A STUDY OF PARASITIC INFECTIONS OF *CLARIAS GARIEPINUS* IN NATURAL WATERS OF OWERRI, IMO STATE, NIGERIA

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ABSTRACT

The major objective of the study was to identify parasitic infections afflicting the fish species *Clarias gariepinus* in two natural water systems, namely, *Otamiri* and *Nworie* rivers, in Owerri, Imo State of Nigeria. The study is located in the Southeastern zone of Nigeria between the latitudes 5° 29'N and 7° 2'N and longitudes 7^0 28'E and 8^0 35'E. A total of ninety (90) fish samples from both rivers were examined for parasitic infections. Purposive sampling was used in the process. Male fish were 46 while female were 44. After parasitological studies involving the use of direct wet mount microscopy and stained smears, parasites were isolated from sixty three fish samples, giving a prevalence rate of 70.0%. The following parasites were recovered from the samples examined - ecto and endo-parasitic protozoa, nematodes, trematodes, and acanthocephalans. Four classes of protozoans - Ciliata, Sarcodina, Flagellata, and Sporozoans, were represented in the findings. The ecto-parasitic ciliates recovered were of the genera: *Trichodina, Chilodonella*, *Tetrahymena* and *Ambiphyra species.*The Acanthocephalans include the eggs of Neoechinorhynchus, adult Moniliformis moniliformis and Paragorgorhynchus species isolated from the intestines.No *Annelida* and *Cestoda* were found.

Keywords: Parasites, infections, *Clarias gariepinus,* fresh water habitat.

INTRODUCTION

The Food and Agriculture Organisation (FAO, 2006), describes the *Clarias gariepinus* as a large size African catfish. Viveen et al (1986) find that taxonomical and biological examination of the African catfish group revealed that *Clarias lazera* and *Clarias gariepinus* so long considered as two separate species, were the same and hence, they came under the same scientific name *Clarias gariepinus,* as confirmed by Janssen (1987, cited in Adam, 2004). *Clarias gariepinus* are freshwater fish found in the tropical regions of West Africa. *Clarias* species of the family Clariidae is commonly called the "Mudfish" and apart from tilapia, is the most cultured fish species in Nigeria. The demand for the fish is very high due to its oily flesh. They spawn naturally in captivity and their induction is easy (Olufayo, 2009).

Fishbase (2007), outlining the Biology of *Clarias gariepinus*, describes the fish as occurring mainly in quiet waters, lakes and pools but may occur in fast flowing rivers and in rapids. The fish is widely tolerant of extreme environmental conditions.

Known as sharptooth catfish in aquaculture, it is a highly recommended food fish in Africa, marketed fresh and frozen, and eaten broiled, fried and baked.

Studies show that the production of *Clarias batrachus* in Thailand and *Clarias gariepinus* in Zambia yielded a standing crop up to 65 to 100 tons /ha, with feed conversion rates of up to 1.05 in experimental least cost diets containing 38 percent crude protein. The global production of catfish as food fish was estimated to be about 320,000 metric tons in 1992 (Losordo et al,1998).

Clarias gariepinus has a high number of gill rakers varying from 24 to 110, the number increasing with the size of the fish; these gill rakers are long, slender and closely set. Two colour patterns can be discerned: the uniform and the marbled pattern . In the uniform pattern, the dorsal surface and the flanks of the body and the dorsal parts of the pectoral and the pelvic fins are generally dark greyish-greenish black, while the belly and the ventral parts of the paired fins are lightly coloured. In the marbled pattern, the specimens show irregular dark blotches on a light coloured

background above and laterally; the belly and the ventral parts of the paired fins are whitish.

Its feeding habit shows it to be a bottom feeder which occasionally feeds at the surface, forages at night on a wide variety of prey, and feeds on insects, plankton, invertebrates and fish but also takes young birds, rotting flesh and plants. It migrates to rivers and temporary streams to spawn and may be caught with dragnets. During intra-specific aggressive interactions, this species was noted to generate electric organ discharges that were monophasic, head-positive and lasting from 5-260 ms (Fishbase, 2007).

The fish species, like other animals, have their own diseases

The problem

Generally, diseases of fish are often over-simplified, which in turn leads to misunderstandings about diagnosis and treatments. This is quite unfortunate, for fish disease can be both highly distressing and in many cases, costly both in treatment and replacement costs. This situation is more so in fishponds, often characterized by their high stocking levels and reliance on biological filtration for good water quality.

Like any animal, fish are susceptible to a range of problems such as tumours, heart and other organ disease, as well as metabolic disorders such as diabetes. However, the overwhelming majority of common health problems involve external parasites, fungus, gill and bacterial infections. According to Fishdoc (2008), any body of water, be it a tank or pond will be teeming with millions of opportunistic bacterial and nearly all fish carry small populations of parasites.

Frances-Floyd (2002) defines fish disease as an abnormal condition characterized by a gradual degeneration of a fish's ability to maintain normal physiologic functions. It is a condition in which the fish is not "in balance" with itself or its environment.

There are two broad categories of disease that affect fish, infectious and non-infectious diseases. Infectious diseases are caused by pathogenic organisms present in the environment or carried by other fish. They are contagious diseases, and some type of treatment may be necessary to control the disease outbreak. In contrast, non-infectious diseases are caused by environmental problems, nutritional deficiencies, or genetic anomalies; they are not contagious and usually cannot be cured by medications.

Infectious diseases are broadly categorized as parasitic, bacterial, viral, or fungal (Frances-Floyd, 2002).

Parasitic diseases of fish are most frequently caused by small microscopic organisms called protozoa which live in the aquatic environment. There are a variety of protozoans which infest the gills and skin of fish causing irritation, weight loss, and eventually death. Most protozoan infections are relatively easy to control using standard fishery chemicals such as copper sulfate, formalin, or potassium permanganate.

Most of the commonly encountered fish parasites are protozoans. Protozoans are single-celled organisms, many of which are free-living in the aquatic environment. Typically, no intermediate host is required for the parasite to reproduce (direct life cycle). Consequently, they can build up to very high numbers when fish are crowded, as is common in culture systems, causing weight loss, debilitation, and mortality.The five most common groups of protozoans are given by Klinger and Francis-Floyd (2002) as: ciliates, flagellates, myxozoans, microsporidians, and coccidians. Parasitic protozoans in the latter three groups can be difficult or impossible to control relative to the former two.

A list of parasitic diseases commonly associated with *Clarias gariepinus* has been given by Fishbase (2007) as follows : *Trichodinosis, Piscicola Infestation (Piscicola sp.) Sanguinicola Disease, Fish Louse Infestation, ProcamallanusInfestation, Quadriacanthus.Infection, Dolops Infestation, Chilodonella Infection, Henneguya infection, Phyllodistomum, Neodactylogyrus, Gyrodactylus.*

MATERIALS AND METHODS

Study area

This study was carried out in Owerri, Imo State, which is located in the South Eastern zone of Nigeria between the latitudes 5° 29'N and 7° 2'N and longitude 7^0 28'E and 8^0 35'E. Specifically, fish samples were collected from the Otamiri and Nworie rivers, in Owerri, Southeastern Nigeria .

The Otamiri River, from its source to its confluence at Emeabiam with the *Uramiriukwa River,* is 30km (Okorie and Acholonu, 2008).

Its watershed covers about 10,000 km^2 with annual rainfall 2250 - 2500 mm. The watershed is mostly covered by depleted rainforest vegetation,

with mean temperatures of 27 °C throughout the year.

Nworie River is a first order stream that runs about a 9.2 km course emptying into the Otamiri River, at Nekede. Nworie River is potentially vulnerable to a variety of polluting influences. According to Okorie and Acholonu (2008), all through its course, there is a steady input of large quantities of detergents from laundry activities.

Test samples

Samples used for this study were adult fishes. A total of ninety (90) fish samples from both rivers were examined for parasitic infections. These fishes varied in sex and habitat. Male fish were 46 while female were 44.

Test reagents and media

The reagents were physiological saline and parasitological stains: Lugol's iodine, Methylene blue, Giemsa and Leishman's stains.

Collection of samples

The fish samples were collected by means of hooks, scoop nets, hand fishnets and cast nets by hired fishermen, into transparent plastic buckets containing water samples from the respective habitat of each fish sample. The buckets were covered with loose plastic covers and transported to the research laboratory where they were examined.

Sterilization of materials

Commercially sterilized surgical blades, cotton wool, gauze and disposable petri dishes were used. The dissecting tools, forceps, dissection boards, test tubes and other materials used for this study were sterilized using the method of the World Health Organisation (WHO) (1991) and Obiajuru and Ozumba (2009). Bench tops and working environments were sterilized with disinfectant and 75% alcohol. Hands were washed intermittently, sterile disposable hand gloves and disposable face - masks were worn to ensure aseptic measures.

Parasitological studies

The samples were examined parasitologically using microscopic techniques (direct wet mounts using physiological saline, Lugol's iodine and methylene blue preparations as well as stained smears using Leishman and Giemsa staining methods) as in Arene (2006) and Obiajuru and Ozumba (2009).

Direct wet mount microscopy

Direct wet smear of each sample was made on grease – free slides using 2 drops each of physiological saline, Lugol's iodine and methylene blue respectively on each slide. The wet smears were covered with coverslips and examined microscopically from edge to edge using low power (x10) and dry high power (x40) objectives.

Stained smears

Each sample was homogenized and duplicate thick films were made on grease – free slides. The films were allowed to air – dry and fixed with absolute methanol. One set was stained by Leishman's staining method and the other set was stained by Giemsa staining method as in Arene (2006) and Obiajuru and Ozumba (2009). They were allowed to drain and dry in the air and examined systematically from edge to edge using oil immersion objective.

Identification of parasites

Cysts and trophozoites of protozoa seen under the microscope were compared with micrographs on standard parasitological atlas for identification purposes as in Chiodini et al (2003), with the aid of expert parasitologists.

Measure of fish length

The age of fish was determined by taking measurement of their lengths and weights. This is the Petersen method elaborated by Bagenal and Tesch (1978).

The total length (TL), and the weight (WT) were the major parameters used to categorise the fish samples.

The total length of fish was obtained using a graduated board. Samples were measured in centimeters beginning from the tip of the snout to the tip of caudal fin. All the samples with a TL range of 15cm and above were regarded as adults. Incidentally, all sampled fish fell within this range.

Fish weights were measured by use of spring balance for large fish and chemical balance for small fish. The average weight was then determined per fish.

Again, all fish in the sample fell into weight range of 250g and above to qualify for classification as adults.

Determination of sex of fish

The sexing of the fish samples was done manually pressing on the genitalia under the lower abdominal region. As pressure is applied, eggs from gravid females extruded through the genital opening confirming them to be matured females. Sex determination was confirmed further by dissection. Ovarian tissues were cut open to expose the

gonads. The males did not release eggs upon application of pressure, and their sex status was equally confirmed on dissection.

Data analysis

The analytical techniques applie to raw data generated from laboratory examination were done using the computational software known as *MINITAB 14*. Results were presented using tables and charts produced from *Microsoft Excel 2007* version.

Results

The parasites isolated from fish samples were from different *phyla* -Protozoa, Nematoda, Annelida, Platyhelminthes and Acanthocephala. A total of 90 fish specimen were examined. Of these, 63 were infected with parasites, giving a prevalence rate of 70.0%. Table 1 gives the general prevalence.

Table 1:Prevalence of parasites in fish samples

Clarias gariepinus	
No. of samples examined	No. of samples infected
90	63 (70.0%)

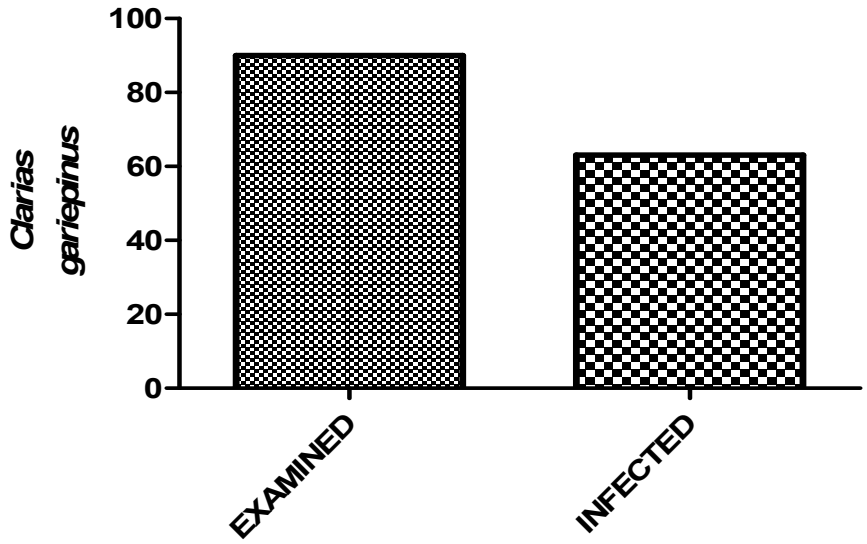

Fig. 1: Bar chart showing examined and infected *C. gariepinus* sample

The protozoans include the ecto-parasitic protozoans of the genus *Trichodina, Tetrahymena, Entamoeba, Ichthyobodo, Chilodonella, Piscinodinium ,Ambiphyra* and endo-parasitic protozoans of the genus *Protoopalina*, *Hexamita, Microsporidia, Balantidium*. The Nematodes include the round worms, *Cucullanus barbi*, *Procamallanus,Camallanus, and Capillaria* species.

The Trematodes include the sporocysts of *Diplostomatid trematode* and sporocyst.

The Acanthocephalans include the eggs of Neoechinorhynchus, adult Moniliformis moniliformis and Paragorgorhynchus species isolated from the intestines.No *Annelida* and *Cestoda* were found.

As already mentioned, the following parasites were recovered from the samples examined - ecto and endo-parasitic protozoa, nematodes, trematodes, and acanthocephalans. Four classes of protozoans were represented: Ciliata, Sarcodina, Flagellata, and Sporozoans. The ecto-parasitic ciliates recovered were of the genera: *Trichodina, Chilodonella*, *Tetrahymena* and *Ambiphyra species.*

For the genus *Chilodonella*, the cilia were arranged in several parallel rows on the concave ventral surface. The macronucleus is round, and the ciliaton is incomplete. The cytostome is distinct. *Chilodonella* is ovoid or kidney-shaped, dorso-ventrally flattened and appears translucent.

The genus *Trichodina* is cap-shaped, with ventrally located ring of denticles as an attachment organelle. This recorded the highest infection. For *Tetrahymena*, the cytostome is distinct. Ciliation is complete and macronucleus is rounded.The genus *Ambiphyra*, previosely called Scyphidia, is a sedentary ciliate protozoa found on the gills and skin of host fish. They are cylindrical in shape and have a row of oval and middle cilia.

For the class sarcodina (Amoebida), the genus recovered is *Entamoeba* species. Neither flagella or cilia were present. They have 'amoeboid' body shape, having pseudopodia for movement. For the class flagellata (*Mastigophora*) they possess one or more flagella. The genus *Ichthyobodo* show a tear-drop shape appearance. They possess flagella for movement. The *Ichthyobodo* are found on the gills, fins and skin of fish . The genus *Piscinoodnium* (sporozoa) were found on the skin and gills of fish samples. They are oval in shape, with amber colour. No organ of locomotion was found (not free-moving).

The endoparasitic protozoans implicated in disease causation in this study include the genus *Protoopalina* which are spindle-shaped (rounded at one end and tapered at the other end). They are ciliates found in the intestine of fish samples. *Opalinids* are apparently non-pathogenic.

The genus Hexamita is a flagellate isolated from the intestine of fish samples. They are pathogenic to fish, having been implicated in causing fish kidney disease. They possess three pairs of flagellum and a pair of eye spots. The genus *Microsporidia* is a sporozoa. They are typically cytozoic parasites. The pathological examination of histological sections of suspected hypertrophic tissues revealed cells containing spores. The spores were extremely small.

The nematode worms isolated from fish samples include the following genera, *Camallanus, Cuculanus, Procamallanus* and *Capillaria.* Nematoda (round worm) were very distinct in shape. They are large and visible to the naked eye, being more noticeable than other endoparasites. *Camallanus spp* were isolated from the intestinal tract. They have smooth, cylindrical and elongated body. They are reddish, thread-like worms. The capillaria are smooth, elongated worms.

Discussion and conclusion

According to Francis- Floyd (2005), parasitic diseases of fish are most frequently caused by small microscopic organisms called protozoa, which live in the aquatic environment. A variety of protozoans are known to be in existence, which generates infections in the gills and skin of fish, with resultant irritation, loss of weight and fish mortalities eventually. Rather fortunately, most protozoan infections are characterized by the fact that they are, relatively, not so difficult to control. Standard fishery chemicals like formalin, copper sulphate, or potassium permanganate have functioned as effective antidote against fish parasitism (Francis – Floyd, 2005; Okpokwasili and Ogbulie, 2001).

The following parasites were recovered from the samples examined - ecto and endo-parasitic protozoa, nematodes, and trematodes. Four classes of protozoans were represented: Ciliata, Sarcodina, Flagellata, and Sporozoans. Fishdoc (2007) states that ectoparasites such as flukes, *Trichodina*, white-spot etc can be causative to skin or gills irritation. These findings associating ecto-parasites with the organs skin and gills corroborate the findings in this study.

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