CONTRIBUTIONS OF HAEMATOLOGICAL FACTORS TO THE DISTRIBUTIONS AND ESTIMATIONS OF *EUSTRONGYLIDES AFRICANUS* LARVAE DENSITIES IN *CLARIAS GARIEPINUS* AND *C. ANGUILLARIS* FROM BIDA FLOODPLAIN OF NIGERIA.

[1] IBIWOYE T. I. I.; [2]BALOGUN, A.M.; [3]OGUNSUSI, R.A AND [1]AGBONTALE J.J.

[1]Aquatic Pathobiology Programme, National Institute for Freshwater Fisheries Research, P. M. B. 6006, New Bussa, Niger State, Nigeria.
[2]Department of Fisheries and Wildlife, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.
[3]Department of Animal Production and Health, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

ABSTRACT

The contributions of haematological factors to the distribution and estimations of Eustrongylides africanus larvae densities in Clarias gariepinus and C. anguillaris of Bida floodplain of Nigeria were documented for the first time. The haematological factors making the most important contributions to the distributions of E. africanus larvae infections in Clarias species are mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and neutrophils count, in descending order of magnitude; having the manifestations for the months of January, March, September and December of the year being closely related. 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 estimations of E. africanus larvae densities in the wild population of Clarias 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 haematological 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, haemoglobin contents, osmotic fragility and differential leucocytes count (Wedemeyer *et al.*, 1983). Haemoglobin determination, red blood cell counts 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 freshwater fishes include those on *Chrysichthys nigrodigitatus*

(Jacob, 1982); *Clarias isheriensis* (Jacob, 1982; Siakpere, 1985); pond-raised *Clarias gariepinus* (C.g), *Heterobranchus longifilis* (H.l), F_1 hybrid (C.g X H.l) 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, Clarias gariepinus, Heterotis niloticus, Hemochromis fasciatus and Tilapia* species (Adedeji *et al.*, 2000) were not related to helminths parasites infestations. Thus, interactions brought about by the changes in the haematological parameter, fish and invertebrate host populations and helminths parasites occurrence might not be understood. *Clarias* are highly priced and valued and also observed all round the year in market of Bida and its environs, therefore, arose the need to investigate the contributions of some haematological factors to the distributions and estimations of *Eustrongylides africanus* in *Clarias* species from Bida floodplain of Nigeria. However, this study provides the first record and report on the contributions of haematological factors to the distributions and estimations of *Eustrongylides africanus* larvae densities in *Clarias* species from Bida floodplain.

MATERIALS AND METHODS

Fish sampled were considered as normal or abnormal on the basis of their external appearance and on the presence absence of obvious signs of helminths 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 the anatomical landmarks described by Klontz and Smith (1968) for adult fish. To avoid mucus and water, their surfaces were carefully wiped dry with tissues. The 2ml disposable sterile syringe with 21-G needle was inserted at right angles to the surfaces of the fish and was slightly aspirated during penetration. It was then pushed gently 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 detatching 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 Neubauer ruling counting chamber ("Ecristallite", Hawkesley and Sons Ltd, London) and $1/5m^2$ 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 types 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) filled up to $5/6^{th}$ sealed with 'Critaseal' or plasticine on one end; spun down at 30,000 rpm for 5 minutes as described by Wedemeyer and Yasutake (1997) in triplicate; readings

made with a microhaematocrit reader and results expressed as percentage or volume of erythrocytes in relation to 100 units or millimetres of plasma in the tubes. The blood sample (0.02ml placed into 4ml of Drabkina's reagent) thoroughly mixed by gentle inversion and allowed to stand for at least 10 minutes for full conversion of haemoglobin to cyanomethaemoglobin (Levinson and Macfat, 1981): the transmittance read on an EEL spectrophotometer at a wavelength of 5–10nm with a reference graph constructed with commercially available artificial standards (British Drug Houses Ltd, Poole, England) for the calculation of the haemoglobin content in gram per 100ml, modified for fish blood as described by Blaxhall and Daisley (1973). The haematological indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) referred to as 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 on four hundred and eighty specimens of *Clarias gariepinus and C. anguillaris* of different sexes, lengths and weights randomly sampled from four fishing localities of Bida floodplain species sampled to determine occurrence of *Eustrongylides africanus* larvae in relation sex and season of the year as described by Margolis *et al.* (1982). Multiple linear regression/correlation analyses were carried out upon the co-ordinates of the first three principal components (PCs): To examine any associations among prevalence, mean intensity and abundance of *Eustrongylides africanus* larvae in *Clarias* species of Bida floodplain and the effects of the twelve haematological factors on their percentage (%) of traces (distributions) subjected to ordination of the months of the year. And, to determine the combined effects of the twelve haematological factors on the percentage (%) contribution of each of the haematological factors to the R^2 for each of the PCs subjected to the coefficient of multiple determinations (R^2). Simple linear regression/correlation analyses were carried out to examine any associations among prevalence, mean intensity and abundance of *Eustrongylides africanus* larvae in *Clarias* species of Bida floodplain and the twelve haematological factors [red blood cell (RBC) count (x_1), RBC size (x_2) and RBC nuclei size (x_3); total white blood cell (WBC) count (x_4); differential WBC count or distribution of RBC types: neutrophils (x_5), lymphocytes (x_6) and eosinophils (x_7); haemoglobin estimate (x_8); haematocrit or packed cell volume measurement (x_9); and haematological indices: MCV (x_{10}), MCH (x_{11}) and MCHC (x_{12})].

RESULTS AND DISCUSSION

The results of the principal component analysis (PCA) ordination of the months based on the twelve haematological factors for the occurrence of *Eustrongylides africanus* larvae in *Clarias* species of Bida floodplain is shown on Table 1. Axis I accounts for 93.5% of the principal component followed by axis II, which account for 6.1% and axis III is 0.4%. Since the accumulated total % of traces for two principal components (axis I and II) accounted for 99.6% out of 100%, therefore, the % trace on axis III is over sighted. And axis II and I were involved in the subsequent analysis carried out. The percentage (%) contribution of the twelve haematological factors to the coefficient of multiple determinations (R^2) for the two principal components (PCs) on axis II and I as shown on Table 2. The haematological factors making the most contributions to R^2 in axis I responsible for 93.5% of traces were MCHC (39.4%), MCH (22.9%), MCV (21.4%) and neutrophils count (3.4%) in order of magnitude. The haematological factors making the most important contributions to R^2 in axis II responsible for 6.1% of traces are neutrophils count (26.2%), MCHC (25.9%), eosinophils count (11.5%) and MCV (8.7%) in order of magnitude.

The correlation coefficients (r) for twelve haematological factors (HFs) with occurrences of *Eustrongylides africanus* larvae infections in *Clarias* species from Bida floodplain is shown in Table 3. Two, three and one out of the 39 combinations of the twelve haematological factors with known correlation coefficients ($r \geq \pm 0.50$) contributed respectively to the estimations of

prevalence, mean intensity and abundance of *E. africanus* larvae densities in *Clarias* species (Table 4) were neutrophils, lymphocytes and eosinophils counts. MCV and MCH as follows:-

ESTIMATIONS FOR PREVALENCE

Y = -0.24x + 19.5 (r = -0 57) with neutrophils count

Y = 0.22x + 80.1 (r = 0.56) with lymphocytes count

When the prevalence is 0% the neutrophils counts is at its maximum of about 82%. Thus, for every 1% increase in prevalence the neutrophils count has a stepwise decrease of about 77%. The % neutrophils counts of the differential white blood cell count were negatively correlated to the prevalence of *E. africanus* larvae in *Clarias* species. Thus the % neutrophils count 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 the lymphocyte count has a stepwise increase of about 352%. The % lymphocytes count of differential WBC count was positively correlated to the prevalence of *E. africanus* larvae in *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 *E. 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 = - 0.54) 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 $0.0\mu^3$ the MCV is at its minimum of about $5\mu^3$ Thus for every 1% increase in mean intensity the MCV has a stepwise increase of about $5\mu^3$ When the mean intensity is $0.0\mu^2$ g when the MCH is at its maximum value of about $9.3\mu^2$ g. Thus, for every 1% increase in mean intensity the MCH has a stepwise increase of about $9.3\mu^2$ 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 % eosinophils 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 % eosinophils 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 $0.0\mu^3$ the MCV is at its minimum of about $4.2\mu^3$. 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 factor that might be suitable in estimate the mean abundance of *E. africanus* in *Clarias* species is MCV.

REFERENCES

Adedeji, O.B.; V.O. Taiwo and S.A. Agbede (2000). Comparative haematology of five Nigerian freshwater fish species. *Nigerian Vetrinary Journal*. 21: 75 – 84.

Anderson, D. and G. W. Klontz (1965). Basic haematology for the fish culturist. *Annual Northwestern Fish Culture Conference*. 16: 38 - 41.

Bhasar, B. R. and K. S. Rao (1990). Use of haematological parameters as diagnostic tools in determining the health of milk fish, *Chanos chanos* (Forskal), in brackish water culture. *Aquaculture and Fisheries Management* 21: 125 - 129.

Blaxhall, P. C. and K. W. Daisley (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*. 5: 771 - 781.

Dacie, J. V. and S. M. Lewis (1975). *Measurement and calculation of size of red cells. practical haematology*. 5th ed. London: Churchill Livingstone. pp 40-43.

Delaney, J. W. and G. Garratty (1969). *Handbook of haematological and blood transfusion technique*. Butterworths, London.

Egwunyenga, A.O.; O.P.G. Nmorsi and A.F. Igbinosun (1999). Haematological changes in cichlids of Ethiope river (Niger Delta Nigeria) due to intestinal helminthiasis. *Bioscience Research Communications*. 11 (4): 361 – 365.

Eroundu, E. S.; C. Nnubia and F. O. Nwdukwe (1993). Haematological studies on four catfish species raised in freshwater ponds in Nigeria. *Journal of Applied Ichthyology*. 9:250 – 256.

Jacob, S. S. (1982). *Osmotic regulation in Chrysichthys nigrodigitatus and Clarias isheriensis*. M.Sc. Dissertation: University of Lagos, Lagos.

Johnson, D. W. (1968). Pesticides and fishes: a review of selected literature. *Transactions of the American Fisheries Society*. 97(4): 398 –424.

Klontz, G. W. (1972). Haematological techniques and the immune response in rain bow trout. In: *Disease of fish* (Ed. Mawdeskley-Thomas, L.E.). Symposium of Zoological Society, London No 30. New York and London Academic Press.

Klontz, G. W. and L. S. Smith (1968). Methods of using fish biological research subjects. In: *Methods of animal experimentation* (Ed. Gay, W. R). London: Academic Press.

Levinson, S. A. and R. P. Macfat (1981). *Clinical laboratory diagnosis*.Philadelphia: Lea and Febiger. 1246pp.

Margolis, L.; G. W. Esch; J. C. Holmes; A. M. Kuris and G. A. Schad (1982). The use of ecological terms in parasitology (Report of an ad-hoc committee of the American Society of Parasitologists). *Journal of Parasitology*. 68:131 – 133.

Mulcahy, M. F. (1970). Blood values in the pike, *Esox lucius* (L). *Journal of Fish Biology*. 2: 203 – 209.

Omoregie, E. (1998). Changes in the haematology of the Nile tilapia *Oreochromis niloticus* Treivas under the effect of rude. *Acta Hydrobiologia*. 40 (4): 287 – 292.

Puchkov, N. V. (1964). The white blood cells. In: *Techniques for the investigation of fish Physiology* (Ed. Pavlovskii, E. N.). Jerusalem: Israel Program for Scientific Translation. pp. 11 – 15.

Sakpere, O. K. (1985). Haematological characteristics of *Clarias isheriensis*. *Journal of Fish Biology*. 27, 259-263.

Smith, C. R (1968). Haematological changes in coho salmon fed a folic acid deficient diet. *Journal of Fisheries Research Board of Canada*. 25 (1): 151-156.

Smith, C. E. and J. E. Halver (1969). Folic acid anaemia in coho salmon. *Journal of Fisheries Research Board of Canada*. 26: 111 –114.

Snieszko, S. F. (1974). The effect of environmental stress on outbreak of infectious diseases of fishes. *Journal of Fish Biology*. 6: 197 - 208.

Svobodova, Z.; D. Pravda and J. Palackova (1986). Unified methods of haematological examination of fish. *Vodany, Czechoslovakia: Research Institute of Fish Culture and Hydrobiology*: 31pp.

Svobodova, Z.; J. Karova; J. Machova; B. Vykusova; J. Hamackova and J. Kouril (1994). *Basic haematological parameters of African catfish (Clarias gariepinus) from intensive warm-water culture*. Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic: 389 (25) 6pp.

Wedemeyer, G. and W. T. Yasutake (1977). *Clinical methods for the assessment of the effects of environment al stress on fish health* . United States Fisheries and Wildlife Services Technical Paper 89: 18 pp. Washington. D. C

Wedemeyer, G. A.; R. W. Gould and W. T. Yasutake (1983). Some potentials and limits of the leucocrit test as a fish health assessment method. *Journal of Fish Biology*. 23 : 711 – 716.

Wintrobe, M.M. (1978). *Clinical haematology*, London, H. Kimpton Press, 448pp.

***Table 1: Result of the principal component analysis ordination of the months based* on twelve haematological factors for the occurrence of *Eustrongylides africanus* larvae infection in *Clarias* from Bida floodplain.**

Axis	Percentage (%) of trace	Accumulated % of trace
I	93.5	93.5
II	6.1	99.6
III	0.4	100.0

Table 2: Percentage contribution of the twelve haematological factors to the coefficient of multiple determinations (R^2)for two principal components.

	Percentage (%) contribution to R^2	
Haematological Factors (HFs)	**Axis 1**	Axis 2
Total red blood cell (RBC) count	1.5	1.1
RBC size	0.4	0.2
RBC nuclei size	0.8	0.0
Total white blood cell count	1.7	4.7
Neutrophils	3.4*	26.2*
Lymphocytes	2.4	8.5
Eosinophils	3.3	11.5*
Haemoglobin content	2.7	5.5
Packed cell volume	0.1	7.2
Mean corpuscular volume	21.4*	8.7*
Mean corpuscular haemoglobin	22.9*	0.5
Mean corpuscular haemoglobin concentration	39.4*	25.9*
Total	**100.0**	**100.0**

*Hfs making the most important contribution to R^2
Axis I = 87.1%
Axis II = 72.5%

Table 3: Correlation coefficient (r) for twelve measured haematological factors with the occurrences of *Eustrongylides africanus* larvae infection in *Clarias* from Bida floodplain of Nigeria.

Parameters		Prevalence	Intensity	Abundance	RBC(T)	RBC size	RBC Nuclei size	WBC (T)	Neut.	Lymph.	Eosi
					X_1	X_2	X_3	X_4	X_5	X_6	X_7
Prevalence		1									
Intensity		0.82	1								
Abundance		0.94	0.96	1							
RBC (T)	X_1	0.14	0.04	0.08	1						
RBC size	X_2	0.18	0.35	0.28	0.44	1					
Nuclei size	X_3	-0.27	-0.29	-0.35	0.16	-0.42	1				
WBC (T)	X_4	0.46	0.16	0.28	0.82	0.32	0.17	1			
Neutrophils	X_5	-0.57	-0.18	-0.34	-0.60	-0.03	-0.31	-0.93	1		
Lymphocytes	X_6	0.56	0.10	0.30	0.59	-0.03	0.16	0.91	-0.97	1	
Eosinophils	X_7	0.32	0.59	0.45	0.11	0.02	0.48	0.23	-0.30	0.11	1
PCV	X_8	0.24	-0.21	0.00	0.62	0.29	-0.17	0.65	-0.56	0.66	-0.5
Hb	X_9	0.30	-0.02	0.11	0.84	0.47	0.13	0.90	-0.78	0.76	-0.0
MCV	X_{10}	0.33	0.57	0.49	0.46	0.94	-0.52	0.29	-0.02	-0.03	0.13
MCH	X_{11}	-0.15	-0.54	-0.39	-0.48	-0.77	0.35	-0.19	-0.11	0.16	-0.3
MCHC	X_{12}	0.29	0.35	0.27	0.40	0.40	0.42	0.63	-0.61	0.42	0.75

X_1 =Total red blood cell count; X_2 =Red blood cell size; X_3 = Red blood cell nuclei size; X_4 = Total white blood cell count;
X_5 = Neutrophils count; X_6 = Lymphocytes count; X_7 = Eosinophils count; X_8 = Haemoglobin estimate; X_9 =Packed cell volume;
X_{10} = Mean corpuscular volume; X_{11} = Mean corpuscular haemoglobin; X_{12} = Mean corpuscular haemoglobin concentration

Table 4.31: Estimation of the occurrence of *Eustrongylides africanus* larvae infection in *Clarias* of Bida floodplain using haematological factors with correlation coefficients ($r \geq \pm 0.50$).

Haematological Factors (HFs)	Equation for Prevalence (Y)	Equivalence of haematological factors (when y=0)	Equivalence of HFs (For 1% change in prevalence)
Neutrophils	$Y = -0.2383x_1 + 19.518$ (r =-0.57)	X = 82	X = -77
Lymphocytes	$Y = 0.2246x_2 + 80.149$ (r =0.56)	X = -357	X = 352
	Equation of intensity	**Equivalence of HFs (when Y = 0)**	**Equivalence of intensity (For 1% change in prevalence)**
	$Y = 0.6501x_3 + 0.0575$ (r = 0.59)	X = -0.1	X = 1.5
Eosinophils	$Y = 13.185x_4 + 65.44$ (r = 0.57)	X = -5.0	X = 5.0
MCV	$Y = -5.435x_5 + 50.656$ (r = -0.54)	X = 9.3	X = -9.2
MCH	**Equation of abundance**	**Equivalence of HFs (when Y = 0)**	**Equivalence of abundance S(For 1% change in prevalence)**
	$Y = 19.417x_4 + 81.996$ (r = 0.50)	X = -4.2	X = -4.2
MCV			

MCV = Mean corpuscular volume
MCH = Mean corpuscular haemoglobin