INVESTIGATION OF NEMATODE *EUSTRONGYLIDES* LARVAE INFECTIONS IN MUDFISH *CLARIAS GARIEPINUS* AND *C. ANGUILLARIS* FROM BIDA FLOODPLAIN OF NIGERIA.

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ABSTRACT

This is the first report and record of the determination whether mudfish Clarias were infected with the larvae of the nematode Eustrongylides. Also, documented the assessment of the socio-economic perceptions of three groups of fisher folks on the economy of infected mudfish Clarias fishing activities. Fifty – six (67.5%) of 83 mudfish Clarias caught by artisan fisher folks were examined for the presence of the larvae of the nematode Eustrongylides. All the 8 sampled fishing localities in Bida floodplain of Nigeria had a mean intensity and abundance of at least 3 and 1 worm per fish per site, respectively. Two hundred and one (96.2%) of 209 worms recovered were from the musculatures at different depths resulting in undulations on the skin surfaces as grub-like presentations. The three groups of fisher folks assessed encountered economic losses from nematode infected mudfish Clarias which attract much debates or rejections during marketing due to its aesthetically displeasing appearance, faster deterioration, higher fragility in smoked form coupled with poorer taste compared to the wholesome ones. Infected female mudfish Clarias had higher worm burden than the males, for each fishing locality.

KEYWORDS: Fisher folks, *Clarias* species, *Eustrongylides* larvae, Bida floodplain.

INTRODUCTION

The nematode *Eustrongylides* larvae infects fish-eating aquatic birds as its final host, with oligochaetes acting as the first intermediate host, and a variety of species of fish as the second intermediate host (Ibiwoye *et al.*, 2004). Adult *Eustrongylides* are apparently not particularly host specific since they have been reported from several species of aquatic birds e.g. cormorants *Phalacrocorax africanus*, herons *Ardea goliath*, snake birds *Anhinga rufa*, pelicans *Pelecanus rufescens*, spoon bills *Platalea leucordia* and cattle egrets *Egretta egretta* (Jaegerskiold, 1909) widely distributed in the African continent. By burrowing through the stomach wall of the bird and into the coelomic cavity, the parasite often causes haemorrhage, scar tissue formation, loss of appetite, retarded growth and often death (Spalding and Forrester, 1993) which can be as high as 80% especially in young birds in breeding colonies (Spalding *et al.*, 1993). The distribution and prevalence of the parasite may be affected by surface water quality and conditions which led to dense populations of oligochaetes intermediate host could set the stage for epizootic of eustrongylodiosis. Hirshfield *et al.* (1993) suggested that high prevalence of *E. ignotus* in the discharge of a power plant in Maryland (USA) were associated with organic enrichment, elevated year-round temperature and consequent high densities of oligochaetes (Measures, 1988).

No studies, however, have been conducted concerning the presence of nematode in mudfish *Clarias* from Bida floodplain, nor its socio-economic perceptions by the fisher folks assessed. This study investigated nematode *Eustrongylides* larvae in mudfish *Clarias* from Bida floodplain. Also, assessed the perceptions of three groups of fisher folks on the economy of *Eustrongylides* larvae infected mudfish *Clarias* fishing activities, for the first time.

MATERIALS AND METHODS

The study area is the freshwater wetlands or floodplains created by rivers Niger and Kaduna around Bida located between longitude 5^0 45' to 6^0 15′ E and latitude 8^0 30′ to 9^0 10′ N within the southern Guinea Savannah zone of Nigeria (Areola *et al.*, 1992).

Eighty-three *Clarias gariepinus* and *C. anguillaris* of different sizes and sexes caught at 8 fishing localities (X = 10.4 fish/locality, SE = 2.1) were collected from major ponds and streams within Bida floodplain including a variety of pristine and human-altered fishing localities. Fish samples were killed in humane manner by cervical dislocation or decapitation, thoroughly examined individually, frozen and dissected under a light binocular dissecting microscope between 10 to 30X magnification for the sites of infection and the larvae of *Eustrongylides* recovered were countered using a manual counter. Its prevalence, mean intensity and abundance on the host (*Clarias*) were determined by standard procedures described by Margolis *et al.* (1982). The socio-economic perceptions of the three groups of fisher folks were assessed by a standard structured questionnaire prepared and administered to a randomly selected fisher folks and owners of reputable restaurants and hotels where large quantities of fresh fish pepper soups were sold on a daily basis. Focus group discussion (Maier *et al.*, 1994) method was also employed at the four (Wuya bridge, Dokomba, Doko and Katcha) randomly selected fishing localities within Bida floodplain of Nigeria.

RESULTS AND DISCUSSION

Worms affecting the 56 (67.5 %) of 83 *Clarias* randomly sampled were identified as nematodes (round worms). Eustrongylid larvae cannot be identified to species. We presumed that all were *Eustrongylides* (Khalil, 1998) based on our description of *Eustrongylides*, which occur in a variety of fish species (Shah-Fischer and Say, 1989; Paperna, 1996; Yanong, 2002; Ibiwoye *et al.*, 2004) as we reported its presence in mudfish *Clarias*. This fish species is ubiquitous in the floodplain, easy to capture in large numbers, highly prized, tasty flesh coupled with high traditional or socio-cultural demands is known to frequently harbour larvae of *Eustrongylides*. Nematodes were found in sheaths (Shah-Fischer and Say, 1989) resulting in undulations on the skin surfaces as grub-like presentations (Yanong, 2002) in the infected fish musculatures and visceral organs, on close inspection these tiny blood-sucking worms were grossly observed at different depths of the muscles. Table 1 showed the sites of infection and worm counts in nematode *Eustrongylides* larvae infected mudfish *Clarias* in Bida floodplain of Nigeria. 201 (96.2%), 4(1.9), 3(1.4) and 1(0.5%) out of 209 worm counts were found in the muscle, gill, gut and kidney respectively. The muscles have the highest affinity for the worms, might be due to the food reserved to sustain them. Females had higher (69.5%) larvae counts than males (23.4%) per site of infection. The relative abundance of nematode larvae varied from one anatomical site of recovery to another. This regional localization could be attributed to several factors including chemo static response, food reserves as well as hydrogen ion concentration (pH). It is evident that female fishes are more frequently infected with parasites than the males from the findings of this study. This observation agreed well with the findings of Mhaisen *et al.* (1988) that female fishes were generally more liable than males to infections with cestode, nematode, acanthocephalan, crustacean and copepod parasites. The mean prevalence, intensity and abundance of *Eustrongylides* larvae in *Clarias* for the dry season are higher than the wet season of the year. Fishes are susceptible to heavy infestation with parasites mainly in the early rain when fishes are weakened by hibernation (a state of exhaustion). Larger fishes are heavily parasites than smaller ones. The intensity and prevalence of parasites infection increases with increasing length, size and age of the fish host. Also, the food amount contained in the intermediate host of the parasite might cause the increase in infection level. The relationship between the fishing localities, worm counts and sex of mudfish *Clarias* with *Eustrongylides* larvae infection in Bida floodplain is shown on Table 2. The nematode count in the fishing localities in descending order of preference was 88, 44, 39, 15, 7.5, and 4 3 for Bida, Katcha, Doko, Wuya Bridge, Badeggi, Lemu, Kusomi and Dokomba. The worm per fishing locality was 25.6 ± 29.9 for the eight fishing localities sampled. It was 22 ± 42, 11.0 ± 22, 9.8 ± 18.2, 3.8 ± 6.5, 1.8 ± 3.5, 1.3 ± 1.0, 1.0 ± 2.0, and 0.8 ± 1.5 worm for Bida, Katcha, Doko, Wuya Bridge, Badeggi, Lemu, Kusomi and Dokomba respectively, in the descending order of preference. The prevalence was 22.9 ± 19.2, 48.1 ± 29.3 and 33.4 ± 4.6 for the males, females and total *Clarias* sampled. The intensity (worm per infected fish) was 1.4 ± 1.4, 2.7 ± 1.5 and 2.8 ± 2.7 for the males, females and total infected *Clarias* sampled. The abundance (worm per fish sampled) was 0.5 ± 0.8, 1.6 ± 1.8 and 1.1 ± 1.2 for the males, female and total *Clarias* sampled. The females had higher prevalence, intensity and abundance than the males this might be due to some physiological phenomenon, physical-chemical factors and seasonal variations in natural fish food and water availability.

The reactions of the fishermen, fishmongers (middlemen) and fish consumers to nematode infected fishes are shown on Table 3. It was observed that the three groups interacted with were at the risk of some obvious socio-economic losses on their dependence on nematode infected fishes. More so, that nematode infected fishes deteriorate faster with poorer taste coupled with the smoked form not as firm in presentation compared to the wholesome ones and tended to attract much debates/rejections during marketing. It appears that those worms might have an important effect on the biology and health of the fish and reduced the populations available to the fishery. Moreover, the aesthetically displeasing appearance of wormy fish certainly caused consumers' rejections, which might affect the economy of the fish industry. The fishermen are yet to notice any drop in the sales of their catches due to worm infestation. To them, the infestation has no impact on the acceptance of their catches. The infected fishes usually 'dressed' by the fishermen who physically remove all the worms that could be easily accessed on the fishes before sale or by mild boiling with hot water to expel the worm prior cooking or smoking for consumption. The fishmongers observed that the fish market is still as viable as ever with more customers coming out in large numbers to buy the fishes. A key informant, fishmonger and fish farmer attested to the continued influx of fish buyers from different places (Ibadan, Onitsha and Calabar). There are different views concerning the state of the worms after consumption. Some believed that cooking kills the worms and render them harmless and had never caused any health problem to anybody not spiritually cursed by fellow human being. There were those who hold the superstitious belief that worms eaten do not die but continue to grow until they are of the length of the consumer's intestine and then caused illness and/or death. These people also have a taboo against *Clarias* consumption. However, the opinion of some fish consumers contrasted very sharply with those of the fishermen and fishmongers that the worms might succeed in eliminating some species of fish, which they long have been used to. They also gave their encounter with worm infected *Clarias* and that they had 'ordered' their wives to eliminate such fish from their menu, thereafter. Most lovers of fresh fish pepper soups in the Bida environ are seriously having a rethinking, extremely careful and selective in the type of fish they consume. The fish farmers are also in a state of dilemma because they do not know how to protect their ponds from being contaminated by these worms.

Figure 1 showed the effect of fishing localities and sex on the prevalence of *Eustrongylides* larvae in *Clarias* from Bida floodplain. The males' prevalence was highest (53.3%) at Bida and lowest (0%) at Dokomba and Kusomi. The females' prevalence was highest (100%) at Bida and Kusomi and lowest (16.7%) at Badeggi. The prevalence for the total sampled was highest (72%) at Bida and lowest (13%) at Lemu. The effect of fishing localities and sex on mean intensity of *Eustrongylides* larvae in *Clarias* from Bida floodplain is shown in Figure 2. The mean intensity for the males was highest (4.4) at Bida and lowest (0%) for Dokomba and Kusomi. For the females, the mean intensity was highest (5.3) for Bida and lowest (1.3) at Kusomi. The total sampled had the highest (5.5) mean intensity at Bida and lowest (1.3) at Lemu and Kusomi. The effect of fishing localities and sex on the mean abundance of *Eustrongylides* larvae in *Clarias* from Bida floodplain is shown in Figure 3. The mean abundance for the males was highest (2.3) at Bida and lowest (0%) for Dokomba and Kusomi. For the females, the mean intensity was highest (5.3) at Bida and lowest (0.3) at Kusomi. The total sampled had the highest (3.5) mean abundance at Bida and lowest (0.2) at Lemu and (Kusomi). Thus the prevalence, mean intensity and abundance were highest at Bida and lowest at Lemu; Dokomba and Kusomi. Doko tended to have its values at about the midway between the highest and lowest values of the prevalence, mean intensity and abundance recorded in the eight fishing localities sampled. This might be to its location which relatively about midway of the eight fishing localities, the water flooding regime and the forage availability.

Eustrongylides have complex, indirect life cycle (Yanong, 2002) with fish as intermediate, paratenic (alternative) or final (definitive) hosts. Where the fish is the intermediate host, the nematode eggs/larvae eaten an invertebrate or a fish intermediate host prior to being eaten by or entering the final host. The final host (which contains the reproductive adult stage of the nematode may be a piscivorous (fish – eating) fish, bird or mammal. Where the nematode has the ability to survive in "alternative" organisms, known as "paratenic" or reservoir hosts, which are not required for completion of the life cycle, but they can contain infective nematode life stages and be a source of infection. They can be fish, worms or other aquatic organisms that can eat the nematode eggs/larvae. Where the fish is the final host, the nematode eggs/larvae enter an aquatic invertebrate intermediate host such as a copepod, annelid worm or insect larva prior to being eaten by or entering the final host fish. The eggs of all *Eustrongylides* are very tough and can easily survive for some time in fishponds. At about 77^{0}F, it can take anytime from 3 – 4 1/2 months from the time fish become infected. This means that, after sterilization of ponds, if fish – eating birds do infect the ponds with *Eustrongylides* eggs, the producer may not see a problem until harvesting the fish, 3 – 4 months later, as this is approximately the time required for the eggs to hatch and become the L3 stage which infects the fish. After this 3 – 4 months period, fish raised on ponds with a population of fish – eating birds have a greater chance of becoming infected as the number of nematode increase overtime. Adults are found in fish – eating birds, the eggs are shed by the aquatic birds into ponds, where they develop into a life stage that is consumed by an oligochaetes or annelid worms where it develops further into a third large stage "L3" which can infect fish when eaten. Once the annelid worms containing the L3 stages is eaten by a fish and digested, the nematodes migrate (within the fish) into the body cavity and, frequency, over the external surface of internal organs such as the liver. Some recent studies, however, suggest that *E. ignotus*, commonly found in mosquito fish, which is a close relative of this *Eustrongylides*, may be able to complete its life cycle without the need for a tubifex worm (Coyner *et al.*, 2002). The first intermediate hosts are oligochaetes or annelid worms. Complementary (reservoir, alternate, paratenic) hosts are fish, where encysted larvae are found. The death of the host might stimulate the nematodes to emerge from their cysts, migrate through the body wall to the surface of their dead host and may survive for over 24hrs when a dampened condition exists. *Eustrongylides* are known from a diversity of fishes and amphibians (Hoffmann, 1999) and their relatively great abundance suggests they are common in the habitats where fish forage. But exceptionally rare parasites of carnivorous mammals associated with riparian or lacustrine habitats. The prevalence recorded in this study may indicate that larvae are particularly abundant in fishes and amphibians available to Rivers Kaduna and Niger and other tributaries supplying water to Bida floodplain, consistent with the potential that humans could be exposed to infection.

All evidences indicated that the larva is *Eustrongylides* and that a wide diversity of aquatic fish-eating birds, which are extremely numerous along the floodplain serve as final hosts with tilapias their predominant food. Adult *Bagrus* and *Clarias* are too large to be eaten by aquatic fish-eating birds or any other potential hosts of *Eustrongylides*. The infection to the final host is apparently transmitted mainly or exclusively through tilapias. *Bagrus* and *Clarias* apparently become infected with larval *Eustrongylides* by ingesting infected tilapias a dominant food item in their diets. The ability of *Eustrongylides* larvae to re-establish in a new intermediate host was experimentally demonstrated with *E. ignotus*. Adult *Eustrongylides* are apparently not particularly host specific since they have been reported from several species of aquatic birds widely distributed on the African continent, e.g. cormorants *Phalacrocorax africanus* (Gmelin), herons *Ardea goliath* (Cretzschmer), snake birds *Anhinga rufa* (Lacepede), pelicans *Pelecanus rufescens* (Gmelin), spoon bill *Platalea leucordia* (Linnaeus) and cattle egrets *Egretta egretta* (Linnaeus) (Jaegerskiold, 1909). *Eustrongylides* can be found within the body cavity, encapsulated on the liver and other organs, but outside the intestinal tract of fish. Eustrongylid nematodes can affect a number of different species, including yellow perch, pumpkin seed, mummichug, guppies, gar, danios and angelfish. Affected fish typically have bloated abdomens (dropsy), as the nematodes frequently migrate into body cavity and can be quite large. Eustrongylids are typically very long, coiled and red (due to the presence of haemoglobin) and an infected fish often has more than one nematode in its body cavity. If a feeder fish containing *Eustrongylides* are fed to other fish, the nematode can migrate out of the feeder fish and into the muscles or other organs of the fish that just consumed them. After migrating into the muscle, this nematode can cause lesions that look superficially similar to a grub. Infection would tend to build up in already infected environment due to re-infected as this worm is viviparous and since contact with other infected fish could be less in the river with the large volume of water than in the floodplains or fish ponds with smaller water volume. The differences in size of nematode larvae might have resulted from the differences in the site of infection. Most piscivorous birds are unlikely to find *Clarias* easy to handle because of its pectoral spines so encapsulation of this larva on *Clarias* might prove to be a dead – end in most cases. The distribution of *Eustrongylides* in fishes have been linked with aquatic habitat having disturbed soil, exogenous nutrient and high densities of oligochaetes (Spalding *et al.*, 1993). Thus, pollution of surface waters may affect the distribution and prevalence of *Eustrongylides* in fish of Bida floodplain. The present investigation shows evidence of heavy nematode *Eustrongylides* larvae infections in *Clarias gariepinus* and *C. anguillaris* from Bida floodplain of Nigeria. This therefore calls for proper fish management in terms of preventing serious outbreaks. The problems of fish infestation by nematode larvae could be best handled through avoidance of overcrowding as well as elimination of conditions that could favour parasitic infestation. Also, the use of animal droppings or organic fertilizers from the adjacent farmlands to the water pools within the floodplain should be averted. Finally, piscivorous birds serving as definitive host of *Eustrongylides* larvae should be prevented or distracted continually coupled with the eliminations of the intermediate hosts (i.e. oligochaetes, small-sized fishes, reptiles, amphibians and mammals). One prediction arising from this hypothesis is that the parasite should be found more commonly in areas of denser, presumably more polluted, human habitation, and should therefore be more prevalent now than in the past. This study gave the first report and record of the determination of nematode *Eustrongylides* larvae in mudfish *Clarias*. Also, documented the assessment of the socio-economic perceptions of three groups of fisher folks on the economy of infected mudfish *Clarias* fishing activities.

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Table 1: Sites of infections and worm counts in mudfish *Clarias* infected with nematode *Eustrongylides* larvae from Bida floodplain of Nigeria.

Sites of infections	1	2	3	4	5	6	7	8	Sub total	Mean	S.D.	% of total
Muscle	85	2	7	3	37	15	4	44	201	24.6	29.2	96.2
Gill	3	0	0	0	1	0	0	0	4	0.5	1.1	1.9
Gut	0	2	0	0	1	0	0	0	3	0.4	0.7	1.4
Kidney	0	1	0	0	0	0	0	0	1	0.1	0.4	0.5
Total sampled	88	5	7	3	39	15	4	44	209	25.6	29.9	100
Mean	22	1.3	1.8	0.8	9.8	3.8	1.0	11.0				
S.D.	42.0	1.0	3.5	1.5	18.2	6.5	2.0	22.0				

1= Bida; 2= Lemu; 3= Badeggi; 4= Dokomba; 5= Doko; 6= Wuya Bridge; 7= Kusomi; 8= Katcha.

Table 2: Fishing localities, worm counts and sex of mudfish *Clarias* infected with nematode *Eustrongylides* larvae from Bida floodplain of Nigeria.

	Males			Females			Total sampled		
Parameters	Sub total	Mean	S.D.	Sub total	Mean	S.D.	Sub total	Mean	S.D.
No of fish sampled	83	10.4	4.6	89	11.1	2.6	172	21.5	6.7
No of fish infected	22	2.8	2.7	42	5.3	3.2	64	8.0	5.8
Worm counts	72	9.0	12.2	137	17.1	17.6	209	25.6	29.9
% of total worm count	187.2	23.4	16.7	614.8	76.8	1.7	100	0.0	0.0
Prevalence (%)	183.4	22.9	19.2	384.5	48.1	29.3	226.4	33.4	4.6
Mean intensity (No/fish)	11.1	1.4	1.4	21.8	2.7	1.5	22	2.8	2.7
Mean abundance (No/fish)	4	0.5	0.8	13	1.6	1.8	9	1.1	1.2

Table 3: Reactions of three groups of respondents to mudfish *Clarias* infected with nematode *Eustrongylides* larvae from Bida floodplain of Nigeria

Fishermen	Middlemen	Fish consumers
Reaction	**Reactions**	**Reactions**
All our catches are sold em-bloc (i.e. without separating the infested from non-infested)	Infested fresh fishes are dressed for sales by nipping of the swollen parts of the body to excise the nematode from the fishes so as to earn more money, eventually. Infested fish are first treated with hot water to facilitate the expulsion of the nematode from the fishes and later subjected to smoking.	Eaters always ask pepper soup sellers whether it is made of nematode infested fishes especially *Clarias* so as to avoid it. Buyers are always sceptical of buying fish pepper soup from any seller where such observation had once been made.
Comments	Comments	Comments
Natives (Nupes): They were more entangled in crop farming than fishing activities at the dry season of the year to earn a living. Non-natives (Hausas and Jukuns): They travel out to their places of origin to engage in dry season crop farming as alternative sources of income. Instead of fishing when the infested fishes are more abundant.	The excised fresh fishes are easy to recognize at sight and tend to attract much debates/rejections during marketing The nematode infested smoked fish were not as firm in presentation as the non-infested ones	Buyers to sellers observe debates/rejections over presentations of nematode infested fresh fishes in pepper soups. Buyers of such infested fresh fishes do lose both their monies. and plates of pepper soup concurrently.

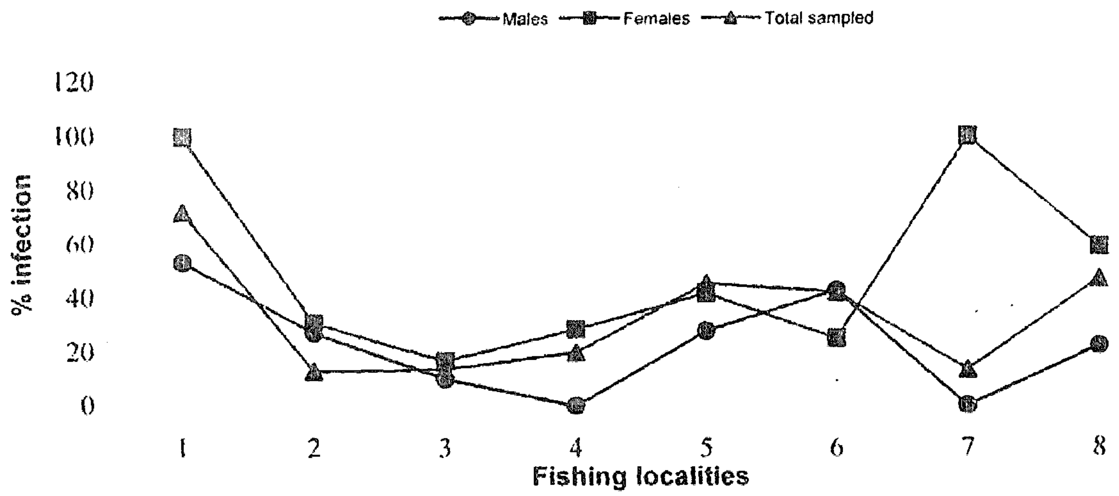

Figure 1: Effects of sex and fishing localities on prevalence of *Eustrongylides* larvae in *Clarias* from Bida floodplain of Nigeria.

Figure 2: Effects of sex and fishing localities on mean intensity of *Eustrongylides* larvae in *Clarias* from Bida floodplain of Nigeria.

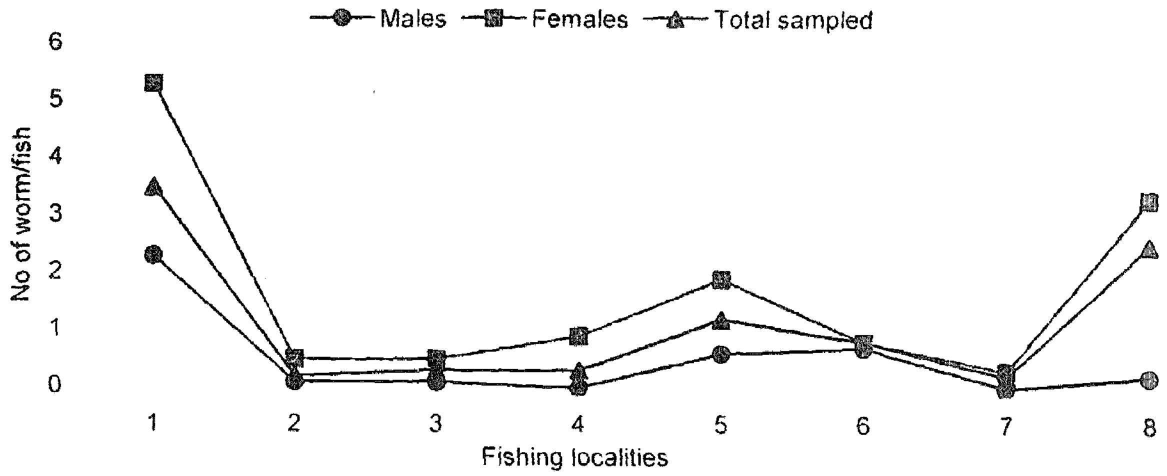

Figure 3: Effects of sex and fishing localitieson mean abundance of *Eustrongylides* larvae in *Clarias* from Bida floodplain of Nigeria.