Helminth communities in Cichlids in natural and man-made ponds in south-west Nigeria.

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ABSTRACT: Three hundred and fifty-four cichlid fishes from a natural reservoir, Eleyele Reservoir (unpolluted station A and polluted station B) and a fish farm, Agodi fish farm in Ibadan, south-west Nigeria were examined for ecto- and endoparasites. *Oreochromis niloticus Tilapia zilli, Hemichromis fasciatus, Sarotherodon melanotheron, Sarotherodon galilaeus, Tilapia mariae* harboured larval trematodes, C*linostormum tilapiae, Neascus* species, *Allocreadium ghanensis, Phagicola longa, Euclinostomum heterostomum, Alloglossidium corti* and Acanthocephalans, *Acanthella* and *Acanthogyrus tilapiae. Hemichromis bimaculatus* haboured no parasites. In the reservoir, males had higher parasitic infections than females but difference was not statistically significant (P>0.05).In the fish farm, females had higher parasitic infections than males and the difference was statistically significant (P<0.05).No infection was recorded in larger sizes of fish examined from all sites. The intestine of the fish hosts at stations A and B of the reservoir, had the highest parasitic load of 24.39% and 36.20% respectively while the body cavity had the highest parasitic load of 43.47% in the fish farm. *O. niloticus* had the highest level of infection (67.03%) and the least level of infection was found in *S. galilaeus* (22.5%). *C. tilapiae* was the most prevalent (66%) while *E. heterostomum* had the least prevalence (1%). [Researcher. 2009;1(3):84-92]. (ISSN: 1553-9865).

Keywords: Cichlids; Fish parasites; Reservoir; Fish farm; Nigeria

INTRODUCTION

Fishing is an important component of aquaculture in Nigeria. Fish is important as a source of protein with low cholesterol level in the diets of the populace and economically as a source of subsistence income (Aken'ova, 2000). With the ever-increasing need for cheap sources of protein, more and more attention is being focused on fish, both from natural waters and fish farming (Khalil and Polling, 1997).

Parasite infections in fish causes production and economic losses through direct fish mortality, reduction in fish growth, fecundity and stamina, increase in the susceptibility of fish to diseases and predation and through the high cost of treatment (Cowx, 1992). Intensive fish culture favours the spread of many diseases and parasites (Anyanwu, 1983)

Knowledge of the disease and pathology of fish in our tropical and sub-tropical waters is far from adequate (Akinpelu, 1983). Studies by Paperna(1980) show that cestodes and trematodes(diplostomatida) are common among cichlids and wild fishes. A close scrutiny of tilapia species for parasites by Meyer (1966) reveal the thorny headed worms, Acanthocephalans, which are common in the intestine of fishes all over the world.

This present work examines the helminthic and acanthocephalan parasites of the cichlids in polluted and unpolluted sites of a natural reservoir and in a fish farm.

MATERIALS AND METHODS

STUDY AREAS

This study was carried out in a natural reservoir, Eleyele Reservoir (with unpolluted station A and polluted station B fishing points) and in a fish farm, Agodi fish farm both in Ibadan city, south-west Nigeria.

Eleyele dam is located on Latitude 7^0 26^1 N and Longitude 3^0 52^1 E in Eleyele area of Ibadan metropolis and with an altitude of 125m above sea level. Seasonal temperature occurs with the mean minimum temperature (24.5^0C) occurring in August when there is dense cloud cover. The mean annual

rainfall is 1262.3mm.It is flood controlled with a maximum depth of 12m during the floods. The polluted point was covered with water hyacinth at the time of this study.

Agodi fish farm is involved in the intensive culture of cichlids. It receives water from the Ogunpa river, a major river in the city.

SAMPLE COLLECTION AND IDENTIFICATION

Live *Tilapia* spp were collected at the three sites during the months of April, May and June fortnightly, cutting across the end of the dry season and the start of the rainy season. Samples were collected between 0700 and 1000 hours at each of the three sampling sites as recommended by Adebisi (1981).Fishes were randomly caught by fishermen using cast nets at stations A and B and drag nets at Agodi farm. The fishes were transported to the laboratory where they were sorted by sizes and species. Identification was done using the atlas by Olaosebikan and Raji (1998). Sexes of fishes were determined by the presence or absence of an intromittent organ on the ventral side just before the anal fin. This was later confirmed by the presence of testes or ovaries observed during dissection.

Length and weight of the fishes were taken using a measuring board and a chemical balance respectively.

EXAMINATION FOR PARASITES

A cut was made on the ventral side of fish from the anal opening to the lower jaw. Two more cuts were made on the lateral side to expose the body cavity and most of the internal organs. Parasitic helminthes that were visible to the naked eyes were looked out for and removed from the fish carcass. These could be cysts, juveniles or larval forms. For better observation, hand lens and dissecting microscope were used. Gills were examined under water, eyes were removed and cut open under water to examine the lens and retina and the body cavity was thoroughly examined. The gall bladder was removed and the content examined on a slide. Squash preparations of the liver, gonads and kidney were made and examined for parasites. Contents and the walls of the swim bladder were also examined. Urinary bladder was removed and opened under water and examined. The stomach and heart were dissected and examined. The abdominal wall was cut laterally to expose the gut. This was opened up into a specimen bottle containing normal saline solution and was left for about 4 hours. The intestines were then teased open from the anterior to the posterior ends in a Petri dish. The surface of the skin was examined and fish flesh was sliced at the dorsal edge to expose the muscles for visible parasite examination.

Helminth cysts were excysted by subjecting them to slight increase in temperature in a bile solution as medium.

PRESERVATION AND IDENTIFICATION OF PARASITES

All helminthes recovered were allowed to die and stretch fully in 0.09% normal saline as recommended by MAFF(1971).They were later preserved in 70% alcohol with one or two drops of glycerine to prevent contraction of the worms and complete evaporation.

The parasites were transferred from the 70% alcohol fixative to Para carmine (1g carminic acid, 0.5g aluminium chloride, 4g calcium chloride, and 100cc 70% alcohol) and left in the stain for one day. They were then washed in 70% alcohol and placed in acid alcohol for differentiation, the process being watched under a microscope. When the preparation was completed, the helminthes were transferred to 70% alcohol. They were then dehydrated in series of alcohol concentrations as follows: three changes of 70% alcohol for 15 minutes each, 95% alcohol for 1 hour and three changes of absolute alcohol for 15 minutes each. They were then cleared in xylol and mounted in Canada balsam (MAFF, 1971).

The specimens were then viewed under the microscope and identified using the keys by Yamaguti(1959).

DATA ANALYSIS

The prevalence and intensity of the parasites were calculated. The chi- square was used to calculate the significant difference between levels of infection at the different stations.

RESULTS

Five species from the three sites sampled out of the seven species of fish hosts belonging to the family Cichlidae, harboured larval trematodes. The larval trematodes were found to infect more than one

host. The two species that did not harbour larval trematodes were *Hemichromis fasciatus* (Peters, 1857) and *Hemichromis bimaculatus* (Gill 1862).

Table 1 shows that *Sarotherodon melanotheron* (Ruppell 1852) had the highest mean number of parasite per host in station A (2.45) and B (4.18) and the highest number of infected hosts in station A (40%) and B (81.8%).This is followed by *Tilapia zilli* (Gervais 1848) with mean number of parasite per host in station A, 0.67 and in B, 1.6 and 27.63% and 45% were infected in stations A and B respectively. At Agodi farm, *Oreochromis niloticus* L. had the highest mean number of parasite per host (3.18) and the highest number of infected host (70.1%). *S.melanotheron, Tilapia mariae* (Boulenger 1899) and *T.zilli* were not found at this site.

All the infected fish species in all stations harboured both trematodes and Acanthocephalans except *T. mariae* which harboured only trematodes at stations A and B and *Sarotherodon galilaeus* L. which also harboured only trematodes at Agodi farm (Table 2).

Table 3 shows that the males had higher percentage of parasitic infection than the females in polluted and unpolluted stations but the difference in parasitic infection and the sex of the fish hosts is not statistically significant ($P > 0.05$). However, this was statistically significant ($P < 0.05$) at the fish farm.

Fish hosts with sizes ranging between 21g – 140.9g recorded the highest percentage of infection while fishes with a size range of 10 – 20.9g and 141 – 470.9g recorded very low or no percentage of infection (Table 4). At station A (Table 5), no infection was recorded in the eyes, operculum and the mouth. The intestine had the highest total parasitic load (24.39%) at a geometric mean of 2.17 and 36.2% at a geometric mean of 2.24 in station A and B respectively (Tables 5). However the total parasitic load was highest in the body cavity (43.47%) at a geometric mean of 2.27 followed by 33.04% at a geometric mean of 1.60 in the gills at the fish farm (Table 5).

Table 6 shows the summary of prevalence of parasite types from all sites. Highest prevalence of parasite was found in the intestine (23.2%) followed by the body cavity (15.2%). *Clinostomum tilapiae* had the highest prevalence in the body cavity (15.2%) followed by the gills (12.4%).

Total parasitic load of the fish hosts decreased from the first sampling (in April) to the sixth sampling (in June) when the rainfall was at its peak at stations (Table 7). *C. tilapiae* maintained the highest percentage of infection throughout the sampling periods, followed by *Acanthella* (Table 7).

Table 1: Infection rate of Fishes examined in all sites

	Eleyele Reservoir				Eleyele Reservoir				Agodi Farm			
	Station A(Unpollted)				Station B(Polluted)							
Fish Host	No. Ex	No. Inf%	Total Parasit	Mean/ Host	No Exm	No Inf%	Total Prst.	Mn Hst	No. Exm	No. Inf%	Ttl Prst	Mn/ Hst
O. niloticus	1	0	0	0	3	0	0	0	87	61(70.1)	277	3.18
S. melanotheron	20	8(40)	49	2.45	11	9(81)	48	4.18	-	-	-	-
S.galilaeus	24	5(20.8)	8	0.33	20	4(10)	4	0.2	14	9(64.3)	21	1.5
T.mariae	1	1(100)	1	1	1	1(100)	1	1	-	-	-	-
H.bimaculatus	1	0	0	0	10	2(20)	2	0.2	14	9(64.2)	24	1.7
T. zilli	76	21(27.6)	51	0.67	71	32(45)	115	1.6	-	-	-	-
Total	123	35(28.5)	109	0.9	116	48(41)	170	1.5	115	79(68.7)	322	2.8

Table 2. Distribution patterns of various parasite types among the cichlids in study areas.

Host	Reservoir St A(Unpoluted) No Ex	No Inf	Parasite Type	Taxa	Reservoir St B(Polluted) No Ex	No Inf	Parasite Type	Taxa	Agodi Fish Farm No Ex	No Inf	Parasite Type	Taxa
O. niloticus	0	-	-		3	0	-	-	87	61	Pl,Ct,Eh, Ac	Trem Acanth
S. melanotheron	20	8	Ag,Ns,Pl, Ct,Al,At	Trem Acanth	11	9	Ag,Ns,Pl Ct,Aa,Ac, At	Trem Acanth	-			
S. galilaeus	24	5	Ag,Ns,Eh, Ac,At.	Trem Acanth	20	2	Pl,Ac,At.	Trem Acanth	14	9	Ct	Trem
T. mariae	1	1	Ct	Trem	1	1	ct	Trem	-			
H. Bimaculatus	1	0	-	-	10	2	Pl,At.	Trem Acanth	*14	9	Ct,Ac.	Trem Acanth
T. zilli	76	21	Ag,Ns,Pl, Ct,Ac,At.	Trem Acanth	71	32	Ag,Ns,Pl, Ct,Eh,Aa Ac,At.	Trem Acanth	-			

Ag-*Allocreadium ghanensis* **H.fasciatus*
Ns-*Neascus*
Pl-*Phagicola longa*
Ac-*Acanthela*
Ct-*Clinostomum tilapiae*
At-*Acanthogyrus tilapiae*
Eh-*Euclinostomum heterostomum*
Aa-*Alloglossidium cortis*

Table 3. Relationship between infection rate of parasites and the sex of cichlids.

Parasites	*O.niloticus*		*S. melan*		*S.galilaeus*		*T.mariea*		**H.bimaculatus*		*T.zilli*	
	M	F	M	F	M	F	M	F	M	F	M	F
A.ghanensis	-	-	-	3	-	2	-	1	-	1	1	-
	-	-	-	1	-	-	1	-	-	-	2	1
	-	-	-	-	-	-	-	-	-	-	-	-
Neascus	-	-	1	-	-	1	-	-	-	-	4	1
	-	-	-	2	-	-	-	-	-	-	1	2
	-	-	-	-	-	-	-	-	-	-	-	-
P.longa	-	-	1	2	-	-	-	-	-	-	2	2
	-	-	2	3	1	-	-	-	-	-	5	10
	1	-	-	-	-	-	-	-	-	-	-	-
Acanthela	-	-	-	2	-	-	-	-	-	-	3	5
	-	-	2	3	1	-	-	-	-	-	8	2
	4	1	-	-	-	-	-	-	1	-	-	-
C.tilapiae	-	-	-	1	-	-	-	-	-	-	1	4
	-	-	1	-	-	-	1	-	-	-	4	2
	43	13	-	-	6	3	-	-	2	6	-	-
A.tilapiae	-	-	1	1	-	1	-	1	-	-	2	5
	-	-	1	3	1	-	-	-	-	-	6	2
	-	-	-	-	-	-	-	-	-	-	-	-
E. Heterostomum	-	-	-	-	1	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	1	-
	3	-	-	-	-	-	-	-	-	-	-	-
A.corti	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	1	-	-	-	-	-	-	-	2	-
	-	-	-	-	-	-	-	-	-	-	-	-

---- Station A; ---- Station B; ---- Station C *(or *H.fasciatus*)

Table 4: SIZE OF CICHLIDS IN RELATION TO INFECTION WITH PARASITES AT STATIONS A, B, AND C.

Group infected	Size(g)	Fish host	No.Examined	No infected	%	Mean No/host	Mean No/infected host
1	10-20.9	*S.melanotheron*	1	1	100	1	1
		S.galilaeus	1	0	0	0	0
		O.niloticus	-	-	-	-	-
		T.zilli	3	0	0	0	0
		H.fasciatus	1	0	0	0	0
		T.mariae	-	-	-	-	-
		H.bimaculatus	-	-	-	-	-
2	21-50.9	*T.mariae*	4	3	75	1.25	1.67
		H.bimaculatus	22	10	45.45	1.09	2.4
		S.melanotheron	7	3	42.86	1.71	4.0
		S.galilaeus	24	5	20.83	0.29	1.4
		O.niloticus	14	7	50	1.07	2.14
		T.zilli	-	-	-	-	-
		H.fasciatus	1	0	0	0	0
3	51-80.9	*S.melanotheron*	9	6	66.67	5.33	8
		S.galilaeus	24	3	12.5	1.5	2
		O.niloticus	37	27	72.97	3.05	4.19
		T.zilli	64	20	31.25	0.67	2.15
		H.fasciatus	9	4	44.44	1.22	2.75
4	81-110.9	*S.melanotheron*	15	6	40	2.06	5.16
		S.galilaeus	7	2	28.57	0.29	1
		O.niloticus	35	24	68.57	3.2	4.67
		T.zilli	51	25	49.01	1.62	3.32
		H.fasciatus	-	-	-	-	-
		T.mariae	1	1	100	1	1
5	111-140.9	*S.melanotheron*	1	0	0	0	0
		S.galilaeus	-	-	-	-	-
		O.niloticus	8	6	75	5	6.67
		T.zilli	3	0	0	0	0
6	141-170.9	*S.melanotheron*	-	-	-	-	-
		S.galilaeus	2	1	50	0.5	1
		O.niloticus	1	0	0	0	0
		T.zilli	1	1	100	10	10
7	171-200.9	*S.galilaeus*	2	0	0	0	0
		O.niloticus	1	0	0	0	0
8	201-230.9	*O.niloticus*	1	0	0	0	0
9	231-260.9	*S.melanotheron*	1	1	100	10	10
10	321-350.9	*T.zilli*	1	1	100	27	27
11	381-410.9	*T.mariea*	1	1	100	1	1
12	441-470.9	*O.niloticus*	1	0	0	0	0

Table 5: Distribution patterns of the parasite types among the various fish organs from all stations.

Station	A					B						C						
Organs	Gd	G	Int	Liv	BC	Gd	G	Int	Liv	M	BC	E	G	Int	Liv	Operc	M	BC
	P GM	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*	*P GM*
Allocreadium ghanensis	- -	- -	*4.8 1.7*	- -	- -	- -	- -	*4.3 1.6*	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
Neascus	- -	- -	*5.9 1.9*	- -	- -	- -	- -	*4.3 1.4*	- -	- -	- -	--	- -	- -	- -	- -	- -	- -
Phagicola longa	- -	- -	*5.7 1.7*	- -	- -	- -	- -	*17.2 1.6*	*1.71.4*	- -	- -	- -	- -	- -	*1.0 0.9*	- -	- -	- -
Acanthela	*0.8 1.0*	*10.6 1.7*	- -	- -	- -	- -	- -	*12.9 2.0*	- -	- -	- -	- -	- -	*5.2 1.8*	- -	- -	--	- -
Clinostomum tilapiae	*0.8 1.0*	*1.6 2.0*	- -	*1.6 2.0*	*2.4 1.0*	*0.9 1.0*	*3.5 1.4*	- -	*2.6 1.6*	- -	*0.9 3.0*	*0.91.0*	*33.0 1.6*	*0.9 1.0*	*5.2 1.2*	*12.2 1.7*	*5.2 1.8*	*43.5 2.3*
Acanthogyrus tilapiae	- -	- -	*8.9 1.9*	- -	- -	- -	- -	*0.86 1.0*	*12.1 1.6*	- -	- -	- -	- -	- -	- -	- -	--	- -
Euclinostomum heterostomum	- -	- -	*0.81 1.0*	- -	- -	- -	- -	*0.86 1.0*	- -	- -	- -	- -	- -	*2.6 9.2*	- -	- -	- -	- -
Alloglossidium corti	- -	- -	- -	- -	- -	- -	- -	*1.72 1.7*	- -	*0.9 21.0*	- -	- -	- -	- -	- -	- -	- -	- -
Total	1.6 2.0	1.6 2.0	24.4 2.2	1.6 2.0	2.4 1.0	0.9 1.0	3.5 1.7	36.2 2.2	4.3 1.5	0.9 21.0	0.9 3.0	0.9 1.0	33.0 1.6	8.7 2.8	6.1 1.2	12.2 1.7	5.2 1.8	43.5 2.3

Table 6: DISTRIBUTON PATTERN OF THE PARASITE TYPES AMONG THE VARIOUS FISH ORGANS FROM ALL THREE STATIONS

PARASITE	EYES		GONADS		GILLS		INTESTINE		LIVER		OPERCULUM		MOUTH		BODY CAVITY	
	Prev%	GMI	Prev%	GMI	Prev%	GMI	Prev%	GMI	Prev%	GMI	Prev%	GMI	Prev%	GMI	Prev%	GMI
Allocreadium ghanensis	-	-	-	-	-	-	3.11	1.65	-	-	-	-	-	-	-	-
Neascus	-	-	-	-	-	-	3.39	1.67	-	-	-	-	-	-	-	-
Phagicola longa	-	-	-	-	-	-	7.63	1.57	0.85	1.26	-	-	-	-	-	-
Acanthella	-	-	0.28	1.0	-	-	9.61	1.85	-	-	-	-	-	-	-	-
Clinostomum tilapiae	0.28	1.0	0.85	1.0	12.4	1.60	0.28	1.0	3.11	1.42	3.96	1.70	1.70	1.82	15.2	2.18
Acanthogyrus tilapiae	-	-	-	-	0.28	1.0	7.06	1.74	-	-	-	-	-	-	-	-
Euclinostomum heterostomum	-	-	-	-	-	-	1.41	3.78	-	-	-	-	-	-	-	-
Allogossidium corti	-	-	-	-	-	-	0.57	1.73	-	-	-	-	0.28	21.0	-	-
Overall parasitic load	0.28	1.0	1.13	1.0	12.4	1.62	23.2	2.27	3.96	1.39	3.96	1.70	1.98	2.58	15.2	2.18

Table 7: DISTRIBUTION PATTERN OF THE PARASITIC HELMINTHES AT VARIOUS SAMPLING PERIODS (FORTH NIGHTLY) FOR ALL THE STATIONS.

PARASITE	SAMPLING PERIODS						
	1	2	3	4	5	6	ENTIRE SAMPLE
	Prev% GMI	Prev% GMI	Prev% GMI	Prev% GMI	Prev% GMI	Prev% GMI	Prev% GMI
Allocreadium ghanensis	7.27 1.57	9.62 1.82	- -	- -	1.49 1.0	1.64 2.0	3.11 1.65
Neascus	5.46 2.0	1.92 10.0	1.67 1.0	1.70 1.0	8.96 1.35	- -	3.39 1.67
Phagicola longa	10.91 1.78	7.69 1.78	8.33 1.89	15.25 1.22	2.99 1.41	4.92 1.82	8.19 1.58
Acanthella	23.64 1.89	3.85 3.87	11.67 1.10	5.09 1.26	4.48 1.59	11.48 2.82	9.89 1.82
Clinostomum tilapiae	32.73 2.79	21.15 1.84	26.67 2.64	15.25 2.70	23.88 1.98	29.51 2.02	24.86 0.26
Acanthogyrus`tilapiae	16.36 2.13	3.85 1.41	6.67 2.63	8.48 1.25	8.96 1.26	- -	7.35 1.70
Euclinostomum heterostomum	- -	1.92 1.0	- -	- -	4.48 9.17	1.64 1.0	1.41 3.78
Alloglossidium corn	1.81 21.0	- -	1.67 1.0	- -	- -	1.64 3.0	0.85 3.98
Overall parasitic load	65.46 3.02	40.39 2.00	46.67 2.32	35.59 2.04	38.81 3.05	44.26 2.29	44.91 0.13

DISCUSSION

The Cichlids species (*Tilapia, Hemichromis and Sarotherodon*) harboured larval trematodes of the genera *Clinostomum, Euclinostomum, Allocreadium phagicola* and *Neascus*. Acanthocephalans and *Acanthella* were also harboured by these cichlids. The flukes proved to be more widespread than the observed Acanthocephalans. Huggins (2000) states that the most frequently observed parasites of fish are flukes. Paperna (1980) also noted that the two Clinostomatids, *C. tilapiae* and *E.heterostomum* recorded in this present study are widespread. The harbouring of both nematodes and acanthocephalans by cichlids in this study is in accordance with the reports by Awachie (1965) and Ukoli (1965) of cichlids of Lake Chad.

The fish farm, had the highest percentage of infection (62.60%) of *C.tilapiae* compared to the unpolluted station A (6.50%) and polluted station B (6.86%). There was no significant difference in the percentage of infection of *E.heterostomum* at the three sites. The percentage infection of *A . ghanensis* was not significantly different at stations A and B and was absent at the fish farm. *Neascus* had a greater percentage infection at station A than B and was not found at the fish farm. *Alloglossidium corti* was found only at station B. Acanthella was observed at all the stations. *Acanthogyrus tilapiae* was found in stations A and B but not at the fish farm.

The man-made pond, fish farm, had the highest percentage of infection of parasites, then followed by station B (polluted) and A (unpolluted). Most Acanthocephalans were found in the fish intestine except for a few number of Acanthella found in the gonad of fish hosts at station A. *A. tilapiae* were found in the gills of fish hosts at station B. This was also reported by Huggins (2000).The intestine had the highest parasitic load in stations A and B.

There was no relationship between the percentage of infection with sex of the cichlids at stations A and B but this relationship existed at the fish farm. Low level of infection in larger sizes of fishes in this study were also reported by Prah (1969) in a dam reservoir in Ghana.

The significant decrease in the percentage of infection at stations A and B from the month of April to June might be due to the fact that molluscan intermediate hosts of parasites might have been swept away by the tide as rainfall increases during the month of June. This was also reported by Ukoli, 1965; Hofman, 1967 and Schell, 1970.

The difference in infection rate and sites of infection in the fishes might be due to the diet. Fishes from stations A and B feed on detritus, benthos, plankton which transmit parasites while fishes at the fish farm are fed with artificial feeds. This could also explain why most of the parasitic helminths of fishes in the wild were found to be harboured in the gut while most of the domesticated tilapiae parasites were found to be more in the body cavity.

The parasites of fish ought to receive more attention and study especially parasites in fish farms where as shown in this study had the highest percentage of infection.

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