

SURVEY OF HELMINTH PARASITES OF FISH IN EBONYI RIVER AT EHA-AMUFU, ENUGU STATE, NIGERIA

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ABSTRACT

Gastrointestinal helminth parasites fauna of fish from Ebonyi River was investigated from February to July 2016. Fish caught from the river were sacrificed and gastrointestinal parasites isolated. A total of 121 fish comprising (and number examined, n) Distichodus engycephalus (n = 28), Labeo senegalensis (n = 34), Auchenoglaris occidentalis (n = 14) and Synodontis clarias (n = 45) were examined out of which 78(65.0%) were infected. S. clarias had the highest prevalence of infection 37(82.2 %), followed by L. senegalensis 21(61.8 %) and D. engycephalus 14(50.0 %). The lowest prevalence of infection was observed in A. occidentalis 6(42.9 %). Prevalence of infection was significantly different between the fish species ($\chi^2 = 11.713$, $p = 0.008$). The parasites isolated were of three species, Camallanus sp., Neoechinorhynchus africanus and Acanthocephalus sp. Overall, prevalence of the three parasites were in the range 30 – 35 %. Overall, Camallanus sp. had the highest mean intensity [4.41 (3.95 – 4.89, 95% CI)]. Mean intensities of N. africanus and Acanthocephalus sp., were only slightly lower: [3.17 (2.81 – 3.48, 95% CI)] and [3.21 (2.79 – 3.66, 95% CI)] respectively. Infection was not significantly dependent on sex of fish or season of sampling ($p > 0.05$). Helminth parasite infection prevalence is high in some fish species of Ebonyi River, Enugu State, Nigeria. This high prevalence of helminths infection may compromise productivity of the fish species infected.

Keywords: Gastrointestinal helminth, *Labeo senegalensis*, *Auchenoglaris occidentalis*, *Synodontis clarias*, Ebonyi River

INTRODUCTION

Fish are one of the aquatic vertebrates that inhabit both fresh and marine waters. Freshwater systems such as streams, rivers, lakes or ponds serve as natural habitats for more than 3000 species of fish in Africa (Khalil and Polling, 1997). Economically fish are very important. Fish and fish products are primary sources of protein to human and fish eating animal (Woo, 1995). According to Conor (2000), fish oils help in blood clotting and maintain the elasticity of arterial walls.

Fish diets contribute immensely to human health and form a staple food for human population including Nigeria where majority of the population are fish consumers (Echi, 2005). Promotion of fish and snail farming has been advocated as one of the means to make-up the short fall in the protein requirements of the ever-growing Nigerian population (Okafor, 2009).

Fish harbour parasites. Many fish species serve as intermediate hosts for these parasites, carrying larval stages and sometimes involving man as the definitive host.

Human infections from fish parasites are serious public health concern in some regions of the world especially southeastern Asia. In Japan, consumption of a delicacy, Sashimi which consists of fresh raw fish may result to anisakiasis, a disease caused by the larval stage of a nematode *Anisakis simplex* (Nawa *et al.*, 2005). Also, Diphyllbothriasis have been reported in connection with consumption of tainted Sashimi (Nawa *et al.*, 2005). Fish parasites often harm their hosts by destroying host tissues, providing site for development of secondary infections or taking blood and cellular fluids from host (MSG, 2017); and heavy infections may result in death of the hosts. Therefore, parasites affect fish production (Batra, 1984).

The aim of this study was to survey the prevalence of helminth parasites of fish in Ebonyi River, Eha-Amufu, Enugu State, Nigeria. Previous studies have reported several fish species of economic importance in Ebonyi River (Nwani *et al.*, 2011; Uneke, 2014, 2015). The species includes *Distichodus engycephalus*, *Labeo senegalensis*, *Auchenoglaris occidentalis*, *Synodontis clarias*, *Tilapia zilli*, *Oreochromis niloticus*, *Oreochromis aureus* and *Clarias gariepinus*. These fishes are consumed as a major source of protein by people in Enugu State. Sales of fish from the River also serve as a source of income to fishermen. The Ebonyi River has been previously reported to be polluted (Ozoko, 2015). Pollution usually alter the immune status of fish predisposing them to infection with parasites. Parasitism interferes with the wellbeing of fish and other aquatic organisms. Presently, there are limited numbers of studies evaluating the parasites of fish of Ebonyi River, Enugu State, Nigeria. Okogwu *et al.* (2011) evaluated the distribution of *Procamallanus laevionchus* in relation to environmental variables in Cross, Asu and Ebonyi Rivers. They reported that the environment and fish host influenced disease prevalence significantly. The present study is intended to add to existing information on parasites of fish in Ebonyi River in particular, and Nigeria waters in general.

MATERIALS AND METHODS

Study Area and Fish Collection: The study was conducted in Ebonyi River, Eha-Amufu, Enugu State, Nigeria which is a tropical freshwater located between 6° 15' - 6° 25' N and 8° 00' - 8° 10' E (Uneke and Jonah, 2017). The soil is porous and subject to erosion. The river has thick marginal vegetation. The climate is tropical. The colour of the water is muddy and has been estimated to have a depth of 6.4 m for the deepest sections and 3.0 m for the shallow parts (Ani *et al.*, 2016). Four species of fish were collected with the assistance of local fishermen using different fishing gear techniques such as cast nets and seine nets (150 – 200 mm mesh size). The collection was done in five different locations namely Umuhu, Camp, Umujiovu, Isu and Aguamde from February to July 2016. The fish species collected were *Distichodus engycephalus*, *Labeo senegalensis*, *Auchenoglaris occidentalis* and *Synodontis clarias*. Fish species were identified using guidelines by Iodo-Umeh (2003) and Olaosebikan and Raji (2013). The standard length (SL) of fish was measured using meter rule, the weight (g) with a three beam Mettler balance. Sex of fish was determined by both morphological examination and observation of the presence of milt or ovary (Ayanda, 2009).

Parasites Isolation and Identification: Procedure for examination of fish for parasites was in line with Paperna (1996) and Marcogliese and PMSC (2011). The buccal cavity of fish was examined. The fish was cut open to expose the internal organs which were separated out and thoroughly examined. The gut was cut into sections, oesophagus, stomach, pyloric caeca, small and large intestine, and placed separately in a Petri dishes containing physiological saline. The sections were further cut longitudinally and gently rinsed in normal saline for parasite dislodgment. Drops from rinse of each section of the gastrointestinal tract were also placed on microscope slide and examined for parasites. Swabs from the stomach and intestinal lining were also smeared on glass slides for examination.

The rinse from each section of the gastrointestinal track was mixed with sodium bicarbonate (NaHCO_3) to enhance parasite search. Squash of stomach, small intestine and large intestine were prepared as wet mount and examined under a compound microscope. Guideline by Yamaguti (1961) and Yamaguti (1963) aided parasite identification.

Statistical Analysis: Prevalence was calculated as Number of hosts infected / number of hosts examined $\times 100$. Chi-square analysis was used for comparison of parasite prevalence. Mean intensity of infection was estimated as number of parasites recovered / number of host infected (Margolis *et al.*, 1982). Confidence interval (CI) of parasite mean intensity was estimated by bootstrapping. Level of significance was set at $p < 0.05$. Data was analyzed using SPSS version 20.0 (IBM Corporation, Armonk, USA).

RESULTS

A total number of 121 fish comprising (and number examined, n) *Distichodus engycephalus* (n = 28), *Labeo senegalensis* (n = 34), *Auchenoglaris occidentalis* (n = 14), and *Synodontis clarias* (n = 45) were examined for helminth infection out of which 78(65.0 %) were infected. *S. clarias* had the highest prevalence of infection (84.9 %), followed by *L. senegalensis* (61.8 %) and *D. engycephalus* (50.0 %). The lowest prevalence of infection was observed in *A. occidentalis* (42.9 %). Prevalence of infection was significantly different between the fish species ($\chi^2 = 11.713$, $p = 0.008$). The parasites were isolated from three sections of the fish: stomach, small intestine and large intestine. *D. engycephalus* harboured helminth parasites only in the stomach and small intestine sections and at prevalence of 28.6 % and 21.4 % respectively; *L. senegalensis* was infected in the stomach, small intestine and large intestine. In *A. occidentalis* all infecting helminth were localized to the stomach and at a prevalence of 42.9 %; all three sections harboured infection in *S. clarias* (Table 1). Helminth parasites prevalence was

not significantly different in all three sections between the fish species.

The parasites isolated were of three species, namely *Camallanus* sp., *Neoechinorhynchus africanus* and *Acanthocephalus* sp. A total of 418 parasites which included 163(39.0 %) *Camallanus* sp., 133(31.8 %) *N. africanus* and 122(29.2 %) *Acanthocephalus* sp. were recovered. The three parasite species were isolated from each of the four fish species collected from Ebonyi River (Table 2). Prevalence of each of the three parasites *Camallanus* sp., *N. africanus* and *Acanthocephalus* sp. in the fish species were > 20 %; except for 10.7 % prevalence of *Camallanus* sp. in *D. engycephalus*. Overall, prevalence of the three parasites were in the range 29 – 39 %. Overall, *Camallanus* sp. had the highest mean intensity [4.41 (3.95 – 4.89, 95% CI)]. Mean intensities of *N. africanus* and *Acanthocephalus* sp. were only slightly lower: [3.17 (2.81 – 3.48, 95% CI)] and [3.21 (2.79 – 3.66, 95% CI)] respectively.

Helminth parasite infection of the four fish species collected from Ebonyi River was not dependent on fish sex (Figure 1). Prevalence of helminth infection was equal (50.0 % each) between male and female *D. engycephalus* (6/12 and 8/16 respectively infected). Prevalence of infection in male (66.7 %, 14/21 infected) and female (53.8 %, 7/13 infected) *L. senegalensis* was high. In *S. clarias* prevalence of infection was higher in female (85.2 %, 23/27 infected) than male (77.8 %, 14/18 infected), but the difference was not significant ($\chi^2 = 0.405$, $p = 0.524$).

Prevalence of helminth parasite infection in the four fish species was not dependent on season, whether rainy or dry (Figure 2). In *D. engycephalus* and *S. clarias* prevalence of helminth parasite was higher in the dry season 62.5 % (10/16 infected) and 84.0 % (21/25 infected) respectively than the rainy season 33.3 % (4/12 infected) and 80.0 % (16/20 infected) respectively. In *L. senegalensis* and *A. occidentalis*, helminth parasite prevalence was higher in the rainy season; though the difference was also not significant ($p > 0.05$).

Table 1: Prevalence of infection in four fish species of Ebonyi River, Eha-Amufu, Enugu State, Nigeria

Fish species	Number Examined	Number infected (%)	Body sections infected (%)		
			Stomach	Small intestine	Large intestine
<i>Distichodus engycephalus</i>	28	14(50.0)	8(28.6)	6(21.4)	0(0.0)
<i>Labeo senegalensis</i>	34	21(61.8)	14(41.2)	5(14.7)	2(5.9)
<i>Auchenoglaris occidentalis</i>	14	6(42.9)	6(42.9)	0(0.0)	0(0.0)
<i>Synodontis clarias</i>	45	37(82.2)	21(46.7)	11(24.4)	5(11.1)
Total	121	78(64.5)	49(40.5)	22(18.2)	7(5.8)
χ^2		11.713	2.402	4.772	4.922
P		0.008	0.493	0.189	0.178

Table 2: Intensity of helminth parasites in four fish species of Ebonyi River, Eha-Amufu, Enugu State, Nigeria

Fish species	Parasite species	Number Infected (%)	Number Recovered	Mean Intensity (95 % CI)
<i>Distichodus engycephalus</i> (n = 28)	<i>Camallanus</i> sp.	3 (10.7)	12	4.00 (3.00 – 5.00)
	<i>Neoechinorhynchus africanus</i>	8 (28.6)	23	2.88 (2.50 – 3.25)
	<i>Acanthocephalus</i> sp.	11 (39.3)	44	4.00 (3.36 – 4.64)
<i>Labeo senegalensis</i> (n = 34)	<i>Camallanus</i> sp.	14 (41.2)	67	4.79 (4.14 – 5.36)
	<i>Neoechinorhynchus africanus</i>	16 (47.1)	53	3.31 (2.63 – 4.00)
	<i>Acanthocephalus</i> sp.	9 (26.5)	21	2.33 (1.56 – 3.00)
<i>Auchenoglaris occidentalis</i> (n = 14)	<i>Camallanus</i> sp.	5 (35.7)	21	4.20 (2.40 – 6.00)
	<i>Neoechinorhynchus africanus</i>	4 (28.6)	15	3.75 (2.50 – 5.25)
	<i>Acanthocephalus</i> sp.	3 (21.4)	11	3.67 (2.00 – 6.00)
<i>Synodontis clarias</i> (n = 45)	<i>Camallanus</i> sp.	15 (33.3)	63	4.20 (3.40 – 4.93)
	<i>Neoechinorhynchus africanus</i>	14 (31.1)	42	3.00 (2.57 – 3.43)
	<i>Acanthocephalus</i> sp.	15 (33.3)	46	3.07 (2.47 – 3.60)
Summary (total for each parasite)				
	<i>Camallanus</i> sp.	37 (30.6)	163	4.41 (3.95 – 4.89)
	<i>Neoechinorhynchus africanus</i>	42 (34.7)	133	3.17 (2.81 – 3.48)
	<i>Acanthocephalus</i> sp.	38 (31.4)	122	3.21 (2.79 – 3.66)
Total		78 (64.5)	418	3.57 (3.30 – 3.84)

CI = confidence interval

DISCUSSION

The overall helminth parasite prevalence of 64.5 % was high. Previous studies have reported similar prevalence of helminth parasite infection in fish. Nwani *et al.* (2008) reported overall endoparasitic helminth prevalence of 41.9 % from four mormyrid species in Anambra River. Uneke and Egboruche (2015) reported overall

prevalence of 75 % for intestinal helminths and protozoan parasites in *Schilbe mystus* from mid Cross River flood system in Ebonyi State; both rivers are situated in southeastern Nigeria where the present study was conducted. Compared to studies that investigated heminth parasite infections in multiple fish species from rivers in southern Nigeria, the prevalence of helminth infection from the present study is relatively higher.

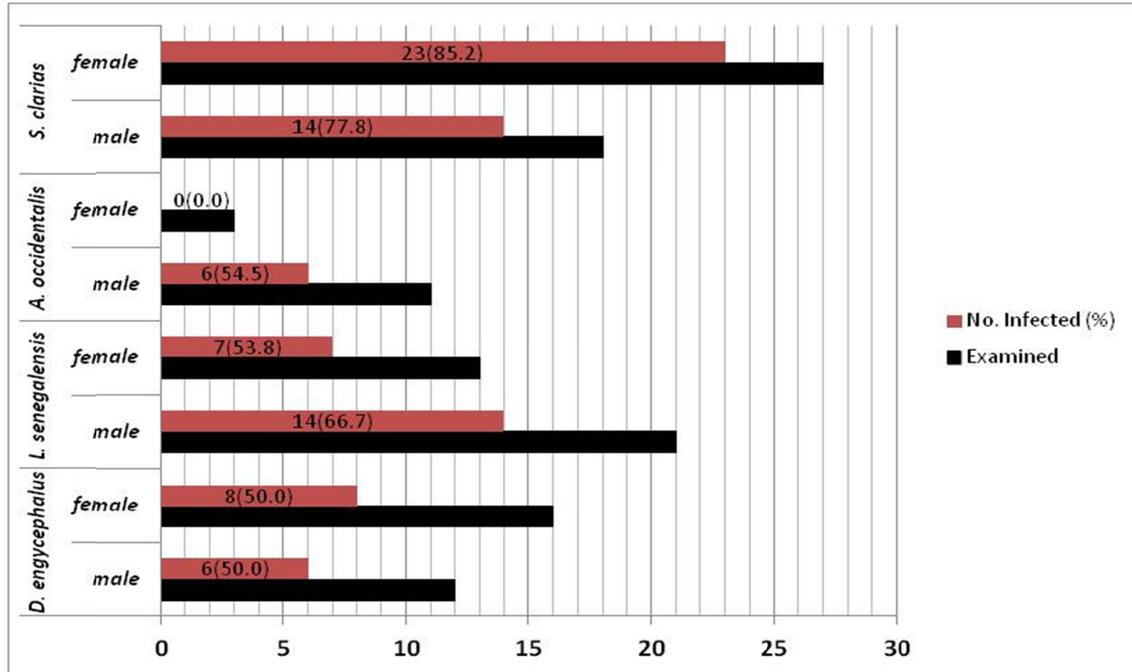


Figure 1: Prevalence of helminth parasites by sex of four fish of Ebonyi River, Enugu State, Nigeria

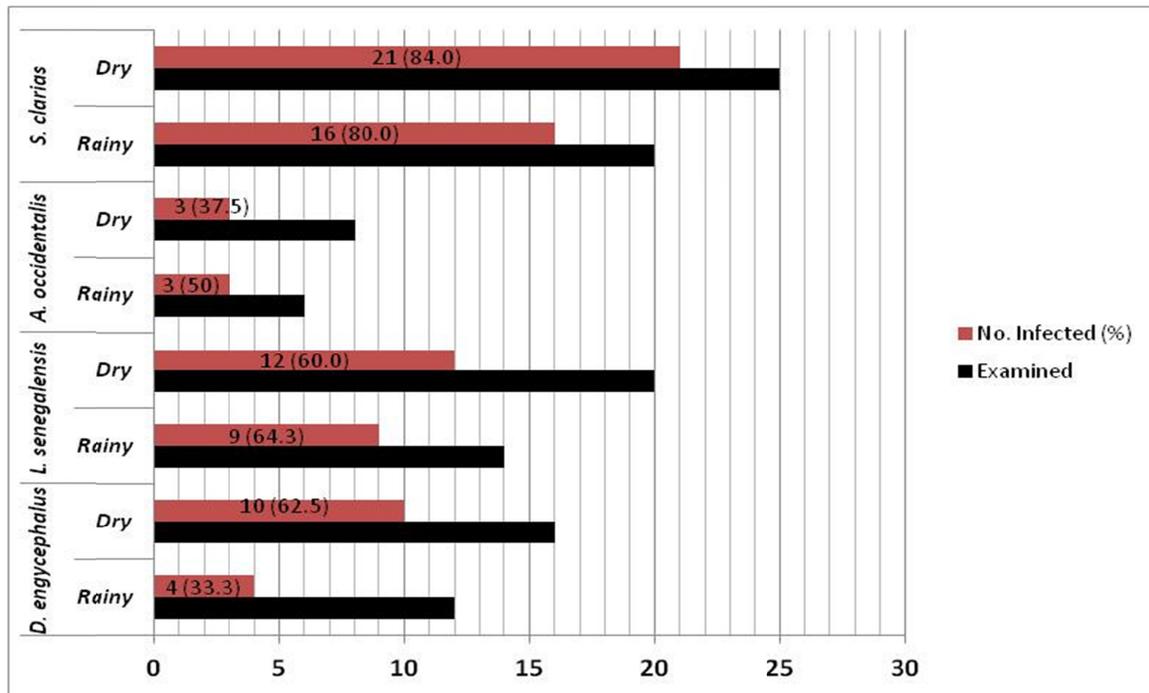


Figure 2: Prevalence of helminth infection by sampling season in four fish species of Ebonyi River, Eha-Amufu, Enugu State, Nigeria

Onyedineke *et al.* (2010), Ejere *et al.* (2014) and Ogbeibu *et al.* (2014) observed helminth parasite prevalence of 60.6 %, 32.9 % and 18.5 % respectively from freshwater fishes in southern Nigeria. This disparity in prevalence of parasitic helminth infection may be attributable to abiotic and biotic factors which are determinants of disease burden (Koskivaara, 1992; Thompson and Larsen, 2004).

Abiotic factors such as increased water temperature may alter the immune status of fish favouring infection and establishment of parasites (Kelly *et al.*, 2010; Rohlenova *et al.*, 2011). Some rivers in Ebonyi State, including the Ebonyi River has been reported as polluted (Abara *et al.*, 2005; Ozoko, 2015). Pollution of the Ebonyi River with heavy metals (Ozoko, 2015) may compromise fish immunity predisposing it to infection by parasites.

Seasonality may also affect parasite prevalence. Usually incidence of infection in freshwater fishes is higher in the dry season as was observed in three of the four fish species in the present study. The reduction in water volume increased rate of contact of fish with parasites (Mikheev *et al.*, 2014). Also temperature changes that characterize seasonal changes may alter fish immunological status predisposing it to infection (Bisset, 1948; Chrzanowski *et al.*, 1988; Iyaji *et al.*, 2009; Karvonen *et al.*, 2013; Abram *et al.*, 2017). Fish sex and sex-skewed demands of reproduction contributed to incidence of disease. Activities such as competition for mate and immune-suppression during spawning by male (Folstad and Karter, 1992), egg production (Simkova *et al.*, 2008; Rohlenova *et al.* 2011) and territorial defence (Reimchen and Nosil, 2001) impose demand on fish that may compromise immunological state. This may be responsible for higher prevalence of helminth infection in *L. senegalensis* and *A. occidentalis* male; and higher prevalence of infection in female *S. clarias* and *D. engycephalus*. Similarly, Omeji (2012) observed more gastrointestinal helminth parasite infection in male *A. occidentalis*, and more infection in female *S. clarias* which were also not significantly different. Prevalence of helminth infection was also higher in *S. clarias* from Warri River (Ejere *et al.*, 2014).

The three parasite species isolated from the fish did not show specificity for fish species. *Camallanus* sp. intensity was however higher in *S. clarias* and *L. senegalensis*. The higher intensity may be a product of feeding habit, immune-suppression, or host suitability for parasite establishment (Kelly *et al.*, 2010; Rohlenova *et al.*, 2011).

Conclusion: The four fish species *D. engycephalus*, *L. senegalensis*, *A. occidentalis*, and *S. clarias* in Ebonyi River harboured helminth parasites. Infection of the four fish species was not significantly sex dependent. Also, seasonality had a minimal influence of parasite prevalence.

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