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Diets and Enteroparasitic Infestation of *Oreochromis Niloticus* (Linné, 1757) (Cichlidae) in Oba Reservoir Ogbomoso, Nigeria

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ABSTRACT

The study was carried out between November 2011 and October 2013, using 707 *Oreochromis niloticus* (Linné, 1757) specimens in a tropical reservoir (8° 3"to 8° 12"N and 4° 6"to 4° 12"E). Standard methods were used to determine food and feeding habit and enteroparasitic infestation of the fish in the reservoir. The fish species was found to be an omnivore, but primarily an herbivore and the feeding on food items of animal origin may be supplementary. The high presence and constancy of sand grains and detritus in the diet showed that the fish though pelagic, do forage regularly into the benthic zone to feed. Intestinal parasites recovered were two acanthocephalans - *Neoechinorhynchus rutili*, *Acanthocentius tilapiae* - and the metacercaria of *Clinestomum tilapiae*. Prevalence of parasitic infestation was higher in the dry season than the rainy season and also higher in females (23.40 %) than in males (19.15 %). The metacercariae were found in the buccal cavity while the two acanthocephalans were found in the intestine, nothing was found in the stomach. Generally, parasites intensity was higher in females than in males and parasitic infestation was found to have an effect on the body weight of *O. niloticus* in Oba reservoir.

Introduction

Fish production is very important in order to meet the proteinous needs of the fastly growing population of Nigeria; it is relatively inexpensive when compared with other sources of animal protein such as cattle, pig, and poultry, whose productions are very expensive due to low technology and poor pasture-lands [1]. In 2007, fish demand has been put at 2.66 million tons in Nigeria, while domestic production amounts to a paltry 620,000 metric tons, leaving a deficit of 2.04 million tons (83.2%); this led to the identification of aquaculture as the next viable solution in order to reduce importation of fish into Nigeria [2]. The word Tilapia formerly refers to three genera of Cichlids – *Tilapia, Sarotherodon, and Oreochromis* -. In 1972, the genus *Tilapia* was separated into two genera based on their reproductive behavior, they are the genus *Tilapia* (the species that brood their young or eggs on the substratum otherwise known as substrate brooders, substrate spawners or guarsers; they have few gill-rakers usually 12 or less on the lower part of the first gill arch) and the genus *Sarotherodon* which was at that time considered to consist of all the mouth breeding Tilapias, all of which have large number of gill-rakers (usually 13 or more on the lower part of first gill arch). In 1983, the genus *Sarotherodon* was again separated into two genera, *Sarotherodon* which includes all the paternal and biparental mouth brooders and *Oreochromis*, the maternal mouth brooders [3].

Several species of Tilapia are cultured commercially, but Nile tilapia (*Oreochromis niloticus*), is the predominant cultured species worldwide [4]. All tilapia are basically herbivores or detritivores, but when food becomes scarce they can feed on zooplanktons, aquatic insects, and crustaceans; as such they occupy an intermediate position between primary producers and piscivores [5]. Studies on natural feeding of fish could provide useful information on the trophic relationships in aquatic eco-systems [6], which could be used in formulating management strategy options in a multi species fishery. Data on different food items consumed by fish may eventually result in identification of stable food preference and in creation of trophic models as a tool to understand complex ecosystems [7], [8].

From an aquaculture perspective, fishes that are infested or infected with parasites are susceptible to stunted growth, low protein content and low reproductive abilities, since the parasites mostly feed on already digested food, blood, body fluids, and tissues [9]. The emanating need to culture fishes for protein consumption for the teeming rapidly growing populations in a developing country like Nigeria have made it necessary to intensify studies on the parasite fauna of the African freshwater fishes [10]. The importance of parasitic infection and infestation on fish production has largely remained an issue of concern to fish farming industry; and some parasites have been discovered to have zoonotic potential in mammalian host including man thereby making them of public health importance [11]. Fishborne zoonotic trematodes (FZT) are transmitted to humans as metacercariae that have encysted in fish [12]. Recent global health assessments have identified FZT as among the most important (and neglected) parasitic zoonotic diseases [12]. This study will use diet indices such as frequency of occurrence, numerical methods/percentage composition by numbers and stomach fullness as described by [13] and [14] to evaluate the stomach content; identify and quantify the resource that the species uses, and provide information on those selected from the choice available in the environment. It will also investigate enteroparasitic species diversity, prevalence, and intensity in the fish species.

Materials and Methods

Study area

The study area was Oba reservoir in Ogbomoso North local government area of Oyo state, Nigeria. Oba reservoir was impounded in 1964 and the tributaries are Idekun, Eeguno, Akanbi - Kemolowo, Omoogun, and Yakun streams.

Procedure for collection of fish specimens

Samples of the fish species (Oreochromis niloticus) were purchased monthly from catches of local fishermen. Fish

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specimens used for morphometric and parasitology studies were purchased from catches of local fishermen using traps, gill nets, and cast nets in the reservoir. The fish specimens used for food and feeding habits were purchased from fishermen using cast nets only, based on the submission of [15]. Also fish specimens used for enteroparasitological investigations could not be used for food and feeding habit studies because the stomach food contents would have been lost in the process of searching for the parasites in the stomach. Collection of fish specimens was done between 06:00 - 08:00 am. All fish specimens were still alive as at the time of purchase. Water from the reservoir was added to the samples at the point of collection before being transported to the laboratory in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria for further investigations.

Species and sex identifications

Identification was done using the characteristic of the family- possession of only a single pair of nostrils - and the alternating dark and light band on the caudal fin (tail) of *O. niloticus* [3]. The sexes were identified by examining the papillae; there are two orifices (openings) in the papillae of female and one in male [3]. The sexes were further confirmed after dissection with the presence of testes (in male) and ovaries (in female).

Immature and mature groups

Fish harvested were divided into two groups – immature and mature -. The minimum sizes of the observable matured gonad in male and female specimens were taken as the maturity sizes.

Seasonal studies

Rainy season was taken as the months of February to end of September and Dry season, between the months of October and January ending.

Morphometric study

Population of fish studied

A total of 308 specimens were studied during the two year period. The first year, November 2011 to October 2012 referred to as (2011/2012), 152 specimens; the second year, November 2012 to October 2013 referred to as (2012/2013), 156 specimen were investigated.

The Length – weight relationship (LWR)

The weight of each specimen was measured using a top loading Metler balance (model PN1200) to the nearest 0.1g after draining excess water with a pile of filter paper, while total length (TL) were measured in centimeter (cm) using a measuring board. Total length was used because no evidence of cannibalism was observed during the pre-data and data collection period.

Analysis

The relationships total length versus body weight for infested and uninfested fish specimens were done by linear regression, [16], and the parameter r^2 (correlation coefficient) were estimated to detect if they had good fit to the line of regression, using the Microsoft – Excel package. The means of the total lengths and weights of infested and uninfested samples for the sexes (male and female) in each year were compared for significance at 95% confidence interval, using independent student ‘t’ test (2-tailed). The analysis was done using SPSS 15.0 for Windows package.

Food and feeding habits

Population of fish studied

Two hundred and four (156 matured, 48 immature) fish specimens were used in 2011/2012, while 195, (160 matured, 35 immature) specimens were used in 2012/2013.

Dissection

The specimens were dissected, and the stomach removed and fixed in a mixture of 10% formaldehyde, glacial acetic acid and 5% ethanol in a ratio of 5:5:90, [17]. The different food items eaten by the fish were identified under the light microscope (at different magnifications as appropriate) by following the keys given by [18] and [19].

Analysis

Analysis was done using diet indices such as frequency of occurrence, numerical methods/percentage composition by numbers as described by [13] and [14] and stomach fullness as described by Williams, [20].

Enteroparasites

Examination for parasites

Examination of fish for parasites, handling and processing followed standard procedure by [21].The buccal cavity was washed with little amount of distilled water and a fine brush into a labelled test tube after which the body cavity was opened ventrally with the aid of scissors. The gut was separated and placed in a Petri dish, stretched out and cut into two regions i.e. the stomach and the intestine; each section was then placed in a separate labelled dish. The separated sections were opened longitudinally to expose the inner surface which was washed with little quantity of distilled water into labelled test tubes. Each labelled test tube containing the residue from the mouth, stomach, and intestine was then examined by placing a drop of the residue on the slide and observed under various magnifications of the light microscope for the parasites. This was repeated until the entire residue has been examined.

Identification of parasite

The recognition of the parasites was enhanced by their wriggling movement on emergence. Parasites found were counted, labelled with the serial number of the fish and placed in physiological saline water overnight to allow them stretch and relax; they were then fixed and stained for identification to species level. Fish specimens found with parasite were given separate serial numbers to differentiate them from those without parasites. Parasites retrieved were identified using information provided by [22], [23], [24].

Processing of recovered parasites

Cestodes and nematode parasites recovered were stained using the procedure of [25]. Fixative used was Formalin acetic acid (FAA). Cestodes were stained using Acetocarmine; Nematodes were stained with Horen’s trichome stain; while Acanthocephalans were stored in weak Erlich’s haematoxylin solution overnight and dehydrated cleared in methyl-salicylate and mounted on a slide in Canada balsam.

Statistical analysis

Infection and infestation of host by parasites were not normally distributed; as such, significant differences of parasitic infestation were tested using a non parametric (Npar.) statistical method, (Kolmogorov-Smirnov K-S test) at 95 % confidence interval using SPSS 15.0 for Windows package. Significant difference between the means of body weight; total length; of infested and uninfested fish specimens were carried out using the student t test (2-tailed) at 95 % level of confidence.

Results

Food and feeding habits

Frequency of occurrence and numerical methods/percentage composition by numbers of food found in the stomach of 204 O. niloticus in 2011/2012 and 195 in 2012/2013 were shown in Table 1. The matured were greater than 16.00 cm (female) and 15.20 cm (Male). Figure 1 showed the percentage composition by number of each food item in immature and matured fish for the two years combined. Analysis of the empty stomachs showed that in the rainy season of 2011/2012, 36.36 % of immature, 34.62 % of matured stomachs were empty, while in the dry season 44.44 %, 36.67 % respectively were empty. In 2012/2013 rainy season, 36.84 % of immature, 32.63 % of matured stomach were empty, while in the dry season 37.50 %, 35.39 % respectively were empty.

Enteroparasites

Different parasites recovered in the stomach and intestine of Oreochromis niloticus and their distribution

Intestinal parasites recovered were comprised of two Acanthocephalan (*Neoechinorhynchus rutili*, *Acanthocentius tilapiae*) and the metacercaria of a Trematode (*Clinestomum tilapiae*). The metacercariae were found in the buccal cavity while the two acanthocephalans were found in the intestine, nothing was found in the stomach.

Prevalence of parasitic infestation in Oreochromis niloticus in sex and seasons of each year

Prevalence of infestation in the two years was 19.15 % and 23.40 % for males and females respectively. The seasonal prevalence was 71.19 % and 28.81 % for dry and rainy seasons of 2011/2012 respectively and 82.19 % and 17.81 % for dry and rainy seasons of 2012/2013 respectively.

Intensities of the various parasites in relation to sex and seasons of the period of study.

Graphical representation of the intensities in relation to sex and seasons in the period of study is shown in Figure 2. A Kolmogorov-Smirnov Z test (p=0.05) between the means of parasitemia in rainy (52.97+9.27) and dry (175.00+25.82) seasons was significant.

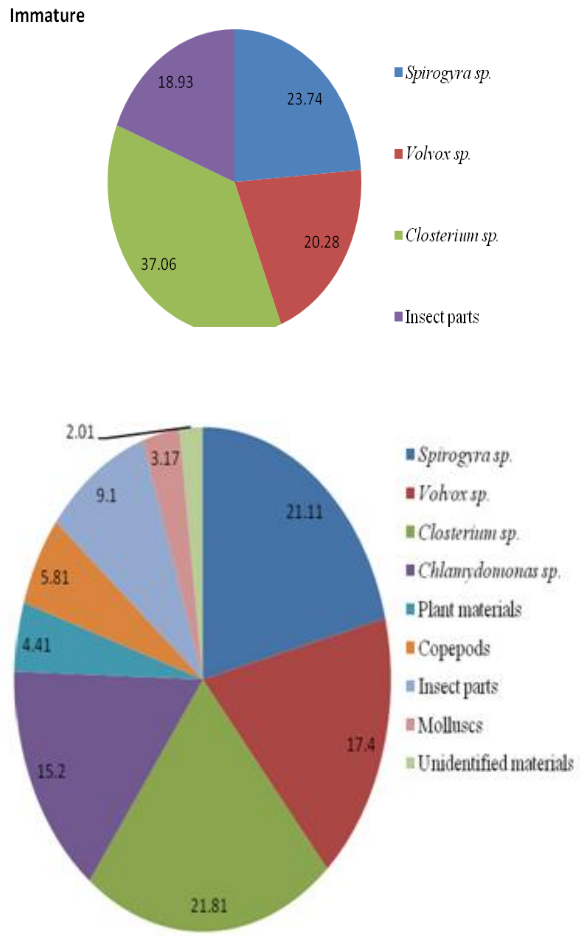

Figure 1. Food items in the stomach of immature and mature *Oreochromis niloticus* in Oba reservoir using percentage composition by number, during the study period.

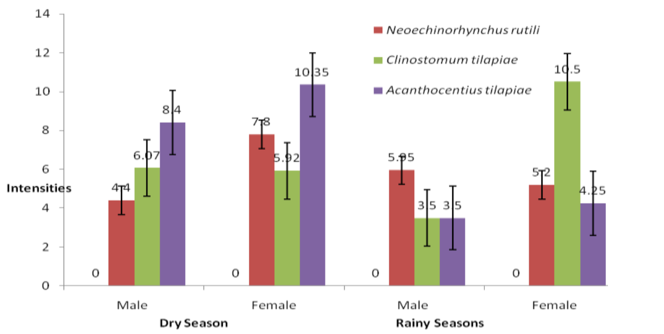

Figure 2. Intensities of Male and Female *Oreochromis niloticus* in Dry and rainy seasons of the period of study.

Relationship of parasitemia and body weight

In 2011/2012, the student t test of body weight of infested males (145.69+18.02 g) and uninfested (165.68+19.01 g) was not significant. In infested females (193.57+21.24 g) and uninfested (230.33+22.60 g) was not significant. In 2012/2013, the significant difference (at p =0.05) between the body weight of infested males (118.30+12.27 g) and uninfested (116.91+7.17 g) was not significant. Whereas in uninfested females (168.78+12.43 g) and infested (116.63+7.16 g) was significant.

Relationship of parasitemia and total length

The student t test at 95 % confidence interval between the total lengths of infested and uninfested male; total lengths of infested and uninfested female in the two years were not significant.

Influence of parasitemia on length – weight relationship

In uninfested male of 2011/2012, r^2 value was 0.970, infested 0.930; in uninfested female it was 0.932, in infested it was 0.926. In uninfested male of 2012/2013, r^2 value was 0.932, in infested it was 0.909; in uninfested female it was 0.926, in infested it was 0.713.

Discussion

The food content of 399 specimens of *O. niloticus* which included 83 immature and 316 matured specimens showed the diet of the matured fish was highly diversified and placed the fish species in Oba reservoir in the omnivore group. The adult diet included *Spirogyra sp.*, *Volvox sp.*, *Closterium sp.*, *Chlamydomonas sp.*, and plant materials as food from plant origin; Copepods, insect parts and Molluscs as food of animal origin; detritus and sand grains from the substratum. In the immature fish specimen's diets *Chlamydomonas sp.*, and plant materials were absent from the plant source while Copepods and Molluscs were absent from the animal source; both detritus and sand grains were very conspicuous. The high presence of sand grains and detritus showed that *O. niloticus* though a pelagic fish species, do forage into the benthic zone to feed, (Table 1). This result is in agreement with the observation of [15] that worked on *O. niloticus* in Ero reservoir, Nigeria and submitted that it was an omnivorous species and high percentage of mud and detritus in the stomach suggested the species are bottom grazers.

The frequency of occurrence and percentage composition by number of food items of plant origin was higher in both immature and mature specimens (Table 1, Figure 1), an indication that the fish species was primarily an herbivore and the feeding on food of animal origin may be supplementary. [26] Observed that the presence of tiny unicuspid teeth in the mouth of the fish suggests that the fish species feed on plants, leaves, buds, and seed of water lilies and are thus herbivorous feeders. Empty stomachs were more in the dry season than the rainy seasons; this may be attributed to the fact that food was more abundant in the rainy season.

Table 1. Frequency of occurrence of seasonal food contents in the stomach of *Oreochromis niloticus* in Oba reservoir

2011/2012						2012/2013					
	Rainy Season		**Dry Season**			**Rainy Season**		**Dry Season**			
Food items IMMARTURE	Frequency	%	Frequency	%	2011/2012	Frequency	%	Frequency	%	2012/2013	The two years
Spirogyra sp.	13	92.86	7	70	83.33	9	75.00	7	70	72.73	78.26
Closterium sp.	14	100	10	100	100.0	11	91.67	10	100	95.46	97.83
Volvox sp.	11	78.57	9	90	83.33	11	91.67	6	60	77.27	80.44
Insect parts	11	78.57	7	70	75.00	8	66.67	8	80	72.73	73.91
Sand grains	10	71.43	9	90	79.17	9	75.00	6	60	68.18	73.91
Detritus	11	78.57	9	90	83.33	11	91.67	8	80	86.36	84.78
Total	SWF =14		SWF =10		SWF =24	SWF =12		SWF =10		SWF =22	SWF =46
MATURE											
Spirogyra sp	61	89.71	33	86.84	88.68	61	95.31	30	71.43	85.85	87.26
Volvox sp.	30	44.12	20	53.63	47.17	19	29.69	8	19.08	25.47	36.32
Closterium sp.	57	83.82	35	92.11	86.79	64	100	38	90.48	96.23	91.51
Chlamydomonas sp.	53	77.94	29	76.32	77.36	47	73.44	34	80.95	76.42	76.89
Plant materials	29	42.65	8	21.05	34.91	15	23.44	8	19.08	21.70	28.30
Detritus	27	39.71	24	63.16	48.11	59	92.19	37	88.10	90.57	69.34
Unidentified materials.	21	30.88	6	15.79	25.47	6	9.38	3	7.14	8.49	16.98
Copepods	27	39.71	12	31.58	36.79	10	65.63	7	16.67	16.04	26.42
Insect parts	39	57.35	11	28.95	47.17	31	73.81	13	59.09	41.51	44.34
Molluscs	7	10.29	5	13.16	11.32	10	15.63	8	19.08	16.98	14.15
Sand grains	40	58.82	23	60.53	59.43	42	65.63	22	52.38	60.38	59.91
Total	SWF =68		SWF =38		SWF =106	SWF =64		SWF =42		SWF =106	SWF =212

Freq. of occ./FOO = Frequency of occurrence; SWF = Stomach with food.

Generally, specimens harvested with food in their stomachs were higher numerically than those with empty stomachs which indicated that food was in abundance in the reservoir throughout the period of study. [27] reported that Nile tilapia originally known to be herbivorous; feeding mostly on algae has diversified its diet to include insects, fish, algae, and plant materials. This choice of diet by the fish species may likely reduce the parasite species diversity and parasitic load that could infest and/or infect the fish species in its habitat. [28] were of the opinion that predatory fish species harbour a greater diversity and abundance of larval helminths than herbivorous and planktivorous species.

The result showed that two Acanthocephalan (*Neoechinorhynchus rutili*, *Acanthocentius tilapiae*) and the metacercaria of a Trematode (*Clinestomum tilapiae*) were found in the enteron of *O. niloticus*. No parasite was able to colonize the stomach of the fish in Oba reservoir, maybe as a result of the acidic content of the stomach of the fish species and/or none availability of nutrients required by the parasites. This result is in accordance with the findings of [29] who reported the presence of the cyst of the metacercariae of C. tilapiae in the skin and pharyngeal region of *O. niloticus* collected in Ibadan, Nigeria [30] reported the presence of the Nematodes (*Procamallanus laevionchus*, *Paracamallanus cyathopharynx*), the Cestode (*Polyonchobothrium sp*.) and the Acanthocephalan, *Acanthocentius tilapiae* in the hybrid and monosex of *O. niloticus* from Egypt. The prevalence of parasitic infestation in *O. niloticus* was higher in females (23.40 %) than in males (19.15 %). This may be due to the physiological state of the female; most gravid females could have reduced resistance to infection and infestation by parasites. This result agreed with [31] who reported higher parasitic infection in female than male. The buccal cavities were infested by *C. tilapiae* only and generally in the two years, the intensity was higher in females than males. This result was in agreement with the findings of [32] who reported a higher intensity of *C. tilapiae* in female *O. niloticus* than male. This trend of higher parasitic intensities in females than males was also observed of *N. rutili* in *O. niloticus*, while intensity of *A. tilapiae* on the other hand was marginally higher in males than in females (Figure 2). Seasonally, prevalence of parasitic infestation in *O. niloticus* was higher in the dry seasons of the two years than the rainy seasons. [33] ascribed the depauperate nature of helminthes in their study of helminthes parasite in *S. galilaeus* and *T. zilli* to the flow of water; [34] were also of the opinion that high water flow of the collecting site was the main factor determining the depauperate helminthes assemblages in fish farm from Mexico. Moreover the molluscan intermediate hosts of parasites might have been swept away by the tide as rainfall increases [31]. However, in this study the high prevalence in the dry season than the rainy season may be due to eutrophication in the rainy season. Eutrophication, normally leads to algae bloom towards the peak of rainy season, which advertently results in an increase in species variety and population of the parasite's intermediate host. This may result in the infestation/infection of fishes that fed on them in the rainy season, thus probably bringing the maturity of the parasites in the fish towards the dry season, depending on the life cycle of individual parasite. Eutrophication often raises parasitism because the associated increase in productivity will increase the abundance of the parasite's invertebrate intermediate hosts, which are mostly fresh water crustaceans, [35]. Another factor may be a drop in water level in the dry season exposing the invertebrates to their fish predators. Piscivorous birds are the definitive hosts for many of the metacercariae found in fish, and bird migration is the greatest contributing factor for dispersal of metacercarial infection [36]. Generally, *N. rutili* and *A. tilapiae* infesting the intestine of *O. niloticus* in Oba reservoir had a higher intensity in the dry season than the rainy season, while *C. tilapiae* infesting the buccal cavity was higher in the rainy season than the dry season.

The knowledge of the life cycle of *C. tilapiae* explains why this is so. The parasite uses two intermediate hosts (fresh water snails and Tilapia) before getting to the final definitive host [32].

The final host of the parasite is piscivorous birds like Herons and Egrets [33]. The eggs of the parasites are passed out with the faeces of the bird into the water when the birds come to the water body to feed on the fish mostly in the dry season when water level is low. The eggs hatch in the water and the miracidium larvae that emerge invade the foot of the snail and encyst in the connective tissues of the freshwater species forming the metacercaria. In the rainy season, when food is abundant the snails forage for food and in the process is consumed by the fish and the fish gets infested; the more infected snail eaten, the more the parasitic load on the fish. The cyst formed in the fish may die off if the life cycle is not completed by predation on the fish by birds, the final host [29]. The fish gets caught by the birds mostly in the dry season when the water level is low and the birds come to the water body to feed. This may reduce the quantity of infested fish in the water body hence low prevalence. Parasitemia was observed not to have any effect on the total length of the fish host.

The influence of parasitic infestation on body weight of *O. niloticus* though not significant, because the student t tests for significance (p=0.05) between the means of the body weight of infested males and uninfested males of 2011/2012, 2012/2013, and infested females and uninfested females of 2011/2012 were not significant. This result was in consonance with the result of [33] who also reported that there was no relationship between parasitic burden and size of fish in their study of helminthes parasites of *S. galila*eus and *T. zilli* from Oshun River, South-West Nigeria. However, in 2012/2013 the influence was observed to be significant. This influence on the body weight was further confirmed from the regression coefficient (r^2) values obtained for infested specimens of both sex in the two years which was lower than the values obtained for uninfested, with the lowest value of 0.713 obtained for infested females of 2012/2013. This showed that the infestation of *O. niloticus* by parasites in Oba reservoir affected the weight of the fish and the effect was more pronounced in the female fish.

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