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Gastrointestinal Helminth Parasites of *Heterotis Niloticus* (Osteoglossiformes: Osteoglossidae) From Anambra River, Eastern Nigeria

Comfort Chidiogo Nwadike[1]., Ebube Charles Amaechi[2*] and Philip Chijioke Okoye Ilozumba[1]

[1]Department of Zoology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria; [2]Department of Zoology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria.

*Email address: ebubeamechi@yahoo.com

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ABSTRACT: Fish diseases especially those caused by parasites are major problems confronting fish farmers globally. This study investigated the gastrointestinal helminth parasites of *Heterotis niloticus* (African Bony tongue fish) from the Anambra River basin over 12 months (January to December, 2013). A total of 294 fish were caught from three locations along the Anambra River, and transported to the laboratory for morphological and parasitological studies. Four species of helminth parasites were isolated comprising one cestode (*Sandonella sandoni*), two nematodes (*Procamallanus laeviconchus* and *Dujardinascaris* species) and one acanthocephalan (*Tenuisentis niloticus*). Out of the 294 fish species examined, the overall prevalence of infection was high (72.8%) with a mean abundance of 0.77 of helminth parasites per fish. Prevalence of helminth infection increased with increasing host weight, although there was no significant relationship (p=0.144). Similarly, there was no significant relationship between the length of fish and helminth infection. The intestine was found to be the site for most of the recovered parasites. More parasites were recovered during the rainy season. It can be concluded that *H. niloticus* is a new host record for *P. laeviconchus*. It is therefore necessary to be aware of the parasitofauna for possible zoonotic disease.

Keywords: Gastrointestinal; Parasites; Helminth; *Heterotis niloticus*; Anambra River.

طفيليات الديدان الطفيلية المعوية من Heterotis niloticus (Osteoglossiformes: Osteoglossidae) نهر أنامبرا، شرق نيجيريا

كومفورت شيديوغو نواديكي 1، إيبوب تشارلز أمايتشي2*. وفيليب شيجيوك أوكوي إيلوزمبا 1

الملخص: تعتبر أمراض الأسماك وخاصة تلك التي تسببها الطفيليات من المشاكل الرئيسية التي تواجه مزارعي الأسماك على مستوى العالم. تناولت هذه الدراسة طفيليات الديدان المعوية لسمكة Heterotis niloticus (أسماك اللسان العظمية الأفريقية) من حوض نهر أنامبرا على مدى 12 شهرًا (من يناير إلى ديسمبر 2013). تم صيد 294 سمكة من ثلاثة مواقع على طول نهر أنامبرا، وتم نقلها إلى المختبر لإجراء الدراسات المورفولوجية والطفيلية. تم عزل أربعة أنواع من طفيليات الديدان الطفيلية تشمل نوع واحد من الديدان الخيطية (*Sandonella sandoni*) واثنين من الديدان الخيطية (نوع *Procamallanus laeviconchus* و *Dujardinascaris*) ونوع واحد من نوع Acanthocephalan (Tenuisentis niloticus). ومن بين 294 نوعًا من الأسماك التي تم فحصها، كان معدل انتشار العدوى مرتفعًا (72.8%) مع متوسط وفرة قدره 0.77 من الطفيليات الطفيلية لكل سمكة. زاد انتشار العدوى بالديدان الطفيلية مع زيادة وزن العائل، على الرغم من عدم وجود علاقة ذات دلالة إحصائية (قيمة الاحتمال = 0.144). وبالمثل، لم تكن هناك علاقة ذات دلالة إحصائية بين طول الأسماك والإصابة بالديدان الطفيلية. وتبين أن الأمعاء هي الموقع الذي تواجدت فيه معظم الطفيليات التي تم شفاؤها. وتم انتشال المزيد من الطفيليات خلال موسم الأمطار. يمكن أن نستنتج أن *H. niloticus* هو تسجيل جديد لعائل P. laeviconchus. لذلك من الضروري أن تكون على دراية بالطفيليات الحيوانية لإحتمالية وجود أمراض حيوانية المنشأ.

الكلمات المفتاحية: الجهاز الهضمي، الطفيليات، الديدان الطفيلية، Heterotis niloticus، نهر أنامبرا.

1. Introduction

The increase in human population and the attendant increase in demand for animal protein has raised the demand for and consumption of, fish and fish products worldwide. Freshwater fisheries have an important bearing on the lives of many African communities primarily as an important source of dietary protein and secondly as a source of subsistence income. Fish provides a good source of high-quality protein and contain many vitamins and minerals [1]. Freshwater and marine fishes all over the world, especially in tropical areas have been faced with challenges of parasitic infections [1,2]. Fish are a host to many larval and adult forms of helminth parasites. Some of these parasites cause diseases to fish affecting their health by lowering their immune capability, altering the biology and behavior of the fish, bringing about morbidity, mortality, reduction in growth and fecundity and also causing mechanical injuries. The African bony-tongue fish *Heterotis niloticus*, family Osteoglossidae is an omnivorous and microphagous fish species found in lakes, swamps, weedy areas of rivers, and creeks where the current is not strong [3]. *H. niloticus* occurs in large rivers and lakes of the Nilo-Sudanean area in Central and West Africa and has been introduced into many rivers and aquaculture stations in Africa [4]. Fish parasite communities are known to be closely associated with the environment, the host fish and the aquatic invertebrate communities. The main factors structuring parasite communities are hydrodynamic variations coupled with seasonal variations in the availability of infectious stages of parasite in a particular environment. One major fish species that occurs regularly in the Anambra River is *H. niloticus* [5]. Quite a few authors have worked on parasites of other fish species in the Anambra River with varying prevalence [6-10]. It is clear from the available literature that no comprehensive study has been made on helminth parasites of *H. niloticus* in the Anambra River, thus it needs further study. The study aimed at providing information on gastrointestinal helminth parasites of *H. niloticus* which is a commercially important fish of the Anambra River, Nigeria and the effect of rainy and dry season on infra communities of parasites.

2. Materials and methods

2.1 Study area

Anambra River is a major tributary of the Niger River. It is linked by numerous tributaries which together form an extensive drainage basin. It is about 207.4 km in length and 14014 km^2 in area. Anambra River Basin lies between latitudes $6^0 10^1$ and $7^0 8^1$ N and longitudes $6^0 30^1$ and $7^0 15$E. The basin has a rainfall of 150-200 cm annually, and an altitude of 1000m above sea level. Temperatures range between 24 to 31°C. The water emerges near Ankpa in the Kogi State of Nigeria, crosses the Kogi/Anambra State boundary north of Ogurugu and meanders through the Ogurugu station to Otuocha, from where it flows down to its confluence with the Niger at Onitsha [11].

The normal rainy season occurs between May and October, with a short break between late July and early August. During the rains, water levels increase in the main river channel. There is also a rise in the levels of the natural depression, lakes, and ponds in the extensive floodplain that lies mainly on the western side of the Anambra River. The rise in the water levels of the river channels is brought about by direct precipitation within the catchment area as well as by inflow from the Niger floodplains. The period of November to April is usually that of the dry season. This season witnesses maximum production of phytoplankton and zooplankton in the lakes and ponds, which occur in the river basin.

2.2 Collection of fish samples

Different fish species were caught from three landing points namely, Nsugbe, Otuocha and Enugwu-Otu from January to December 2013 using nets of various sizes (25 mm – 100 mm), hooks and line, caste nets, gill nets as well as local traps. Some fishermen were engaged to catch the fish specimens.

2.3 Identification of fish samples

Identification of fishes to species level was done in the Zoology laboratory of Nnamdi Azikiwe University, Awka and some were done in situ in the field. Mean morphometric features and mean meristic characters were used for proper identification of *H. niloticus*. Specific morphometric features used were the length of the lateral line, dorsal fin length, peduncle fin length, caudal length and anal fin length. Mean meristic characters used included the number of spiny fins on the left and right sides, the number of sensory pits on the right and left sides and the number of pelvic fin rays on the left and right sides. These were compared with standard keys with taxonomic descriptions and indices as in Idodo-Umeh [12].

2.4 Parasitological studies

Live fish specimens were transported to the laboratory in fish tanks and were killed by pithing. Occasionally however, live fish were maintained in circular metallic fish tanks. Before the examination, the fish were weighed to the nearest gram using an Adams electronic weighing balance; model AQP 1600. The total and standard lengths of each fish were determined using a measuring board calibrated in centimetres. The alimentary canal was excised at the anterior limit of the esophagus and the anal end of the cloaca and was placed in a clean dissecting dish containing clear tap water. In opening the alimentary canal, a continuous longitudinal slit, starting from the cloacal end was employed in order to reduce the chances of cutting long helminths such as cestodes which may extend from one section into another. As the alimentary canal was opened, the contents of each section of the gut (esophagus stomach, intestine, and rectum) were emptied into separate Petri dishes which contained normal saline and were examined thoroughly for helminth parasites. The gut wall in each section was also carefully scraped with a scalpel onto a microscope slide and thoroughly searched for attached or adhering helminths.

2.5 Identification of Parasites

The identification of helminth parasites was based on a comparison of distinctive body shapes/morphological features of the collected specimen and those described in the literature using an identification guide [13]

2.6 Statistical analysis test

Statistical analysis was performed using Statistical Product and Service Solution (SPSS) version 21. Results were presented using means, percentages, and frequencies and expressed in figures and chats. Variables were further analyzed using Chi-Square to test for the association between Fish hosts and the prevalence of infection at a 0.05 level of significance. In addition, Pearson correlation analysis was used to assess the relationship between the weight and length of fish and the prevalence of infestation among these fish. Differences, associations, and relationships were considered significant if $P<0.05$.

Previously mentioned terminology [14] was employed in the analysis of the data which includes,

Abundance – 'Is the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected'.

Mean Abundance – 'Is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts)'.

Mean Intensity – 'Is the average intensity of a particular species of parasite among the infected members of a particular host species. In other words, it is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite'.

3. Results

During the study period from January to December 2013, a total of 294 *H. niloticus* fish species were examined for the prevalence of gastrointestinal helminth parasites. A total of 214 fishes were infected (72.8%) with four species of metazoan parasites (1 Cestode, 2 Nematodes and 1 Acanthocephalan) which were collected mostly from the intestine of the host fish. The total number of parasite individuals was 904, among them were 318 cestodes,76 nematodes, and 510 acanthocephalans (Table 1).

Table 1. Prevalence and abundance of Endohelminth parasites in *Heterotis niloticus* caught from Anambra River.

Parasite taxa	Parasite species	N.E	N.I	N.P.R	P (%)	M.I.I	M.A
Cestode	*Sandonella sandoni*	294	110	318	37.41	2.90	1.10
Nematode	*Procamallanus laeviconchus*	294	8	40	2.72	5.00	0.14
	Dujardinascaris species	294	4	36	1.36	9.00	0.12
Acanthocephalan	*Tenuisentis niloticus*	294	92	510	31.29	5.54	1.73
Total			214	904	72.8%		

KEY: N.E – Number Examined; N.I – Number Infected; N.P.R – Number of Parasites Recovered; P – Prevalence (as a percentage); M.I.I – Mean Intensity of Infection; M.A – Mean Abundance.

The prevalence of helminth infection increased with increasing host weight although it declined in weight groups 200- 299g and 600- 699g. However, correlation analysis revealed that there was no significant relationship ($P=0.144$) between fish weight and prevalence of infection. This implied that the prevalence of helminth infection in *H. niloticus* did not correlate with the weight of the fish. Similarly, correlation analysis revealed that there was no significant relationship between the weight of *H. niloticus* and the mean intensity of infection and mean abundance (Table 2).

Table 2. Relationship between fish weight and helminth infection.

Fish species	Fish weight (g)	N.E	N.I	P (%)	N.P.R	M.I.I	M.A
H. niloticus	0-99	44	16	36.36	64	2.00	0.73
	100-199	100	44	44.00	164	1.90	0.82
	200-299	48	20	41.67	164	4.10	1.71
	300-399	30	16	53.33	52	1.63	0.90
	400-499	16	10	62.50	228	11.40	7.13
	500-599	8	8	100.00	132	8.30	8.30
	600-699	36	34	94.45	880	12.94	12.22
	700-799	4	0	0	0	0.00	0.00
	800-899	-	-	-	-	-	-
	900-999	-	-	-	-	-	-
	1000-1099	4	4	100.00	16	2.00	2.00
	1100-1199	4	4	100.00	108	13.50	13.50

KEY: N.E-Number Examined; N.I-Number Infected; N.P.R – Number of Parasites Recovered; P-Prevalence; M.I.I - Mean Intensity of Infection; M.A-Mean Abundance.

The prevalence of helminth infection in *H. niloticus* did not follow a steady increase with host length. All the 8 fishes examined in the length group 10-19 cm were infected and 12 worms were recovered, giving a prevalence of 100.00 %, M.I.I. of 3.00 and M.A. of 3.00. Among the 196 fishes examined in the length group 20-29 cm, 80 (40.82%) were infected and 93 worms were recovered giving a prevalence of 40.82 %, M.I.I. of 2.33, and M.A. of 0.95 (Figure 1).

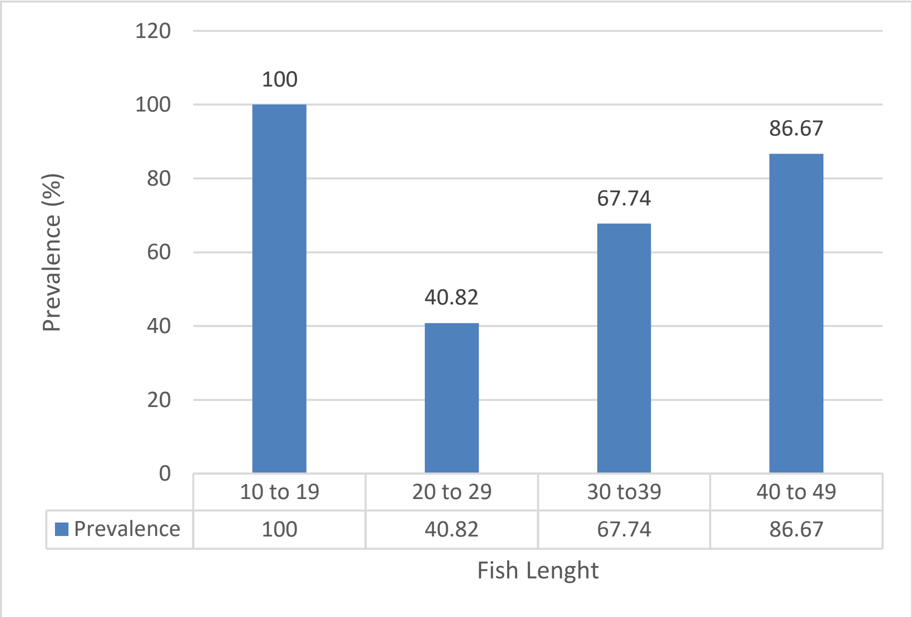

Figure 1. Prevalence of helminth infection among *H. niloticus* across the various lengths.

Table 3. Prevalence of helminth infections in the microhabitats of *Heterotis niloticus* caught from Anambra River.

Parasite Taxa	Parasite Species	N.P.R	Oesophagus	Stomach	Intestine	Rectum	Caecum	Pyloric caeca
Cestode	*Sandonella sandoni*	159	0	0	90(56.6)	0	0	0
Nematode	*P. laeviconchus*	20	0	0	6(30.0)	14(70.0)	0	0
	Dujardinascaris species	18	0	0	12(66.6)	6(33.3)	0	0
Acanthocephalan	*Tenuisentis niloticus*	255	0	0	41(16.0) 184(72.2) 18(7.1)		0	12(4.7)

KEY: N.P.R – Number of Parasites Recovered.

Prevalence of helminth infections in relation to microhabitat of fish host showed that out of 159 of *S. sandoni* worms, 90 (56.60 %) were removed from the posterior intestine while 69 (43.40 %) were removed from the mid- intestine. A total of 20 *P. laeviconchus* were removed from the intestine and rectum of the affected host.

A total of 255 *T. niloticus* were removed from *H. niloticus*, 41(16.08 %) were removed from the anterior intestine, 184 (72.16 %) from mid-intestine, 18 (7.06%) from post intestine and 12(4.71 %) from the pyloric caeca (Table 3). Seasonal variation in the prevalence of helminth infection of *H. niloticus* in Anambra River showed *S. sandoni* was removed from *H. niloticus* in the dry and rainy season. In the dry season, 78 out of 212 fishes examined were infected and 135 worms were removed, giving a prevalence of 36.79 %, M.I.I of 3.50 and M.A of 1.30. In the rainy season, 32 out of 82 fishes examined were infected and 24 worms were removed, giving a prevalence of 39.02 %, M.I.I of 1.50 and M.A of 0.60 (Figure 2). Out of 82 fishes examined, 8 were infected with *P. laeviconchus* and 20 worms removed in the rainy season, giving a prevalence of 9.76 %, M.I.I of 5.00 and M.A of 0.50. None of the examined fishes were infected in the dry season (Figure 3).

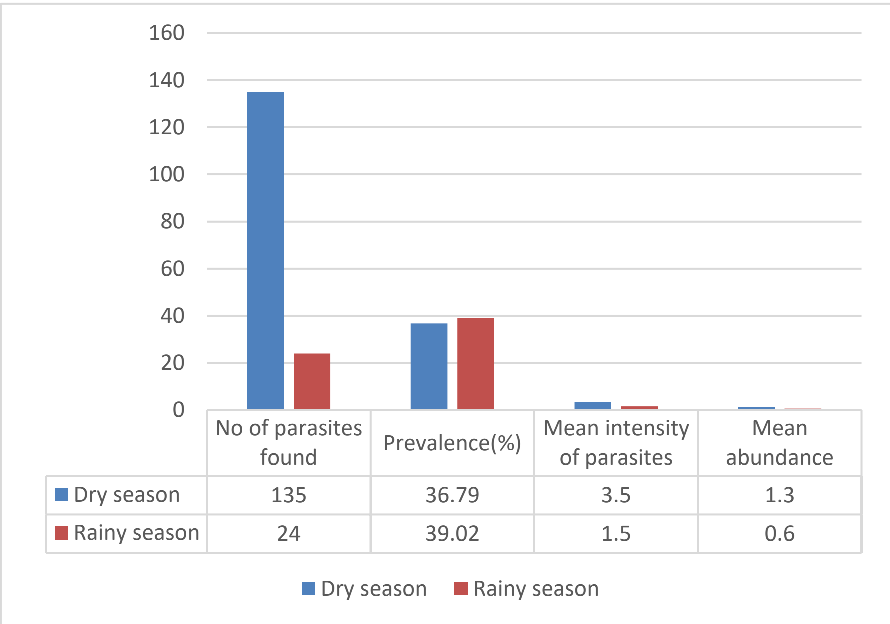

Figure 2. Seasonal variation of *S. sandoni* recovered from *H. niloticus* during the study period.

In the dry season, *T. niloticus* was removed from 86 out of 212 *H. niloticus* examined and 249 worms were removed, giving a prevalence of 40.57 %, M.I.I of 5.80 and M.A of 2.40. In the rainy season, 4 out of the 82 fishes examined were infected and 6 worms were removed, giving a prevalence of 4.88 %, M.I.I of 3.00 and M.A of 0.12 (Figure 4).

Dujardinascaris species were removed from 4 out of 82 *H. niloticus* examined in the rainy season and 28 worms were removed, giving a prevalence of 4.88 %, M.I.I of 6.00 and M.A of 0.44. No parasites were removed from *H. niloticus* in the dry season.

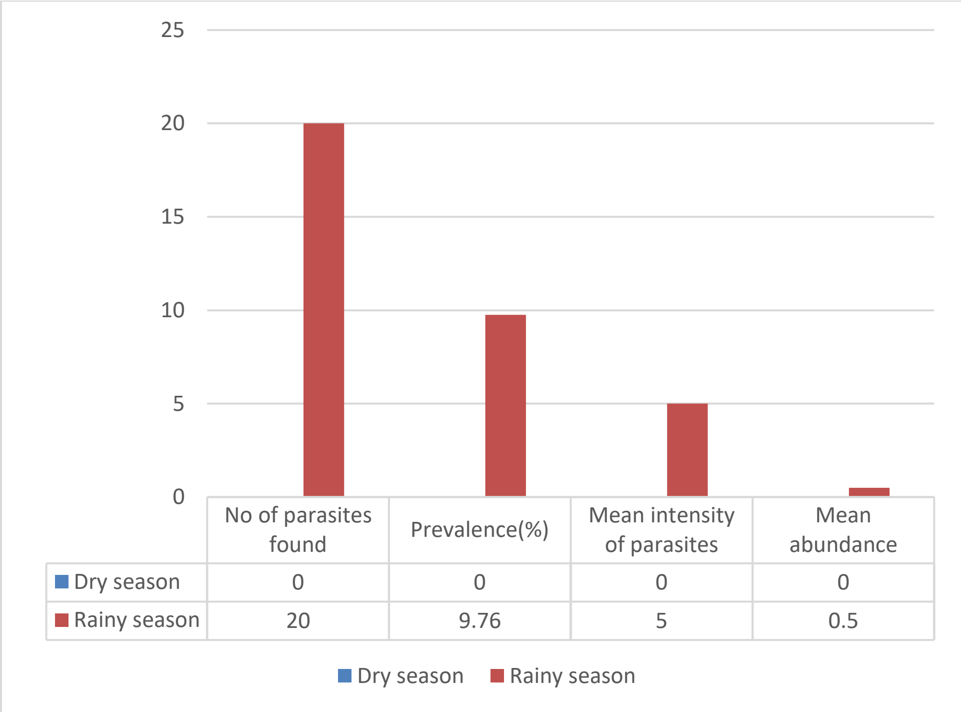

Figure 3. Seasonal variation of *P. laevinonchus* recovered from *H. niloticus* during the study period.

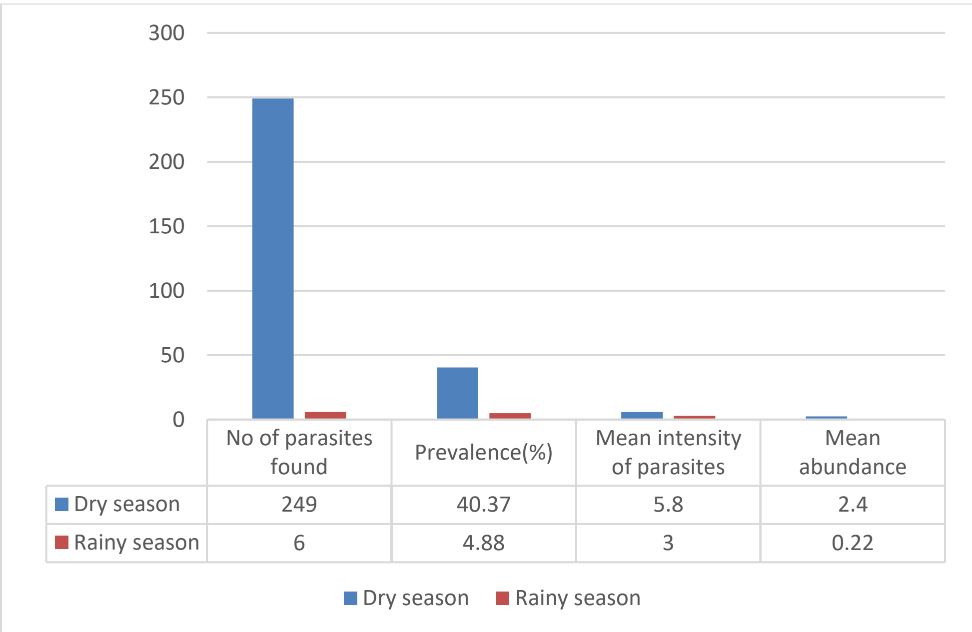

Figure 4. Seasonal variation of *T. niloticus* recovered from *H. niloticus* during the study period.

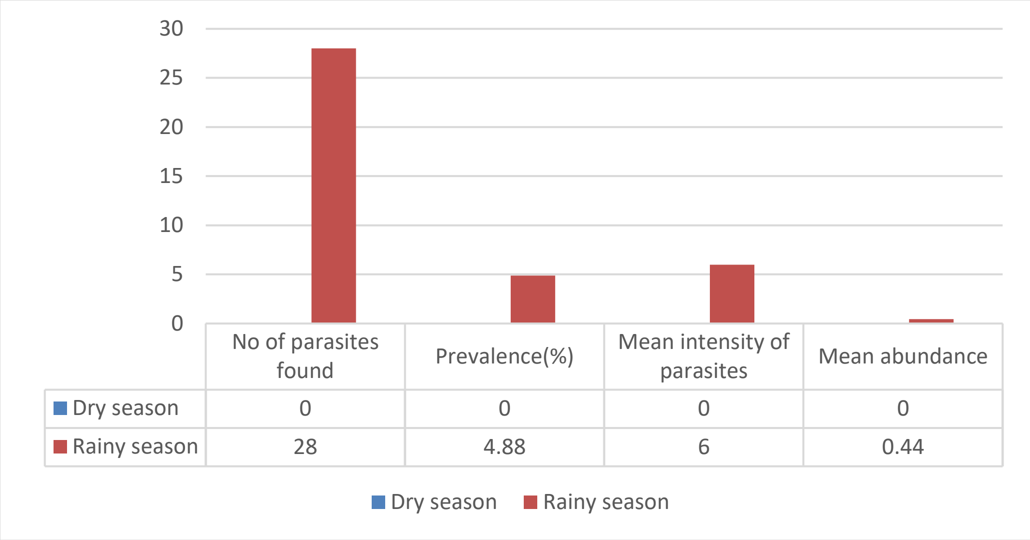

Figure 5. Seasonal variation of *Dujardinascaris sp*. recovered from *H. niloticus* during the study period.

4. Discussion

A total of 294 *H. niloticus* (Osteoglossidae) fish species were collected from three different stations of the Anambra River and examined for gastrointestinal helminth parasites. The overall prevalence of helminth infection was 72.8% which is higher than results obtained by other authors in other studies within Nigeria [3,15,16]. The level of parasitic infection observed in this study may be connected with the omnivorous and microphagous feeding habits of the fish host, primarily consuming a combination of two or more crustaceans, insects, plankton, and plant detritus. *H. niloticus* has a high adaptiveness to the natural diet which is responsible for its success in various habitats [17].

In this study, *H. niloticus* was observed to have a rich gastrointestinal helminth community. Three parasite species (of the groups of cestodes, nematodes, and acanthocephalan) were found in the examined fish species regardless of the station. The presence of *S. sandoni* in *H. niloticus* is not new as previously mentioned [18] removed the same parasite from the same host in River Anambra with a prevalence of 15.78 %. Similarly, [3] also removed *S. sandoni* in *H. niloticus* from Lekki Lagoon, Lagos. It was reported that *S. sandoni* is a relatively frequent parasite of *H. niloticus* [19]. *P. laeviconchus* was also removed from *H. niloticus* in this study and *H. niloticus* may be seen as a new host record for this parasite. *P. laeviconchus* is widely distributed in several fish hosts in Africa [20-22]. *Dujardinascaris* species which was recovered from *H. niloticus* agrees with the findings of [18]. The finding of *T. niloticus* in *H. niloticus* is not new as it has been earlier reported from Anambra River and from Lekki Lagoon, Lagos [3,18]. This parasite could be said to be an important parasite of *H. niloticus* when compared with other parasites recovered from *H. niloticus,* in terms of fisheries management in this river system.
Factors that determine fish parasite fauna as well as the intensity and prevalence in an aquatic habitat include; the diet of the host, lifespan of the host, mobility of the host throughout its life including the habitat it comes into contact with, population density and the size of the fish [2].

In the present study involving *H. niloticus*, a positive correlation exists between fish weight and the helminth infection tested. This means that as the weight of the fish increases, the prevalence of the helminth parasite tends to increase. This agrees with the report of [23] that bigger fishes cover wider areas in search of food than the smaller ones and as a result, they take in more food than the smaller ones and this tends to expose them more to infestation by parasites. The relationship between *H. niloticus* length and helminth infection in the present study shows no significant relationship between the fish length and helminth infection. This result agrees with [3] who recorded that worm burden and intensity of infection in *H. niloticus* in Lekki Lagoon, Lagos was independent of fish size. However, our present observation does not agree with the findings of [16] who reported a significant relationship between fish size and level of helminth infection. A plausible explanation for this could be linked to the availability of intermediate hosts which influences parasite composition in the host population and this varies between geographically distinct habitats.

The result of the findings on the microhabitat of the parasite showed that the cestode *S. sandoni* was recovered from the intestine of the fish host. This is similar to the findings of [3] who recovered *S. sandoni*, from the intestine of *H. niloticus.* The findings are as would be expected since tapeworms lack a digestive tract and absorb nutrients which are usually more abundant in the intestine of vertebrates using the tegument. In all, the removal of cestode parasites from the intestine would be attributed to the lack of a digestive system in cestode and so they obligatorily depend on the end product of digested food in the host which is absorbed through the body surfaces, hence they are localized in the host intestine where their nutritional requirements are satisfied.

The camallanid nematode, *Procamallanus laeviconchus* was recovered from the intestine and rectum of *H. niloticus* at a prevalence of 30.0% and 70.0% respectively, implying that the intestine is the preferred microhabitat of this parasite in the host. This could be due to the conducive nutritional advantage presented by the host's intestine to the parasites and the availability of the intermediate host, mesocyclops (a copepod) in the environment as similar findings were reported by [21, 24].

Tenuisentis niloticus recovered from *H. niloticus* in the anterior (16.08 %), mid (72.16 %), posterior regions (7.06 %) and pyloric caeca (4.71 %) shows that the mid-intestine is the preferred microhabitat of *T. niloticus* which is a common parasite of *H. niloticus.*

Generally, the acanthocephalan parasites are predominantly found in the intestine of the fish species parasitized which agrees with the findings of [25,26] in fishes from Kainji Lake, River Niger, and Owa stream respectively. This would be attributed to the lack of a digestive system in acanthocephalans and so they depend on the end product of digested food in the host. The result of seasonal variation in the prevalence of helminth infections in *H. niloticus* shows that seasonal patterns of infection varied from one parasite to another. While Acanthocephalan parasite (*T. niloticus*) has its highest infection rates in the dry season, Cestodes and nematodes (*S. sandoni*, *P. laeviconchus* and Dujardinascaris species) have the highest infection rates during the rainy season. Based on the observation of the present study, the occurrence of higher number of recovered parasites (cestodes and nematodes) in the rainy season and lower number in the dry season can be explained on the basis of the availability of food and high temperature during the rainy season, making the movement of the fishes to increase thereby disturbing the turbidity rate of the water. This muddy situation is an indicator of pronounced polluted environment which in turn predisposes the fish to infection. The higher level of parasitic infection recorded by Acanthocephalan (*T.niloticus*) could be linked to eutrophication which occurs in the rainy season and often increases parasitism because the associated increase in productivity will increase the abundance of the invertebrate intermediate hosts, mostly freshwater crustaceans [27]. Eutrophication leads to algal blooms at the peak of the rainy season, which result in an increase in species variety and population of the parasite's intermediate host, towards the end of the rainy season. This may result in the infection of fishes that feed on them, and thus probably bring about the maturity of the parasites in the fish towards the dry season depending on the life cycle of individual parasites. Another factor may be a drop-in water level in the dry season exposing the invertebrates to their fish predators and leading to an increase in host density and greater overlap of intermediate and definitive hosts.

5. Conclusion

This study is on the gastrointestinal helminth parasites of *H. niloticus* of Anambra River Basin in Nigeria. Four species of helminth parasites have been isolated comprising cestode, nematode and acanthocephalan parasites. A high overall prevalence of parasitic infestation of 72.8% was found with a mean abundance of 0.77 of helminth parasites per fish. Prevalence of helminth infection was also found to be increasing with the increase of host weight. The intestine of the fish was found to be the major infective site for most of the parasites recovered. With the results obtained it is highlighted that the studies of parasitofauna in fish is required in considering probable zoonotic transmission of parasitic diseases from fish**.** The study concluded that *H. niloticus* is a new host record for *P. laeviconchus.*

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Conflict of interest

The authors declare no conflict of interest.

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