Characterization of Nematode Infestation on Parachanna obscura (Gunther, 1861) (Channidae) and Infection in the Blood

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Abstract

Parachanna obscura commands high food value and quite critical in the control of tilapia population in ponds. The health status of fish is assured upon proper diagnosis of the blood parameters. Research on fifty P. obscura from Great Kwa River, Nigeria investigated, showed 24 (48%) were infested with nematode and infected the blood while 26 (52%) were not. The mean blood Pack cell volume (PCV), Lymphocyte, Monocyte, Leucocyte, Neutrophils, and Eosinophils for infected(non-infested) P. obscura respectively were 20.8% (21.6%); 44.4% (39.3%); 1.9% (1.92%); 4.18x10^3 mm^-3 (4.48x10^3 mm^-3), 51.7% (57.5%). and 1.58% (1.2%). No positive correlation existed between infested and non-infested. The lower PCV, Leucocyte, and Neutrophils are indications of lower immunity. Prevalence rate increased with size from 26% (22-26.9cm), 56.5% (27-31.9cm) to 66.7% at class range 32-36.9cm, while Incidence of infestation decreased with increase in length, 2.75, 2.25 and 2.0 respectively for 22-26.9cm, 27-31.9cm and 32-36.9cm. The class 12-16.9cm did not follow any pattern. The parasitic burden occurred at the weaker/more vulnerable part-anal pore (61.7%), followed by fish intestine (29.8%), cardiac (4.3%) and caecum (4.3%). The mean physicochemical parameters of the study area across the three tidal intervals ranged from 5.11±0.04-6.72±0.00 for pH, conductivity 18.70±0.20µS-37.95±1.05 µS, dissolved oxygen 2.60±0.00 mg/l- 4.00±0.60 mg/l, temperature 25.50±0.50°C-27.00±0.00°C, salinity 0.01±0.00mg/l-0.03±0.01mg/l and total dissolved solid (TDS) 23.19±0.25mg/l- 47.06±1.30mg/l.

Introduction

Parachanna obscura popularly known as snakehead belongs to the family channidae and is commonly found in natural freshwater/swamp of the southern part of Cross River State. It inhabits lowland rivers, streams, creeks, swamps, canals, lakes and ponds (Teugels, Breine & Thys van der Audernaede, 1984; Guseva, 1990; Teugels, Reid & King, (1992). This species of fish constitutes a highly cherished member of the fish community in Cross River State, Nigeria and Bangladesh with superb taste and high-quality flesh which commands a high market value (Ama-Abasi & Anthony, 2010; Akther, Hakkani, Moni, & Mohanta, 2018). In fish culture, P. obscura exhibits an enormous potential in Tilapia sp. population control being a voracious predator (Bassey & Ajah, 2010). According to Satheeshkumar, Ananthan, Senthil, Kumar, and Jagadeesan, (2010); Pradhan, Patra, and Pal, (2014), the use of haematological parameters in diagnosing the state of
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health of natural fish is gaining worldwide acceptance as a tool in the management of fish both in the natural and artificial environment. Changes in haematological parameters due to infection by parasites such as nematodes are one of the indices of the ill health of the fish (Clauss, Alistair, & Arnold, 2008; Adeyemo, Okwilagwe, & Ajani., 2009). The parasitic form of nematodes lives in the host gut, blood (e.g. Wuchereria bancrofti, Loa loa, Onchorcercia volvolus and Dracunculus sp. as well as other filarial hosts), tissue nematodes (Trichurius spirasis), intestinal nematodes (T. trichuria), and lymph vessels most of which are macroscopic (Conver, Spadling, & Forester, 2003). The parasitic infection of fish leads to economic losses due to not only mortality but also treatment costs, decreasing growth that reduces the expansion of aquaculture (Akther, Hakkani, Ashaduzzaman & Mohanta, 2017; Gado, Mahfouz, Moustafa, & Lolo, 2017; Oladipo, Sunday & Ogunbiyi, 2019). Diseases caused by parasites make fish culturing a high-risk investment (Kumari & Perven, 2017). Stress in fish may be induced by both biotic and environmental factors (Labello, Saunder, & Crawford, 2001; Witeska, 2005; Ahmed & Sheikh, 2020). Ajah and Ukutt (2018 a, b) observed that water soluble fraction from crude oil can stress a fish and lead to alteration of the haemotological characteristics of starved or fed catfish. This study therefore analysis the blood of infested and non-infested P. obscura as a tool to determine its state of health and the aquacultural implications following Kori-Siakpere and Egbor (1997; Kori-Siakpere, Ake, & Idoge, 2005).

Materials and Methods

Study Area

Fifty life specimens of the African Snakehead, Parachanna obscura were obtained from the Great Kwa River (a tidal river) within the Esuk Mba axis of the Akpabuyo Local Government Area of Cross River State, Nigeria. The Great Kwa River covers a distance of 60 km in length and an average of 1.66 km in width. It lies along latitude 8.22°N and Longitude 4.45°E.

Physicochemical Parameters of Esuk Mba Water for the Three Tidal Cycles

The mean results from analyses of different water quality parameters carried out in the study area at different tidal intervals are as follows: the mean pH ranged between 5.11±0.04 to 6.72±0.00, Conductivity 18.70±0.20µS and 37.95±1.05µS, Dissolved Oxygen 2.60±0.00mg/l to 4.00±0.60mg/l, water temperature range from 25.50±0.50°C to 27.00±0.00°C, Salinity level wasn’t lower than 0.01±0.00mg/l nor higher than 0.03±0.01mg/l and Total dissolved solid (TDS) ranged between 47.06±1.30mg/l and 23.19±0.25mg/l across the tidal intervals.

Fish Samples

Fifty (50) samples of P. obscura (Figure 1) were caught by means of fish trap and put in aerated containers for onward transportation to the Zoology and Environmental Biology Laboratory, University of Calabar, Nigeria. The samples were stored in two reinforced plastic water tanks of 200l capacity each for a minimum of two (2) weeks following Gupta and Banerjee (2011). During this period, they became adjusted to the laboratory conditions and were fed with artificial (pelleted) feeds and ground shrimps obtained locally to avoid possible distortion of the haematological parameters due to effect of starvation. The fish were weighed using Kitchen scale (Model: Ashton Meyers Scale 0.1 g - 5 kg) and recorded in grams (g). A metre rule was used to measure the length in centimeters (cm).

Figure 1. Acclimatization of Parachanna obscura in the tank

Table 1. Prevalence and incidence of Nematode infestation in Parachanna obscura

<table>
<thead>
<tr>
<th>Length class</th>
<th>Sample size (%)</th>
<th>P. obscura infested (%)</th>
<th>Nematodes in Infested P. obscura</th>
<th>Incidence of infestation</th>
<th>Prevalence per class (%) /Total population (%)</th>
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<td>32 – 36.9</td>
<td>6(12)</td>
<td>4(16.7)</td>
<td>9</td>
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from the snout to the caudal fin. In similar experiment, Jerônimo, et al., (2014) used 60 fish, 30 per season to investigate the haematological and histopathological parameters in South American fish *Piaractus mesopotamicus* parasitized by monogenean (Dactylogyridae) while Mazumder, Fivelstad, Ghaffar, and Das (2019) analyzed theirs using 40 blood specimens of juvenile snapper fish (*Lutjanus molabaricus*).

### Blood Collection

The fish was anaesthetized with MS-222 (Imanpoor, Bagheri & Hedayati, 2010) before the fish belly was placed upward. Blood samples were obtained from the lamellae around the gill arch with the aid of a heparinized 2 cm³ disposable plastic syringes (Figure 2) and 21-gauge disposable hypodermic needle as described by Lavanya, Ramesh, Kavitha, and Malarvizhi (2011). The use of the blood plastic syringe is a necessary precaution with fish blood because contact with glass results in decreased coagulation (Smith Lewis & Kaplan, 1952). The site chosen for puncture was thoroughly wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted directly to where the gills originate from and was gently aspirated during penetration (Figure 2). It was then pushed gently down until blood started to enter as the needle punctured the blood vessel. Blood was taken until about 2ml has been obtained. Thereafter, the needle would be withdrawn, and the blood gently transferred in heparinized specimen (EDTA) bottles (Lavanya, et al., 2011) shown as Figure 3 and 4 to act as anti-coagulant; the samples were then mixed gently but thoroughly. Some blood samples were used for the measurement of packed cell volume (PCV), white blood cell count (WBC), Erythrocyte count and Leucocytes count.

### Blood Analysis

Red blood cells (RBC) count was done on blood samples appropriately diluted with Hayem’s fluid in RBC diluting pipette. The method described by Lewis and Ward (1975) was adopted. A 1:20 blood sample dilution was discharged into the improved Neubauer chamber.
and viewed with a light microscope. The cells were counted using x40 magnification with observation of the margin rule.

White blood cells (WBC) count was done in a manner similar to RBC count. Blood samples were diluted (1:20) with Turk’s fluid and introduced into the chamber and counted. The numbers of cells/ mm³ were obtained by multiplying by 50 (Dacie & Lewis, 1984).

The capillary tube was 2/3 filled with anti-coagulated blood and spun at 12,000 g for 5 minutes using micro-haematocrit centrifuge. The PCV was read using micro-haematocrit reader as a percentage following Adebayo, Fagbenro, Ajayi, and Popoola (2007).

0.02ml of sample was placed in Sahli’s tube (90 – 140 g/dl) holding 0.1N HCL in its 1in unit mark and allowed to stand for 5 minutes. The brown precipitate acid-haematin was developed to match the unfading standard colour (Dacie & Lewis, 1984). The volume of the solution in the graduated Sahli’s tube was then converted to haemoglobin concentration in g/dl. The haematological indices included the following; mean packed cell volume (PCV), mean leucocyte, mean Eosinophills, mean Neutrophills calculated from the equations given by Anderson and Klontz (1965).

Statistical Analysis

The following parameters were calculated following Margolis, Esch, Holmes, Kuris and Schad (1982) as adopted by Akther, et al (2017):

- Prevalence per size group;
  \[
  \frac{\text{No. of host infested in the class}}{\text{Total No. of host in the class}} \times 100
  \]

- Prevalence per population;
  \[
  \frac{\text{No. of parasites in the class}}{\text{Total No. of host examined}} \times 100
  \]

- Incidence of infestation;
  \[
  \frac{\text{Number of parasites}}{\text{Number in the host class infested}}
  \]

- Condition factor (CF);
  \[
  \frac{100P}{L^3}
  \]

  where: P is weight (g), L is length (cm)

IBM SPSS Statistics 20 software package was used to (1) compare the means using ANOVA and test of linearity to determine the level of homogeneity and skewness at α = 0.05 level test, and (2) determine the coefficient of correlation of parameters between infested and non-infested *Parachanna obscura* at 0.05 and 0.01 level of significant.

\[
\text{Co-efficient of correlation } r = \frac{\sum(x-x)(y-y)v\sum(x-x)^2\sum(y-y)^2}{\sum(x-x)^2\sum(y-y)^2}
\]

Results and Discussion

A total of fifty (50) fishes were examined and the size ranges taken as well as the frequencies of occurrence in relation to size variation are as shown in Table 1. The specimens were divided into five (5) size groups ranging from 12 cm to 37 cm. There was a preponderance of specimens with standard length ranging from 22 cm to 32 cm over others accounting for 88 % frequency of the total population of specimens examined (Table 2).

All size groups were examined for nematode infestation and a total of twenty-four or 48% of the *Parachanna* were infested while twenty-six or 52% were non-infested as shown in table 3. Percentage of
Table 3. Combined Correlations coefficients of the haematological parameters between infested and non-infested *P. Obscura*

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*Correlation is significant at the 0.05 level (2-tailed). &nbsp;Infested and 2 non-infested.

**Correlation is significant at the 0.01 level (2-tailed)."
incidence of infestation was 60% with the size groups ranging from 17cm to 21.9cm.

The size groups 17-21.9cm, 22-26.9cm, 27-31.9cm and 32-36.9 cm respectively had an average of 1.33, 2.75, 2.25 and 2.0 nematodes per fish. Oden, Ama-Abasi and Ndome (2015) observed 12 worms per fish in the dry season and 6 worms per fish in the wet seasons as variations in incidence of nematode parasite on snakehead of the lower Cross River with no variation with size. In the present study, the parasitic load was on the weakest or most vulnerable part (anal pore having 61.7% of nematodes) followed by the intestine (29.8%) while cardiac and caecum had 4.3% each which is opposed to Oden, et. al., (2015), Moravec (2000) and Ibiwoye, et al., (2006) who rather had the highest occurrence at the gut though they never examined the anal region. The World Book Encyclopedia (1994) reported that researchers have found more than 90,000 nematodes in a single rotting apple which goes to confirm why the concentration around the anus (smelling region). The incidence of parasitism decreased with size of Parachanna with 22-26.9cm having 2.75 followed by 27-31.9cm having 2.25 and then 32-36.9cm with 2.0. Invariable no defined pattern was followed because at 12-16.9cm length group the incidence was as low as 1.33. Conversely, the incidence of parasitic infestation may increase or decrease with size since other factors like nutrition, age and environment (presence of other parasites, water temperature and pollution, etc) could increase or lower the parasitic load.

The highest prevalence rate (66.7%) of infestation in Parachanna obscura was obtained with the length class (32-36.9cm) followed by 60% for length class (17-21.9cm) while the length class (22-26.9cm) had the least prevalence rate of 26.7% as shown in table 1 and 2. Length-class-wise, three out of every five Parachanna of the length size 17-21.9cm were infested, 4 out 15, 13 out of 23, and 4 out of 6, respectively, at 22-26.9cm, 27-31.9cm and 32-36.9cm group were infested with nematodes. Prevalence based on population referred to the number of nematodes in a particular length group over the total Parachanna population (50). Thus prevalence did not follow any regular pattern with length. Akther, Hakkani, Ashaduzzaman and Mohanta (2017) stated that prevalence of infection of parasite vary considerably depend on seasons, environment and management practices. Both Gado, Mahfouz, Moustafa and Lolo (2017) and Khan, Khartoon, Muhammad and Shafi (2019) observed that prevalence of some ecto-parasitic diseases in African catfish and fish Johnius dussumieri were also seasonally dependent. Gado, et al., (2017) had 15.5% and 15% as highest prevalence of infestation of ectoparasites on Clarias gariepinus in both autumn and winter and spring and summer periods respectively while Akther. et al., (2017) had prevalence rates ranging from 4.5% in the stomach to 48.04% on the skin and monthly prevalence range from 50% to 92.30% on Channa punctatus. Oladipo, et al., (2019) and Sorour and Hamouda (2019) got varying prevalent rates ranging from 11% and 60.32% on Gnathonemus senegalensis (Trunkfish) and 0.55 to 100% on C. gariepinus by different ecto- and endo-parasites.

Infected blood of Parachanna obscura revealed oval, penial shape and Bi-concave Red cells. The Red cells appeared in clusters and the Lymphocytes were not normal, while the Non-infested blood of P. obscura, both the. Red cell and the Lymphocyte appeared normal.
The results of haematological characteristics of Parachanna obscura are as shown in Table 3. The value of pack cell volume (PCV %) was higher for non-infested than infested P. obscura. It ranged from 12 to 33% and 17 to 31% for infested and non-infested respectively. The mean total PCV were 20.84 and 21.6% for infected and non-infested. This reduced value on the infected portends negative signal to the aquaculturist who should guard against any further loss of blood by employing some chemotherapeutic measures (Obiekezie, & Ajah, 1994).

The mean lymphocyte ranged from 21 to 65% and 21 to 76% for infected and non-infected Parachanna obscura respectively. The overall mean lymphocyte for infested and non-infested P. obscura was 44.38% to 39.3% respectively. Such sudden rise from 39.3% to 44.38% is an indication that the fish is fighting an infection which causes the lymphocytes to multiply rapidly thereby producing characteristic swelling of the lymph. At this point the aquaculture candidate is at the risk of survival or at the least loses in economic value. The value on mean monocyte ranged from 0 to 6% for non-infested and 0 to 5% for infested P. obscura with overall mean of 1.9% and 1.92% for non-infested and infested respectively. Monocytes as a type of leucocytes (white blood cells) that help to build immunity in fish. The slightly lower level in the infected shows that the fish’s immunity level is being compromised and calls for urgent attention. The mean Eosinophils was very low and ranged from 0 to 7% in both infected and non-infested P. obscura. The overall mean Eosinophils was 1.58% for infected and 1.2% for non-infested. The increase in level of Eosinophil being one of the combatants against parasitic infection could most likely be to cub the infection rate in Parachanna. The value of mean Neutrophils ranged from 24 to 78% for infested P. obscura. as against 33 to 76% for non-infested. The overall mean Neutrophils were 57.5% and 51.67% for non-infested and infested P. obscura respectively. The mean Leucocyte \( (x^{10^9/mm^3})\) was 4.18 for infected and 4.48 for non-infested P. obscura with a range of 3.4 – 5.4 for infected and 2.4 to 6.2 for non-infested P. obscura. The lower the neutrophilic value, the lower the immunity level.

The mean condition factor (k) was higher as the fish length increased with infestation being (0.71) slightly lower than that of the non-infested fishes (0.82) (Table 4). The lower condition factor shows reduced immunity Nazeef and Abubakar (2013) opined that k below one is not good. Coincidentally, the infected had k below one showing reduced wellness probably due to parasitic infection.

The test of means using anova amongst the infested showed positive linearity only between Lymphocyte and Eosinophils \( (\chi^2_{5}=22.002; \ 6.279)\) and between Leucocytes and Monocytes \( (\chi^2_{5}=0.075; \ 3.620)\). Deviations from linearity were 0.353 and 0.530 respectively. The rest did show any form of homogeneity. Besides, none of the pairs showed any positively significant \( (P<0.05)\) skewness - change or alteration in any particular direction.

Table 3 shows the correlation coefficient between all the haematological parameters in both the infested and non-infested Parachanna. None of the parameters showed any positive significant difference between infested and non-infested. However, when one parameter in either infested was compared to non-infested, Leucocyte in infested had moderate positively \( (P<0.05)\) significant correlation with infested Neutrophils while PCV2 vs Lym2 had mild positive significant correlation \( (P<0.05)\). Parameters with mild negative significant correlation at 0.05 level (2 tailed test) included Leu2 vs Neu2, Leu2 vs Lym2, Leu1 vs Lym1, PCV1 vs Lym1, PCV2 vs Neu2, Mono1 vs Mono2 Lym1 vs Eosino1. At \( \alpha = 0.01 \) and 0.001 only the following had very strong negative significant correlation-Neu1 vs Lym1, Neu2 vs lym1, and Neu2 vs Lym2. The positive correlation implies that as one parameter goes up the other rises whereas with the negative correlation means that a decrease in a parameter results to commensurate decrease in the value of the other. Highly positive significant correlation \( (P<0.01; \ 0.9255)\) existed between length and weight of P. obscura (Table 5) which was very high for infected than non-infected Parachanna obscura. Correlation between length and condition factor \( (k)\) was negative (Table 2). Other correlations, that is, between Length and Leucocyte, Length and PCV, Weight and Leucocytes were positive but very poorly correlated. This study agrees with Boon, Zuxu and. Boom (1990) that parasitic infections reduced the PCV and Leucocyte values. This work also corroborates with Adebayo, et. al., (2007) where the Leucocytes value showed a negative correlation with PCV. Intensification of infestation and hence higher degree of infections evidenced by lower pcv, leucocytes and neutrophils, will invariably, not only reduce the immunity but will also lower the economic values of the aquaculture candidate.

The blood analysis of infected and non-infected Parachanna obscura presented in table 3 based on the 50 fish samples showed a slight variation in their haematological values. These variations may be attributed to the presence of parasites (nematodes). In some of these specimens it was observed that those fish harbouring nematodes weighed more than the non-infested ones and the incidence of infestation was highest for length classes 22cm to 32cm probably due to increase in size of the specimens. The abundance of these length classes may be attributed to the selectivity of the gear being used especially in the swamps/tributaries where the species were more abundant. Garcia, Echevarrin, Martinez, and Zamora, (1992), opined that fish size can cause variation in haematological values.

The effects of nematode infestation in Parachanna obscura were pronounced in parameters
such as Pack cell volume (PCV), Lymphocyte, Monocytes, Eosinophills, Leucocytes and Neutrophills. It has been observed that the PCV was lower in infested (20.8%) than non-infested (21.6%) specimens and tend to increase with length and weight. The parasitic effects of nematode on fish can result in anaemic condition in the fish. This situation was a reverse of Leucocytes which were higher in infested (44.38%) than non-infested (39.3%) P. obscura. The present study established the fact that as the level of parasitic infestation increased, the PCV reduced and caused variation in blood index values. Besides, the previous studies by Lavanya, et al., (2011); Pradhan, Patra, Mohanty, and Pal (2012); Pradhan, Patra, and Pal (2014); and George, Akinrotimi, and Nwokoma (2017) attributed such variations to factors such as age, fish size, nutritional state, season (Pradhan, et al., 2012), spawning, sex (Collazos, Ortega, Barriga, & Rodriguez, 1998), malnutrition (Ighwela, Ahmad, & Abolmunafi, 2012), environmental stress (Singh & Tandon, 2009; Ahmed & Sheikh, 2020), and genetic variation in different fish species, parasitic infestation is hereby implicated.

The presence of Neutrophills caused more serious stress in the infested (51.7%) than non-infested (57.5%) Parachanna obscura because it provided the fish with defense against invading nematodes, a process known as chemotaxis. The Leucocyte was lower in infested (4.18 x 10^6/mm^3) than non-infested (4.48 x 10^6/mm^3) P. obscura. This is understood because the leucocytes in infested P. obscura are always engaged in defending the fish against foreign bodies and in the process may be destroyed. George, et al., (2017) observed that the introduction of two pesticides - atrazine and metolachlor to Clarias gariepinus resulted in significantly higher positive difference over the control in white blood cell (WBC), neutrophils, monocytes, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC). Jerónimo, et al., (2014), stated that hematological alterations might be related to physiological characteristics as well as disease or culture conditions. They added that in the host-parasite relationship, the severity in health alterations might be associated to attachment mechanisms of the parasite, its life cycle and mainly, the number of parasites.

**Conclusion**

The haematological parameters - Pack cell volume (%), Leucocyte (%), Monocyte (%), Eosinophills and Neutrophills (%) values were lower in the infested while the Lymphocyte (%) was higher in the non-infected P. obscura specimens obtained from Great Kwa Rivers, Cross River estuary depicting the extent of damage parasitic nematode had on P. obscura. More parasites constituting, 61.7% and 29.8%, respectively, were found in the fish anal pore and intestine whereas only 4.3% were each found in the cardiac and caecum region. This study creates awareness of the probable negative impact parasite infestation could have on the fish industry, hence the obvious need to administer prophylactic treatment or chemotherapeutic when already manifested. Prevalence and incidence of infestation moved in opposite direction in P. obscura. The immune system of Parachanna were affected negatively due to parasitic infestation exemplified by the reduced values amongst key parameters like Neutrophils, leucocytes and PCV etc in infested compared with non-infested. The aquaculture implication of such reduction is low immunity and economic losses to the fish farmer.

**References**


