Haematological Profile of the African Lungfish, *Protopterus annectens* (Owen) of Anambra River, Nigeria

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Abstract: Haematological studies of the African lungfish, *Protopterus annectens* were carried out in order to establish a normal range of blood parameters which would serve as baseline data for assessment of the health status of the fish as well as reference point for future comparative surveys ..Blood parameters such as erythrocyte, leucocyte and thrombocyte counts, haemoglobin contents, Mean Corpuscular volume, Mean Corpuscular haemogbobin, Mean Corpuscular haemoglobin concentration, blood osmolality, pH, haematocrit, glucose, urea, uric acid, creatinine and ionic concentrations were determined in the various reproductive stages (fingerlings, juveniles, intermediates and adults) of *P. annectens.* Intraspecies haematological relationships in *P. annectens* indicated a high positive correlation between haematocrit and both erythrocyte counts and haemoglobin contents respectively in all sizes of *P. annectens;* with mean r –values of 0.860 and 0.843 ($p < 0.05$) for Hct/EC and Hct/Hb4 respectively. A number of factors which might influence haematological characteristics of *P. annectens* were discussed. [Journal of American Science 2010;6(2):123-130]. (ISSN: 1545-1003).

Key words: Haematology, Profile, African, Lungfish, Anambra River, Nigeria

1. Introduction

The African lungfish, *P. annectens* is a highly prized food fish in Nigeria (Otuogbai, 2001; Otuogbai and Ikhenoba, 2001). It is distributed in shallow parts of rivers and lakes of some West African countries ranging from Senegal to Cameroon where it contributes to a relatively high percentage of artisanal fisheries (Otuogbai, 2001; Otuogbai and Ikhenoba, 2001; Okafor 2004a). Intensive studies on the biology of *P. annectens* have just begun (Otuogbai, 2001; Otuogbai and Ikhenoba, 2001; Okafor and Chukwu, 2005a). However, only few studies have been carried out on the haematological characteristics of *P. annectens* (Okafor, 2004abcd; Okafor and Chukwu, 2005b).

The determination of haematological values of fishes are carried out for a variety of purposes: to establish a 'normal range' of blood parameters (Etim, *et al*., 1999), to investigate conditions that might lead to alterations of some of these values such as sampling methods, temperature, sex, maturity, disease condition or nutrition of the fish (Clarks *et al*., 1979; Barham *et al*., 1980) and to ascertain the effects of certain chemical pollutants (e.g. insecticide) and sublethal strength of some toxicants (such as heavy metals e.g. lead) on blood values (Mathiessen, 1981; Etim *et al*., 1999).

In the light of the above, it was considered worthwhile to undertake a study on some selected haematological parameters of various sizes of the African lungfish, *P. annectens* (fingerlings, juveniles, intermediates and large). This would form a baseline data for assessment of the health status of the fish as well as reference point for future comparative surveys.

2. Materials and Methods

Fish Samples

A total of 152 live specimens of the African lungfish, *P. annectens* comprising fingerlings (mean length 20.5cm ± 3.5, mean weight 50.5g ± 10.4), juveniles (mean length 32.5cm ± 6.0, mean weight 223.8g ± 15.3), intermediates (mean length 39.9cm ± 2.8; mean weight 380.2g ± 24.4) and large (mean length 48.7cm ± 3.5; mean weight 956.6g ± 32.6) were obtained from Anambra river at Otuocha in Anambra State, Nigeria. The fishes were transported to the Zoology laboratory of the University of Lagos, Lagos – Nigeria in plastic buckets (27 x 15cm) containing water got from Anambra river. In the laboratory they were acclimatized for three weeks during which they were provided daily with insect larvae, fish feed obtained from the Nigerian Institute of Oceanography and Marine Research (NIOMR) Victoria Island, Lagos as well as boiled rice and beans to avoid the possible effect of starvation on any of the haematological values.

Fish were examined for any sign of infection or disease condition (Obiekezie, 1988) and only those fishes considered to be healthy were used for the study.

Blood Collection

Blood was collected from the caudal blood vessels of the fishes using the method of Kori-Siakpere and Egor (1997).

Ethylene diamine tetracetic acid (EDTA) was the anti-coagulant employed, because unlike heparin, it did not cause the blood cells to shrink.

Blood Analysis

The Erythrocyte count (EC) was done in an Improved Neubauer haemocytometer following the method of Baker and Silverton (1982). The total Leucocyte count (TLC) was determined in the same Improved Neubauer haemocytometer (used for red cells) following the same method of Baker and Silverton (1982). The microhaematocrit method of Blaxhall and Darsley (1973) was employed in the determination of blood haematocrit. The haemoglobin content of blood samples was determined by cyanmethaemoglobin method (Wharton and McCarty, 1972; Blaxhall and Daisley, 1973). The Thrombocyte count (TC) was determined in an Improved Neubauer haemocytometer using a phase contrast microscope (Okafor and Chukwu, 2005b). The blood osmolality, ionic concentrations, and pH were determined using a Karl Kolb osmometer, flame photometer and pH metre respectively, following the method of Chukwu and Odiete, (1999). The blood urea, uric acid, creatinine and glucose levels were determined following the standard methods of Dyer 1965; Wharton and McCarty, 1972 and Brewer *et al*., 1974.

The haematological Indices: Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were calculated using the formula of Baker and Silverton (1982) given below:

$$\text{Mean Corpuscular Volume (MCV µm)} = \frac{\text{Haematocrit (\%) x 10}}{\text{Erythrocyte count (mm}^3\text{)}}$$

$$\text{Mean Corpuscular Haemoglobin (MCH pg)} = \frac{\text{Haematocrit (g\%) x 10}}{\text{Erythrocyte count (mm}^3\text{)}}$$

$$\text{Mean Corpuscular Haemoglobin Concentration (MCHC) \%} = \frac{\text{Haemoglobin (g\%) x 100}}{\text{Haematocrit (\%)}}$$

Statistical Analysis

Regression analyses were employed between the various parameters measured. The coefficient of regression (r) was checked for statistical significance by the student t-test at 0.05 level of significance (Zar, 1984).

3. Results

The haematological profile of *P. annectens* under normal conditions: erythrocyte, leucocyte and thrombocyte counts, haematocrit, haemoglobin contents, MCV, MCH, MCHC, pH, Osmolality, glucose, Urea, Uric acid, and Creatinine levels, Na^+ and K^+ concentrations are presented in Table 1.

The Erythrocyte, Leucocyte and Thrombocyte counts in the fingerlings ranged from 54,000 to 85,000mm^3, 1500 to 3700mm^3, and 7.8 to 15.0mm^3 respectively, while in the adult specimens they were 60,000 to 97,000 mm^3, 1400 to 1800mm^3 and 5.0 to 11.6mm^3 respectively (Table 1).

The mean blood levels of urea and uric acid in the fingerlings were 21 ± 2.6 and 0.2 ± 0.1 respectively, while in the adult specimens they were 45.1 ± 5.6 and 0.4 ± 0.2 respectively **(Table 1)**.

There is a high positive correlation between haematocrit and both erythrocyte counts and haemoglobin contents respectively in all sizes of *P. annectens;* with mean r –values of 0.860 and 0.843 ($p < 0.05$) for Hct/EC and Hct/Hb4 respectively **(Tables 2– 4)**.

High positive correlation was recorded between osmolality , urea and uric acid levels respectively in all sizes of *P. annectens* with mean r- values for Osmolality/urea, = 0.897, and Osmolality/uric acid, = 0.573 ($p < 0.05$) respectively **(Tables 2 – 4)**.

4. Discussion

The significance of fish haematology in disease aetiology of fishes cannot be over emphasized (Kori-Siakpere and Egor, 1997). It is also necessary to establish normal haematological characteristics of a particular species of fish which would serve as reference for future comparative studies.

Blaxhall and Daisley (1973) for instance, have reported the essence of using haematocrit to detect anaemic conditions in fishes. Several reported values for fish haematocrit fall between 20% and 35% and rarely do values above 50% been reported (Clarks *et al*., 1976; Etim *et al*., 1999). The mean haematocrit values for *P. annectens* of all sizes (fingerlings, juveniles, intermediates and large) fall within this range. That is 27.7%, 28.1%, 28.8% and 29.2% for fingerlings, juveniles, intermediates and large specimens of *P. annectens* respectively.

Das (1965) reported that both the haemoglobin contents and Erythrocyte counts tend to increase with length and age of the fish. In the present study, the haemoglobin contents and Erythrocyte counts of *P. annectens* were higher in the large and intermediate sized specimens than in the fingerlings and juveniles (Table 1). Preston (1960) also observed such similar findings in the Plaice, *Pleuronectes platessa*.

Table 1: The Normal Haematological Profile of *Protopterus annectens*

Size Group	Mean Total Length (cm)	Mean Body Weight (g)	Haematocrit (%)	Erythrocyte Count (mm^3)	Leucocyte Count (mm^3)	Thrombocyte Count (mm^3)	Haemoglobin contents (g%)	PH	Osmolality (mOsmol/l)	Glucose (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Mean Corpuscular Volume (MCV) (µm)	Mean Corpuscular Haemoglobin (MCH) (pg)	Mean Corpuscular Haemoglobin Concentration (MCHC) (%)	Na+ (mg/l)	K+ (mg/l)
Fingerlings	20.5±3.5 (15.8-27.5)	50.5±10.4 (37.2-74.5)	27.7±1.9 (23.5-37.3)	60,000±5000 (54000-85,000)	2200±250 (1500-3700)	11.4±3.0 (7.8-15.0)	7.4±0.4 (7.1-8.7)	7.5±0.2 (7.2-7.8)	255±20.2 (230-320)	71±7.5 (60-95)	21±2.6 (18.0-27.0)	0.2±0.1 (0.1-0.3)	0.4±0.3 (0.2-0.8)	461.7±18.5 (346.8-501.5)	123.3±10.2 (98.5-168.2)	26.7±2.8 (23.1-32.3)	99.5±3.8 (95.2-108.6)	8.0±1.0 (7.8-9.5)
Juveniles	32.5±6.0 (24.5-39.0)	223.8±15.3 (80.0-250.0)	28.1±1.8 (24.1-38.2)	62,000±5000 (55,000-90,000)	20,00±220 (1400-2200)	9.3±2.9 (6.1-13.1)	7.4±0.4 (7.1-8.7)	7.5±0.2 (7.1-7.8)	249±18.8 (215-330)	68±8.5 (55.0-94.0)	27.4±3.4 (23.1-31.6)	0.3±0.2 (0.1-0.7)	0.4±0.3 (0.2-0.8)	453.2±17.6 (339.2-498.7)	119.4±9.6 (92.7-188.1)	26.3±2.4 (22.7-31.9)	99.6±6.2 (90.1-110.5)	8.2±1.3 (7.2-11.5)
Young Adults	39.9±2.8 (36.9-43.9)	380.2±24.4 (250-550)	28.8±2.1 24.4-33.1	64,000±5000 (55000-95000)	1750±150 (1400-2000)	9.2±2.8 (6.2-12.3)	7.5±0.4 (7.0-8.8)	7.5±0.2 (7.0-7.6)	241±20.6 (205-330)	67±12.0 (52-90)	29.6±5.1 (24.0-31.5)	0.3±0.2 (o.1-0.7)	o.8±0.2 (0.5-1.3)	450±16.7 (336.0-496.4)	117.2±8.8 (90.3-179.3)	26.0±2.2 (22.4-31.6)	100.4±4.6 (93.5-110.0)	8.6±1.1 (7.9-8.4)
Old adults	48.7±6.5 (43.0-60.9)	956.6±232.6 (550.0-1622.0)	29.2±2.2 (23.0-32.0)	69,000±5000 (60,000-97,000)	1700±160 (1400-1800)	8.3±3.2 (5.0-11.6)	7.5±0.4 (7.0-8.8)	7.5±0.2 (7.0-7.6)	239±21.1 (195-340)	65±8.8 (55-90)	45.1±5.6 (25.2-55.1)	0.4±0.2 (0.2-0.7)	0.8±0.4 (0.5-1.1)	423.2±16.2 (309.2-469.6)	108.7±8.4 (81.8-170.8)	25.7±1.7 (22.1-31.3)	100.2±4.8 (94.0-111.0)	8.4±0.9 (7.0-11.3)

Table 2: Intraspecies Haematological relationships in *Protopterus annectens* (p<0.05) (Finger lings)

	Total Length	Weight	EC	TLC	TC	Hct	Hb4	pH	Osmolality	MCV	MCH	MCHC	Glucose	Urea	Uric Acid	Creatinine	Na^+	K^+
Total Length																		
Weight	0,71																	
E C	0.75	0.61																
TLC	0.51	0.48	0,10															
TC	0.54	0.51	-0.38	0.15														
Hct	0.76	0.77	0.89	-0.10	-0.31													
Hb4	0.84	0.73	0.95	0.28	-0.09	0.83												
pH	0.09	-0.06	-0.07	-0.05	-0.08	-0.07	-0.10											
Osmolality	0.19	0.39	0.12	-0.09	0.10	0.05	-0.55	-0.50										
MCV	0.82	0.71	0.93	0.08	0.11	0.92	0. 78	-0.12	-0.21									
MCH	0.85	0.79	0.94	0.07	0.05	0.75	0.91	-0.11	-0.18	0.88								
MCHC	0.77	0.75	0.78	0.05	0.03	0.96	0.95	-0.08	-0.23	0.79	0.90							
Glucose	-0.63	0.22	0.18	0.14	0.11	0.17	0.20	0.06	0.55	0.09	0.08	0.10						
Urea	0.85	0.88	0.16	0.06	0.05	0.11	0.09	0.10	0.91	0.14	0.13	0.09	0.08					
Uric Acid	0.71	0.73	0.05	0.04	0.13	0.10	0.08	0.18	0.59	0.11	0.07	0.06	0.11	0.82				
Creatinine	0.58	0.56	0.03	0.04	0.06	0.09	0.08	0.11	0.49	0.20	0.16	0.09	0.10	0.68	0.66			
Na^+	-0.31	0.19	0.11	0.12	0.08	0.15	0.05	-0.68	0.53	0.09	0.08	0.11	0.06	0.33	0.28	0.19		
K^+	-0.23	0.18	0.16	0.15	0.05	0.10	0.09	-0.61	0.50	0.11	0.12	0.10	0.09	0.25	0.22	0.16	0.80	

EC = Erythrocyte count in million /mm^3
TLC = Total leucocyte count in thousand / mm^3
TC = Thrombocyte count in thousand / mm^3
Hct = Haematocrit (%)
Hb4 = Haemoglobin contents (g%)
MCV = Mean Corpuscular Volume (µm)
MCH = Mean Corpuscular Haemoglobin (g)
MCHC = Mean Corpuscular Haemoglobin Concentration (%)

urea in mg/dL
uric acid in mg/dL
Creatinine in mg/dL
Na^+ in mg/L
K^+ in mg/L
Glucose in mg/dL

Table 3: Intraspecies Haematological relationships in *Protopterus annectens* (at p<0.05) (juveniles)

	Total Length	Weight	EC	TLC	TC	Hct	Hb4	pH	Osmolality	MCV	MCH	MCHC	Glucose	Urea	Uric Acid	Creatinine	Na^+	K^+
Total Length																		
Weight	0,69																	
E C	0.71	0.63																
TLC	0.52	0.50	0,08															
TC	0.49	0.51	-0.37	0.13														
Hct	0.80	0.81	0.85	-0.06	-0.26													
Hb4	0.82	0.75	0.93	0.17	-0.09	0.84												
pH	0.07	-0.08	-0.09	-0.05	-0.08	-0.09	-0.09											
Osmolality	0.18	0.33	0.07	-0.10	0.09	0.07	-0.51	-0.53										
MCV	0.79	0.74	0.96	0.10	0.12	0.90	0. 78	-0.11	-0.20									
MCH	0.84	0.80	0.94	0.09	0.03	0.70	0.89	-0.08	-0.14	0.87								
MCHC	0.78	0.77	0.78	0.08	0.04	0.91	0.93	-0.06	-0.19	0.81	0.93							
Glucose	-0.58	0.21	0.15	0.18	0.09	0.12	0.16	0.05	0.55	0.09	0.09	0.09						
Urea	0.83	0.86	0.11	0.05	0.03	0.11	0.08	0.12	0.89	0.13	0.13	0.07	0.08					
Uric Acid	0.75	0.69	0.09	0.08	0.12	0.10	0.09	0.11	0.57	0.12	0.08	0.06	0.10	0.83				
Creatinine	0.55	0.56	0.05	0.09	0.06	0.09	0.10	0.10	0.50	0.18	0.19	0.08	0.09	0.69	0.68			
Na^+	-0.30	0.18	0.11	0.11	0.09	0.16	0.05	-0.67	0.59	0.10	0.12	0.09	0.07	0.38	0.30	0.15		
K^+	-0.29	0.20	0.15	0.15	0.07	0.09	0.10	-0.63	0.48	0.10	0.08	0.10	0.09	0.22	0.27	0.16	0.78	

EC = Erythrocyte count in million /mm^3
TLC = Total leucocyte count in thousand/mm^3
TC =Thrombocyte count in thousand / mm^3
Hct = Haematocrit (%)
Hb4 = Haemoglobin contents (g%)
MCV = Mean Corpuscular Volume (µm)
MCH = = Mean Corpuscular haemoglobin (pg)
MCHC = Mean Corpuscular haemoglobin Concentration (%)
Glucose in mg/dL
Urea in mg/dL
Uric acid in mg/dL
Creatinine in mg/dL
Na^+ in mg/L
K^+ in mg/L

Table 1: Intraspecies Haematological relationships in *Protopterus annectens* (at p<0.05) (adults)

	Total Length	Weight	EC	TLC	TC	Hct	Hb4	pH	Osmolality	MCV	MCH	MCHC	Glucose	Urea	Uric Acid	Creatinine	Na^+	K^+
Total Length																		
Weight	0,74																	
E C	0.64	0.66																
TLC	0.60	0.52	0,08															
TC	0.51	0.56	-0.41	0.20														
Hct	0.79	0.75	0.84	-0.10	-0.32													
Hb4	0.88	0.83	0.90	0.25	-0.10	0.86												
pH	0.10	-0.05	-0.07	-0.05	-0.08	-0.08	-0.09											
Osmolality	0.21	0.35	0.11	-0.09	0.09	0.08	-0.53	-0.54										
MCV	0.80	0.73	0.93	0.09	0.10	0.92	0. 77	-0.11	0.20									
MCH	0.84	0.78	0.96	0.08	0.06	0.76	0.88	-0.10	-0.16	0.90								
MCHC	0.79	0.73	0.81	0.06	0.03	0.94	0.93	-0.07	-0.22	0.76	0.86							
Glucose	-0.59	0.18	0.17	0.10	0.11	0.15	0.19	0.08	0.52	0.08	0.08	0.09						
Urea	0.88	0.88	0.14	0.05	0.05	0.09	0.08	0.11	0.89	0.11	0.11	0.10	0.07					
Uric Acid	0.75	0.75	0.08	0.04	0.11	0.16	0.09	0.17	0.56	0.10	0.08	0.06	0.10	0.85				
Creatinine	0.54	0.49	0.06	0.05	0.06	0.08	0.09	0.08	0.50	0.18	0.19	0.08	0.10	0.67	0.65			
Na^+	-0.28	0.17	0.10	0.10	0.08	0.11	0.05	-0.63	0.58	0.10	0.12	0.11	0.08	0.37	0.30	0.16		
K^+	-0.32	0.18	0.20	0.13	0.09	0.10	0.10	-0.68	0.57	0.10	0.11	0.10	0.06	0.21	0.27	0.20	0.89	

EC = Erythrocyte count in million /mm^3
TLC = Total leucocyte count in thousand/mm^3
TC =Thrombocyte count in thousand / mm^3
Hct = Haematocrit (%)
Hb4 = Haemoglobin contents (g%)
MCV = Mean Corpuscular Volume (µm)
MCH = = Mean Corpuscular haemoglobin (pg)
MCHC = Mean Corpuscular haemoglobin Concentration (%)
Glucose in mg/dL
Urea in mg/dL
Uric acid in mg/dL
Creatinine in mg/dL
Na^+ in mg/L
K^+ in mg/L

Eisler (1965) had suggested that there was a correlation between haemoglobin concentration and activity of fish. The more active fishes tend to have high haemoglobin values than the more sedentary ones. Consequently, *P. annectens* being a relatively quiet and sedentary species (Okafor, 2006) has a slightly lower haemoglobin concentration than other more active African teleosts such as *Clarias buthupogon* whose mean hemoglobin concentration is as high as 9.88g/dL (Kori-Siakpere and Egor, 1997).

The blood urea and uric acid levels of the fingerlings and juveniles were slightly lower than those of intermediate and adult specimens because these fingerlings depend less on ureotelism and uricotelism as means of nitrogenous excretion than the adults.

The wide range of blood Osmolality observed in *P. annectens* is an indication of high degree of tissue tolerance and this of great value when encountering the estuarine or brackish water environment.

Acknowledgements

We wish to acknowledge the technical assistance given to us by Mr Bayo Adeluyi and Mrs I.A. Aderenle, of Zoology Department, as well as Mr Isaac Arukwe of the Department of Biochemistry, all of the University of Lagos, Nigeria. We are also grateful to Mr. Eddy Onuoha of the Department of Veterinary Microbiology and Pathology of the University of Nigeria, Nsukka-Nigeria for his own contributions toward the success of the study.

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References

1. Baker FJ and Silverton RE. *Introduction to Medical Laboratory Technology*. 5th ed. Butterworth & Co. (Publishers) Ltd. London. 1982; 549pp.
2. Barham WT, Smith GL and Schnoobe HJ. The effect of bacterial infection on erythrocyte fragility and sedimentation rate of rainbow trout, *Salmo gairdneri* (Richardson). *Journal of Fish Biology*. 1980; **16**: 177 – 180.
3. Blaxhall PC and Daisley KW. Routine haematological methods for use with fish blood. *J. Fish Biol.* 1973; **5**: 771 – 781.
4. Brewer JM, Pesce AJ and Ashworth RB. *Experimental techniques in Biochemistry.* Prentice Hall Inc., Englewood Cliffs, New Jersey, 1974.
5. Clarks S, Whitemore DH Jr. and McMahon RF. Consideration of blood parameters of largemouth bass, *Micropterus salmoides. Journal of Fish Biology*, 1979; **14**: 147 – 154.
6. Chukwu LO and Odiete WO. Osmotic and ionic performance of the euryhaline teleost, *Elops lacerta* (Val) in freshwater, estuarine and marine habitats. *J. Sci. Res. and Dev*. 1999; **4**: 1 – 8.
7. Das BC. Age-related Trends in the blood chemistry and haematology of the Indian Carp, *Catla catla*. *Geronotologica*, 1965; **10**: 47 – 64.
8. Dyer JR. *Applications of Absorption Spectroscopy of Organic Compounds.* Prentice-Hall, New Jersey, 1965.
9. Eisler R. Erythrocyte count and Haemoglobin content in nine species of Marine Teleost, *Chesapeake. Sci*. 1965; **6**: 116 – 120.
10. Etim L, Ekanem SB and Utin A. Haematological profile in two species of catfish, *Chrysichthys nigrodigitatus* (Lacepede) and *Chrysichthys furcatus* (Gunther) from the Great Kwa River, Nigeria. *Global Journal of Pure and Applied Sciences*. 1999; **5**(1): 1 – 4.
11. Kori-Siakpere, O. and Egor, V.E. (1997). Haematological characteristics of the African mudfish, *Clarias buthupugon* (Pisces : Clariidae). *Bull. Sci. Assoc. Nig.* **21**: 177 – 185.
12. Mathiessen P. Haematological changes in fish following aerial spraying with endosulfan insecticide for tse-tse fly control in Botswana. *Journal of Fish Biology*, 1981; **18**: 461 – 470.
13. Obiekezie AI. *Henneguya chrysichthi* sp. nov. (Protozoa: Myzozoa) parasitic on the African estuarine catfish, *Chrysichthys nigrodigitatus* (Lacepede) from the Cross River Estuary, Nigeria. *Journal of Fish Biology*, 1988; **32**: 207 – 221.
14. Okafor AI. Changes in blood pH and haematocrit due to salinity in the African lungfish, *Protopterus annectens* (OWEN). *Afric. J. Sci.* 2004a; **5**(1): 986–993.
15. Okafor AI. Some haematological adjustments to salinity stress in the African lungfish, *Protopterus annectens* (Owen). *World J. Biotech*, 2004b**; 5**: 842 – 846.
16. Okafor AI. Blood haematocrit and pH alterations during aestivation in the African lungfish, *Protopterus annectens* (Owen). *J. Sci. Engr. Tech.* 2004c**; 11**(2): 5607 – 5614.
17. Okafor AI. The effects of aestivation on blood cell counts and haemoglobin contents of the African

lungfish *Protopterus annectens* (OWEN). *J. Appl. Sci.* 2004d**; 7**(1): 4008 – 4014.

18. Okafor AI. Ecophysiological studies on osmotic regulation and aestivation in the African lungfish, *Protopterus annectens* (Owen) from Anambra river, Nigeria. Ph.D thesis, University of Lagos, Nigeria, 2006; In Preparation.
19. Okafor AI and Chukwu LO. The effect of salinity stress on buccal ventilatory rate in the African lungfish, *Protopterus annectens* (Owen). *Anim. Res. Int.* 2005a**; 2**(1): 252- 254.
20. Okafor AI and Chukwu LO. Thrombocythaemia in an aestivating African lungfish, *Protopterus annectens* (Owen) from Anambra river, Nigeria. *J. Expt. and Clin. Anat.* 2005b**; 4**(2): 35 – 39.
21. Otuogbai TOS. Aestivation peculiarities of the African lungfish, *Protopterus annectens* (Owen). *J. Appl. Sci.* 2001; **4**(4): 2180 – 2189.
22. Otuogbai TOS and Ikhenoba A. Food and feeding habits of the lungfish, *Protopterus annectens* (Owen) and the possible role of the paired fins in feeding. *World J. Biotech*. 2001; **2**(2): 233 – 238.
23. Preston HA. Red Blood values in the Plaice (*Pleuronectes platessa*. L). *J. Mar. Biol. Assoc.* U.K. 1960; **39**: 681 – 687.
24. Wharton DC and McCarty RE. Experiments and methods in Biochemistry. Macmillan Publishing Company, New York, 1972.
25. Zar JH. *Biostatistical analysis*. 2nd edition. Englewood Cliffs. J.N. Prentice Hall, 1984; 717pp.

14/09/2009