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Incidence of nematode parasites in snakehead, *Parachanna obscura* of the lower Cross river system, Nigeria

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Abstract
The incidence of nematode parasites in African snakehead, *Parachanna obscura* of the lower Cross River system was investigated. The objective was to determine the prevalence and intensity of the infection with size, gender and season of the fish species. A total of 510 specimens of *Parachanna obscura* was sampled from July 2009 to January 2010. The fish were processed through standard parasitological manipulations. There was 53.9% prevalence of infection and mean intensity of 8 nematodes per fish in the Great Kwa River. In the Cross River 96 fishes were infected with a total of prevalence and mean intensity was highest in the lower length-classes. Mean intensity was lower in females than in males due to their reproductive requirement. Generally, there was no significant difference in prevalence by size and gender at $p > 0.05$. Mean intensity and nematode abundance, were higher in Great Kwa River than the Cross River. There was no correlation between the total numbers of nematodes and the infected *P. obscura* by size and gender. The prevalence of infection by season in Great Kwa River was variable; with a mean intensity of 12 worms per fish in dry season and 6 worms per fish in wet season. The low level of helminthes infection in *Parachanna obscura* means that it is safe as aquaculture candidate and this should be vigorously pursued.

Keywords: Nematodes, *Parachanna obscura*, Size, Gender, Season, Lower Cross River system.

1. Introduction
African snakehead, *Parachanna obscura* is an emerging candidate for aquaculture. The species is known to have sweet flesh with highly acceptable market value [1]. It is one of the subsistence species in the Cross River system and good food condiments for the people of the Cross River Basin. There is an ongoing screening of the species for aquaculture because of its hardiness, good quality fillet, and high nutritional value. Ama-Abasi *et al.*, [1] reported high proportion of protein and fats for varying nutritional and energy requirements for people with different health challenges. Previous work on the species include Ama-Abasi and Ogar [2] on proximate composition, Ama-Abasi and Affia [3] on food and feeding habit, Adebayo and *et al* [4] on haematological profile of the species.

On the other hand the greatest setback to aquaculture and its growth has been fish parasites and diseases [5, 6]. African snakehead and other fish species are known to be hosts of parasites and diseases. Akinsanya *et al.*, [7] reported on parasites of *Parachanna obscura*, from Lekki Lagoon, Lagos to include two helminthes, *Procamallanus* sp (*Spirocamallanus*) and *Contracaecum sp* and a trematode, *Clinostomum* metaceria, with prevalence of 5.9%. There is no documented evidence of snakehead parasites in the Cross River system in spite of the fact that snakehead fishery is popular and all year round in the river system with a significant economic interest. It was the objective of this study therefore to ascertain the incidence and prevalence of nematode infection of *Parachanna obscura* from the lower Cross River system as a further screening of its aquaculture potentials.

2. Materials and Methods
2.1 Study area
The Cross River system is formed from numerous tributaries arising from the western slopes of the Cameroun Mountains which have two spurs into Nigeria as the Oban Hills in the south and the Obudu Hills in the north. When the main river enters Nigeria from the Cameroun, it flows first in a westward direction and then turns southwards and enters the Atlantic Ocean

with a limited delta formation, but forming a wide estuary. The whole Cross River system lies approximately between longitude 7°30/E and 10°00/E and latitude 4°00/N and 8°00/N. Three major rivers that constitue the Cross River system are the Cross River, Calabar River and the Great Kwa River. The Study was conducted specifically from the Cross River and the Great Kwa River (Fig 1.).

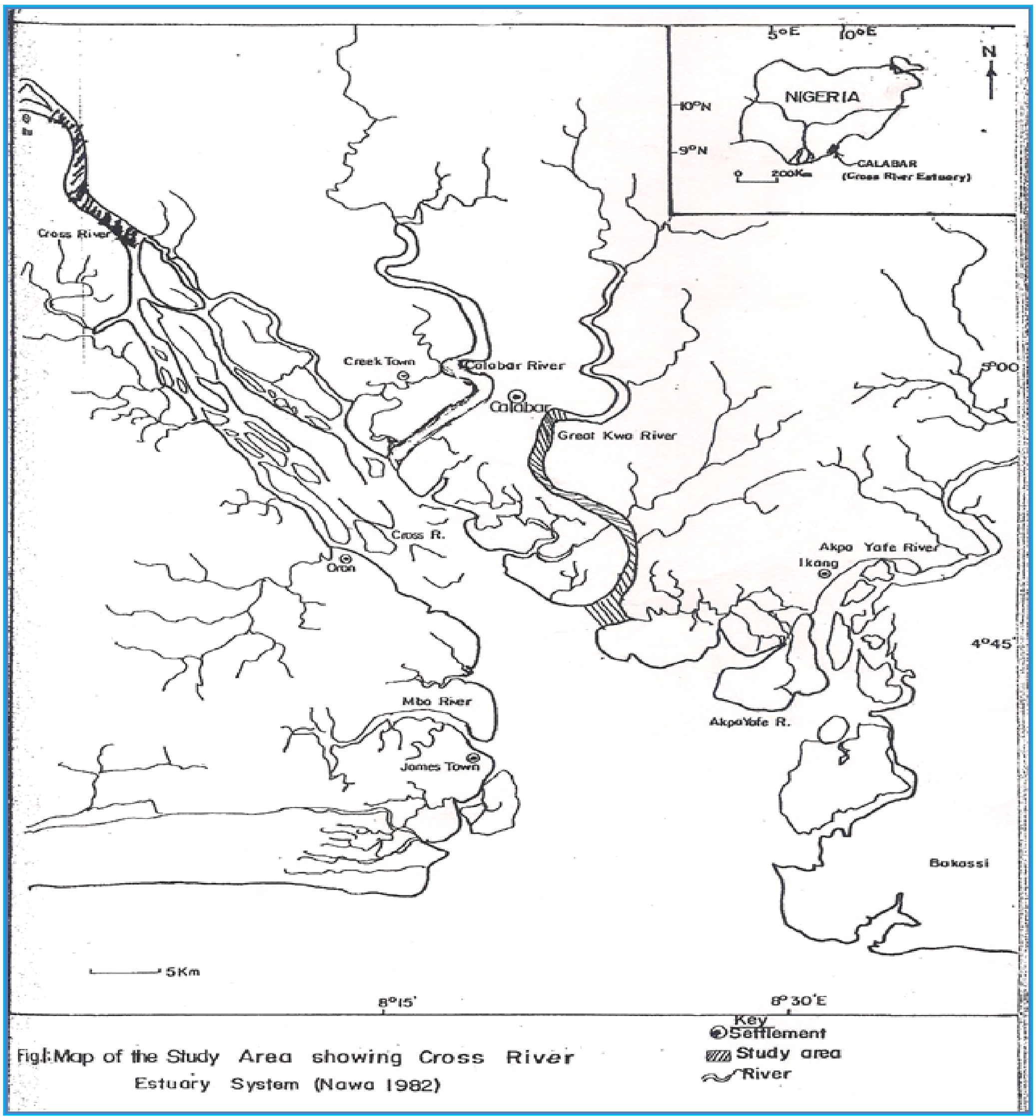

Fig 1: Map of the Study Area Showing Cross River Estuary System [12]

Key
ɘ Settlement
\\\\\ Study Area
~ River

2.2 Specimen collection and methodology

Live specimens of *Parachanna obscura* were purchased from artisanal fishermen at Ayadehe, Bridge head, about 54 km from Calabar on the Cross River and also Obufa Esuk beach on the Great Kwa River. The specimens were transported in 10 litre buckets half-filled with water back to the Central Biological Oceanography laboratory in Institute of Oceanography, University of Calabar. The fish were kept in water troughs for 3 days to acclimatize. This method was adopted to ensure that fish were alive and were sacrificed with adequate time interval to carry out parasitological analysis to avoid the death of the parasites were the fish to die before the analysis.

Total length measurements of the fish were taken using a graduated wooden measuring board to the nearest 0.1 cm. Weights were taken using the Trip weighing balance. (Balance – Ohaus Havard Mechanical Trip Balance with readability of 0.1 g and capacity of 2000 g). Fishes were necropsied in accordance with ethical procedures according to the method of Adebisi [8]. *Parachanna obscura* was dissected, degutted and dissected organs placed in Petri dishes with 0.85% physiological saline and inspected for nematodes. The entire fish was thoroughly examined and worms carefully extracted under a stereozoom microscope. Nematodes present per fish were further isolated, counted and their numbers recorded. They were washed with glycerol and transferred into slides for microscopic examination.

The method of Rafique *et al*., [9] was used for microscopy. Photographs of live fish and nematodes were taken and recorded using a digital camera (Dimensions: 2048 × 1536). All nematodes obtained from the intestine of the *P. obscura* were killed by gentle heating and fixed in 4% formalin. After being cleared in 2% glycerol the larvae were examined by light microscopy. Some nematodes were preserved in Gilson fluid. *Parachanna obscura* was identified using the diagnostic features described by Courtenay and Williams [10]. The adult nematodes were measured with a meter rule while the larvae

and some special features of the adults were measured under the microscope, using the stage and ocular micrometer.
Samples were collected for twenty months from January, 2007 to September, 2008. Juveniles and adults, females and males of the fish species were randomly sampled and nematode infection investigated. The sexes of the fish were determined according to the method of Olurin and Somorin [11]. Analysis of variance and Chi-square were used for statistical analysis.

Plate 1: *Parachanna obscura* males with dark blotches and females with light blotches

3. Results

Two species of nematodes were identified. The nematode species identified were *Neocamallanus* sp. and *Paracamallanus cyathopharynx*. Nematodes were extracted from mainly fish gut, gills and muscles. There was the preponderance of infection in the gut. Nematode infection prevalence and intensity by size, gender and season are given in Tables 1, 2 and 3; Fig 2 and 3. Nematodes occurred in both viscera and muscle tissues as in Tables 4; and in all the length classes. The length class of 10-15 cm apparently had the highest prevalence of 100% with mean intensity of 8.4 worms/fish. Prevalence was lowest in length class of 22- 27 cm (39.3%), with a mean intensity of approximately 11 worms / fish. The Chi square test showed that, there was no significant difference in prevalence by size (X^2, > 0.05, 4).

In the Cross River, 421 *Parachanna obscura* specimens were sampled from August, 2007 to September, 2008. The analysis of the internal organs of all fishes for nematode infection was done and observations recorded. 96 fishes were infected with a total of 440 nematodes extracted

Table 1: The prevalence of nematode infection in *Parachanna obscura* by size in the Great Kwa River (%).

Length classes (cm)	**10-15**	**16-21**	**22-27**	**28-33**	**34-39**	**40-45**	**Total**
Sample size	5.6(5)	15(14)	31.5(28)	41.6(37)	5.6(5)	-	89
Number of fish infected	10.4(5)	18.8(9)	22.9(11)	41.7(20)	6.3(3)	-	48
Number. of Nematodes	11(42)	27.6(99)	33.1(119)	21.2(76)	6.4(23)	-	359
Prevalence %	100	64.3	39.3	54.1	60	-	53.9
Mean Intensity	8.4	11.0	10.8	3.8	7.7	-	7.5

Actual number of specimens in parenthesis.

Table 2: The prevalence of nematode infection in *Parachanna obscura* by size in the Cross River (%).

Length class (cm)	**10-15**	**16-21**	**22-27**	**28-33**	**34-39**	**40-45**	**Total**
Sample size	4.3(18)	34(143)	38.2(168)	19(80)	2.6(11)	0.2(1)	421
Number of fish infected	1(1)	35.4(34)	43.8 (42)	17.7(17)	1(1)	1(1)	96
Number of nematodes	0.5(2)	40(168)	43.2(190)	17.2(76)	0.5(2)	0.5(2)	440
Prevalence %	5.6	23.8	25	21.3	9.1	100	22.8
Mean intensity	2.0	4.9	4.5	4.5	2.0	2.0	4.6

Actual number of specimens in parenthesis.

Table 3: The prevalence of nematode infection in *Parachanna obscura* by gender in the Great Kwa River and Cross River (%).

	Great Kwa River			Cross River		
Gender	**Females**	**Males**	**Total**	**Female**	**Males**	**Total**
Sample size	66.3(59)	33.7(30)	89	82.2(346)	17.8(75)	421
Number of fish infected	66.7(32)	33.3(16)	48	80.2(77)	19.8(19)	96
Number of nematodes	61.8(222)	38.2(137)	359	69.5(306)	30.5(134)	440
Prevalence %	54.2	53.3	53.9	22.3	25.3	22.8
Mean intensity	6.9	8.6	7.5	4.0	7.1	4.6

Actual number of specimens in parenthesis

There was no significant difference in prevalence by gender (X^2= 0.00113). There was however significant difference in z the prevalence of nematode infection in *P. obscura* by season ($P < 0.05$, $X^2 = 14.76965$, 3df) in Cross River.

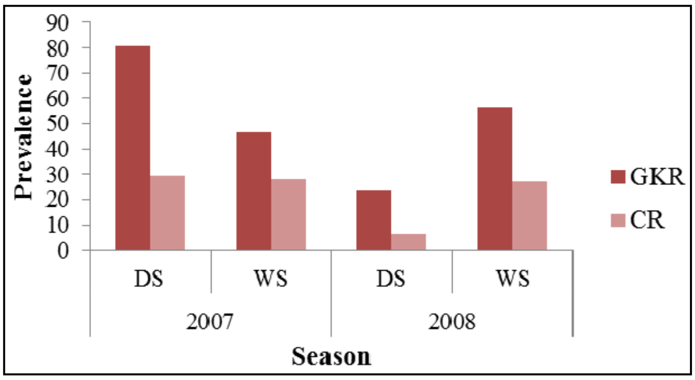

Fig 2: Prevalence of nematode infection in *P. obscura* by season in Great Kwa River / Cross River

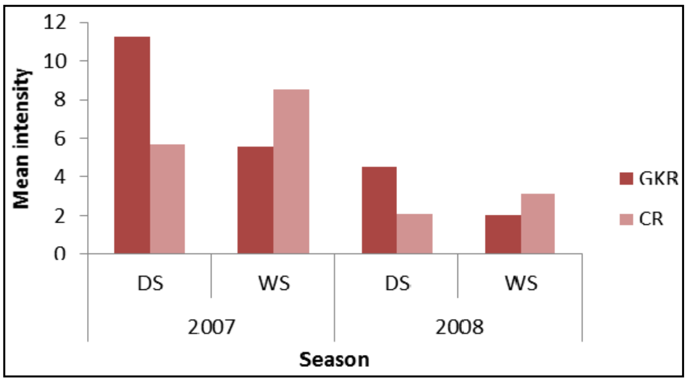

Fig 3: Mean intensity of nematode infection in *P. obscura* by season in Great Kwa River /Cross River

Table 4: The distribution of nematodes in various organs of *Parachanna obscura* from Cross River system

Site	Total number of *Parachanna obscura* infected	Mean body length (cm)	Mean body weight (g)	Total number of nematodes extracted	Gut	Muscle fillet	Gills	Gonads	Liver	Pericardial carvity	Eye	Swim bladder
Great Kwa River	48	**26.01**	**163.8**	359	336	23	0	0	0	0	0	0
Cross River	96	**23.7**	**112.10**	440	385	37	4	0	1	5	0	8
Total	144			799	721	60	4	0	1	5	0	8

4. Discussion

The number of *P. obscura* infected does not depend on the size of the fish within the limit of samples analyzed. The lack of correlation between nematode infection and the size of *Parachanna obscura* is quite different from what is reported from other fish parasites. Obiekezie and Ekanem [5] reported severe infection and damage caused by *Trichodina maritinkae* to the population of *Heterobranchus longifilis* fry. The damage and prevalence showed strong correlation with age, with the youngest suffering more damages than the older ones. This lack of correlation between size and parasitic impacts is probably because all the specimens of snakeheads were adult and sub-adult and had already acquired strong immunity against infection and so did not show great variation in prevalence compared to Obiekezie and Ekanem [5] which reported on fry of catfish.

The highest concentration of nematodes in the gut of *Parachanna obscura* gave a clear indication of the preference of the intestine to any other site for attachment. This could be attributed mainly to the availability of food in this region. Fish parasites generally preferred alimentary canal for various reasons to include presence of semi- digested food, hidden nature of the alimentary canal and the absorbent nature of the intestine thereby providing easy attachment for habitation. Although the presence of nematode parasites in the intestine of fish has no dramatic adverse effects on the host, overcrowding and blockage of the lumen of the alimentary canal was obvious in some of the fish samples; this probably could lead to poor feeding and growth. Such fish may not do well in close culture. The present result is similar to the earlier work of Moravec [13], Ibiwoye, *et al.*, [14]. They iterated that worms have preference for region of attachment in the alimentary canal of fish. Furthermore, nematodes perhaps penetrated the mucosal wall and created passages for the flow of blood and tissue fluid; which accumulated in the intestine and caused bloating of the abdomen, haemorrhages and lesions at the sites of attachment.

The main factors determining the distribution of fish parasites; prevalence, intensity and abundance of infection in aquatic environments are, the diet of the host, the life span, mobility of

the host throughout its life; and a variety of habitats encountered, its population density and the size attained.
Prevalence of nematode infection was above average, 53.9% with a mean intensity of 8 worms per fish. This shows that, generally, more than half of the population of *Parachanna obscura* were infected with nematodes in Great Kwa River. Perhaps, the omnivorous and microphagous feeding habits of *P. obscura* juvenile on zooplanktons, was a contributory factor. As they grow into juveniles, they feed on insect larvae, small crustaceans, and fry of other fishes [15, 16, 3]. Gale [17] reported that, juveniles actively migrate in schools, hunting foods like zooplanktons, small insects and crustaceans; while adults are solitary feeders, eating both terrestrial and aquatic birds, fishes, frogs, tadpoles.
The low prevalence in the length-class 22-27 cm could be due to parasite immunity. A similar observation was reported in the study of the parasitic helminth fauna of *Parachanna obscura* from Lekki Lagoon, Lagos, Nigeria; the length groups 21-2 5 cm had low prevalence, while the highest length groups 26-30 cm recorded zero prevalence of infection [17]. Rahman and Bakri [18] reported high prevalence (73.2%), of nematode infection, in *Channa striata* in Kedah peninsular in Malaysia.
In the Cross River, infection prevalence and mean intensity were generally low compared to that of Great Kwa River. Fishes were probably more confined to marginal vegetation in Great Kwa River with abundant decaying organic matter, which supported a great number of dense zooplankton communities feeding on phytoplankton. However, the Cross River habitat is mainly floodplain with a wider dispersal of aquatic communities.
Since all length classes of *P. obscura* habour nematodes, no size of fish from the wild is safe for stocking in aquaculture. If other *Channa* species play host to gnathostomes, especially considering the migratory tendencies of exotic species in global fishery; *P. obscura* may likely be a potential host of gnathostome; and consumers dependent on capture fishery stand the risk of infection. Therefore, proper screening of fish of all sizes will be required. More research will be needed on identification of nematodes of *P. obscura* from Cross River system. And proper education on safe methods of preparation of fish.
The nematode infections of the different gender categories were also recorded. Female fishes appeared more in the entire sampling regime from both sites. Generally, the prevalence, mean intensity and abundance of nematode infection of *P. obscura* in both sites, showed that the proportion of females and males infected in the population was gender bias. Males carried more nematodes than females. This could be due to territoriality. Schmidt [19] pointed out that in addition to the dissolved oxygen content which affects embryonic development in the eggs of parasites, factors like muddy bottom water and feeding habits might also contribute to the low incidence of helminth parasites in the fish. Gravid female fishes were about 40% in the total sampling regime and probably were more confined to their territory than the males; and digested the nematodes for food, during reproduction. Similarly, Alam *et al*., [20], observed that, the male of *Channa punctatus* harboured more endoparasites in polluted water. In Cross River, males harboured more nematodes than females; although the case of pollution of the river was not established. Similar findings have been reported in many freshwater fish species [21, 22] and the main reason for the differences in parasitic load with sex is thought to be physiological. However, endoparasites have been reported to infect the two sexes differentially because male and female fish often have different feeding habits [23]. Al-Zubaidy [24] analyzed the infection rate and intensity of *Contracaecum* sp. larvae by host sex, out of sixty-nine males examined 16 (23.2%) were infected with 32 larvae (2 larvae per fish); on the other hand, eighty-nine females, 25 (28.1%) were infected by 94 larvae (3.8 larvae per fish). There was a slight variation in mean intensity with sex of the host, where females showed heavier parasite burden, though this was not statistically significant ($P < 0.05$).
Prevalence of nematode infection in *P. obscura* varied with season. Great Kwa River recorded a remarkably high value of 80.8%; in the dry season, and a low value of (46.7%) in the rains, of the year, 2007. In this same year, the prevalence record in Cross River, was higher in the dry season than the rains (29.7% and 28.2%) respectively; although relatively low, with greater sample sizes. Many factors are responsible for the fluctuations in number and availability of infective nematodes. The key factors governing infective parasite populations are temperature and moisture in the external environment and flooding. Great Kwa River and Cross River exhibited fluctuations in temperature. Nematodes are capable of developing and maintaining populations of infective larvae over considerable ranges of temperature and moisture [25]. On the other hand parasites respond differently to the same environmental variables during different phases of their life cycle. This explains the inconsistency in the dry and rainy seasons in the infection of nematodes in *P. obscura*. Moravec and Scholz [26] studied the seasonal dynamics of some nematode species, *Rhabdochona hellichi* in its definitive host, the barbel (*Barbus barbus*) in the Jihlava River (the Danube basin), Czech Republic. The parasite occurred in barbel throughout the year (overall prevalence 93% and intensity of infection 1–1384 (mean 78) nematodes per fish, but there were distinct seasonal fluctuations in prevalence and mean intensity values associated with the parasite's seasonal cycle of maturation. The strictly seasonal maturation of this nematode may be associated with the temperature regime in the locality and seasonal cycles of maturation of its intermediate hosts.
Although, there was an obvious decrease in prevalence and mean intensity values in the dry season of 2008, in both sites. Environmental factors such as temperature, salinity, pH and dissolved oxygen are the main factors influencing the distribution of nematodes in tropical mangroves as well as estuarine habitat. Temperature is considered to be an important factor controlling the nematode abundance and distribution in the present study.

5. Conclusion
This study has established incidence of nematode infection in *Parachanna obscura* of the lower Cross River system. The intensity of infection is low and does not pose a threat to its aquaculture. There is no significant difference in infection amongst the various size classes. In aquaculture enclosure with good water quality the rate of infection can be depressed or totally eradicated. We strongly recommend snakehead, *Parachanna obscura* of the lower Cross River system as aquaculture species in view of its hardiness and apparent ability to resist infection by helminthes.

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