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# Prevalence of Henneguya Chrysichthys (Flagellated Protozoa: Cyst) and Haematological Changes Due to the Infection in Chrysichthys Nigrodigitatus *(Pp. 124-134)*

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**Abstract**

*Four Hundred (400) samples of Chrysichthys nigrodigitatus were examined for Henneguya chrysichthys using methods described for gills examination and haemopatholgy. 135 (33.8%), Chrysichthys nigrodigitatus were infected by Henneguya chrysichthys. The infection was seen on the gill filament as an oval cyst somewhat white and visible to the naked eyes. 27 (20%), 88 (65.2%) and 20 (14.8%) were recorded for low, moderate and high infection respectively. Lowest (22.7%) and highest (45.5%) prevalence were observed in the months of December and May respectively. Prevalence was higher in wet season (54.1%) than in dry season (45.9,%) More female fish (51.1%) had infection than the male fish (48.8%).Haematological examination revealed changes in blood cell count, erythrocyte sedimentary rate and Haematocrit counts. Hemoglobin (per 100ml) remains the same in infected and uninfected fish. Erythrccyte count ($X10^6/mm^3$) was 1.28±1.11 and*

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*1.0±0.53 while leucocytes count (per mm$^3$) was 2.36±1.0 and 43200±0.60 for uninfected and infected fish respectively. Leucocytosis in infected fish was marked by lymphocytes (35.11±0.32), Neutrophils (26.32±1.65), plasmocytes (23.06±0.9 1) and monocytes (16.0±0.21). Corresponding values of these classes of Leucocytes are 7.38±0.69, 21,86 ± 1.71, 6.90± 1.12 and 7.24± 1.09 respectively in uninfected fish. Infected fish appeared weak and emaciated.*

**Keywords:** - Infected, uninfected, Prevalence, Haemotological, Leucocytosis

## Introduction

*Henneguya chrysichthys* is a flagellate protozoa that is parasitic in *Chrysichthy nigrodigitatus*. Many forms of protozoa infecting fish have been reported by Ama - Abasi and Obiekezie (2002) and Klinger and Francis-Floyed. (2002). They include *Icthyophthirus multifis, Chilodonella sp*, *Trichodina sp* and *Apiosoma sp*. Others are *Hexamita sp, Icthyobodo sp* and *Cryptobia sp.*

Many protozoa parasitic 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 fish. For instance, *Hexaminta sp* was reported by Obiekezie (1983) to cause malnutrition and emaciation, *Chilodonella sp* is responsible for respiratory and Osmoregulatory inbalance, *Myxobolus cerebralis* in brain of fish leads to whirling disease. The host brain cells at the point of invasion of this parasite, undergoes enormous hypertrophy forming xenoma which sometimes are several millimeters in diameter.

*Henneguya chrysichthys* was first reported by Obiekezie (1983) from Cross River Estuary. In contrast to the work of Casal *et al.,* (2002) on *Hennoquya friderici n. sp.* on the Amazonian teleostean fish*, Leporinus friderici* in which adult forms of the parasite were isolated, only the cyst of *Henneguya* where recovered from the gills of *Chrysichthys nigrodigitatus* by Obekezie (1983). The cysts were isolated from the proximal ends of the filamental rays of the fish. The bony materials of the rays and central cartilage were destroyed. A cross section of infected gills displayed perforation of filamental rays. No host reaction was observed. Cyst growth was described as synchronic following observation of mature cysts with young plasmodia. As many as 14 large cysts occurred along a single filament. The infection gave rise to leucocytosis and low Red blood corpuscles count.

Another flagellate infecting blood of fish is *Trypanosome. sp.*

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Woo (1995) reported increase number of leucocytes (Leucocytosis) and decrease number of erythrocytes and low hemoglobin content as symptoms of the infection in fish. Another pathobiology reported was increase in serum globulins. Pathological changes were related to parasite intensity or burden. Hypoglycemia and hypercholesterolemia were observed in fish with heavy *Trypanosome* burden.

The objective of this work is to observe seasonal prevalence of the parasite and the effects of this parasitic flagellate on the haematological parameters of the fish.

**Materials and Methods**

Cross River Estuary takes its rise from Cameroon Mountains and flows west ward into Nigeria, then Southward into Atlantic Ocean at the gulf of Guinea. It occupies an area of 54,000km$^2$ with 39000km of the area in Nigeria (Ama-Abasi and Holzlobner, 2002 and Akpan and Ofem, 1993). The Estuary is located approximately between latitude 4° and 3°N and Longitude 7° 30 and 10°E in the South East of Nigeria.

**Collection of Sample**

Four Hundred (400) samples of *Chrysichthys nigrodigitatus* were collected from Cross River Estuary and examined for *Henneguya* sp using methods described below.

**(A) Examination of Gills for *Henneguya sp***

Each fish was placed on a dissecting board. The opercula cavity was cut open with the aid of a pair of forceps and scissors. The gills were removed into Petri dish. Sections of gills were cut and observed under the microscope for parasites. Gills arches with parasites were excised and fixed in 10% formalin. The gill filaments were then scrapped into Petri dishes containing 4% formalin. Sediments were filtered out and smeared on micro slide and examined under microscope.

**(B) Blood Examination (Cheesbrough, 2005)**

Fish samples were immobilized by a sharp blow on their cranium. The first few drops of blood from several peduncles were discarded and later drops of blood collected for examination. Collected blood was allowed into 10ml test tubes internally coated with heparin as anticoagulant before the eventual assessment of the parameters. The heparin bottles were examined to ensure that there were no clots formed.

Fresh blood was also obtained from fish liver in two ways.

i. Severance of fish caudal peduncle

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ii. Directly from the heart through the soft tissue located below the gill region by means of syringe.

Thin blood films were immediately made, fixed in methylalcohol and later stained with Giemsa stain. Stained films were examined for parasites under the oil immersion objectives (XL 00) of the microscope.

Also a drop of whole blood was placed on a slide and allowed to clot, contraction of the clot left a circle of cleared serum into which parasites migrated. Slides were declared positive or negative after critical examination.

**(C) Packed Cell Volume (PCV)**

Haematocrit measurements (PCV) were made by means of commercially available non-heparinized capillary tubes (Hawskley and Sons Ltd, London) Microhaematocrit tubes were filled with fish blood by capillary actions to about ¾ full and one end was sealed with plasticine. Tubes were spun at 1500 r. p. m for 5 minutes using an haematocrit. Haematocrit was read off with a Hawsley microhaematocrit reader. The results were expressed as percentages of red cells in relation to the plasma in the tubes using a graduated scale and alternatively, using a microscope fitted with graduated Ocular micrometer.

**(D) Erythryocyte Sedimentation Rate (ESR)**

This parameter was determined using westergrene's sedimentation tube. A 4.1 dilution of fish blood was made with 3.8% sodium citrate solution (anticoagulant) and loaded in the westergren's tube.The tube was made to stand vertically by means of a clamp. The ESR was expressed as the distance the column of erythrocytes fell in the westergren's tubes per hour standing vertically.

**(E) Blood Cell Counts**

Neubauer ruled Chamber (Haemocytometer) was used in counting blood cells. The chamber grid was $9mm^2$ and depth of 0.1mm. Care was taken to ensure that no fluid over flowed into the moats bordering the chambers. An EDTA anticoagulated blood sample was taken in capillary Pasteur pipette. Any blood samples that showed the slightest sign of the presence of a clot was discarded. Counting was done immediately after diluting the pipette using a phase contrast microscope. All the blood cell types were easily recognized and counted at the same time using these fluids.

For the leucocyte counts, four large squares on the edges of the ruled ends compromising 16 squares in all were counted. Eighty (80) small squares in the central area were counted for the erythrocytes.

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**(F) Categorization of Infection**

Categorization of infection into low, moderate and high infection was done based on international standard as reported by Kliniger and Francis Floyed (2002). Parasite infection below 5 cysts per gill was taken as low infection. 5 — 9 cysts per gill were moderate and above nine (9) cysts per gill were categorized as high infection.

**(G) Parasite Intensity**

Parasite intensity/Burden was taken as the mean number of cycts on gill of *Chrysichthys nigrodigitatus.*

**Results**

*Henneguya chrysichthys* was observed as an oval cyst somewhat white in colour and visible to the naked eye on the filamental rays of the fish (Plate:1) 135 (33.8%) of the 400 samples examined were infected by *Henneguya.* Lowest (22.7%) and highest (45.5%) monthly prevalence were observed in the months of December and May respectively (Table 1). 27(20.0%) 88(65.2%) and 20(14.8%) of all observed cases were low, moderate and high cases respectively (Tables 2, 3 and 4) 73 (54.1%) and 62(45.9%) were recorded in wet and dry season respectively. While 69(51.1%) and 66(48.9) cases were constituted by female and male fish respectively.

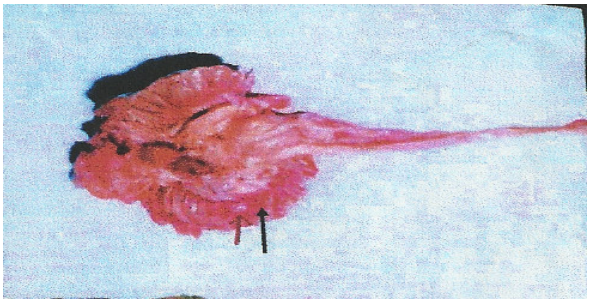

Plate 1: Gill of *Chrysichthys nigrodigitatus* infected by *Henneguya chrysichthys*
Arrows point to cysts of *Henneguya chrysichthys* on gill filaments of *Chrysichthys nigrodigitatus*

In the low infection category length class 11-20cm and 21 – 30cm had zero prevalence. Lowest 4(5.0%) prevalence was observed in length class 31 - 40cm while highest prevalence (26.7%) in this category was observed in length class 71 — 80cm. Parasite intensity/burden (mean number of cysts per gill of fish) decreased with increasing fish length throughout the three (3) categories of infections (Tables 2, 3 and 4) Intensity ranged of 1-4, 6-8 and 11-14 cysts per gill in the low, moderate and high infection. Generally parasites intensity was highest during wet season months.

Zero prevalence was also recorded for moderate infection category at 11 — 20cm and 21 — 30cm lengths. 13.8% and 88.0% prevalence were recorded for 31 - 40cm and 61 — 70cm lengths as lowest and highest prevalence respectively (Table 3) All fish from 11cm to 40cm had no infection in the high infection category. 5.0% and 28.0% prevalence were recorded at 41 .50cm and 61 — 70cm lengths as lowest and highest prevalence respectively (Table 4)

**Haematological Changes**

Effect of *Henneguya chrysichthys* on blood parameters of *Chtysichthys nigrodigitatus* was observed in fish with parasites intensity above 11 parasites per field of view (X100 objective). Below this intensity no change in blood parameter was observed, Changes were observed in erythrocyte count, erythrocyte sedimentary rate, leucocytes count and haematocrit (PVC). No change was observed in haemoglobin concentration (Table 5).Leucocytosis in infected fish was marked by lymphocytes (35.11±0.32), Neutrophils (26.32±1.60), plasmocytes (23.06±O.91 )and monocytes (16.01±1.21).Corresponding values of these cIases of leucocytes in uninfected fish were 7.38±0.64, 21.86 ± 1 . 71, 6.90 ± 1 .12 and 7.24 ± 1.00 respectively. The fish appeared weak and emaciated.

**Effects of *Henneguya* on gills of *Chrysichthys***

Red blood cells were absent in gill areas surrounding the spots where *Henneguya* cysts were found .These spots appeared colourless or white with no gill filament.

**Discussion**

Observation of *Henneguya chrysichthys* in *Chrysichthys nigrodigitatus* agrees with the report of Obiekezie (1983) and Casal *et al*; (2002). 33.8% prevalence of the infection reported in this work is high and calls for examination of harvest before presentation of fish for public consumption. Higher Seasonal Prevalence (54.1%) Observed in wet season agrees with

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available literature on fish parasitism which always is in favour of wet season. During wet season salinity is considerably reduced along with increasing sewage (Asuquo and Offem, 1993). Low salinity encourages parasite survival and possibility of parasite infecting fish. Increase in parasite prevalence in fish along with increasing pollutants makes parasites, especially macro parasites, biomakers for pollution in aquatic environment (Schluderman *etal;* 2003).

Female fish had higher parasite prevalence then male fish irrespective of the category of infection. This may be as result of differences in feeding habit, relative abundance of the female fish and habitat selection tendencies of female fish especially the spawning sizes. However Thomas, (1964) had suggested testostesone, immunosuppression, Corticosteroid — based immune suppression and differences between the size and behaviour of sexes as reasons for higher female parasitism in trout.

Fish 11 — 30cm lengths were not infected by *Henneguya chrysichthys*. The reason may be their limited habitat exploitation tendency due to size and vulnerability to predators which often cause them to be confined to predator free areas in the sea. Most parasites were isolated in fish 31 — 90cm length. This group has fully developed locomotary appendages and can explore almost all niches in the estuary. Thus they are exposed to greater risk of parasite of infection.

Parasite' intensity decreased with increasing fish length. Overall observation showed higher intensity during wet season months. This suggested most parasites invading fish body during wet season. The length of fish is usually related to age of fish. Older fish are larger than young fish (Longhurst, 1966). Older fish are better adapted to aquatic environment and can detect areas of danger. Thus, where meals are contaminated, predators are numerous with low dissolve oxygen larger fish can easily detect these conditions and avoid such areas.

**The presence of *Henneguya Chrysichthys* on Gills of *Chrysicthys***
*Nigrodigitatus* resulted in lost of red blood cells in the gill region. Difference between Erythrocyte count for uninfected (1.28±11) and infected (1.0 ± 0.53) fish were found to be significant, using t-test at P> 0.05. Thus 4.69±0.66mm/hr and 5.98±0.71mm/hr were recorded as sedimentary rates respectively. Lost of blood cells resulted in emaciation and poor growth due to low oxygen supply to fish tissue for oxidation of ingested nutrients.

Excessive number of leucocytes (43200±0.60) observed in infected fish against that observed in uninfected fish. (2.36±1.0) is believed to be a defensive provision against the presence of *Henneguya* cysts. The defensive network can be explained in terms of functions of the different classes of leucocytes. Monocytes and neutrophils function in defence of the body. Sembuilding and monocytes are precursors of tissues phagocytes such as Kupfer cells which can engulf foreign bodies and digest them. Lymphocytes are responsible for development of cellular immunity (by T-lymphocytes) and humoral immunity (B- Lymphocytes). These together had no observable effect on the cyst due to large size of cyst.

The combined affects of erosion of gill filament where the cysts of *Henneguya chrysichthys* occurred on the gills of *Chrysichthys nigrodigitatus* was lost of blood erythrocytes resulting in low oxygen supply to tissues and organs of the fish, less nutrient distribution, leucocytosis and emaciation in infected fish. Fish presented for Publication consumption need to be regularly checked to ensure only high valued fish carcase are presented for public consumption. Also commercial fish farmer are encouraged to embark on regular examination of fish stock to reduce the effect of parasites which might lead to late spawning, low yield and financial lost.

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**Table 1:** Monthly prevalence and intensity of *Hennaguya chrysichthys* in *Chrysichthy nigrodigitatus*

| **Month** | **No fish Examined** | **No. of Fish infected** | **Monthly Intensity (Per/ml)** |
|---|---|---|---|
| January | 22 | 81(36.4) | 6 |
| February | 44 | 16 (36.4) | 6 |
| March | 45 | 12(26.6) | 6 |
| April | 44 | 14(32.8) | 7 |
| May | 44 | 20(45.5) | 7 |
| June | 46 | 14(30.4) | 8 |
| July | 44 | 15 (34.0) | 8 |
| August | 22 | 9(40.0) | 7 |
| September | 22 | 6(27.3) | 6 |
| October | 22 | 9 (40.9) | 7 |
| November | 23 | 7(30.4) | 7 |
| December | 22 | 5 (22.7) | 7 |
| **Total** | **400** | **135(33.8%)** | |

**Table 2:** Monthly prevalence and intensity of *Hennaguya chrysichthys* in *Chrysichthy nigrodigitatus at low infection*

| Length Class (cm) | No of fish examined | | | No (%prevalence of infected fish | | | Means intensity (per/ml) | Cond. factor |
|---|---|---|---|---|---|---|---|---|
| | M | F | Total | M | F | Overall no (% prev) | | |
| 11-20 | 40 | 70 | 110 | 0.(0.0) | 0(0.0) | 0(0.0) | - | 0.02 |
| 21-30 | 31 | 52 | 83 | 0(0.0) | 0(0.0) | 0(0.0) | - | 0.05 |
| 31-40 | 36 | 44 | 80 | 4.(11.1) | 0(0.0) | 4(50) | 4 | 0.02 |
| 41-50 | 18 | 22 | 40 | 6(33.3) | 2.(9.1) | 8(20.0) | 4 | 0.01 |
| 51-60 | 15 | 18 | 33 | 3(20.0) | 2(11.1) | 5(15.2) | 3 | 0.007 |
| 61-70 | 10 | 15 | 25 | 1(10.0) | 3(20.0) | 4(16.0) | 2 | 0.007 |
| 71-80 | 7 | 8 | 15 | 3.(42.9) | 1(12.5) | 4(26.7) | 2 | 0.005 |
| 81-90 | 5 | 9 | 14 | 2(40.0) | 0(0.0) | 2(14.3) | 1 | 0.05 |
| ∑ | 162 | 238 | 400 | 19(11.7) | 8(3.4) | 27(6.8) | 18 | 0.169 |

**Table 3:** Prevalence Intensity and condition factor of *Henneguya sp* in different length class of *Chrysichthys nigrodigitatus* at moderate infection

| Length Class (cm) | No of fish examined | | | No (%prevalence of infected fish | | | Means intensity (per/ml) | Cond. factor |
|---|---|---|---|---|---|---|---|---|
| | M | F | Total | M | F | Overall no (% prev) | | |
| 11-20 | 40 | 70 | 110 | 0.(0.0) | 0(0.0) | 0(0.0) | - | 0.05 |
| 21-30 | 31 | 52 | 83 | 0(0.0) | 0(0.0) | 0(0.0) | - | 0.05 |
| 31-40 | 36 | 44 | 80 | 9.(25.0) | 2(4.5) | 11(13.8) | 8 | 0.01 |
| 41-50 | 18 | 22 | 40 | 7(38.9) | 10(45.5) | 17(14.2) | 7 | 0.07 |
| 51-60 | 15 | 18 | 33 | 10(40.0) | 11(61.1) | 21(63.6) | 6 | 0.007 |
| 61-70 | 10 | 15 | 25 | 9(90.0) | 13(87.7) | 22(88.0) | 6 | 0.005 |
| 71-80 | 7 | 8 | 15 | 6.(85.7) | 5(62.2) | 11(73.3) | 6 | 0.005 |
| 81-90 | 5 | 9 | 14 | 2(40.0) | 4(44.4) | 6(42.9) | 7 | 0.145 |
| ∑ | 162 | 238 | 400 | 43(26.5) | 45(18.9) | 88(22.0) | | |

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**Table 4:** Prevalence Intensity and condition factor of *Henneguya sp* in different length class of *Chrysichthys nigrodigitatus* at High infection

| Length Class (cm) | No of fish examined | | | No (%prevalence of infected fish | | | Means intensity (per/ml) | Cond. factor |
|---|---|---|---|---|---|---|---|---|
| | M | F | Total | M | F | Overall no (% prev) | | |
| 11-20 | 40 | 70 | 110 | 0.(0.0) | 0(0.0) | 0(0.0) | - | 0.02 |
| 21-30 | 31 | 52 | 83 | 0(0.0) | 0(0.0) | 0(0.0) | - | 0.05 |
| 31-40 | 36 | 44 | 80 | 0.(0.0) | 0(0.0) | 0(0.0) | - | 0.02 |
| 41-50 | 18 | 22 | 40 | 2(11.1) | 0(0.0) | 2(5.0) | 14 | 0.01 |
| 51-60 | 15 | 18 | 33 | 0(0.0) | 6(33.3) | 6(18.2) | 13 | 0.007 |
| 61-70 | 10 | 15 | 25 | 1(10.0) | 6(40.0) | 7(28.0) | 10 | 0.007 |
| 71-80 | 7 | 8 | 15 | 1.(14.3) | 2(25.0) | 3(20.0) | 10 | 0.005 |
| 81-90 | 5 | 9 | 14 | 0(00.0) | 2(22.2) | 2(14.3) | 11 | 0.005 |
| ∑ | 162 | 238 | 400 | 4(2.5) | 16(6.7) | 20(5.0) | | |

**Table 5:** Haematological Changes Observed in *Chrysichthys nigrodigitatus* infected by *Hanneguya sp*

| **Blood Parameters** | | **Uninfected Fish** | **Infected Fish** |
|---|---|---|---|
| Erythrocyte (RBC) count ($x10^6/mm^3$) | | 1.28±1.1 | 1.0± 0.53 |
| Leucocyte (WBC) count (per $mm^3$) | | 2.36±1.0 | 43200±0.60 |
| Erythrocyte Sedimentary rate (E.S.R) (mm/hr) | | 4.69±0.66 | 5.98±0.71 |
| Haematocrit (Pcv) | | 40.71±1.0 | 32.50±1.1 |
| Diff. Leucocyte Count (%) | Lymphocytes | 7.38±0.69 | 35.11±0.32 |
| | Monocytes | 7.24±1.0 | 16.01±1.21 |
| | Neutrophils | 21.86±1.71 | 26.32±16.01 |
| | Eosinophils | 0.2±2.93 | 0.10±0.60 |
| | Plasmocytes | 6.90±1.12 | 23.06±0.91 |
| | Macrophage | 0.00±0.00 | 0.81±0.99 |

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