

## Haematological changes in African lungfish, *Protopterus annectens* after aerial exposure

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**Abstract:** Specimens of the west African lungfish, *Protopterus annectens* (mean weight 294.3 g mean length 38.1 cm), procured from Agulu lake at Agulu in Anambra state, Nigeria were removed from the ambient water and exposed to complete aerial life for a period of seven consecutive days after which some of their haematological changes were determined. There were significant polycythaemia, leucocytosis, thrombocythaemia and elevation of haemoglobin contents ( $p < 0.05$ ). The importance of these haematological adjustments which the fish makes whilst subjected to complete aerial habitat prior to aestivation are discussed.

**Key word:** Aerial habitat, Leucocytosis, Polycythaemia, Thrombocythaemia  
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### Introduction

The west African lungfish, *Protopterus annectens* lives in shallow parts of some rivers and lakes of several West African countries from Cameroon to Senegal and beyond (Otuogbai, 2001; Otuogbai and Ikhenoba, 2001; Okafor and Odiete, 2002; Ndukuba *et al.*, 2006; Okafor *et al.*, 2006). It is an obligatory air-breather because even though it uses the gills to breathe oxygen from the ambient water, it rises to the water surface at intervals to use its paired lungs to breathe atmospheric oxygen and when denied of access to air, it dies (Okafor, 2004a). However, when the ambient water dries up completely as is usually the case during the peak of dry season, *Protopterus* has evolved a means of escaping desiccation by excavating a burrow in the sediment where it retires in a sleep-like fashion throughout the dry season, in fact until the water reappears, usually during the next rainy season.

Previous reports from Nigeria (Okafor, 2004a, b, c, d); Okafor and Chukwu, 2005) and abroad (Janssens, 1964; Johansen and Cohen, 1968; Delaney *et al.*, 1974; Lomholt and Johansen, 1975), dwelt on some physiological adjustments which accompany this state of dormancy, otherwise known as aestivation in *Protopterus*. However, studies on physiological adjustments which *Protopterus* undergoes when the ambient water has totally dried up before it begins to aestivate in the mud are rather scanty (Chew *et al.*, 2003). The present report aims to precisely provide information on some haematological changes when exposed to a complete aerial life prior to aestivation. This would probably give us an insight on some haematological adjustments which occurred amongst the early fishes.

### Materials and Methods

Live specimens of the West African lungfish, *P. annectens* were collected from Agulu lake, Nigeria and transported to laboratory

in large perforated polythene bags containing some water from the lake.

The standard lengths and weights were quickly determined before they were acclimated at room temperature of  $25.8 \pm 6^\circ\text{C}$  for two weeks. The fish were fed *ad libitum* on insect larvae, boiled rice, palm nuts and pelleted fish feed obtained from the Nigeria Institute of Oceanography and Marine Research (NIOMR). The water in all tanks was renewed thrice weekly to remove waste materials.

After acclimation, the standard lengths and weights of all surviving specimens were re-determined. Then, twenty specimens (randomly selected from amongst the survivors) were introduced in the five tanks (0.60 x 0.30 x 0.30 m) whose water contents were completely emptied. They were left inside these tanks for seven days. The remaining 24 live specimens were maintained continuously in the five remaining tanks (0.60 x 0.30 x 0.30 m) each of which contained four litres of dechlorinated tap water for seven days.

All specimens in both groups were weighed daily and provided with food during that one week of experiment. At the end of seven days of complete aerial exposure of one group of the specimens, the standard lengths and weights of both groups (aquatic and aerial) were re-determined. The blood cells erythrocyte count (EC), leucocyte count (LC), thrombocyte count (TC) and haemoglobin content (HC) in both groups were estimated following the methods of Blaxhall and Daisley (1973).

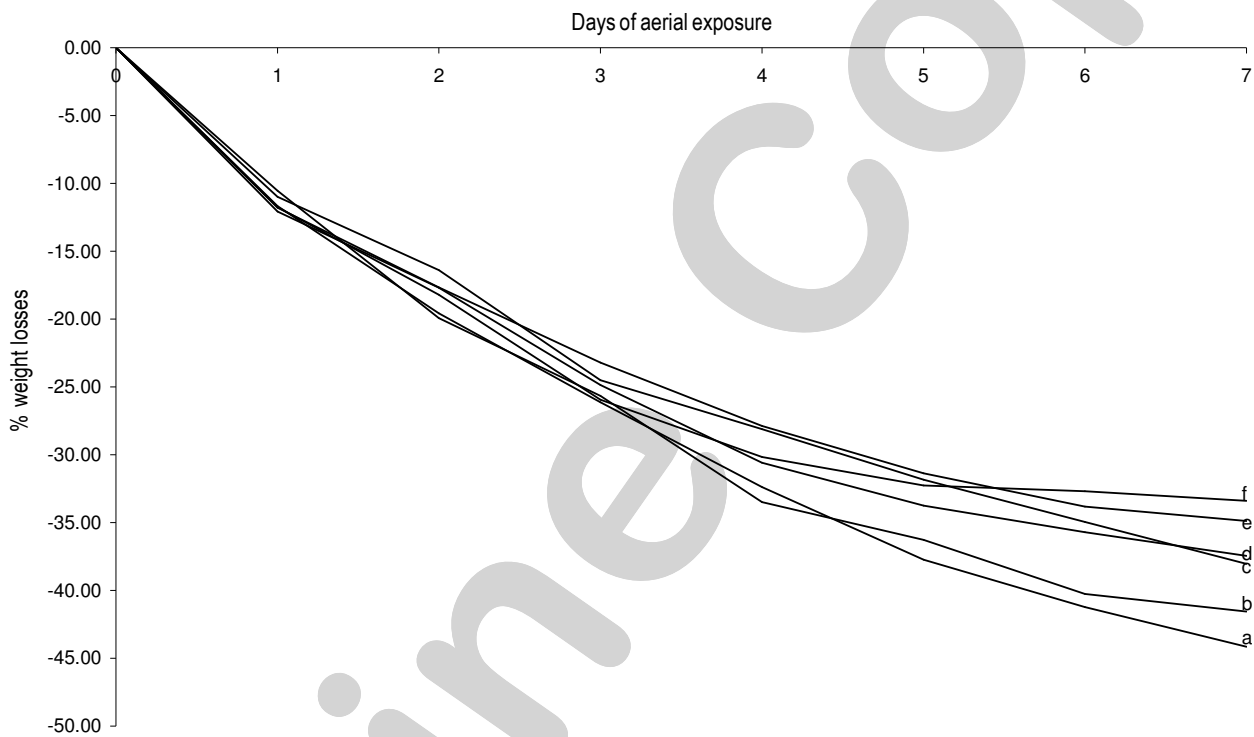
Thus 1.0 ml of blood was drawn from the caudal blood vessels of each specimen using 2 ml disposable syringe and immediately transferred into 5 ml test tube containing 0.1g of ethylene (-) diamine (-) tetracetic acid (EDTA) as anticoagulant.

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**Table - 1:** The mean erythrocyte, leucocyte and thrombocyte counts as well as mean haemoglobin contents of various sizes of *Protopterus annectens* exposed to aerial and aquatic habitats respectively

Range of standard lengths (cm)	MTBW (g)	Aerial				Aquatic			
		Erythrocyte counts (mm <sup>3</sup> )	Leucocyte counts (mm <sup>3</sup> )	thrombocyte counts (mm <sup>3</sup> )	Haemoglobin contents (g%)	Erythrocyte counts (mm <sup>3</sup> )	Leucocyte counts (mm <sup>3</sup> )	Thrombocyte counts (mm <sup>3</sup> )	Haemoglobin contents (g%)
(a) 16.1-27.0	50.4 <sup>A</sup>	116.000 <sup>G</sup>	5200 <sup>H</sup>	24.1 <sup>I</sup>	11.5 <sup>J</sup>	60.000 <sup>K</sup>	2400 <sup>L</sup>	11.5 <sup>M</sup>	7.4 <sup>N</sup>
(b) 27.1-31.0	194.2 <sup>B</sup>	125.000 <sup>G</sup>	5100 <sup>H</sup>	23.4 <sup>I</sup>	11.5 <sup>J</sup>	63.000 <sup>K</sup>	2400 <sup>L</sup>	10.8 <sup>M</sup>	7.4 <sup>N</sup>
(c) 31.1-34.0	224.1 <sup>C</sup>	130.000 <sup>G</sup>	4700 <sup>H</sup>	21.5 <sup>I</sup>	12.7 <sup>J</sup>	67.000 <sup>K</sup>	2200 <sup>L</sup>	9.6 <sup>M</sup>	7.4 <sup>N</sup>
(d) 34.1-37.0	289.3 <sup>D</sup>	135.000 <sup>G</sup>	4800 <sup>H</sup>	21.9 <sup>I</sup>	12.8 <sup>J</sup>	69.000 <sup>K</sup>	20.00 <sup>L</sup>	9.3 <sup>M</sup>	7.5 <sup>N</sup>
(e) 37.1-40.0	345.6 <sup>E</sup>	140.000 <sup>G</sup>	4400 <sup>H</sup>	20.5 <sup>I</sup>	12.9 <sup>J</sup>	70.000 <sup>K</sup>	20.00 <sup>L</sup>	8.7 <sup>M</sup>	7.5 <sup>N</sup>
(f) 40.1-43.0	618.5 <sup>F</sup>	135.000 <sup>G</sup>	4100 <sup>H</sup>	20.2 <sup>I</sup>	12.8 <sup>J</sup>	74.000 <sup>K</sup>	1900 <sup>L</sup>	8.2 <sup>M</sup>	7.5 <sup>N</sup>

MTBW = Mean total body weights, means (3 and 4 numbers of samples) followed by the same superscript letter in a column are not significantly different in the student newman keul's test (p<0.05)



**Fig. 1:** The daily mean percentage weight losses in six groups standard length specimens of *Protopterus annectens* during one week of exposure to an aerial habitat

By the use of a haemoglobin pipette, 0.02 ml blood were drawn from each extracted blood sample into 5.0 ml of formal citrate (sodium citrate, 3.0 g; formaldehyde, 1.0 ml and distilled water 100 ml) in a bijou bottle to give a dilution ratio of 1:250. A few drops of mixture were loaded into the Improved Neubauer haemocytometer. The red cell number was estimated with the aid of a light microscope.

A second 0.02 ml of blood were drawn from each extracted blood sample into 0.4 ml of Turk's solution (1% glacial acetic acid) in a bijou bottle to give a dilution ratio of 1:20. Some drops of the mixture were again loaded into the same improved Neubauer haemocytometer and white cell number was determined by using a light microscope.

A third 0.02 ml of blood were drawn from each extracted blood sample using a haemoglobin pipette into 0.4 ml of 1% ammonium oxalate in a bijou bottle to give another dilution ratio of 1:20. The mixture was loaded into the Improved Neubauer haemocytometer. The blood platelet number was estimated with a phase contrast microscope (Biostar B<sub>4</sub>, Exacta and Optech) by employing methods described by Blaxhall and Daisley (1973) as well as Okafor and Chukwu (2005).

Finally, 0.02 ml of blood were drawn from the remainder of each extracted blood sample using a haemoglobin pipette into 5.0ml of Drabkin's fluid (potassium cyanide 0.2 g; potassium ferricyanide 0.2; sodium bicarbonate 1.0 g; and one litre of distilled water) of pH



7.0 contained in a bijou bottle. This gave a dilution ratio of 1:250. Then 1.0ml of this mixture was poured into a cuvette and its optical density (OD) read in a photoelectric colorimeter at absorbance of 540nm, zeroing with blank. The haemoglobin content of each blood sample was calculated as:

$$Hb_4 = \frac{T \times C \times D}{A \times 1000} \text{ g } 100 \text{ ml}^{-1}$$

T = test absorbance

A = standard absorbance

C = concentration of cyanmethaemoglobin standard (mg 100 ml<sup>-1</sup>)

D = dilution factor = 250

1000 = converts mg 100 ml<sup>-1</sup> to g 100 ml<sup>-1</sup>

The student *t*-test was used to compare the results of the two groups at 5% level of significance.

### Results and Discussion

The changes in body weights of the surviving aerial group of *P. annectens* after every day of exposure to a complete aerial habitat are illustrated in Fig. 1. These aerial group of specimens lost a mean weight of 37.72% after seven days of aerial exposure. The aquatic specimens had a mean body weight increase of 0.1 ± 0.08%. The mean differences between the values recorded for EC, LC, TC and HC of the aquatic specimens were compared with those of aerial specimens. It was found that one week exposure of *P. annectens* to a complete aerial life (without water) led to a statistically significant polycythaemia (*p*<0.05), leucocytosis (*p*<0.05), thrombocythaemia (*p*<0.05) and elevated haemoglobin concentration (*p*<0.05) Table 1.

There is reduction in oxygen absorption of air-breathing freshwater teleosts exposed to complete aerial life (Waddell, 2007). The paper opined that since oxygen intake was reduced during exposure to complete aerial life (without water at all) there was the need for polycythaemia. Since the erythrocytes contain the oxygen carriers, haemoglobin, polycythaemia would ensure the elevation of haemoglobin concentration that would deliver more oxygen to the tissues. Okafor (2004a) had reported polycythaemia and its associated hyperhaemoglobinaemia in *P. annectens* that was exposed to dilute concentrations of seawater ranging from 1.8 to 14.0‰.

Polycythaemia had also been reported during aestivation of *P. annectens* when its oxygen supply was reduced (Okafor, 2004b).

Similarly polycythaemia and hyperhaemoglobinaemia were reported by Okafor *et al.* (2006) in *P. annectens* when the oxygen content of the ambient acid water (pH = 4.6) was low.

The observed leucocytosis could be attributed to strong emotion due to change of habitat, from its normal freshwater habitat to a life completely out of water. Wilkinson and Cuthbertson (1976), Clausen (1977), Kinney and Felig (1979) have all reported that leucocytosis could be caused by strong emotion. Smith (1984) recorded a significant rise in leucocyte counts amongst students preparing to take a difficult examination. The fish was then faced with

emotional threat of survival in the aerial habitat and consequently leucocytosis ensued. Leucocytosis was reported by Okafor (2004a) to occur in *P. annectens* exposed to brackish water of salinity between 1.8 and 10.5‰. Okafor and Chukwu (2005) found that there was leucocytosis in *P. annectens* when its burrow was being excavated and attributed it to threat of tissue injury or septic infections that might have occurred due to such an exercise. Okafor *et al.* (2006) had also found that there was leucocytosis in the African lungfish due to exposure to acid water of pH ranging from 4.6 to 5.8.

Studies have shown that thrombocythaemia occurs whenever there are either body wounds or serious threats to body wounds (Hampton and Brinkhouse, 1976; Zucker, 1980). Thus the blood platelet number increased significantly during complete aerial exposure in order to increase capillary resistance and prevent any likelihood of serious bleeding that may result from desiccation or from body damage during burrow excavation. Okafor and Chukwu (2005) had established the occurrence of thrombocythaemia in the African lungfish, *P. annectens* just after three weeks of aestivation in the mud. This was to heal body wounds that might have occurred because of digging of burrow.

Nkedife (2008) found that the blood platelet number was elevated in juvenile African catfish, *Heterobranchus bidorsalis* that received several bites from their adult counterparts during confinement. Okafor (2008) reported the proliferation of blood platelets in *P. annectens* when exposed to brackish water (S = 10.5‰).

Due to dehydration that occurred amongst those specimens exposed to complete aerial life the observed polycythaemia, leucocytosis and thrombocythaemia could also be partly attributed to haemoconcentration.

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