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Metabolic Adjustments of the West African Lungfish, *Protopterus Annectens* in Brackish Water

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Abstract

African lungfish, *Protopterus annectens (P. annectens;* mean weight, 338.8g and mean length, 39.5cm) procured from Imo River at Umuna in Imo State, Nigeria, were, after acclimation in dechlorinated water, transferred into each of the following concentrations of seawater:-0%0, 1.9%0, 3.7%0, 5.6%0 , 7.4%0 , 9.3%0 , and 11.1%0 , and placed there for four weeks after which the levels of their blood nitrogenous wastes (urea, uric acid and creatinine) were determined . There was a significant positive linear correlation between salinity and mean blood urea, uric acid and creatinine concentrations respectively.

The study strongly suggests some metabolic adjustments that occur whilst maintaining or culturing *P. annectens* in brackish or estuarine water regimes.

Keywords: Salinity, Nitrogenous wastes, *Protopterus annectens*

1. Introduction

The African lungfish, *P.annectens* lives in shallow and swampy parts of some Rivers and Lakes of some West African countries such as Cameroun, Nigeria, Togo Republic, Ghana, Senegal etc (Laberge and Walsh, 2011; Loong *et al,* 2012 a,b; Lopez *et al,* 2012; Nakamuta *et al,* 2012) Recent studies have shown that it can tolerate seawater up to a maximum of 10.5%o (Okafor, 2004, 2008) Thus, the fish has got the potential of being cultured in brackish water. When some freshwater fish species are cultured in brackish water, they exhibit enhanced hatching, growth and survival rates (Holliday, 1969; Akingbe, 2012) Consequently, there is immense need to understand certain physiological adjustments by which *P. annectens* can be cultured in brackish or estuarine waters. There is very little or no existing information on the nitrogenous excretion of lungfish in brackish or estuarine waters. In order to fill the lacunae in knowledge, the study aims to determine precisely the changes in serum nitrogenous wastes when *P. annectens* is transferred from freshwater into different concentrations of dilute seawater and

maintained there for a while. The information obtained may serve as valuable resource in osmoregulatory studies of the species in brackish or estuarine ecosystems.

2. Materials and Methods

2.1 Procurement of Lungfish

Live specimens of the African lungfish, *P. annectens* procured from Imo River at Umuna-Okigwe of Imo State, Nigeria were transported into Physiology laboratory of Animal and Environmental Biology Department, Abia State University, Uturu-Nigeria.

2.2 Laboratory Acclimation of Lungfish

After the determination of their standard length and weights, they were allowed to acclimate at room temperature of 26.7$\pm$ 0.9^{0}C for three weeks in seven plastic tanks (0.5 x 0.3. x 0.4 m) which were neither covered nor aerated and each of which contained 5 litres of dechlorinated water.

The fish were fed on fish feed obtained from the Regional Aquaculture Centre , Aluu in Rivers State, Nigeria *ad libitum* until used for the experiment. The water in all tanks was renewed daily to prevent the accumulation of excess or uneaten food, waste materials and the fish's mucous secretions.

2.3 *Collection and Serial Dilution of Sea Water*

Seawater (S=37%0) was collected from the Naval Base, Borokiri in Port Harcourt (Atlantic Ocean) and filtered with a fine 0.5mm sieve.

Five litres of each of the following percentages of seawater were prepared and ascertained with both a hand refractometer (Model: Automatic RFM 700) and a Beckman salinometer; model RS 5-3); 0%0, 1.9%0, 3.7%0, 5.6%0, 7.4 %0, 9.3%0, and 11.1%0,. This was carried out by diluting the seawater (S= 37 %0,) with the appropriate volume of clean dechlorinated water.

Table 1. The preparation of various concentrations of dilute seawater, each with a volume of 5 litres.

Salinity of the medium (%0)	Volume of dechlorinated water (litres)	Volume of undiluted seawater(litres)	Volume of prepared dilute seawater(litres)
11.1	3.5	1.5	5.0
9.3	3.8	1.2	5.0
7.4	4.0	1.0	5.0
5.6	4.3	0.7	5.0
3.7	4.5	0.5	5.0
1.9	4.8	0.2	5.0
0.0	5.0	0.0	5.0

It was not necessary to prepare seawater above 11.1%0 concentration since *P. annectens* cannot tolerate indefinitely seawater above 10.5-11.1%0 (Okafor , 2004,2008).

2.4 Introduction of Lungfish in Tanks Containing Seawater

Thirty six specimens were selected from amongst the survivors of acclimation and introduced into twelve plastic tanks (0.5x0.3x0.4 m) containing the above concentrations of seawater at a stocking density of three specimens per tank. Only healthy and active fishes which had no obvious signs of physical injuries or disease were selected for the study.

All fishes were fed and all tanks were neither covered nor aerated with air pumps throughout the four week period of immersion in dilute seawater.

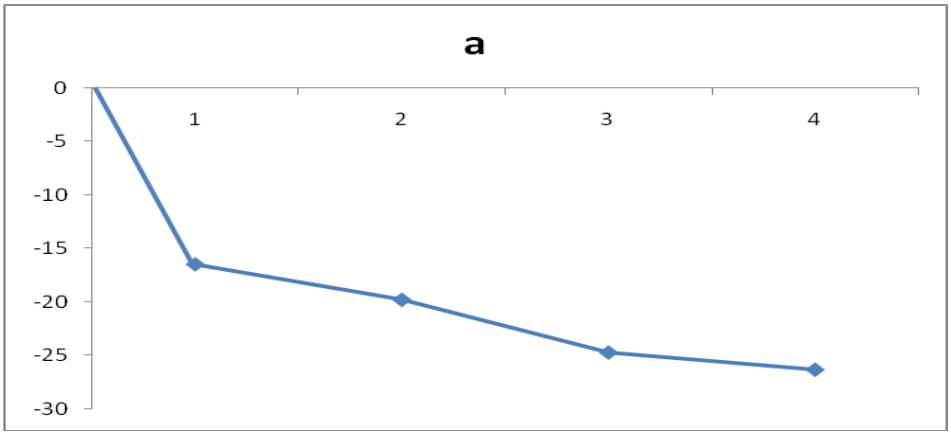
2.5 Determination of Rate of Ammonia Production and the Concentration of Serum Nitrogenous Wastes

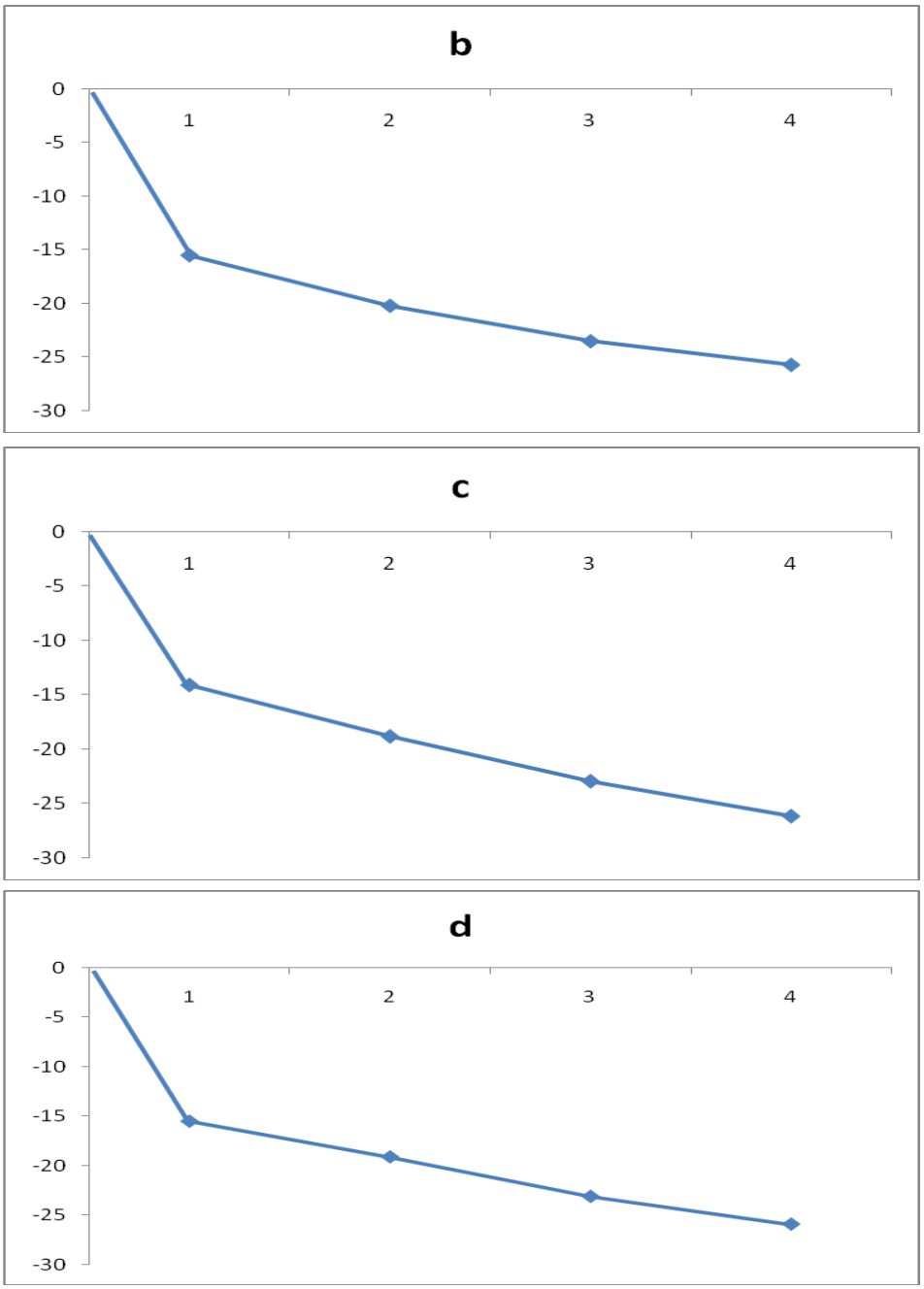
After 28 days of immersion in various concentrations of dilute seawater (1.9-11.1%0) ammonia production rate over a 12 hr period of every *P. annectens* specimen was investigated, following the method of Okafor (2008).

Then all fishes were mildly anaesthesized with chloroform and by the use of 1.0ml heparinized disposable syringes, about 0.5ml of blood was drawn from the caudal blood vessels of each specimen. The serum urea, uric acid and creatinine concentrations of each sample were determined by the use of Diacetylmonoxine, Folin/Trimble and Jaffer's standard methods respectively. (Dyer, 1965., Wharton and McCarty, 1972; Brewer *et al*, 1974; Cheesbrough,2007).

3. Results

The mean changes in body weights of various size groups of *P. annectens* during the four weeks of immersion in seawater (S=1.9 to 11.1%0) are shown in Fig 1.





Key
a = SL 31.1-34.0
b = SL 34.1-37.0
c = SL 37.1-40.0
d = SL 40.1 -43.0

Fig 1. The mean changes in body weights during immersion of *P. annectens* in various concentrations of dilute seawater.

Ammonia production rate decreased as standard length of fish increased. (Table 2).

Table 2. Ammonia production rates and the mean levels of urea, uric acid and creatinine in the blood of two groups of *Protopterus annectens(**aquatic*** and ***saline**)* after four weeks of immersion of one group in saline water.

		SALINE			AQUATIC			
Range of standard length (cm)	Mean body weights (g)	Mean urea level (mg/dl)	Mean uric acid level (mg/dl)	Mean creatinine level (mg/dl)	Mean urea level (mg/dl)	Mean uric acid level (mg/dl)	Mean creatinine level (mg/dl)	Ammonia production rate ($mgNH_3/hr/g$)
31.1-34.0	168.8	365	0.6	1.2	28	0.2	0.4	0.00036
34.1-37.0	261.5	415	0.9	1.6	30	0.3	0.5	0.00021
37.1-40.0	345.2	510	1.2	1.9	35	0.4	0.6	0.00009
40.1-43.0	796.4	550	1.3	2.3	46	0.4	0.8	0.00005

As salinity of the media increased where *P. annectens* was placed, there was a corresponding increase in the mean serum levels of urea, uric acid and creatinine respectively.

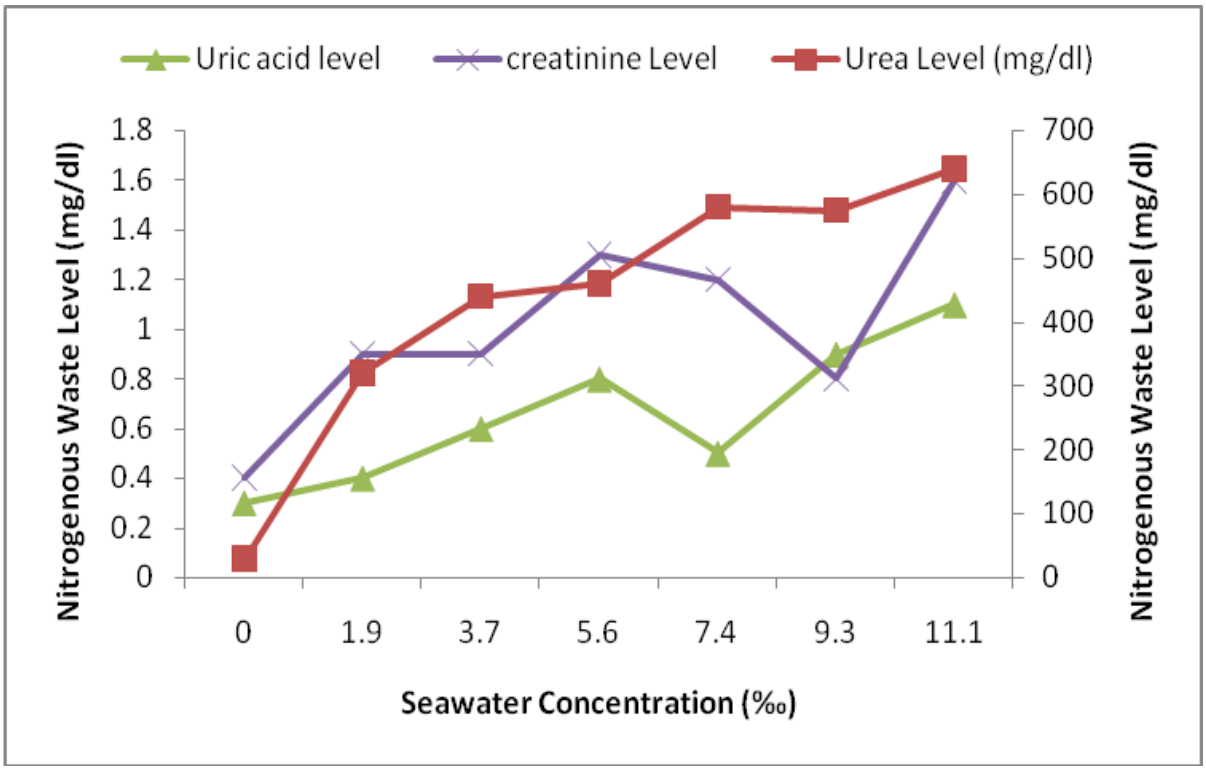


Fig 2. The mean serum urea, uric acid and creatinine levels (mg/dl) of *P. annectens* (large) after four weeks of introduction in various concentrations of dilute seawater.

The mean serum urea , uric acid and creatinine levels of various ranges of standard length of *P. annectens* in both the saline water (S= 1.9 to 11,1%0) and ordinary water (S = 0%0) are also shown in Table 2.

Regression analysis shows a significant positive linear correlation between salinity and urea,

uric acid and creatinine levels respectively. Salinity/urea, r=0.964682; salinity/uric acid, r= 0.906157; salinity/creatinine r = 0.865184.

4. Discussion

In its normal freshwater habitat, *Protopterus,* just like many freshwater fishes was able to get rid of its nitrogenous wastes in the form of ammonia (Patel *et al*, 2009a,b; Loong *et al*, 2005, 2008) However, the removal of metabolic wastes in the form of ammonia entails the elimination of much volumes of water (Balinsky and Baldwin, 1961) Ever since *P. annectens* was introduced into dilute seawater, it was losing much body water (Fig 1.). Consequently it could not afford to lose too much water through the excretion of ammonia. On the other hand, ammonia is not supposed to be stored in the body fluids since it is poisonous (Chew, *et al* , 2004) The only alternative was to detoxify some ammonia and convert it into non poisonous urea and uric acid. Tissue protein was also catabolized in saline water to yield non poisonous creatinine. All the enzymes of the ornithine , uricolytic and creatininolytic pathways have been detected in the liver of lungfish (Janssens and Cohen, 1966; Loong *et al*, 2005, 2008; Hung *et al*, 2009; Laberge and Walsh 2011; Oguejiofor, 2012) Balinsky (1981) had also reported the accumulation of some nitrogenous wastes in amphibia when subjected to a hyperosmotic environment.

This paper therefore suggests that when *P. annectens* is exposed to saline water, there is a slight re-adjustment of the lungfish's metabolic pathway so that it is not only ammonotelism, but also includes ureotelism, uricotelism and even creatininotelism as well.

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