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Protozoan Parasites of *Chrysichthys nigrodigitatus* (Lacepede: 1803) in the Mid-Cross River Flood System, South Eastern Nigeria

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Abstract
The presence, classes and level of protozoan parasite infection in *Chrysichthys nigrodigitatus* from mid-Cross River flood system, Southeastern Nigeria was determined. Analysis was carried out using Wet Mount Method and Light Microscopy. A total of 300 *C. nigrodigitatus* comprising of 155 males and 145 females were examined for protozoan parasites infection, 98 (32.7%) were not infected, 202 (67.3%) were infected with protozoan parasites. This study reviews that *Ichthyobodo necator* (36.4%) was the most abundant found in *C. nigrodigitatus*, *Cryptobia iubilans* (29.0%), *Eimeria chrysichthyii* (21.3%), *Piscinoodinium pillulare* (5.0%), *Chloromyxum auratum* (3.3%), *Chilodonella uncinata* (1.8%), *Hexamita intestinalis* (1.8%) and *Encephalitozoon intestinalis* (1.5%) were found in *C. nigrodigitatus*. Among the body parts of the sampled fishes, the gills had the highest parasitic load 43.2%. Female fishes had significantly more parasites than male fishes. The prevalence rate is alarming and calls for proper fish management in terms of preventing serious outbreaks.

1. Introduction

C. nigrodigitatus represents one of the most imperiled groups of animals and exhibits some of the highest rate of extinction. Freshwater fish in developing countries of Asia and Africa are more at risk of extinction than in other developing countries (Araoye, 1999 and Craig, 2000). Yanong (2002), highlighted in his work one of such factors contributing to the decline of *C. nigrodigitatus*, which is the negative impact of parasites on host-growth and survival has been demonstrated in several parasite-fish host system in both aquaculture and in natural population. Piscine parasites cause profound pathological changes which lowers the growth rhythm considerably and affect the quality of the fish and often leads to death of the fish which results in enormous economic losses to the fish industries (Geets and Ollevier, 1996). Klinger and Francis-Floyd (2002), pointed out that some piscine parasites such as protozoa are transmissible to man and other fish-eating domestic and non-domestic animals. Some fish parasites would develop in humans if the fish is eaten raw, but none would be harmful if the fish is thoroughly cooked. Most fish especially in the wild population are likely to be infected with parasites, but in the great majority of cases, no significant harm to the host may be identified, thus there are only few reports of parasites causing mortality or serious damage to the fish population, but this may be largely because such effects go unnoticed. All fish carry pathogens and parasites.

Usually, this is at some cost to the fish. If the cost is sufficiently high, then the impacts can be characterized as a Disease. However, disease in fish is not well understood (Enayat, 2011). Differential symptoms of parasites by the consumption of raw or slightly preserved fish such as: - sashimi, sushi, gravalax; all have gastrointestinal symptoms such as: - vomiting, diarrhea, abdominal pain, nausea. More than 70 species of protozoa and helminth parasites can reach humans by food and water that is, through the consumption of sea and/or fresh water foods such as fishes, mollusks, frogs, tadpoles, camarons, crayfishes (Ray Sahelian, 2012). According to Dykova and Lom (1981), protozoa are a vast assemblage of eukaryotic organisms and that most of the commonly encountered piscine parasites are protozoa, which with practice, are the easiest to identify and control. In general, protozoa are one of the major sectors of fish parasites that have been long neglected because of its inherently difficulty in studying compared to other larger parasites. Among protozoa, ecto and endoparasitic protozoa occupy a very important sector has one of the hazardous threat to fish health. Many protozoa parasites in fish are considered to be commensals; however, pathogenic protozoa such as *Trichodina sp* are known to cause mortality in both wild and cultured fish species. For instance, a heavy infection of *Trichodina sp* has been reported to cause mortality in Sphareroides (Obiekezie, 1983). Mortality due to protozoa infection arises as a result of severe alteration of morphology and physiology of the fish. Thus this study seeks to provide information on the protozoan parasites of *C. nigrodigitatus* for the proper management of the bagrid fishery in terms of parasite infection.

2. Materials and Methods

2.1. Study Area

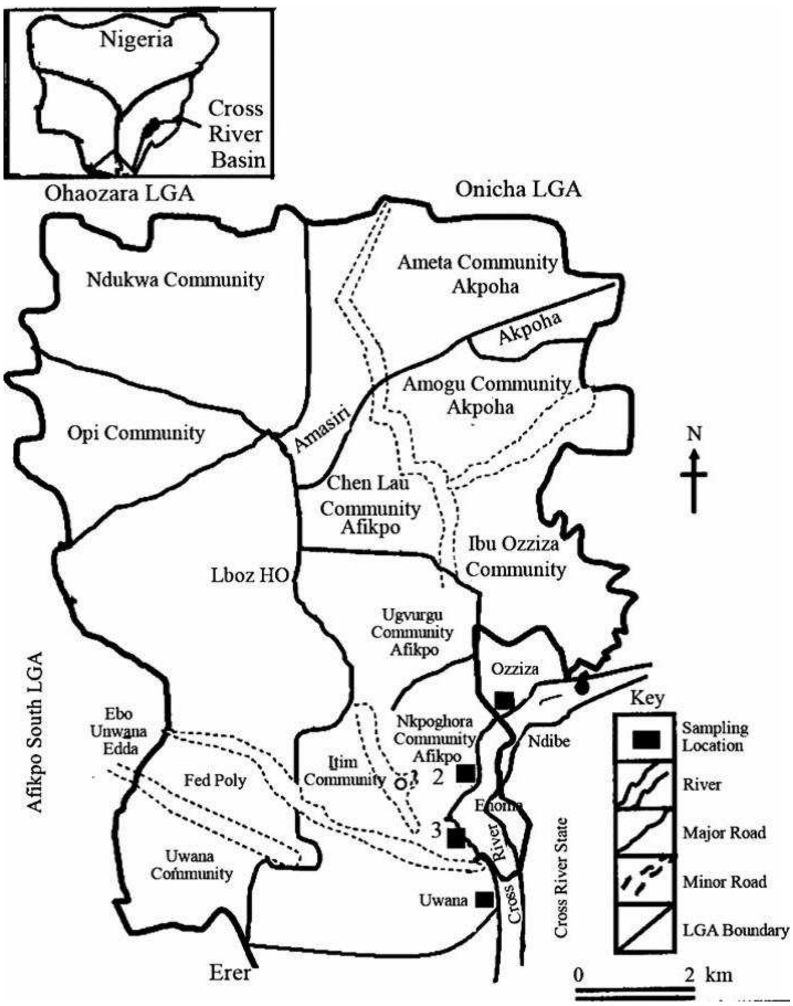

Fig. 1. *Map of Afikpo North Local Government Area showing the sampling locations in the Cross river flood system (Okoh et al., 2007).*

The study area is mid-Cross River flood system which lies between $5^0 57$” latitude $5^0 30$’20” North and$7^0 58$” longitude $5^0 30$’20” East (Okoh*et al*., 2007). The river system covers an area of 54000km^2, with 14000km^2 in Cameroun and 39000km^2 in Nigeria (Moses 1979; Lowenberg and Kunzel, 1992; King, 1996). Fish samples were collected at a landing point (Ndibe beach) within the Mid-Cross River flood system. The ecological zone in this area is rainforest. Along the banks of the mid-Cross River flood system, patches of riparian vegetation are evident. This vegetation serves as breeding grounds for a wide variety of animals like fishes, reptile, amphibians, insects and microorganisms. The riparian vegetation plays a role in natural purification of the river system due to the presence of tall leave that filter debris from the river, thus keeping the river system relatively transparent. The climate is humid and is defined by a dry season and a rainy season. The river overflows its banks during the rainy season, but decreases in volume as the dry season approaches.

2.2. Sampling Techniques

The river system contains several species of freshwater fishes of different families. The specimens were identified using images from websites such as; - www.fishbase.se; www.aquqmonster.org; www.planetcatfish.com and identification keys by Olaseobikan and Raji (1998).

2.3. Laboratory Analysis

The fish samples were placed dorso-ventrally on the dissecting board. Incision was made using the scalpel blade from the dissecting kit to expose and remove the stomach and entrails of the fish for the parasite examination. The stomach and entrails were placed in Petri dishes and preserved with ethanol to prevent the decay of the visceral organs. The gills of the fish sample were dissected with the scalpel blade, placed in Petri dishes and preserved in ethanol for parasite examination.

2.4. Method of Parasite Examination

The stomach and entrails of each specimen were aspirated into Petri dishes containing physiological saline solution. Two drops of the solution were mounted on the glass slide and smeared with the wire loop. Cover slips were placed over the smear. The glass slide were placed on a Light Microscope and observed using the objective lens for endoparasites. The gills of each fish specimen were placed in Petri dishes containing 10ml of physiological saline, later removed and smeared on a glass slide using a wire loop with 1-2 drops of physiological saline solution added and the smear was viewed with a Light Microscope. The parasites were identified by making their sketches as observed with the microscope and compared with pictorial guide on fish parasites by Poudere*t al,* (2005); Durborow (2003); Klinger and Francis-Floyd (1987). The parasites observed on the Light Microscope were counted and recorded.

3. Results

Result of the 300 samples, 145 females (48.33%) and 155 males (51.67%) of *C.nigrodigitatus*from wild used for the study are as shown in Table 1. Out of the 300 samples used, 98 (32.67%) were not infected by protozoan parasites, while 202 (67.33%) of the samples were infected with protozoan parasites and are observed to harbour a total of 456 protozoan parasites. Out of the 98 non-infected fish samples, 61 (62.24%) were males and 37 (37.76%) were females. Out of the 202 infected fish samples, 94 (46.35%) were males and 108 (53.47%) were females from the wild (Table 1). Infected samples of *C. nigrodigitatus*with weight (1-100g) were 37 (12.3%) and those with weight (101-700g) were 165 (55.0%) (Table 2). Among the parasites found on the parts of the specimen, *Ichthyobodo necator* (*pyriformis*) was the most abundant 161 (35.5%), followed by *Cryptobia iubilans* 132 (28.9%), *Eimeria chrysichthyii* 102 (22.3%), *Piscinoodinium pillulare* 23 (5.0%), *Chloromyxum auratum* 15 (3.3%), *Chilodonella uncinata* 8 (1.8%), *Hexamita intestinalis* 8 (1.8%) and *Encephalitozoon intestinalis* 7 (1.5%). It was observed that the gills had the highest parasitic load of protozoan parasites 192 (42.1%) followed by the stomach 139 (30.5%) and the intestine 125 (27.4%) (Table 3) (Fig. 2). Results of the percentage prevalence of protozoan parasites based on phyla are as shown in Table 4. The results show that parasites of the phylum Euglenozoa are most abundant in *C. nigrodigitatus* while Microspora is the least abundant phylum.

Table 1. *Infection rate in relation to the sex of C. nigrodigitatus.*

Sex	Number Examined	Number Infected	Percentage Infected (%)
Male	155	94	31.3
Female	145	108	36.0
Total	300	202	67.3

P = 0.05

Table 2. *Infection rate in relation to the weight (g) of C. nigrodigitatus.*

Weight (g)	Number Examined	Number Infected	Percentage Infected (%)
1-100	80	37	12.3
100-200	110	88	29.3
200-300	10	6	2.0
300-400	35	37	12.3
400-500	45	28	9.3
500-600	10	4	1.3
600-700	10	2	0.7
Total	300	202	67.3

P = 0.05

Table 3. *Parasite location and percentage prevalence in relation to the sex of C. nigrodigitatus.*

Parasites	Location of isolated parasite	Prevalence (%)		
		Male	Female	Total
Piscinoodinium pillulare	Gills	8(4.1)	15(5.7)	23(5.0)
Ichthyobodo necator	Gills	68(35.2)	93(35.4)	161(35.3)
Chilodonella uncinata	Gills	0(0.0)	8(3.0)	8(1.8)
Encephalitozoon intestinalis	Stomach	1(0.5)	6(2.3)	7(1.5)
Cryptobia iubilans	Stomach	59(30.6)	73(27.8)	132(28.9)
Hexamita intestinalis	Intestine	8(4.1)	0(0.0)	8(1.8)
Chloromyxum auratum	Intestine	4 (2.1)	11 (4.2)	15 (3.3)
Eimeria chrysichthyii	Intestine	45(23.3)	57(21.7)	102(22.3)
Total		193	263	456

Table 4. *Prevalence of protozoan parasites based on phyla.*

Phylum	Prevalence (%)
Myxozoa	3.3
Dinoflagellata	5.0
Apicomplexa	21.3
Euglenozoa	65.4
Metamonada	1.8
Microspora	1.5
Ciliophora	1.8

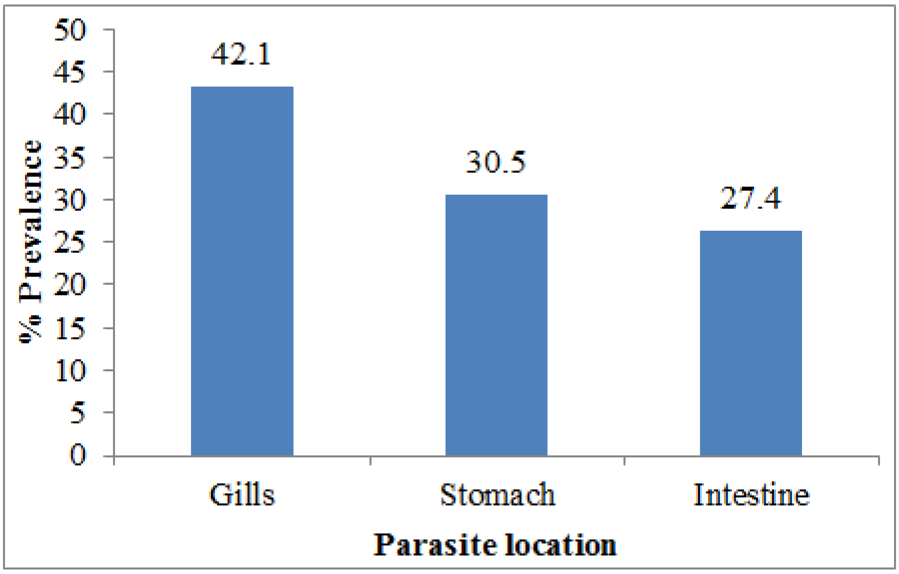

Fig. 2. *Percentage prevalence of parasites on various locations in C. nigrodigitatus.*

4. Discussion

Of the three hundred (300) samples of *C. nigrodigitatus*, two hundred and two (202) samples were infected with different species of protozoan parasites.Female samples were more infected than the male samples. *C. nigrodigitatus* samples with larger weight (101-700g) had more parasites than those with smaller weight (1-100g). Different kinds of protozoan parasites were observed to be present on different locations of *C. nigrodigitatus*. *Ichthyobodo necator, Chilodonella uncinata, Piscinoodinium pillulare* occurred on the gills where chronic infections of the fish were observed, *Eimeria chrysichthyii* and *Chloromyxum auratum* were found on the intestine while *Encephalitozoon intestinalis and Cryptobia iubilans* were found on the stomach. Gills were also observed to harbour the highest number of protozoan parasites. This observation agrees with the works of Omeji*et al.,* (2011), who reported that the highest load of protozoan parasites in the gills of *Clarias gariepinus*. This could be because the gills are the center of filter feeding and are the sites of gaseous exchange. This observation agrees with the reported works of Emere and Egbe (2006), who reported highest load of protozoan parasites in the gill of *Synodontis clarias* and Nyaku *et al*. (2007) who reported highest load of protozoan parasites in the gills of *Auchenoglanis ocidentalis*, *Oreochromis niloticus*, and *Bagrus bayad* of River Benue, Nigeria. Investigation by Woo (1987) and Woo (2003) had shown the gills to be infected by different protozoan parasites.

According to Somerville (1984), the sieving ability of the gill rakers may help to trap some organisms, and this could be attributed to the presence of the protozoan parasites there. The heavy load of parasites on the gills relative to other parts of the body impaired the gills from functioning well as an organ of respiration, hence could result to death. This agrees with the reported works of Borg (1960), Omoniyi*et al*. (2002), and Rahman*et al*. (2002). *Cryptobia iubilans* infected the stomach of *C. nigrodogitatus*. This observation agrees with the work of Omeji *et al* (2011) who reported that *Cryptobia iubilans* infected only the intestine and stomach of *Clarias gariepinus*. Omeji *et al* (2011) reported in contrast that the occurrence of *Cryptobia iubilans* in the intestine to be greater than in the stomach either might be due to the presence of digested food present in the intestine or due to the greater surface area presented by the intestine. In agreement, the observation made in this study, reveals greater number of *Cryptobia iubilans* in the stomach than in the intestine. Francis-Floyd (2011) reported that large numbers of *Cryptobia iubilans* in the stomach were responsible for extreme weight loss in African Cichlids. According to Dykova and Lom (2007), histopathological lesion infections are confined primarily in the stomach ranging from isolated granulomas to diffuse granulomatous gastritis in cichlids. Bigger fishes were observed to have higher rate of protozoan parasites than the smaller ones in this study. This might be because the bigger ones cover wider areas in search of food. As a result, they take in more food than the smaller ones, and this exposed them more to infection by parasites. In addition, they are omnivorous and feed on anything that comes their ways (Yanong*et al.,* 2004). Emere and Egbe (2006) had made similar observation in *S. clarias*. In this study, female fishes had greater rate of protozoan parasite infestations than the male counterparts. This might be connected to the physiological state of the females. Most gravid females could have had reduced resistance to infection by parasites. In addition, their increased rate of food intake to meet their food requirements for the development of their egg might have exposed them to more contact with the parasites, which subsequently increased their chance of being infected. Wickins and Marcfarlene (1973) relate it to availability of more food which possibly harbours the infective stage. Aken'Ova, (1999) is of the view that higher infection during the raining season may be due to increase in host population due to spawning, abundance of food and increase host activity in larger volume of water hence increase encounter with the infective agent. In conclusion the present result shows significantly high infection in *C. nigrodigitatus*by protozoan parasites. This could be as a result of the indiscriminate use of the river-basin. Such uses include; Domestic activities such as; bathing along the course of the river-basin, washing and dumping of refuse in the water body. These activities could reduce the resistance of *C. nigrodigitatus*to parasite infestation and diseases, as a result of the destruction of habitat and breeding ground. The prevalence rate is alarming and calls for proper fish management in terms of preventing serious outbreaks. With the increase interest in aquaculture, it is vital to have facilities and services for diagnosis, treatment and control of fish diseases. Various strategies that could be employed in the conservation and management of *C. nigrodigitatus* include: closed fishing seasons, habitat restoration and enhancement, implementation of fishing laws such as; Inland Fisheries Act, Endanger Species Act, public enlightenment and education about conservation. Also, the settlement located around the study area is rural. Great progress in the conservation of *C. nigrodigitatus* will be achieved if the government sees the intense necessity in developing the area.

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