and *Drosophila melanogaster* with high reproductive success in those that have the favoured traits (Silva *et al*., 1992; de Bono and Bargmann, 1998; Sokolowski, 1998). Suggestive pressures on selection for the fittest. Natural selection would favour *C. complanatum* in *T. guinensis* during the cause of time.

The metacercariae of these species had predilection for the mesenteries of blood capillaries in the buccal cavity, this shows higher prevalence and abundance of the parasites around this area. This could be the presence of blood in this area which contains the nutrients the parasites feed on through passive, active or facilitated diffusion. Relative larger morphometric body length of *C. complanatum* would be more influx of nutrients than the other two species. Malek and Mobedi (2001), the higher prevalence and abundance of parasites in the area under the mouth to behind the operculum are probably due to the presence of blood, which *Clinostomum complanatum* feed on. It disagrees with the finding that the main habitat of *Clinostomum complanatum* in the *Carassius* sp is the muscles around the gills (Aohagi and Shibahara, 1994).

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