

# ACID TOLERANCE OF THE AFRICAN LUNGFISH, *PROTOPTERUS ANNECTENS* (OWEN)

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## ABSTRACT

The tolerance of the African lungfish, *Protopterus annectens* (Owen) to acid water was investigated under laboratory conditions. Healthy adult specimens of the African lungfish, *Protopterus annectens* (Owen); mean length  $38.3\text{cm} \pm 2.4$ , and mean weight  $294.3\text{g} \pm 5.2$  were subjected to acid waters of concentrations that ranged from pH 3.8 to pH 7.4. *P. annectens* tolerated acid water from neutral pH 7.4 down to a pH of 4.6. It is possible that *P. annectens* was able to maintain almost a constant level of blood pH in acid waters due to the possession of acidophilic cells in the gills which actively secrete  $\text{H}^+$  from the blood into the water medium.

**KEYWORDS:** Acid water, pH buffered solutions, Serum pH

## INTRODUCTION

There were many species of lungfish that existed in the past but most are now extinct. (Ahlberg *et al*, 2003) However, only three genera, *Lepidosiren*, *Protopterus* and *Neoceratodus* have managed to survive until today. The latter are discontinuously distributed in rivers and lakes of South America, Africa and Australia respectively. (Johansen *et al* 1967; Funkhouser *et al* 1972; Okafor and Chukwu, 2005).

The lungfish is of biological interest due to its peculiar mode of life. For instance it is able to live in the water during the wet season breathing with both gills and lungs but during the dry season when the ambient water has dried up completely, it can excavate a burrow in the soil where it stays and resorts to breathing with only the lungs until the next wet season. (Funkhouser *et al*, 1972; Okafor and Odiete, 2002a).

There are four species of the African genus, *Protopterus* that are distributed in various waters of African continent.

*P. amphibius* (Peters) is found in rivers and lakes of Kenya (Bear *et al* 1992), *P. aethiopicus* (Heckel) inhabits lake Victoria in Uganda. It is also distributed in lakes and rivers of Tanzania and Sudan (Delaney *et al*, 1974). *P. dolloi* (Boulenger) is found in Gabon, Congo and Zaire (Forster and Goldstein, 1966).

*P. annectens* (Owen) is distributed in rivers and lakes of Senegal, Gambia, Ghana, Republic of Benin, Nigeria, Chad, Camerouns (Otuogbai and Ikhenoba 2001; Okafor and Odiete 2002b, Okafor, 2004; Okafor and Chukwu 2005b).

Due to the fact that not much work has been done on acid tolerance of African fishes, this study was therefore undertaken. In fact there is no available information meanwhile on acid tolerance of the African lungfish, *Protopterus*. A Knowledge of acid tolerance limits of fishes is of ecological significance in determining how they can be distributed as well as their impact on ecosystems.

The findings would guide us in the culture of this fish, especially in areas where human utilization of water contributes to high concentrations of carbon dioxide in that body of water such as near sewage and some industrial and even thermal effluents. It is also hoped that the information obtained would provide baseline information for regulatory agencies in setting up national standard for water quality management.

## MATERIALS AND METHODS

Live specimens of the West African lungfish, *P.*

*annectens* procured from Oguta lake, in Oguta, Imo State of Nigeria were brought to the Animal and Environmental Biology laboratory of Abia State University, Uturu, Nigeria.

## Acclimation and Maintenance of *P.annectens* in the Laboratory

Their standard lengths which were quickly determined ranged from 27.2cm to 42.0cm, mean  $38.3 \pm 2.4$ ; while their weights ranged from 156.6 to 460.4g, mean  $294.3\text{g} \pm 5.2$ .

They were introduced into 12 plastic tanks that measured  $0.54 \times 0.38 \times 0.30\text{m}$ , each of which contained 3 litres of dechlorinated water where they acclimated for 28 days. During this period, they were maintained at room temperature and fed on insect larvae, palm nuts, biscuits and boiled rice.

The water in all tanks was renewed thrice a week to prevent accumulation of excess or uneaten food, waste materials and the fish's mucous secretions.

## Preparation of various pH buffered solutions

Two and a half litres each of the following pH buffered solutions were prepared: 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, 6.6, 7.0, and 7.4. This was done by using water obtained from Oguta lake in mixing a certain volume of 0.1 M citric acid monohydrate (Molecular mass, 210.14) with a corresponding volume of 0.2 M disodium hydrogen orthophosphate. (Molecular mass, 141.98). The actual pH of the prepared buffer solution was ascertained with a pH meter at  $25.7^\circ\text{C}$  (Table 1). The prepared pH buffered solutions were transferred into 10 plastic tanks that measured  $0.54 \times 0.38 \times 0.30\text{m}$ , each tank containing a particular pH solution.

**Table 1:** The preparation of 2½ L of citric acid/disodium hydrogen orthophosphate buffer of various pH values.

Volume of 0.1 M citric Acid ( $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ ) (Litres)	Volume of 0.2 M disodium hydrogen orthophosphate ( $\text{Na}_2\text{HPO}_4$ ) (Litres)	pH of the mixture at $25.7^\circ\text{C}$
1.613	0.887	3.8
1.465	1.035	4.2
1.331	1.169	4.6
1.213	1.287	5.0
1.106	1.394	5.4
0.989	1.511	5.8
0.848	1.652	6.2
0.681	1.819	6.6
0.441	2.059	7.0
0.229	2.271	7.4

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### Selection of fishes and determination of acid tolerance limits of *P. annectens*.

Twenty healthy and active specimens of *P. annectens* which had no visible signs of disease or wounds were selected from amongst those that survived acclimation and introduced into the above 10 tanks at a stocking rate of two specimens per tank. All fishes were fed and all tanks were neither covered nor aerated throughout both the period of acclimation and the period when they were immersed in various pH buffered solutions. Their acid tolerance limits in the various pH buffered solutions were noted.

### Blood collection from specimens of *P. annectens* acclimated in acid waters.

In order to determine the effect of acid water on serum pH, blood was extracted from each of the fishes after 2 weeks of introduction inside the various pH buffered solutions. This was carried out first by mildly anaesthetizing each

specimen with chloroform and then by the use of 5ml disposable heparinized syringes, blood was drawn from their caudal blood vessels. The extracted blood was immediately centrifuged in a Gallenkamp centrifuge at 3000 revolutions per minute for 30 minutes. The supernatant was collected and the pH of the supernatant was determined using pH metre (Blaxhall and Daisley, 1973).

### Gradual acclimation of *P. annectens* in acid water

A second set of experiment was later carried out to monitor the gradual acclimation of *P. annectens* to acid water. Eight specimens of *P. annectens* of about the same size were placed in pH buffered solution of 6.0. After a week, they were transferred to pH buffered solution of 5.0. After another week, they were transferred to pH buffered solution of 4.6, and in the same manner to that of 4.5, 4.4 and 4.3 respectively. Their acid tolerance limits as well as their serum pH levels in the various buffered solutions were determined. (Table 2).

**Table 2 : The serum pH values in specimens of *Protopterus annectens* when introduced in acid water of various concentrations.**

Sp	SL	B.W	Serum pH in 6.0pH water	Serum pH in 5.0 pH water	Serum pH in 4.6 pH water	Serum pH in 4.5 pH water
A	38.9	299.6	7.4	7.4	7.0	D
B	37.9	342.5	7.3	7.4	7.1	D
C	39.1	238.5	7.4	7.3	7.0	D
D	38.2	309.1	7.5	7.4	7.0	D
E	37.0	295.6	7.4	7.4	7.0	D
F	39.3	285.6	7.4	7.2	7.0	D
G	36.8	241.4	7.4	7.4	7.0	D
H	37.6	301.3	7.2	7.4	7.0	D

Sp = Specimen  
SL = Standard Length in cm  
BW = Body Weight in gms

D = Death of specimen before 24 hours

### Histological analysis of the gills

Histological studies of the gills were made to ascertain the structural basis for result interpretation. Live specimens of *P. annectens* were killed by chloroform inhalation. The gills were removed and fixed in Bouin's fluid, and then dehydrated through graded series of alcohol, cleared in Xylene and embedded in molten paraffin wax. Thin sections of about 5 to 6  $\mu$ m thick were stained in Alcian blue, after the method of Cook; (1974).

### RESULTS

#### Toxicological analysis

*P. annectens* survived for over 2 weeks of the

experiment in pH buffered solutions of 7.4 down to 4.6. Mortality occurred before 24 hours in pH buffered solutions of 4.5 and below. When placed in pH buffered solution of 4.5, for instance, the fish initially made some struggling movements for about 15 minutes and then went into a state of 'acid coma' before death.

The mean serum pH values and the corresponding buffered solutions are as shown in Table 2. Regression analysis shows a positive linear correlation between mean water pH and mean serum pH values.  $r = 0.9832641$

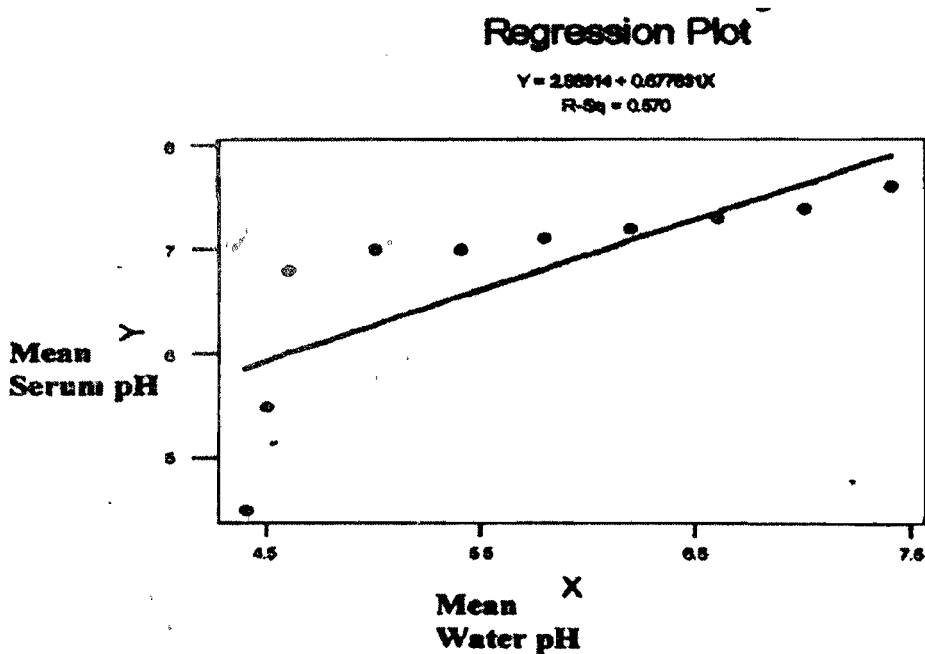


Fig. 1: The mean serum pH values in various concentrations of acid water.

Table 3: Water and serum pH values

(Water pH) X-axis	(Serum pH) Y-axis
4.4	4.5
4.5	5.5
4.6	6.8
5.0	7.0
5.4	7.0
5.8	7.1
6.2	7.2
6.6	7.3
7.0	7.4
7.4	7.6

**Histological Analysis**

The gills were vestigial and weighed 0.64% of body weight. Large sized acidophylic cells were present on the gill epithelium. (Plate 1).



Plate 1: Histological analysis showing large scale acidophilic cells

**DISCUSSION**

*P. annectens* was able to tolerate acid water from pH of 7.4 down to pH of 4.6. Below a pH of 4.6, when the mean serum pH level was below 6.8 and when water pH was also further reduced to 4.5 and below, all specimens died. Thus extreme reduction in water pH led to an alarming increase in the amount of H<sup>+</sup> in the blood. Excessive H<sup>+</sup> in the blood inhibit the excitability of neurons (Kuffler and Nicholls, 1976).

The result also indicates that *P. annectens* can thrive in moderate acid waters like in water bodies near sewage and certain industrial effluents. But if the acidity of the water goes above a certain level, death follows. The presence of acidophilic cells in the gills suggest the active secretion of H<sup>+</sup> from the blood into the surrounding water. (Chukwu and Odiete, 1999).

In this study, acidity was considered to be the cause of mortality. However, there might be some other factors that could play crucial roles in ameliorating or exacerbating acid tolerance. Such factors may include water quality (especially the nature of several other ions) water conditions (especially temperature) as well as biological variables such as sex, age, size, stage in life cycle, origin, previous exposure to acid waters etc.

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