International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 3; Issue 3; July 2018; Page No. 60-64

The Prevalence, intensity and seasonal variation of parasitic helminths of freshwater fishes collected from Osinmo Reservoir, Ejigbo, Osun State, Nigeria

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Abstract
This study was carried out to investigate the fish community in Osinmo Reservoir, Ejigbo, Osun State, Nigeria for parasitic helminth infections, and to determine the prevalence, intensity and seasonal variations of parasites in the fishes. Fish collection was carried out once a month from March 2013 to February 2014. The fishes caught were kept in open plastic buckets in water obtained from the pond. They were transported directly to the laboratory and examined for parasites within an hour of being caught, using standard laboratory procedures. 417 fish specimens belonging to nine species, *Clarias gariepinus* (11), *Heterotis niloticus* (12), *Hepsetus odoe* (41), *Malapterurus electricus* (7), *Mormyrus rume* (40), *Oreochromis niloticus* (41), *Parachanna obscura* (79), *Sarotherodon galileus* (9), and *Tilapia zilli* (177), were caught and examined. Five of the fish species were infected and five helminth species were recovered from them. The helminths include metacercaria of a trematode *Neascus sp.* Found in *O. niloticus* (2.4%), and four nematodes, *Procamallanus leavionchus* recovered from *C. gariepinus* (27.3%), *Rhabdochona congolensis* from *Heterotis niloticus* (33.3%), *Ascarpos strongylina* found in two fish hosts, *Parachanna obscura* (8.9%) and *Hepsetus odoe* (2.4%) and an unidentified nematode larva in H. odoe (2.4%). In all the fishes, the juveniles were uninfected. Among the adult fishes, prevalence was usually significantly higher among the males than the females ($P<0.5$). Fishes of higher standard lengths also consistently had significantly higher prevalence of infection than the ones with lower standard lengths ($P<0.5$). The mean intensity of infection varied from 3 for *Neascus sp*. in *Oreochromis niloticus* to 9 for *R. congolensis* in *Heterotis niloticus*. Males generally carry more worm burden than females. Fishes of lower standard lengths tend to carry higher worm burdens except in *C. gariepinus* where fishes of higher standard lengths are more heavily infected. The nematode, *Procamallanus leavionchus*, occurs only in the dry season while *R. congolensis* and the trematode *Neascus sp.* were found only during the wet season. *A. strongylina* was found in *H. odoe* only during the wet season while the worm occurs in *P. obscura* in both wet and dry season.

Keywords: prevalence, intensity, seasonal variation, parasitic helminths, osinmo reservoir

1. Introduction

Fishes are the most numerous groups of the vertebrates, accounting for more than half of the vertebrate species [15] there are at least 20,000 known species and more than 58% are found in marine environment. More recently [9] stated that there are almost 28,000 known extant species, of which 27,000 are bony fish, with 970 sharks, rays, and chimeras and about 108 hagfish and lampreys.

Fish serves as good source of animal protein for man and his livestock [7]. A breakdown showed that fish accounts for more than forty percent of the protein diet of two-third of the global population [19]. Fish oil contains omega-3-essential fatty acids necessary for the proper functioning of the brain, heart and immune system [20]. Due to increase in the human population the demand for fish as a source of protein is on the rise [3]. Fish is important to the human population in trade and economy. It is of importance in the diet of different countries especially in the tropics and sub tropics where malnutrition is common [5].

Parasites are capable of causing harm to the fish host notwithstanding the species, either through injury to the tissues or organs, in the process of burrowing or consuming Food or the removal of digested food in the gut of the fish as well as the secretion of proteolytic enzymes.

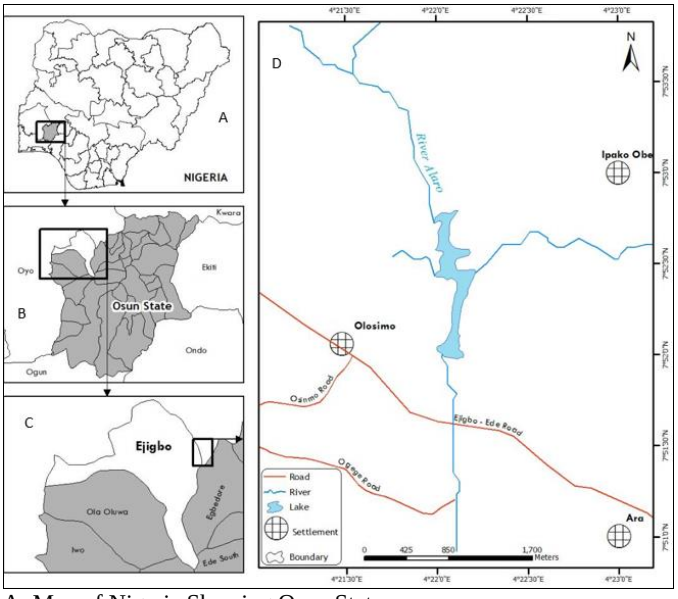
Fish parasites result in economic losses not only from mortality but also from treatment expenses, growth reduction during and after outbreak of diseases, and they militate against expansion of aquaculture [17].

In Nigeria adequate awareness of the impact of disease to aquaculture is lacking as revealed by numerous reports by [4], [2] and [3]. Parasitic diseases of fish are common all over the world and are of particular importance in the tropics [16]. Hence the continuous need to sample fish for parasites in Nigeria. In order to bring about a control of these parasitic infections, there is the need for baseline information and proper documentation of the types and geographical distribution of these parasites upon which a control plan could be based. There has not been a previous study on the parasite fauna of fishes in Osinmo Reservoir, Ejigbo local Government Area, Osun State, which is a source of fish to the surrounding local communities. The work proposed in the current study will survey the helminth fauna of the fishes in this reservoir. The results will provide useful new information on our knowledge of parasitic helminth infections of fish in Nigeria.

2. Materials and Methods

2.1 Study Area

Osinmo reservoir in Ejigbo Local Government Area of Osun State (Fig.1) was created in 2005 by the impoundment of the Ataro River which took its source from Iwaye River in Oyo State. Many streams unite to form the river. The catchment area is about 102 km^2. It occupies the area from longitude 004°21.2′ E to 004°21.7′ E and latitude 07°52.8′ N to 07°53.2′ N. The surface area of the reservoir is about 0.78 km², while the mean maximum depth is 3.2 m. The climate is characterized by rainy season from May to October and dry season from November to April. The area occupies an undulating terrain with the highest altitude of about 365.76 m above the sea level. The vegetation area is lowland rain forest. There is heavy rainfall between July and September of each year with annual rainfall of 52.35 inches. Osinmo reservoir was built primarily to supply potable water to inhabitants of Ejigbo and Ara. The reservoir provides a number of ancillary benefits among which is production of fish to these communities [18].



A: Map of Nigeria Showing Osun State
B: Map of Osun State Showing Ejigbo Local Government Area
C: Map of Ejigbo LGA Showing location of Osinmo Reservoir
D: Map showing Osinmo Reservoir and River Ataro

Fig 1

2.2 Collection of fish specimens

Fish collection was carried out once a month from March 2013 to February 2014. The fishes were caught fresh directly from the reservoir in the morning between 6.00am to 9.00am using cast nets and gill nets of one inch or two inch mesh size. The fishes were put in a bucket with some of the reservoir water to preserve them alive and covered with a net and then transported directly to the laboratory at the Department of Zoology, Obafemi Awolowo University, and Ile Ife for examination. In the laboratory the fishes were identified and sorted into species using the keys of [13]. Each fish was then given an identification (ID) number. Thereafter, the dimensions and the weights of the fishes were taken, their sexes determined, and were then examined for parasites. The standard length and total length of each fish were determined using the standard meter rule and recorded in centimetres, and the weight measured using salter balance and recorded in grams.

2.3 Sex determination

The sexes of the fish were determined by either pressing the abdomen of each fish specimen for the extrusion of sperm for males and eggs for females or they were dissected and observed for the presence or absence of testes and ovaries. The presence of testes signified a male while the presence of ovary signified a female.

2.4 Examination of fish for parasites

The external body surfaces, the scales, gills, fins and mouth of each fish were carefully examined for ectoparasites, with the use of a hand lens. The ectoparasites found on the fish were carefully removed from the tissues to which they were attached with the aid of a pair of scissors and forceps and then placed in a petri dish containing 0.9% saline. The parasites recovered were counted and transferred into different specimen bottles labelled with the fish ID number and fixed for one hour using formaldehyde. The parasites were then preserved in 70% alcohol. The fish abdomen was slit open by a longitudinal incision. The gut was dissected out and cut into stomach and intestine. Each segment was placed in a different petri dish containing 0.9% saline, where the contents were expressed. Each organ was examined under a dissecting microscope, on a black surface background to make the parasites more visible. The nematodes recovered from the stomach and intestine of the fishes were transferred from saline to acetic acid in a clean petri dish to get them stretched and were preserved in 70% ethanol in different specimen bottles labelled with the fish ID number according to types. The specimens of each species of worms recovered from the two organs in each fish were counted and the numbers recorded against the ID number of each fish. The body cavity of the fish was also examined using a hand lens The muscles of each fish were also examined and dark spots examined were teased to see if they contain parasite cysts.

2.5 Preparation of permanent slides of the parasites

Permanent whole mounts of some of the parasites were prepared on clean glass slides. The preserved digenetic trematode larvae removed from the scales were stained in Delafield's Haematoxylin, counter stained in eosin and mounted on glass slides using Canada balsam. The nematodes recovered were removed from 70% alcohol, cleared and mounted in lactophenol without staining and the edges of the cover slips were sealed with Glycerol.

2.6 Examination and identification of prepared specimens

Prepared slides of the mounted parasites were examined under a light microscope with objective lenses of x10 and x40.
Measurements of the whole length of the worms and organs were made using calibrated light microscope. Identification of the parasites was by the use of features observed in the mounted specimens and was based on the descriptions [20].

2.7 Data analysis
The data collected were coded and entered into SPSS for version 17(SPSS Inc., Chicago, IL) and Microsoft excel and were used for data analysis using descriptive statistics (such as frequency tables, means and percentages) as appropriate. Inferential statistics like Chi-squared test were used for comparative analysis of parasite prevalence and mean intensity with respect to sex and size.

3. Results
3.1 parasitic helminths recovered
Five helminth species were recovered from the fishes examined in this study. These include four nematodes and one trematode. Three of the nematodes were identified as *Ascarpos strongylina*, *Rhabdochona congolensis* and *Procamallanus leavionchus*, while the fourth one was unidentified and was in the larval stage. The trematode was identified as *Neascus* sp. and the specimens recovered were all metacercariae Only five of the fish species (*Clarias gariepinus, Heterotis niloticus, Hepsetus odoe, Oreochromis niloticus and Parachanna obscura*) were infected while no infection was observed in the other four species. The parasitic helminths recovered from each of the five infected fish species are depicted in Table 2. Each of the infected fish species was infected with only one helminth species except for *Hepsetus odoe* in which two different species was observed (Table 2).

3.2 Prevalence and intensity of helminth infection in the fishes
Of the 417 fish examined, only 16(3.8%) were positive for helminthes. Prevalence among male fishes (4.8%) was higher than the females (2.7%). Among the individual infected fish species, prevalence ranged from a minimum of 2.4% by each of *A. strongylina* and the unidentified nematode larva in *Hepsetus odoe* and of *Neascus sp.* in *O. niloticus*, to a maximum of 33.3% in *Heterotis niloticus* infected with *R. congolensis* (Table 2).

None of the juveniles of the various fish species was infected. The only specimen of *O. niloticus* infected with *Neascus sp*. was an adult female (31-35cm). Also only one specimen of *Hepsetus odoe* was infected with *A. strongylina* and one specimen of the same fish species was infected with the unidentified nematode, both of which were females. These result in a prevalence of 2.4% in the three cases (Table 2). In adults of *Parachanna obscura*, prevalence of infection with *A. strongylina* was 9.9% (Table 3). Prevalence of infection among the male fishes (9.5%) is similar to that in the females (10.3%). Prevalence was significantly higher in fishes of lower standard length (4.8%) (P>0.05). Prevalence of *R. congolensis* in *Heterotis niloticus* was 33.3% (Table 3). Only male fishes were infected. Prevalence ranged from 27% among fishes of lower standard lengths to 100% among those of higher standard length (Table 3) (P>0.05). The infection patterns observed among the different fish species shows that generally prevalences of helminth infections are higher among the males than the females and among the fishes of the higher standard lengths than the smaller fishes.

R. congolensis infection was found only in male *Heterotis niloticus*. The mean intensity of infection of this worm, as shown in Table 4 was 9. The mean intensity of infection in fishes with standard length 25-30cm (10) was higher than that in fishes with standard length 31-35cm (6) (state sig. diff). Only males of *Clarias gariepinus* were infected with *P. leavionchus* with a mean intensity of 5.7. The worm burden in the smaller fishes of standard length 16-35cm was lower than 6.5 recorded in the larger fishes with standard length 35-40cm. a mean intensity of 3.5 of the worm recorded for *A. strongylina* in *P. obscura*. The mean intensity of 3.5 of the worm recorded in male *P. obscura* is higher than 1.7 recorded in the females of the fish. The worm burden is also higher (3.7) among *P. obscura* of lower standard lengths (11-30cm) than the burden of 2 in fishes of higher standard length (31-40cm). Only one female *O. niloticus* was infected with three specimens of *Neascus sp*.

3.3 Seasonal distribution of helminth parasites
A summary of the months of the year in which the fish species which showed helminth infections were caught and the months in which parasites were found in some of them is shown in Table 5. *Clarias gariepinus* was caught in September during the rainy season, and in December and February. This implies that the parasites tend to be prevalent in *C. gariepinus* in the dry season. *Rhabdochona congolensis* on the other hand was recorded in *Heterotis niloticus* only in the July and August during the wet season while fishes caught in March and February were not infected. Only one specimen of *Ascarpos strongylina* was recovered from a specimen of *Hepsetus odoe* and this was at the peak of the wet season in July, although the fishes were caught in seven months spreading across the wet and dry seasons. On the other hand, *A. strongylina* was recovered in both the rainy season (April, May and August) and dry season (January and February) from *Parachanna obscura*. The larvae of the trematode *Neascus sp*. was recovered from *Oreochromis niloticus* only in September during the wet season and none in December, the only dry season month when the fish was caught.

Table 1: Numbers of Various Fish species caught during the study

Fish species	Juveniles	Adults			Total (Adult and Juvenile)
		Male	Female	Both sexes	
Clarias gariepinus	0	6	5	11	11
Heterotis niloticus	0	7	5	12	12
Hepsetus odeo	3	25	13	41	41
Malapterurus electricus	2	4	1	7	7
Mormyrus rume	6	28	6	40	40
Oreochromis niloticus	0	27	14	41	41
Sarotherodon galileus	0	3	6	9	9

Tilapia zilli	42	88	47	177	177
Parachanna obscura	8	42	29	79	79
Total	61	230	126	417	417

Table 2: Prevalences of helminth parasites in different fish species

Fish species	No examined	Helminth infection	No. of fish infected	Prevalence
Clarias gariepinus	11	*Procamallanus leavionchus*	3	27.3
Heterotis niloticus	12	*Rhabdochona congolensis*	4	33.3
Oreochromis niloticus	41	*Neascus sp.*	1	2.4
Parachanna obscura	79	*Ascarpos strongylina*	7	8.9
Hepsetus odoe	41	Unidentified nematode larvae	1	2.4
		Ascarpos strongylina	1	2.4

Table 3: Prevalence

Fish Std length (cm)	Male		Female		Both sexes	
	No exam	% infected	No. exam	% infected	No exam	% infected
I. *Rhabdochona congolensis* in *Heterotis niloticu*						
26-30	6	50	5	0	11	27
31-35	1	100	0	0	1	100
Total	7	57.1	5	0	12	33.3
II. *Procamallanus leavionchus* in *Clarias gariepinus*						
16-35	4	25	4	0	8	12.5
36-40	2	100	1	0	3	66.7
Total	6	75	2	0	11	27.3
III. *Ascarpos strongylina* in *Parachanna obscura*						
11-30	35	5.7	27	3.7	62	4.8
31-40	7	28.6	2	100	9	44.4
Total	42	9.5	29	10.3	71	9.9

Table 4: Intensity

Fish Std length (cm)	Male			female			Both sexes		
	No exam	No infected	Mean intensity	No. exam	No. infected	Mean intensity	No. exam	No. infected	Mean intensity
I. *Rhabdochona congolensis* in *Heterotis niloticus*									
26-30	6	3	10	5	0	0	11	3	10
31-35	1	1	6	0	0	0	1	1	6
Total	7	4	9	5	0	0	12	4	9
II. *Procamallanus leavionchus* in *Clarias gariepinus*									
16-35	4	1	4	4	0	0	8	1	4
36-40	2	2	6.5	1	0	0	3	2	6.5
Total	6	3	5.7	5	0	0	11	3	5.7
III *Ascarpos strongylina* in *Parachanna obscura*									
11-30	35	2	10	27	1	1	62	3	8
31-40	7	2	4	2	2	4	9	4	4
Total	42	4	3.5	29	3	1.7	71	7	2.7

4. Discussion

The study showed a low overall infection rate of 3.8% in all the 417 fish examined. This is unlike the high infection rate reported elsewhere (59.15%) in River Niger [14]. The wide differences in the helminth infection rates observed in the fishes in this present study and that of [14] could be attributed to differences in the environmental conditions of the two water bodies where the studies were carried out. The sanitary condition of Osinmo Reservoir a relatively small water body of less economic importance cannot be compared to that of River Niger, one of the two major water bodies in Nigeria which collects effluents from several smaller water bodies scattered all around Nigeria. [6]noted that a water body that is being used as a source of drinking water is likely to be relatively better, compared to a larger river which serves as a collecting basin receiving water and all kinds of organic wastes from various sources which are usually unclean and thus capable of harbouring different kinds of organisms including parasites vectors. The number of isolated nematodes species as well as their absolute number was higher than trematodes in this study, this can be attributed to the fact that nematodes which have a free living stage in their lifecycle are able to form a cyst around itself and survive extremely harsh weather conditions until a suitable host is located. The dominance of nematode species in this study is in agreement with the findings of [10] who recorded nematodes as the most

common parasites, infecting 18.6% of the fish population he examined from Okhuaihe River Edo State. The nematode *Procamallanus leavionchus* was found only in the fish *Clarias gariepinus* in this study, the host specificity of *P. leavionchus* to *C. gariepinus* as observed in this study is a strong indicator of a close evolutionary relationship between this parasites and this host. The occurrence of *P. leavionchus* in *C. gariepinus* is a common occurrence in the freshwaters of Nigeria and Africa as a whole. This claim can be supported by the works of several researchers who have repeated encountered this parasite in *C. gariepinus*. The level of infection between male and female fishes in this study was in the ratio 16:13. The sex ratio of infection found in this study can be in direct response to the fact that more males than females occurred in the sampled fish population; similarly a higher number of helminth parasites were found in males than the females. The difference in the level of infection among the sexes could also be attributed to the fact that male fishes are able to forage further for food than their female counterparts who slowed by the process of reproduction which hinders them from moving far away from their hatchlings.

The feeding pattern of fish is an important factor in their infestation with parasites. In this study carnivores and omnivores fishes recorded the highest rates of helminth parasites infection while herbivores and detritus feeders were least infected. This could be as a result of the natural predisposition of carnivorous and omnivorous fishes to the eggs and larvae of parasite species in their diets which is majorly made up of smaller fishes and zooplanktons which serve as intermediate, reservoir and transport host for most of this parasite species. This claim agreed with the findings of [8] who reported that predatory fish species harboured a greater diversity and abundance of larval helminths than herbivorous and planktivorous species.

Infections in the juvenile fish collected in this study were nil in comparison to those of the adult fishes. The fact that the adult fishes were the only ones infected showed the susceptibility of older fishes to parasite species as they age and their level of immunity against parasites and diseases begin to dwindle. It could also be as a result of older fishes losing their strength as they age and not been able to handle the various anthropogenic stress.

A seasonal pattern of parasitic infection recorded showed peak infection in the rainy season (August) and low infection rate in the dry season (January). During the rainy season fishes are exposed to several kinds of pollutants which are carried into the water body from excess runoffs from various sources, hence making the fishes vulnerable to foreign materials.

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