CONTRIBUTIONS OF HAEMATOLOGICAL FACTORS TO THE ESTIMATIONS OF *EUSTRONGYLIDES AFRICANUS* LARVAE DENSITIES *CLARIAS GARIEPINUS* AND *CLARIAS ANGUILARIS* FROM BIDA FLOODPLAIN OF NIGERIA

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Abstract
The contributions of haematological factors to the estimations of *Eustrongylides africanus* larvae densities in *Clarias gariepinus* and *C. anguillaris* of Bida floodploain ;of Nigeria were investigated. Five haematological factors (neutrophils, lymphocytes and eosinophils counts; MCH and MCV) having positive or negative correlation coefficient (r) between 0.50 and 0.85 contributed to the estimation of *E. africanus* larvae densities in the wild population of Claria species.

Key words: *Eustrongylides africanus* larvae, *Clarias* species, haematological factors, Bida floodplain.

Introduction
Disease aetiology is a triad complex (Snieszko, 1974), which includes the host (fish), the parasite (agent) and the micro- and macro- habitats (environment). Haematological characteristics provide useful indices of dietary sufficiency, pathological status and physiological response to environmental stress (Svobodova *et al.*, 1986; 1994) in addition to the definition of haematologicakl norms for a variety of teleosts used in aquaculture (Bhasar and Rao, 1990). Considerable efforts have been directed towards the development of standard procedures for the sampling and analysis of fish blood. Haematocrit, erythrocytes count and haemoglobin concentration are the most readily determined haematological parameters under the field and hatchery conditions (Bhasar and Rao, 1990). The micro-haematocrit represents the parameters most often studied perhaps because it is easily undertaken and interpreted. The haematocrit value is not easily altered as other parameters, and should be used in conjunction with erythrocyte and leucocytes count, hemoglobin contents, osmotic fragility and differential leucocytes count (Wedemeyer *et al.*, 1983). Hemoglobin determination, red blood cell count and haematocrit are recommended as check on the health of the stock (Anderson and Klontz, 1965).
Most of the several contributions towards a better understanding of fish haematology deal with marine species (Johnson, 1968). Scanty information available in the literature on the haematology of the Nigerian firewater fishes include those on *Chrysichthys nigrodigitatus* (Jacob, 1982); *Claria isheriensis* (Jacob, 1982; Siakpere, 1985); pond-raised *Claria gariepinus* (C.G), *Heterobranchus longifilis* (H.I), F1 hybrid (C.G X H.I) and *C. nigrodigitatus* (Erondu *et al.*, 1993); *Oreochromis niloticus* (Omoregie, 1998); *Hemochromis fasciatus*, *Chromidatilapia guntheri*, *Tilapia mariae* and *T. zilli* (Egwunyenga *et al.*, 1999); and *Cyprinus carpio*, *Claria gariepinus*, *Heterotis niloticus*, *Hemochromis fasciatus* and *Tilapia* species (Adedeji *et al.*, 2000) were related to helminths oarasites infestations. Thus, interactions brought about by the changes in the haematological parameter, fish and invertebrate host populations and helminthes parasites occurrence might not be understood. *Clarias* are highly priced and found all year round in market of Bida and its environs. The aim of this study therefore is to investigate the contributions of some haematological factors to the estimations of *Eustrongylides africanus* in *Clarias* species from Bida floodplain of Nigeria. This study will provide base line information on the contributions of haematological factors to the estimations of *Eustronggylides africanus* larvae densities in *Claria* species from Bida floodplain in Nigeria.

Material and Methods
Fish sampled were considered as normal or abnormal on the basis of their external appearance and on the presence absence of obvious sign of helminthes parasites infestation; killed in humane manner by a sudden gentle cervical dislocation or decapitation and thoroughly examined individually. The sites chosen for the cardiac puncture was about half an inch behind the apex of the 'V' formed ;by the gill covers and isthmus anatomical described by Klontz and Smith (1968) for adult fish. To avoid mucus and water, their surface were carefully wiped dry with tissues. The 2ml disposable sterile syringe with 21-G needle was inserted at right angle down until blood started to enter as the needle punctures the heart. Blood was taken under gentle aspiration until 0.5ml has been obtained, then the needle was withdrawn. After detaching the needle from the syringe, the blood was mixed well in a vial containing anticoagulant potassium salt of ethylene diamine tetra-acetic acid (EDTA) ,to give a final concentration of 5mg EDTA per ml of blood. The caudal peduncles (Klontz, 1972) severed for juvenile fish and the freely flowing blood was collected into dry containers containing 0.1g of ethylene diamine tetra-acetic acid disodium salt (EDTA). Thin blood smears were prepared for all samples, care being taken to prevent the entry of tissue fluid into the glass slides.
The erythrocytes were enumerated using the Hedrick's fluid (Smith and Halver, 1969) to introduce the suspension into an improved Neubaur ruling counting chamber ("Ecristallite", Hawkesley and Sons Ltd, London) and 1/5m2 counted by an ocular eyepiece micrometer. The collected blood was introduced into the counting chamber of the haemocytometer to enable the differentiation to be made between leucocytes; erythrocytes and thrombocytes count under the microscope at 100x objective using the

appropriate avian diluting fluids (Mulcahy, 1970) according to methods described by Blaxhall and Daisley (1973). Thin blood smears as for human were prepared for all samples, stained with Giemsa diluted one part in ten with buffer (Puchkov, 1964). Counting a total of 200 leucocytes, the relative numbers of the types (lymphocytes, neutrophils, eosinophils, basophils, monocytes and granulocytes) in the peripheral blood were recorded and the results expressed as a percentage of the white blood cell population. Typical blood smears prepared from each fish sample were stained using Leishman stain (in distilled water buffered at PH 6.8 as diluents); examined and the dimensions of fifty representative red and white blood cell chosen at random by means of an eyepiece (ocular) micrometer (Dacie and Lewis, 1975) to determine the respective average values. The well-mixed blood was drawn into commercially available heparinised microhaematocrit capillary tubes (Hawksley and Sons Ltd, London) filed up to 5/6th sealed with 'Critaseal' or plasticine on one end; spun down at 30,000rpm for 5 minutes as described by Weedemeyer and Yasutake (1997) in triplicate; reading made with a microhaematocrit reader and results expressed as percentage or volume of erythrocytes in relation to 100 units or millimeters of plasma in the tubes. The blood sample (0.2ml olaced into 4ml of Drabkina's reagent) thoroughly mixed by gentle inversion and allowed to stand for at least 10 minutes for full conversion of hemoglobin to cyanomethaemglobin (Levinson Macfat, 1981); the transmittance read on an EEL spectrophotometer at a wavelength of 5-10nm with a reference graph constructed with calculation of the hemoglobin content in gram per 100ml; midified for fish blood as described by Blaxhall and Daisley (1973). The haematological indices: mean corpuscular volume MCV, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) plasma referred to as to the "absolute values" were obtained for each fish sample according to the formulae given by Anderson and Klontz (1965), Delaney and Garratty (1969) and Wintrobe (1978).

Routine examinations were carried out on four hundred and eighty specimens of *Clarias gariepinus* and *C. anguillaris* of different sexes, lengths and weights. The specimens were randomly sampled from four fishing localities of Bida floodplain species to determine occurrence *Eustrongylides africanus* larvae in relation to sex and season of the year as described by Margolis et al (1982). Sample linear regression/correlation were carried out to examine any association among prevalence, mean intensity and abundance of *Eustrongylides africanus* larvae in *Clarias* species of Bida floodplain and the twelve harmatological factors [red cell blood cell (RBC) count (x1), RBC size (x2) and RBC nuclei size (x3); total white blood cell WBC count (x4); differential WBC count or distribution of RBC types: neutrophils (x5), lymphocytes (x6) and eosinophils (x7); hemoglobin estimate (x8); haremetocrit or packed cell volume measurement (x9); and haematological indices: MCV (x10), MCH (x11) and MCHC (x12)]

Results and Discussion

The correlation coefficients (r) twelve haematological factors (HFs) with occurrence *Eustrongylides africanus* larvae infections in *Clarias* species from Bida floodplain is shown in Table 1. Two, three and one out of the 39 combinations of the twelve haematological factors with known correlation coefficients ($r > \pm 0.50$) contributed respectively to the estimations of prevalence, mean intensity and abundance of *E. africanus* larvae densities in *Clarias* species (Table 2) were neutrophils, lymphocytes and eosinophils counts, MCV and MCH as follows:

Estimations for Prevalence

Y =- 0.24 x 19.5 (r =-0.57) with neutrophils count
Y = 0.22 x 80.1 (r =0.56) with lymcocytes count

When the prevalence is 0% the neutrophils counts is at its maximum of about 82%. Thus, for every 1% increases in prevalence the neutrophils count has a stepwise decrease of about 77%. The % neutrophils counts of the differential white blood cell count were negative correlated to the prevalence of *E. africanus* larvae in *Clarias* species. Thus the % neutrophils counts increases as the prevalence decreases, and vice versa. When the prevalence is 0% the lymphocytes count is at its minimum of about 35%. Thus for every 1% increase in the prevalence in the lymphocyte count has a stepwise to the prevalence of *E. africanus* larvae I *Clarias* species. Thus the % lymphocytes count increases as the prevalence, and vice versa. The two haematological factors that might be the most suitable to estimate the prevalence of africanus larvae in *Clarias* species are neutrophils and lymphocytes count in descending order of preference.

Estimations for Mean Intensity

Y = 0.65 + 0.06 (r = 0.59) with eosinophils count
Y = 13.2x = 65.40 (r = 0.57) with MCV
Y = -5.4x + 50.70 (r = -054) with MCH
Y = 0.65 + 0.06 (r = 0.59) with eosinophils count
Y = 13.2x = 65.40 (r = 0.57) with MCV
Y = -5.4x + 50.70 (r = -054) with MCH.

When the mean intensity is 0.0% the eosinophils count is at its minimum of about 0.1%. Thus, for every 1% increase in mean intensity the eosinophils count has a stepwise increase of about 1.5%. when the mean intensity is 0.0µ3 the MCV is at ist minimum of about 5µ3. Thus for every 1% increase in mean intensity the MCV has a stepwise increase of about 5µ3. When the mean intensity is 0.0µ2 g when the MCH is at maximum value of about 9.3µ2 g. Thus, for every 1% increase in mean intensity the MCH has a stepwise increase of about 9.3µ2 g. The eosinophils of the differential WBC count and MCV were positively correlated to the mean intensities of *E. africanus* larvae in *Clarias* species. Thus, the % eosinphilos count and MCV increase as the mean intensities of *E. africanus* larvae in *Clarias* species increase, and vice versa. The MCH was negatively correlated to the mean intensity of *E. africanus* larvae in *Clarias* species decreases and vice versa. the three haematological factors that might be most suitable to estimate the mean intensity of *E. africanus* larvae in *Clarias* species is MCH, MCV and % eosinphils counts in descending order of preference.

Estimations for Mean Abundance

Y = 19.4x + 82.0 (r = 0.50) with MCV

When the mean abundance is 0.0µ3 the MCV is at its minimum of about 4.2µ3 . The MCV is positively correlated with the mean abundance of *E. africanus* larvae in *Clarias* species. Thus MCV increases as the mean abundance of *E. africanus* larvae in *Clarias* species increases, and vice versa. The only haematological factors that might be suitable in estimate the mean abundance of *E. africanus* in *Clarias* species is MCV.

Conclusion

Many fish farmers, as well hatchery operators, often need to know the health status so as to make management decisions

like how weight gain' feed intake and utilization, and also administer the right dosage of medication. Reasonable skill in estimating parasitic infection density is necessary for fishery workers as it will frequently be necessary to estimate parasite density when facilities to measure haematyological factors are not readily available or their use are not practicable. This study has shown that haematological factors, as media of for proper physiological activities, could be used to estimate the density of parasitic infections in freshwater fishes. Thus, a simple device for quick and accurate estimate of the density of parasites in fishes could be derived for research and development purposes.

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