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Multiple infections of Helminths in the alimentary system of *Clarias gariepinus* (Burchell, 1822) in a tropical reservoir

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The study was carried out in Erinle Reservoir accessed through Oore (Latitude 7° 58' 12" North; Longitude 4º 33' 36" East), in Osun State, Nigeria, between August 2011 and November 2013. A total of 103 live fishes purchased from local fishermen were examined. In the laboratory, morphometric parameters were measured, the sexes determined and the fish species *Clarias gariepinus* were dissected to separate the alimentary system. Parasites recovered were identified and counted and the data were subjected to statistical analyses. The parasites recovered were *Procamallanus laevionchus, Paracamallanus cyathopharynx* (Nematodes.), *Anomotaenia* sp., *Monobothrium* sp., *Polyonchobothrium clariae*. (Cestodes) and *Neoechinorynchus rutili* (Acanthocephalan). Using a non parametric (NPar) (Kolmogorov-Smirnov; K-S) test, infection was significant at p = 0.05 within seasons, and female fishes were more infected than male. *Monobothrium* sp. had the highest range of infection (0 to 44) and intensity (18.5±2.65) while *Anomotaenia* sp. had the least (0 to 1) and (1.00±0.02) respectively. Six parasites were found in the intestine, while four were in the stomach. There was high prevalence in medium and large sized fish and a direct linear relationship exist between length and intensity. The body weight was significant in relation to infection at p = 0.05 (K-S test) and was also significant in sex. Multiple infections were common, which showed a positive correlation between most of the parasites except *Anomotaenia* sp. which showed negative correlation with *P. clariae.*

Key words: *Clarias gariepinus,* morphometric, parasite, prevalence.

INTRODUCTION

In many parts of the world, studies of fish parasites and diseases are very advanced, resulting in the accumulation of an enormous amount of literature and information. Studies in Africa vary considerably from area to area and the parasites are mostly mentioned, as part of the fulfillment of the biology of the host fish species. Paperna (1996) published a concise update of the parasitic diseases of fish in Africa, which described the

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concurrence and geographical distribution, life cycles, pathology, epizootiology and control of the parasites. Khalil and Polling (1997) also produced a check list of the Helminthes parasites of African freshwater fishes. Numerous parasites were associated with *Tilapia zilli, Clarias anguillaris* and *Clarias gariepinus* during the study of the biology of the fish species in their natural environments (Eyo and Olatunde, 2001; Ovie and Ovie, 2002; Olofintoye, 2006).

From an aquaculture perspective, it is noteworthy that disease and parasite infestation have been poorly studied in Africa due to the low level intensity of aquaculture in the region. At present, the paucity of research in fish diseases in Africa is not seen as a factor that will negatively impact on aquaculture development and as such is not a target research area. The emanating need to culture fishes for protein consumption for the teeming rapidly growing populations in the developing countries like Nigeria have made it necessary to intensify studies on the parasite fauna of the African freshwater fishes (Akinsanya et al., 2007).

Fish parasites are important because they affect fish production particularly under culture systems, by decreasing their yield, aesthetic value, marketability, palatability and reproductive potential (Ibrahim et al., 2001; Oniye et al., 2004), and if left un-curtailed, may lead to mass mortality of fish, or in some cases, emergence of zoonotic species. They are therefore studied with a view to understanding their population biology and elucidate their life cycles in order to develop an efficient approach of controlling them (Olufemi, 2008).

The occurrences of Helminth parasites in fishes have been studied in various water bodies in Nigeria, with most of the work done primarily from the morphologic and morphometric descriptions, but factors that may limit the ability of parasites to co-exist in multiple infections in a host fish species had in most studies been neglected. This study is focused on the level of co-existence/concurrent infection between identified parasites in *C. gariepinus*, the factors that might limit this relationship and the effects it has on *C. gariepinus*.

MATERIALS AND METHODS

The study area was Erinle Reservoir accessed through Oore (Latitude 7° 58' 12" North; Longitude 4° 33' 36" East), in Odo-otin local government area of Osun State, Nigeria. Erinle Reservoir was impounded in 1958 and expanded in 1980. The tributaries are Oyan, Otin, and Erinle streams, in Osun State Nigeria. Two seasons are recognized in the study area - dry season from October to March and rainy season from April to September each year.

A total of 103 fishes were examined between August 2011 and November 2013. The fishes were purchased from fishermen, transferred into a plastic container with water and transported to the research laboratory. Transportation was done in the morning to avoid undue stress due to temperature rise. Dead fishes were removed from the collections and examined immediately while the live ones were preserved in a plastic aquaria containing water from the reservoir, and examined subsequently as the investigation progresses. The fish was killed by cervical dislocation. The standard / body length (from the tip of the snout to the end of the caudal peduncle) were measured in centimeters with the aid of a measuring board and recorded for each fish specimen. The weight for each fish was obtained by using a Metler balance. Sexes were determined, the male possess a distinct sexual papilla that is conspicuously located behind the anus, the sexual papillae are absent in females (Akinsanya and Otubanjo, 2006).

Dissection

The body of each fish was examined for abnormalities (if any), and placed on a dissecting board. The body cavity was opened with the aid of scissors and the mesentery and connective tissues, connecting loops of the gut and the liver were cut and the organs separated. The gut was then placed in a large Petri dish, stretched out and cut into two regions, that is, the stomach and the intestine. Each section was then placed in a separate labeled dish. The separated gut sections were opened by longitudinal incision to expose the inner surface which was washed with very little quantity of distilled water into labeled test tubes. A drop of the residue was placed on the slide, and observed under ×10 and ×40 objectives of the light microscope for the various parasites, a process that was repeated until the entire residue has been examined. Most of the parasites were easily identified by their wriggling movements, parasites found were counted, labeled with the serial number of the fish and placed in physiological saline overnight to allow them stretch and relax.

Cestodes, Nematode and Acanthocephalan parasites recovered were stained using the procedure of Khalil (1991). Identification of specimen to species level were undertaken and confirmed with the assistance of Oniye et al. (2004) and Akinsanya and Otubanjo (2006) who had earlier confirmed the identity of the parasites through the assistance of the British Museum (Natural history), United Kingdom. The terms prevalence and mean intensity were applied as defined by Margolis et al. (1982), while range refers to zero to maximum number of endoparasites retrieved from a fish host.

Statistical analysis

Infection of host by parasites was not normally distributed; significance of parasitic infection was tested using a non parametric (Npar.) statistical method, (Two independent sample Kolmogorov-Smirnov K-S test at p=0.05) and presented pictographically using SPSS version 10.

Pearson correlation analyses was carried out using the computer-based SPSS version 10 to determine if a pair of parasites in multiple and concurrent infection within hosts were linearly correlated and significant (p = 0.01).

RESULTS

Parasites recovered

The gastro-intestinal helminth parasites recovered comprised two nematodes *(Procamallanus laevionchus, Paracamallanus cyathopharynx.*), three cestodes

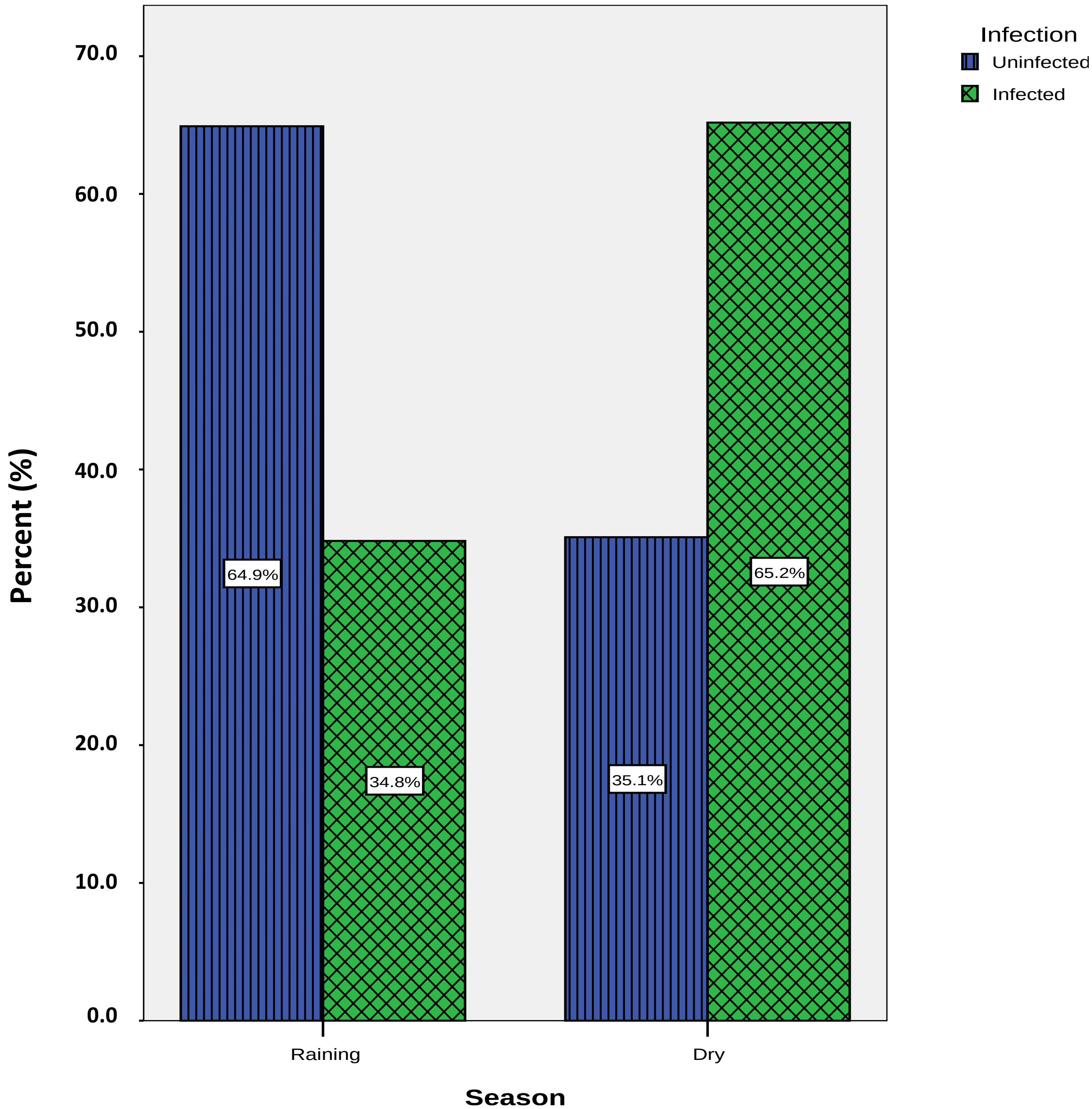

Figure 1. Differences in infection between rainy and dry seasons.

(*Anomotaenia* sp., *Monobothrium* sp., *Polyonchobothrium clariae*.) and one acanthocephalan (*Neoechinorynchus rutili*).

Prevalence and intensity of parasites in *C. gariepinus*

A total of 103 fishes were examined out of which 55 were infected giving 53.4% prevalence. There was no significant difference in the gastro-intestinal Helminth infection while Helminth intensity in the intestine was higher than in the stomach.

Seasonal distribution of parasites

There was a significant difference (p = 0.05; K-S test), in infected *C. gariepinus* over seasons as more fishes were infected in the dry season than the rainy season (Figure 1).

Distribution of parasites

Monobothrium sp. had the highest range of infection, with a maximum abundance of 44 in a single host specimen

Table 1. Prevalence, range and intensity of parasites in the stomach and intestine of *Clarias gariepinus*.

Parasite	Prevalence %		Range of parasites		Nos. of parasites		Intensity	
	STO	INT	STO	INT	STO	INT	STO	INT
Anomotaenia sp.	1.94	4.85	0 - 1	0 - 1	2	5	1.00±0.02	1.00±0.02
Neoechinorynchus rutili	0.00	12.62	0	0 - 4	0	25	0.00	1.92±0.07
Procamallanus laevionchus	0.00	8.74	0	0 - 3	0	14	0.00	1.56±0.05
Polyonchobothrium clariae	2.91	39.81	0 - 12	0 - 12	20	215	6.67±1.06	5.24±0.40
Paracamallanus cyathopharynx	16.50	38.83	0 - 12	0 - 20	91	434	5.35±1.04	10.85±1.45
Monobothrium sp.	36.89	41.75	0 - 20	0 - 44	406	796	10.68±1.35	18.5±2.65

STO = Stomach; INT = Intestine.

and an intensity of 18.5±2.65 in the sampled specimens. *Anomotaenia* sp. had the least range (0 to 1) and an intensity of 1.00±0.02 (Table 1).

Six parasites were found in the intestine, while only four were in the stomach. *P. laevionchus* and *N. rutili*, were absent in the stomach (Table 1).

Although *N. rutili* was not found in the stomach, its presence in the intestine varied with season and sex. Intensity of infection in dry season (0.263± 0.055) was higher than rainy season (mean 0.038±0.053), and female fishes (0.381±0.071) were more infected than male (0.220±0.079). Infection of the intestine by *P. laevionchus* was higher in the rainy season (0.334±0.043) than dry season (0.048±0.045). *P. clariae* infected both the stomach and intestine generally, but the infection was not affected by season. *Monobothrium* sp. has the highest occurrence, both in stomach and intestine; its distribution was normal in both seasons and sex.

Generally, the prevalence and intensity of parasitic infection in the stomach and intestine showed significant difference (p = 0.05; K-S test.) with a reduced level in the stomach. There was a significant difference between infected males and females (p = 0.05; K-S test). In all infected specimens examined, 43.8% were males while 56.2% were females.

Relationship between body length and degree of infection

There was high prevalence in medium (20 to 29.9 cm) and large (30 to 39.9 cm) sized *C. gariepinus* and a direct linear relationship between length and intensity (Figure 2). Most of the infected fish specimens had longer body lengths than uninfected specimens (the mean body lengths for uninfected and infected fishes were 20.14 cm ±0.40 and 24.38 cm ±0.40 respectively within sex; male uninfected 19.82 cm ±0.57, infected 24.01 cm ±0.63; female uninfected 20.45 cm ±0.67, infected 24.76 cm ±0.57). Also larger size fishes were mostly infected in the rainy (22.98 cm ±0.40) than the dry season (21.54 cm ±0.40).

Relationship between body weight (g), sex and degree of infection

Figure 3 showed that majority of sampled specimens infected with helminthes were within the same weight groups, (30 to 70 g). Infection of *C. gariepinus* by parasites results in reduction of weight of the fish and generally females have more weight than male in the sampled specimens. Within the same total length range (20 to 39.9 cm), the mean weight of uninfected (66.48 g ±1.97) samples was considerably higher than infected (50.70 g ±1.89). The female (uninfected, 70.04 g ±3.01; infected, 53.31 g ±2.53) samples were found to have more weight than their male (uninfected, 62.92 g ±2.55; infected, 48.08 g ±2.82) counterpart; the general mean weight was 64.40g ±2.07.

Concurrent/multiple parasitic infection

Multiple infection of host by the identified parasites was a common occurrence. Table 2 showed that ability to successfully co-inhabit with one another in the same host was positively correlated between most of the parasites. Although, *N. rutili* and *P. laeviochus* could not co-inhabit with one another, *Anomotaenia* sp. will only appear concurrently with only *P. laevionchus.*

DISCUSSION

Prevalence and intensities of parasites in the gut of *C. gariepinus*

Prevalence of Helminthes was higher in the intestine than the stomach, similar to the findings by Mohammed et al. (2009) and Bichi and Yelwa (2010). They argued that

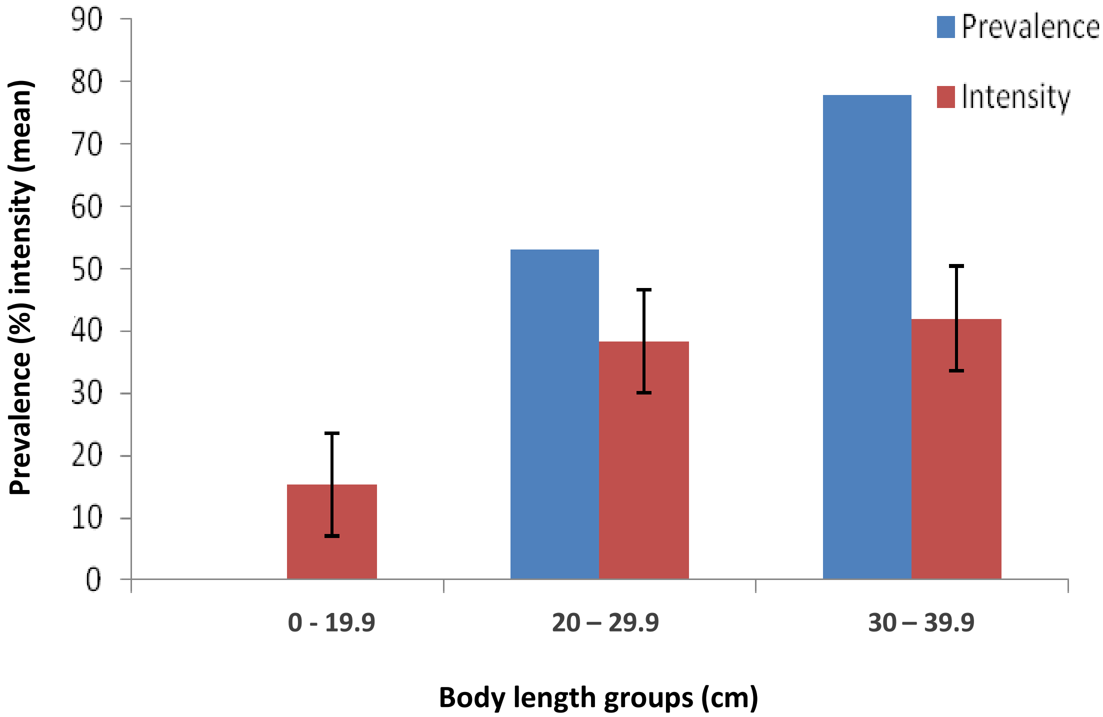

Figure 2. Relationship between body length, prevalence and intensity.

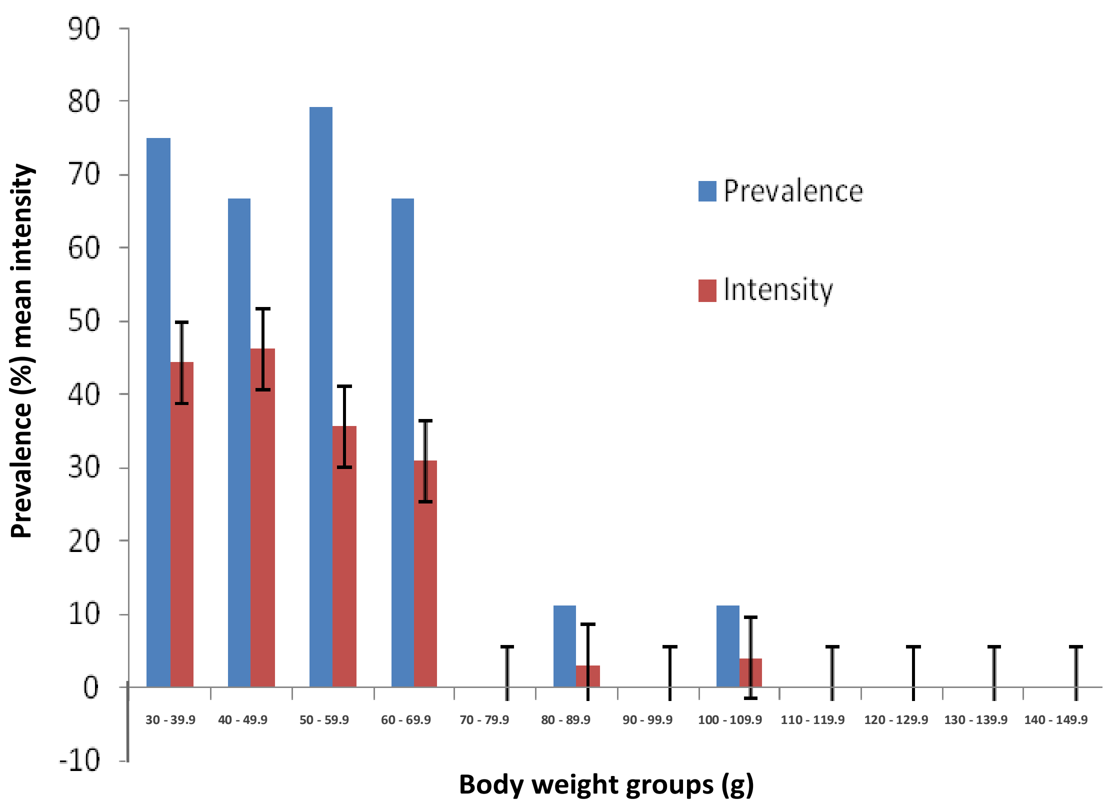

Figure 3. Relationship between body weight, prevalence and mean intensity.

Table 2. Simulation matrix for correlation between multiple infections by parasites in the stomach and intestine of *Clarias gariepinus* in Erinle Reservoir.

Parasite	*Anomotaenia* sp.	*N. rutili*	*P. laevionchus*	*P. clariae*	*P. cyathopharynx*
Neoechorynchus rutili	0.142				
Procamallanus laevionchus	0.256**	0.051			
Polyonchobothrium clariae	-0.006	0.361**	0.234**		
Paracamallanus cyathopharynx	0.005	0.354**	0.304**	0.726**	
Monobothrium sp.	0.025	0.429**	0.266**	0.726**	0.819**

Pearson correlation;** Correlation is significant at the 0.01 level (2-tailed).

regional localization in the gut can be attributed to several factors, such as hydrogen ion concentration, chemotactic response as well as food reserve. Onwuliri and Mgbemena (1989) reported that Helminthes differ in their nutritional and respiratory requirements which may influence their choice of habitat. However, the major factor that may have contributed to high parasitic prevalence in the intestine than in the stomach was that most parasites found in the reservoir were Cestodes that lacked digestive system. Obligatorily, they had to depend on the digested food in the intestine of their host, which they absorbed through their thin body tegument (that is syncytial and highly absorptive). These parasites may find an acidic medium as presented by the stomach not conducive.

The high prevalence is further supported by the findings from other studies that reported a high prevalence in the wild population of *C. gariepinus* (Anosike et al., 1992; Oniye et al., 2004; Olofintoye, 2006; Dankishiya and Zakari, 2007; Olufemi, 2008; Ajala and Fawole, 2012). The varying percentages reported from the West, through the Middle Belt to the far Northern part of Nigeria showed a pattern of decreasing prevalence from Western to Northern Nigeria. This may be attributed to a reduction in the parasite's intermediate host species diversity which may be dependent on types of forest/vegetation available in each geographical ecosystem. Western Nigeria is populated by tropical rain forest, the Middle Belt by savanna woodland/ Sudan savanna and Northern Nigeria by Sahel/dry savanna. Parasite intensity was also higher in the intestine than the stomach for majority of the parasites except Anomotaenia sp, which further confirmed the likely preference of the intestine by the parasites to the stomach.

Parasitic infection in relation to sex

There was a significant difference (p = 0.05) in the infection of male and female in the specimens examined. Emere (2000) reported differences in the incidence of infection between male and female fish, and argued that this may be due to differential feeding, either by quantity or quality of food eaten or as a result of different degrees of resistance and infection. The female samples had more weight than their male counterpart, which supported the opinion of differential feeding, either by quantity or quality of food eaten which predisposes the females to infection. Emere and Egbe (2006) reported that due to physiological state of the female, most gravid females could have reduced resistance to infection by parasites.

Effects of seasonal variation on parasitic infection

The prevalence and intensity of infection was higher in the dry than the rainy season. The factor responsible for this is eutrophication, which often raises parasitism because the associated increase in productivity will increase the abundance of the invertebrate intermediate hosts, mostly fresh water crustaceans (Lafferty and Kuris, 1999). Eutrophication leads to algal bloom at the peak of rainy season which results in 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 fed 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 parasite. Another factor may be a drop in water level in the dry season exposing the invertebrates to their fish predators. Fawole and Akinsanya (2000) reported a similar prevalence of infection in *Sarotherodon galilaeus* by plerocercoid larvae of pseudophyllidean cestode in Opa Reservoir in Ile-Ife, Nigeria, and higher prevalence of infection was recorded in the dry than the rainy season.

Degree of infection of the gut of *C. gariepinus* by parasitic nematodes

Moravec (1974, 1975) studied the life cycles of *P. laevionchus* and *P. cyathopharynx* and obtained a development of the first three larval stages in *Mesocyclops leuckarti*. It was observed that the *callanidae* give birth to the first stage larvae, which are ingested by the copepods. The larvae of *P. laevionchus* reached the third stage after two moults in eight to nine days at temperatures of between 23 and 24°C. *P. cyathopharynx* is an ovoviviparous camallanid nematode whose larvae are liberated into the gut of the host and passed out with the faeces. The first moult of the parasite takes place in the Copepod intermediate host and the last two moults in the fish. It is speculated that the utilization of the copepods as food by the catfishes is responsible for their infection by the nematodes. Although Oniye et al. (2004) did not report the presence of *P. cyathopharynx*, the occurrence of *P. laevionchus* at a very low prevalence (0.83%) in the intestine was reported.

The intensity of *P. cyathopharynx* found in this study was comparable to that obtained by Ayanda (2009) who reported a similar occurrence in *C. gariepinus*. Although both parasites are nematodes, the absence of *P. laevionchus* in the stomach may be adduced to differences in physiological tolerance to the acidic medium prevalent in the stomach. Even though P. *cyathopharynx* was found both in the stomach and intestine, they preferred the intestine as portrayed by higher intensity and range displayed. Williams and Jones (1994) suggested that parasitism varied from one aquatic ecosystem to the other and this was influenced by the interplay of mixed biotic and abiotic factors. Akinsanya and Otubanjo (2006) also reported the presence of *P. cyathopharynx* in the intestine of *C. gariepinus* in the Lagos Lagoon; this suggested that *P. cyathopharynx* may also possess physiological adaptability/tolerance to salinity fluctuations.

P. laevionchus intensity was higher in the rainy than dry season, which may be deduced from the life cycle of the parasite as argued by Moravec (1975) - that the larvae reached the third stage after two moults in eight to nine days at temperatures of between 23 and 24°C in the Copepod intermediate host. This temperature range fell within the rainy season so that within nine days of reaching infective stage, fish host feeding on the copepods become infected. Dry season of higher temperature, may not support the development of the parasite larval stages in the invertebrate host, hence its low prevalence in the dry season.

Degree of infection by parasitic cestodes

Cestodes formed the bulk of the parasites recovered; Singh et al. (2013) also reported cestodes as having maximum prevalence in *C. batrachus* and *C. gariepinus.* Anatomically, cestodes lack digestive system and obligatorily have to depend on the digested food of their host, which they absorb through their body surfaces, hence they are better found in the part of the host intestine where their nutritional requirements can be met. Host body secretions and immune system may not be a threat as Buchmann and Lindenstrom (2002) suggested that parasites have an in-built molecular disguise to avoid the host hostile secretions present in its microhabitat.

P. clariae infection showed a higher prevalence in the intestine than the stomach but the intensity was a reversal. Although prevalence was low in the stomach, the intensity was high, which suggested *P. clariae'*s preference for both stomach and intestine. This observation was also reported by Oniye et al. (2004) and Barson and Avenant-Oldewage (2006). The high intensity in the stomach may be due to the anatomical structure of the stomach of *C. gariepinus,* which had a depression (at its exit to the intestine-pyloric region) in which already digested food from the stomach remain for some time before proceeding to the intestine (Oniye, Personal communication). The acidic level at this portion of the stomach may have become diluted to a value midway between stomach and intestinal pH. Here, food is not absorbed by host thus reducing the competition between parasite species residing in the intestine and host, thus giving the foraging cestodes from the intestine an ample opportunity to feed.

Bichi and Yelwa (2010) also reported *Anomotaenia* sp. and an unidentified species of *Procamallanus* in *C. gariepinus* as dominating the stomach only. *Anomotaenia* sp. a cestode which was expected to be prominent in the intestine than the stomach, as a result of their nutritional requirement (already digested food) seemed to be an exception.

Monobothrium sp's presence in stomach and intestine suggested an ability for a wide physiological adaptation and probably resistance to host defense system. This gave the parasite an opportunity for high prevalence and intensity. The difference in infection between the stomach and intestine could be attributed to high acidity of the stomach since the most resistant parasites occur comfortably there (Alfred-Ockiya, 1985).

Degree of infection by parasitic acanthocephalan

N. rutili was found in the intestine of the fish and its prevalence was low. It was not recovered in the stomach which signified the possibility of its not being able to survive in an acidic medium. This supported Oniye et al. (2004), Olofintoye (2006) and Ayanda (2009) who reported very low prevalence of the worm in the intestine of its host; while Nnadi (2012) reported the presence of its eggs in the intestine.

Body length in relation to parasitic infection and season

Infection was directly proportional to body length, bigger fish specimens were recovered in the rainy season which predisposes them to infection. Oniye et al. (2004), Olofintoye (2006) and Ayanda (2009) reported that prevalence of intestinal Helminth infection in *C. gariepinus* increases with their standard length. A plausible explanation for this may be the change in diet from weeds, seeds, phytoplankton and zooplankton as juveniles to insect larvae, crustaceans, worms and fish at sub-adult and adult stages. These invertebrates may serve as intermediate hosts to some of these parasites, while eutrophication which is seasonal will boost productivity in the rainy season, thereby increasing the population of those invertebrate organisms that served as food for the sub-adult and adult stages.

Body weight in relation to parasitic infection, season and sex

The mean weight of uninfected samples was considerably higher than infected which suggested a weight loss as a result of infection. This was evidenced in Bichi and Yelwa (2010) who reported the damage inflicted by infection of two different species of *Polyonchobothrium* (*Polyonchobothrium polypetri* and *Polyonchobothrium* sp.) in the gut of *C. gariepinus.* They were found blocking and attached firmly to the intestinal lining thus, inducing lesion at site of attachment. This also, likely suggested the absence of infective organisms in the type of diet of the fish while young. Omeji et al. (2013) observed heavier fishes as being infected more than low weighted fishes.

Generally female samples were found to have more weight than their male counterpart, which may have predisposed the females to a higher prevalence than their male counterpart due to their feeding habits. Furthermore, bigger fishes were recorded more in the rainy season which may be due to the high water level that made it difficult for fishermen to go fishing thereby giving the fish opportunity to grow coupled with eutrophication that increased invertebrate population and a change in diet on the part of the fish to more nutritive invertebrates.

Multiple and concurrent infection by parasites

Relationship between *Anomotaenia* sp. and *P. clariae,* suggested that *Anomotaenia* sp. will probably not survive in an acidic medium like the stomach and when found in the intestine with *P. clariae* an intense competition probably ensued, as a result of same physiological demand. *P. clariae* may likely have an advantage thereby diminishing the population of *Anomotaenia* sp. Relationship between *Anomotaenia* sp. and *P. cyathopharynx* was not significant though positive but very low; this also applied to *Monobothrium* sp. and *N. rutili. P. laevionchus* will easily co-exist with the remaining parasites but its relationship with *N. rutili,* though positive was low and not significant. Both parasites were absent in the stomach. This result likely pointed to similar physiological needs and sensitivity between the two parasites especially to certain existing factors in their immediate micro environment which may instigate fierce competition. Apart from the mentioned exceptions, positive linear association among the identified parasites occurred freely.

Conclusion

The gastro-intestinal helminth parasites in the gut of *C. gariepinus* include two nematodes *(P. laevionchus, P. cyathopharynx.*) three cestodes (*Anomotaenia* sp.*, Monobothrium* sp.*, P. clariae*) and one acanthocephalan (*N. rutili*). *Monobothrium* sp. had the greatest intensity of infection while *Anomotaenia* sp.*,* had the least intensity. Sex and season were found to possibly influence the level of infection while diets; also, may likely contribute to the stage at which parasitic infection occurs in *C. gariepinus.* Fishes of small sizes and weight were not infected. *P. laevionchus* and *N. rutili* were absent in the stomach, maybe as a result of their sensitivity to an acidic environment as presented by the stomach. The cestodes formed the bulk of parasites found in the gut of *C. gariepinus*. The possibility of multiple and concurrent infection of different species of parasites in a fish was established and poses a health risk of zoonotic transmission to consumers.

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