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RESEARCH ARTICLE

IDENTIFICATION, CLASSIFICATION AND PREVALENCE OF INTESTINAL PARASITES OF DIFFERENT FISH SPECIES FOUND IN AFIKPO NORTH LOCAL GOVERNMENT AREA FRESH WATERS

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ABSTRACT

Identification, classification and the prevalence rate of intestinal parasites of different fish species found in Afikpo North Local Government Area fresh waters were carried out. A total of 58 fishes were collected at random locations in Afikpo North Local Government Area of Ebonyi state. They were sampled, identified and classified into 28 species. Seven (7) common fishes among them were examined for gastrointestinal parasites. A total of twenty one (21) parasites of Seven (7) species were isolated from the fishes which comprising of Six (6) *Diphyllobothrium spp*, Four (4) *Contracaecium spp*, Three (3) *Acanthocephalan*, One (1) *Ornithodiplostoniummetacoracariae*, Three (3) *Polyonchobothriumclariae*, Three (3) *Procamallusspp* and One (1) *Anisakis simples*. Those whose standard length falls between the range of 18.4-19.5cm have the prevalence rate of 47.62% and those that falls between the range of 23.5-34.7cm have the prevalence rate of 52.38%. Among the parasites isolated, *Diphyllobothriumspp* recorded the highest prevalence rate of 28.6% while *Anisakis simples* and *Ornithodiplostonium metacoracariae* recorded lowest rate of 4.76%.

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INTRODUCTION

The numerous freshwater bodies of Nigeria with over 270 fish species are the richest in fish diversity in West Africa (Holden and Reed, 1972). Most of these fish species have been published mainly in scientific journals. These diversities of fishes of Freshwater are found in Afikpo North which is surrounded by freshwaters and serves as the major supplier of local fishes to Afikpo and its environment. Fish identification and classification becomes very important in order to find out those fish diversity and have them properly documented. Parasites of fish constitute one of the major problems confronting the modern fishes both in the wild and at culture and pathological condition arising from parasitic infections assume high magnitudes especially under crowed conditions (FAO, 2006 and Okwuosa, 2011). All fishes are potential host to many different species of parasites that causes significant mortalities among captive and wild fish stocks.

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Accurate identification of parasite is therefore important so that a build-up of parasite number can be prevented. Information about the moods of transmission and potential intermediate hosts is offer crucial to select the most appropriate management action to reduce the problem. Present approach to treatment of parasites disease is largely limited to those on external surface and the intestinal lumen. So blood parasites and encysted worms cannot be treated effectively and economically, there by remain among the major causes of human misery and death in the world and are important obstacles to the development of economically less favored countries. The purpose of this research is therefore, to identify, classify and determine the prevalence rate of intestinal parasites of different fish species found in the Afikpo North L.G.A. In the study area little or no work has been done to identify, classify and determination of the prevalence rate of intestinal parasites of different fish species that is why this study is very necessary. The internal or endoparasites of fish inhabits the digestive factor other organ in the body while external or ectoparasites attach themselves to the gills, skins and fins of fish (Paperna, 1991 and Okwuosa, 2011).

MATERIALS AND METHODS

Study area

Afikpo town, is located on 6^0 North latitude and 8^0 East longitude with an area of about 64 square mile ($164km^2$). The town comprises two local Government areas namely Afikpo North and Afikpo South Local Government Area respectively. The study was carried out in AfikpoNorth L.G.A which is a hilly area despite occupying a region low in attitude, which rises 350 feet above sea level, it is a transitional area between open grassland and tropical forest and has an average annual rainfall of seventy seven inches (198cm). It is surrounded with various bodies of freshwaters. The areas where samples were collected include Enohia beach, Unwana beach, Ndibe beach, Ebujimgborn stream, Iyi-Obasi, Ubeyiriver and Eke market, AhiaOgo, and park market randomly in the following towns and villages such as Amasiri, Unwana, Afikpo, Enohia, Oziza, Enohia Item, Kpogrikpo, EnohiaNkalu all in Afikpo North L. G. A, Ebonyi state Nigeria.

Collection of sample

The studied samples were obtained from fishermen (with prior arrangement) at Ndibe beach, Unwana beach, Enohia beach, Ebujimgbom, Iyi-Obasi, Ubeyirier, Uyi, and some other places, and some were bought from the market such as Eke market, AhiaOgo, Park market randomly all in Afikpo North L. G. A, Ebonyi state. The samples were caught by the fishermen with gill nets (mesh size, 3cm) set in the evening and retrieved the following morning. Fishes were placed in an ice-chest and transported to the Laboratory for identification processing and examination for parasitic helminthes.

Identification of fish samples

Fish samples were identified to the species level using taxonomic key (Holden and Reed, 1972). The measurements taken were total length (T.L) and standard length (S.L), using a calibrated dissecting board, the weight of each fish was taken using a sensitive weighing balance (metter ®). Each fish was assigned a reference number during dissection to ensure a proper documentation of records obtained (Marfinez *et al.,* 2004).

Processing and preservation of fish

As each fish was dissected, the intestine was all removed using appropriate dissecting tools. These organs were then placed in saline water contained in petri-dishes to aid the emergence of parasites. The petri-dishes were then thoroughly examined for parasitic helminthes.

Processing and preservation of parasites

Parasites obtained were cleared by washing them in saline for thirty minutes to remove mucus, and the worms relaxed in distilled water for ten minutes. The relaxed in distilled water allowed the parasites to void their eggs. With the *Acanthocephalans*, the distilled water also caused the proboscis to be extended. An applicator rod was also used to exude the proboscis of the *Acanthocephalans*. After relaxation parasites were killed and fixed in Alcohol-formalin Acetic acid (AFA) solution. The parasites were left in the fixative for 24 hour and then transferred to a 70% alcohol solution. Non-staining method were used in the treatment of the parasites. The parasites were dehydrated in 70% alcohol, 85% alcohol, 95% alcohol, and absolute alcohol for a period of ten minutes each. After dehydration, the parasites were cleared in xylene and mounted on a slide warmer for ten minutes. The slides were then observed under a light microscope and the parasites identified using information provided by Yamaguti (1963), Ukoli (1966), Khali, L.F. (1991), Bundley and Williams (1994), Juan and Windsore (2006), and Edoh *et al.* (2008).

Collection data and statistical analysis

Relationships between parasite burden and other variable (length and Weight) were compared using correlation analysis and t-test (Steel and Torrie, 1981).

RESULTS

Fish identification

A total of fifty eight (58) specimens of fishes were sampled as shown on Table 1 below. Samples were randomly collected from Enohia Beach, Unwana Beach, Ndibe Beach, EbujiMgbom Streams, Iyi–Obasi, Ubeyi River, Eke Market, AhiaOgo and Park Market in Enohia Town, Unwana, Afikpo Town, along Unwana Road and their various markets all in Afikpo North Local Government Area in Ebonyi State Nigeria, between November and December 2015.

Table 1. Fish Identification

S/N	Common Name	Local Name	Number Of Occurrence	Standard and Local Length (cm)	Specie Name
1	Black mangrove fish /cichlid or spotted tilapia	Apupammiya	2	Standard =16 Local = 13	*Tilapia mariae*Boulenger, 1899
2	Cichlid (Tilapia)	Okpea	2	Standard=19.2 Local = 16.0	*Tilapia dageti* (Thys Van de Audenaede, 1971) Synonym: T. *Melanopleura*Thys Van de (Dumeril, 1859)
3	Catfish	Arira	1	Standard=18.2 Local = 12.2	*Heterbranchus spp.*
4	Cichlid(Red Belly Tilapia)	Apupa	2	Standard=25.6 Local = 22	*Tilapia zili* (Gervais, 1848) Synonym: *H. elongates* (Guichenof, 1861)
5	Black mangrove fish or cichlid or spotted tilar	Agbammeya	2	Standard=14.2 Local = 11.5	*Tilapia mariae*Boulenger, 1899 Synonym: *Tilapia*MeckoPelligin, 1911
6	Cichlid (Tilapia)	Okpokorommanu	1	Standard = 21 Local = 18	*Tilapia dagsti* (Thys Van de Audenaeve)1971 Synonym: *T. Melanopleucan* Thys Van de (Dumeril 1859)

7	Cichlid (Nile Tilapia)	IkitaIyi	2	Standard=13.5 Local = 10.4	*Oreachromisniloticus*(Linnaecus,1758) Synonym: *Tilapia miloticus* (Linnaeus,1758)
8	Catfish	Erum-edo	2	Standard=16.3 Local = 14	*Synodontisomias* Gunther, 1864
9	African River Pike	Okoro	2	Standard	*Genius hepsetus*Swinson, 1883
10	Stout fish	Apupa-oriewa	2	Standard=10.3 Local =7.5	*Petrocephalusbovei* (Cuvier and Valenciesnnes,1846)
11	Cichlid (NileTilapia)	Ahuiyielugwe	2	Standard = 30 Local = 27.5	*Oreochromisniloticus* (Linnaeus, 1758)Symnonym: *Tilapia niloticus* (Linnaeus,1758)
12	Prawn (giant tiger prawn)	Iko	1	Standard = 10.5 Local = 7.4cm	*Penaeus monodon*Fabricous, 1798 Synonym: *Senisulcatus* De Haan, 1844
13	Cichlid	Ehia	2	Standard=12.4 Local = 8	Genus: *Tylochromis* Regan, 1920Specie: *Tylochromisgentinki* (Steindachner,1894) Synonym:Paratilapia (Pelmatochomis) Jenkinkiisterinda Chinner,1894
14.	Crayfish	Oyaransielu	1	Standard= 12 Local= 8.9	Genu: *Astacidae* Species:*Austropotamoobi-nspallipss*
15.	Parrot grunt	Edoo	3	Standard=18.5 Local = 15	*Pomadasysperoteti* (Cuvier, 1830)
16.	Buffer fish	Eturutakpa	2	Standard= 24 Local = 20	*SchilbsUranoscopus*Ruppell, 1832Synonym: *Schilbsisidori*(Cuvier and Valenciennes, 1839)
17.	Elephant Snout fish	Apupa	3	Standard=19.5 Local= 17.3	*Mormyrusrume*Cuvier and Valenciennes, 1846
18.	Puffer fish (Gobe)	Okono	1	Standard=12.3 Local= 10.2	Genus: *Tetraodon* (Linnaeus, 1758) Specie: *Tetradilonfahaka*strigusus Bennet, 1834 Synonyms:*Tetraodonfahaka*(Ruppel, 1829), *Tetraodon* Linnaeus, 1758
19.	Cichlid	Okpea	3	Standard=14.5 Local = 12.2	Specie:*Tilapiasparmansi*(Holly,1925)Synonyms: *Acerina* Gervais 1848 *T.shariensis*Fowler, 1949
20.	Pig snout grnt	Otinii	2	Standard=18.5 Local = 15	*Pomadosysrogeri* (Curvier, 1830)
21.	African carp	Ihii	2	Standard=21.5 Local= 17.3	*Labeopseudocoube*Blanche and Milton, 1960
22.	Flathead groy mullet	Ehiaakpankogiri	2	Standard=18.4 Local = 15	*Mugicephalus,* Linnaeus, 1758 Synonym: *Mugiashanteeusis*, Bleeker, 1863
23.	Cray fish	Okpoto	1	Standard=13.6 Local= 10.5	*Parastacoidae,* Huxley 1879
24.	African bony tongue	Okpokoro	3	Standard=23.2 Local = 20.5	Genus:*Heteroti*, Ruppal, 1829 Specie:*Heterotisniloticus* Cuvier, 1829
25.	Niger perch or Nile Perch	Oyara	2	Standard=14.5 Local = 12.3	Genus:*Lates* (Cuvier, 1828Specie: *Latesniliticus* (Linnaeus,1758)
26.	African Tiger fish		1	Standard=24.5 Local= 20.9	*Hydrocynuslineatus*Bleeker, 1863 Synonyms: *Hydrocyannusviltatus*(Castelnan,1861),*Hydroc-yon vittatus*Boulenger, 1898
27.	Moon fish		1	Standard = 30 Local= 27.2	Genus: *Citharinus,* Curvier 1817 Specie:*Citharinuscithanus*and *C. latus* Species: Citharinuscithanus (Geoffrey stiffilaire, 1809)
28.	Catfish (Mud fish) or sharp tooth catfish		2	Standard = 18.9 Local = 16	*Clariasgariepinus* (Burchell, 1822) Synonyms: *Claris* (Azera, Valenciennes, 1840 *Claris maeraeanthus,* Gunther 1864) *Clariastsanensis*Baulenger 1902
29.	Moryrid or trunk fish	Mallet Etionuogorogo or ubohonuogorogp	4	Standard= 30 Local= 27.5	*Mormyropsdelicosus* (Leach, 1818) Synonym: *Mormyropsangulloides*(Linnaeus, 1758) *Mormyropsangulloides*Voltae Roman, 1966 *Mormyropscurviceps,* Roman196
30.	African bony tongue	Okpuo	2	Standard=27.4 Local = 24	Genus: *Heterotis*Ruppel, 1829Specie: *Heterotisniloticus*Cuvier, 1829

They were subjected to fish identification procedure using fish identification kit. This result obtained as shown in table 1 below and followed by parasitological investigations. Table 4.1 shows the classification of the 58 fishes that were sampled and subjected to standard fish identification using fish identification kit. There various local names, standard and local lengths were recorded too in the table as well as species names and number of occurrence.

Intestinal parasite identification

The Results of the investigation reviewed a total of 4 classes which arecestrode, nematodes, trematodes and phylum acanthocephalan as shown in Table 2.

Table 2 further reviewed the standard weight and length of various fishes that were examined for intestinal parasites as well gives the stages of life of the parasites found in the intestine. Table 3 recorded the number of occurrence of various parasites in the seven (7) fishes examined and their prevalence rates. Table 4 reviewed the intestinal parasites prevalence rate based on their various standard lengths.

DISCUSSION

The results of this investigation have shown that 58 fishes were sampled and were collected from various places randomly in Afikpo North Local Government Area of Ebonyi.

Table 2. Relationship between parasitic burdens, length of Fish and weight of fish

S/N	Fish Common Name	Specie Name	Standard Length (cm)	Standard Weight (g)	Helminth	Stages		
						Ova	Larva	Adult
1.	Cichlid (*Guenthe's* mouth broader)	Chromic tilapia guetheri (Sauvage, 1882)	30	543.10	*Diphylloboth-riu spp.*	✓	✓	✓
						✓	✓	X
		Synonym: *Pelmatochromisguentheri* (Sauvage, 1882)			*Contracaecum spp.*	✓	✓	*
		Pellegrinni(Boulenge, 1902)						
2.	Cat fish	*Synodontiswaterloti*, Dagst, 1962	34.7	407.90	*Contracacecium spp.*	✓	✓	*
					Acanthocaphalan	✓	✓	✓
3.	Barb	*Barbuscellipterus*Boulenger, 1907	30	127.10	*Diphylobothrium spp.*			
					Orinithodiplostoniummetaceracariae	✓	✓	✓
						✓	✓	✓
4.	Parrot grunt	*Pomadosysperotetis* (Curier, 1830)	18.5	149.60	*Polyonchobothriumclariae*	✓	X	
					Diphyllobothrium spp.	✓	*	✓
					Procamallus spp.	✓	✓	✓
					Acanthocephalan	✓	✓	*
						✓	*	✓
5.	Pigsnout	*Pomadasysrogsri*(Cuvier, 1830)	19.5	127.40	*Diphyllobothrium spp.*	✓	✓	✓
					Procamallus spp.	✓	✓	*
					Crontacaecium spp.	✓	✓	✓
								✓
								✓
6.	Flat head grey Mullet	*Mugilcephalus*, Linnaeus, 1758	18.4	72.60	*Polyonchobothriumclariae*	✓	✓	✓
		Synonym: Mugiashantensis, Bleeker, 1863			*Diphyllobothrium spp.*			
					Acanthocephalan	✓	✓	✓
						✓	*	*
7.	African bony tongue	Genus*: Heterotis*Ruppel, 1829	23.5	73.90	*Diphyllobothrium spp.*	✓	✓	✓
		Specie:*Heterotinilotocis* Cuvier, 1829			*Contacaecium spp.*			
					Anisakis simples	✓	*	✓
					Polyonchobothriumclariae	✓	✓	*
						✓	✓	✓

Table 3.Helminths of different species of fish from Afikpo North Local Government Area, Ebonyi state

S/N	Helminth	Host	Number Infected	Prevalence %	Range	Intensity (Mean ±Sd)
1	*Diphyllobothrium spp.*	Cichlid, Barb, Parrot grunt, Pig snout, Flat head grey mullet, African bony tongue, Cat fish	6	28.6	1 – 2	3.5 ± 0.417
2	*Contracaecium spp.*	Cichlid, Barb, Parrot grunt, Pig snout, Cat fish, African bony tongue,	4	19.04	2 – 5	5.25 ± 0.3125
3	*Acanthocephalan*	Cat fish, Parrot grunt, Flat head grey mullet,	3	14.28	1– 3	7 ± 1.333
4	*Ornithodiplostonium metacoracariae*	Barb	1	4.76	2– 5	21 ± 20
5	*Polyonchobothriumclariae*	Parrot grunt, Flat head grey mullet, African bony tongue	3	14.28	2– 4	7 ± 1.333
6	*Procamallus spp.*	Parrot grunt, Pig snout grunt, Barb	3	14.28	1– 5	7 ± 1.333
7	*Anisakis simples*	African bony tongue	1	4.76	3– 6	21 ± 20
	TOTAL		21	100%		

Table 4.The prevalence (%) of the intestinal parasite in relation to standard length of various fish

Body	18.4– 19.5cm	23.5 – 34.7cm	Total
Number Examined	3	4	7
Number Infected	3	4	7
Prevalence Of Infection (%)	47.62	52.38	100%

Relationship between parasite burden body weights in fish

Table 5. Using t-test analysis

Table 5.1. One-sample statistics

	N	Mean	Standard deviation	Standard error of mean
Number infected	7	3.0000	1.73205	0.65465
Standard weight	7	214.5143	187.71720	69.81654

Table 5.2. One- sample test

Test value =0.05

	T	df	Significance (2-tailed)	Mean difference	95% confidence interval of the difference	
					Lower	Upper
Number infected	4.506	6	0.004	2.9500	1.3481	4.5519
Standard weight		6	0.022	214.46429	43.6294	383.2992

Such as, t=test df= Degree of freedom

Table 6. Using correlation co-efficient (r)

	Correlations	Number infected	Standard weight
Number infected	Pearson correlation	0.828	1
	Sig.(1-tailed)	0.011	
	N	7	7
Standard weight	Pearson correlation	1	0.828
	Sig.(1-tailed)		0.011
	N	7	7

Relationship between burden and body length of fish

Table 6. Using t-test analysis

Table 6.1. One-sample statistics

	N	Mean	Standard deviation	Standard error mean
Number infected	7	3.0000	1.73205	0.65465
Standard length	7	24.8714	6.68374	2.52622

Table 6.2. One-sample test

Test value = 0.05

	t	Df	Sig.(2-tailed)	Mean difference	95% confidence interval of difference	
					Lower	Upper
Number infected	4.506	6	0.004	2.95000	1.3481	4.5519
Standard length	9.826	6	0.000	24.82143	18.6400	31.0029

Table 7. Using correlation co-efficient (r)

	Correlations	Number infected	Standard weight
Number infected	Pearson correlation	1	0.586
	Sig.(2-tailed)		0.083
	N	7	7
Standard length	Pearson correlation	0.586	1
	Sig.(2-tailed)	0.083	
	N	7	7

Such as, t = test df = degree of freedom

N/B: All the calculations were done with spss-software = statistical program for social science.

After a scientific identification and classification, these 58 fishes fall into 28 species of fishes. The result of this investigation also revealed the occurrence of five (5) species of gastrointestinal helminthes parasitizing seven (7) common fish species found in fresh waters in Afikpo North Local Government Area in Ebonyi State. These are the phylum nematode, such as *Procamallanus spp.* with a prevalent rate of 14.28%, *Contracaecium spp.* with a prevalence rate of 19.04%, *Anisaks simples* with a prevalence rate of 4.76%, *Phylum platyhelminths* such as class – cestodes like *Polyonchobothrium clappas* with a prevalence rate of 14.28%, *Diphyllobothrium spp.* with a prevalence rate of 28.60%, and class – Trematodes like *Orinithodiplostorium spp.* with a prevalence rate of 4.76%, and *Phylum acanthocephan* with a prevalence rate of 14.28% (*Neoechinorhynchusrutili*).

The high infection rate recorded in this investigation is in agreement with observation of Ukoli (1987); Ndifon and Jimeta (1990); Anta *et al*., (2000) which they subscribed to this observation. The commonest infection that have high intestinal parasitic load of the fish was caused by a class – Cestodes that is *Diphyllobothrium spp,* which have six (6) occurrence rate hence prevalence rate of 28.6% (in terms of number of parasites found in the intestine), followed by phylum Nematodes that is *Contracaecium spp.* which recorded four (4) number of fish infected with prevalence rate of 19.04% and the lowest infection that have intestinal parasitic load of fish cause by *Ornithodiplostorium metaceracariae* and *Anisakis simples*, which recorded one (1) as number of fishes infected with prevalence rate of 4.76% as recorded in table 3 above and illustrated. According to Okwuosa (2011), some species of fish

such as *Clariae spp.*, Cichlids etc are bottom dwellers/feeders, they feed on what is most available and close to them such as detritus, water invertebrates, there may be intermediate hosts of various parasites which may develop into adults in the gut of fish after consumption especially if is by a proper definite host (birds, such as gray heron). Judging by the fish, the intermediate host (mesocyclops la copepod) in case of *Diphyllobothrium spp.* are common in the environment, Royce (1972) concluded that the presence of cestodes in fish lead to decline in population in their natural environment although, this study did not investigate their assertion and there are needs to carry out research more on this issues and conditions in Afikpo North Local Government Area, Ebonyi State. The distribution of helminthes (intestinal parasites) in the intestine, stomach, fish gut and also other part of fishes like Cichlid, barb, parrot grunt, pig snout, flat head grey mullet, African bony tongue, catfish etc, showed that the majority of the parasites occurred in the intestine. Similar finding were reported by Khali (1973); Ugwuzor (1987); Ndifon *et al*., (1990); Auta *et al*., (2000); and Oniya *et al.,* (2004). This could be due to the conducive nutritional advantage presented by the host's intestine to the parasites (Bunkley *et al.,* (1994).

Onwulini and Mgbemena (1989) observed that helminthes sometimes differ in their nutritional and respiratory requirement. In this study, cestodes commonly found in the intestine, this suggest that food/diet is probably responsible for the burden in parasite species as reported by Oniya (2004) and Emere (2006).Based on our results fishes whose length fall between the range of 30 – 34.7 have lesser number of parasites while those that falls between the range of 23.5 – 18.4 have higher number of parasites, as illustrated in Table 4.2 above, which is contrary to the findings of Bishop and Margolis (1955) reported that the high infection observed in larger fishes in their works i.e. research may be due to the fact that the larger or adults fish provide greater surface for infection than smaller or juveniles fishes. But in our finding, it is obvious that this could depend on the level of pollution of the water that those fishes are found since those organisms (parasites) prevalence rate are dependent of level of contamination of the organism's habitat and food.

Roberts (1978) showed that the number of parasite increases with fish length and suggested that the parasites increases in parasitization could be to parasitic larvae accumulation from year to year as the fish grows older, but in this study, this is contrary, that the number of parasites does not depend on the length and weight of the fishes rather the food intake, environment of which the fish leave i.e. part of water, hence this calls or needs for further fish histographical research on the whole body or intestinal parasitic investigation and intestinal parasitic prevalence in Afikpo North Local Government Area, Ebonyi State. The present investigations shows evidence of parasite helminth infection of different fish species. The presence of these parasite might elicit some pathological effects on the fishes by retarding their growth, causing tissue disruption and even death. However, it might be said that parasitic infection of fish does reduce their productivity, as shown by several studies (Onwuliri and Mgbemena (1987) and Anosike*et al*., 1992).

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