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Eco-physiological adaptation of the land snail *Achatina achatina* (Gastropoda: Pulmonata) in tropical agro-ecosystem

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Abstract The survival of land snails in an adverse environmental condition depends on the integral physiological, morphological and behavioural adaptations. These adaptations are essential in understanding the species-specific habitat requirements and in predicting their environmental responses. In this study, the monthly and the periodic patterns of eco-physiological adaptation of land snail, *Achatina achatina* in Nsukka tropical agro-ecosystem were assessed from December 2012 to July 2013. Standard methods were employed in sampling the land snail and determination of the water content, biochemical fuel reserves and enzyme concentrations of the samples. The present results showed that lipids were high at the beginning of aestivation and depleted as the aestivation progressed. Glycogen was significantly low throughout the aestivation months (December–March) and increased in the active months (April–July). Protein content recorded a definite pattern all through the months studied. Catabolism of lactate and a decrease in activity of LDH during aestivation and substantial increase upon activation were observed. Data showed that transaminase and aspartate enzymes depleted during the aestivation months indicating that the snails may have developed potential cell injury due to oxidative stress and thermal heat. A disassociation between the physiological responses and climatic data was recorded. The physiological adaptation of *A. achatina* ensures regular adjustment under extreme conditions and compensates for its metabolic regulation in the tropics. It is concluded that survival of *A. achatina* is not environmentally

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predicted; rather it depends on the species-specific inherent process in predicting responses for survival.

Introduction

The adaptation to stressful environment by land snails is made possible due to a series of morphological, behavioural and physiological responses to homoeostatic cues. The investigation of the adaptive and compensatory processes is useful in understanding the physiological ability of these organisms during extreme conditions such as intense heat, and thermal death due to water loss. These adaptive modifications had aided the success of gastropod snails in terrestrial habitats, especially the *Achatina achatina* of the tropical agro-ecosystem.

The snail, *A. achatina* is the prominent species of land snails endemic to West Africa. It is popularly referred to as giant African land snail (Cobinah, 1992). It belongs to the family Achatinidae. *A. achatina* is distributed widely across West African tropical rainforest and savannah regions within the coasts of West African countries. It is found at various altitudes, inhabiting log of decayed woods, rock exercise, tree trunks and leaf litters and forest floors. This snail has two distinct periods in each seasonal cycle: a long active period during the rainy season (Late March–October) and an aestivation period during the dry season (Late October–early March). It reproduces during the rainy season upon activation from aestivation. The snail aestivates throughout the dry season that extends from November till end of March, by sealing the shell aperture with calcareous mucus layers called epiphragm to reduce water loss (Cook, 2001).

The mechanisms of survival of land snails and their regulation of metabolism for adaptation to the tropical ecosystems depend on the species-specific inherent processes and habitat requirements for predicting responses to environmental changes. The variation in these behavioural patterns and the physiology of land snails is related to repeated sequences of photoperiod, temperature, humidity and water availability (Machin, 1975; Prior, 1985; Cook, 2001; Storey, 2002). The investigation of adaptive and compensatory processes that determine the physiological ability of organisms under extreme conditions can be useful in understanding habitat requirements of these organisms.

The key factors for ensuring survival of land snails during adverse environmental conditions and hypo-metabolic state are fuel reserves and water retention (Storey, 2002). The natural habitat of land snails is damp with vegetation undergrowth. Being cold blooded, land snails are sensitive to changes in atmospheric humidity and temperature (Chinaka and Wilson, 1995). Therefore, they thrive well in moderate temperatures (23 °C–31 °C) and humidity (48 mmHg–82 mmHg); and can survive many months and even years without food and water during aestivation, through its inherent physiological processes.

According to Rees and Hand (1993), one specialisation that is well developed among pulmonate land snails is the capacity to enter the dormant state of aestivation during periods of hot and dry environmental conditions. There are limits to the duration of aestivation that can be tolerated by land snails and mortality eventually increases as aestivation is prolonged. While physiological alterations that occur during aestivation have received much interest over the past few decades (Barnhart, 1986), major questions remain unanswered regarding the metabolism of aestivating snails.

All previous studies on the physiology of land snails as well as their ecological implication have been limited only to microhabitat set up in laboratories, an approach that does not reflect the environmental influences on its physiology. Relatively, little information is available on the eco-physiology of land snails (Riddle, 1983; Arad, 1990; Withers et al., 1997; Giokas et al., 2005, 2007; Arad et al., 1992, 1998). However, no data are available on the ecophysiological adaptation of the widespread pulmonate snail family in Nigerian tropical ecosystem. The present study therefore is to unravel the ecological and physiological responses of *A. achatina* in the tropical savannah region and the possibility of using this species as an indicator of climate change in a variable tropical savannah region of West Africa.

Materials and methods

Study area

The study area was Nsukka agro-ecosystem, located on the northern part of Enugu State Nigeria (N 6° 5′ 23′ N; 7° 23′ 44′ E, 456 m asl). The area belongs to the tropical rainforest belt of Nigerian vegetation with an overlap with the guinea savannah. It is a hilly geographic site flanked by grassy

Table 1 Monthly climatic data for Nsukka area.

Climatic variables	Months					
	December	January	February	March	June	July
Mean rainfall (mm)	0.00 ± .000	0.00 ± .000	0.0009 ± .036	0.0016 ± .041	0.0038 ± .056	0.0051 ± .064
Mean solar radiation (kW/m^2)	154.79 ± 215.33	137.17 ± 192.52	154.10 ± 222.58	185.32 ± 267.83	152.12 ± 221.20	144.74 ± 216.20
Mean ambient temperature (°C)	25.12 ± 4.54	25.89 ± 3.95	27.27 ± 3.36	28.66 ± 3.62	25.14 ± 2.67	24.37 ± 2.39
Mean soil temperature (°C)	25.67 ± 3.56	25.34 ± 4.35	25.35 ± 5.34	24.88 ± 2.87	22.88 ± 4.38	23.56 ± 2.47
Mean relative humidity (mmHg)	37.12 ± 17.34	41.50 ± 23.98	64.01 ± 17.36	60.32 ± 17.64	78.00 ± 10.72	79.94 ± 10.32

lowlands with a plateau physiographic view and a high central zone of peak mountains/hills. Rainy season lasts from April to October while dry season lasts from November to March of the next year. Other climatic data of the area are shown in Table 1. The vegetation/terrain is montane guinea savannah and patches of relic high forest and forest outliers along the plateau. Trees with thick canopies are present as well as a large proportion of plantain and Banana plantations. The soil is mainly made up of shallow and strong lithio-sub found on the steep.

Sampling

The snail sampling was done by using a combination of quadrat count and a direct search technique every month from December, 2011 until July 2012, during the third week of each month at approximately 07.00 h in the morning and 19.00 h in the night. The number of adult and juvenile snails, including their state whether active or aestivating was recorded. Snails collected were taken to the laboratory immediately after sampling.

Determination of water content

The fresh tissue from each individual ($n = 3$) was weighed on a Mettler balance (Germany) after removing the shell and the fresh mass was recorded. The tissue was then placed in a roast-oven at 42 °C and weighed every 12 h until a constant mass was obtained. The water content was expressed as a percentage of dry weight.

$$\text{Water content } (\%) = \frac{\text{fresh mass} - \text{dry mass}}{\text{dry mass}} \times 100 \qquad (1)$$

Extraction of tissues for biochemical assays

Individual snails were weighed before and after removing the shell. The foot muscle tissues were cut and preserved at −15 °C to retain the metabolites until used for biochemical analysis. Haemolymph samples were collected by inserting a 25 – gauge needle into the heart of the snails. The haemolymph was held on ice for no longer than 10 min until use. Haemolymph sample from individual snails was used in all experiments.

Determination of biochemical fuel reserves and activity of LDH

Lactate was determined according to Marti et al. (1997). Total lipid content was determined using solvent extraction (chloroform–methanol) method as described by Folch et al. (1957). The glycogen content was determined using the method of Dreiling et al. (1987). Total protein was determined by the Biuret method (Reinhold, 1953) while the activity of LDH was measured according to Ward et al. (1969).

Determination of biochemical enzyme concentrations

The biochemical enzymes assayed include alkaline phosphatase (ALP), aspartate amino-transferase (AST) and Alanine amino-transferase (ALT). Alkaline phosphatase was measured using Fishman and Lerner (1953) method. Assay for aspartate amino-transferase and alanine amino-transferase was determined according to the methods of Reitman and Frankel (1957). All the biochemical enzymes were assayed using Randox Enzyme Kits.

Data analysis

The data accumulated were subjected to a one-way analysis of variance (ANOVA). Student *t*-test was used to separate the measured variables into active and non-active (aestivation) periods. Stepwise discriminant analysis (SDA) was used to determine which of the factors/parameters accounted most for the differences between the two periods. Correlation was used to identify relationship between mean monthly measured variable with climatic data. Interrelationship among the measured variables and with climatic data was determined using principal component analysis (PCA). Data obtained were recorded as mean ± SD. Duncan's multiple range test was used to separate means of groups. Level of significance was set at $p < 0.05$.

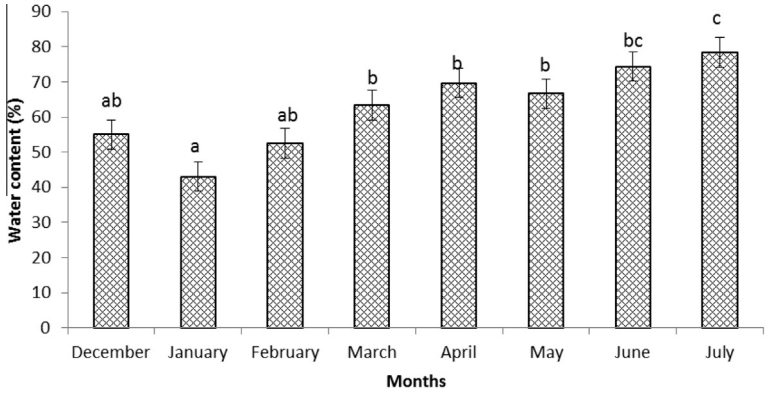

Fig. 1 Monthly changes in water content of *A. achatina*. Mean values with different numbers as superscripts on each bar are significantly different at $p < 0.05$.

Results

Behaviour

The snails were found individually buried in underground burrows below tree shrubs, leaf litters; and in crevices of old cement block with thick white calcareous epiphragm during aestivation. It was observed that arousal from aestivation was stimulated when climatic conditions became favourable (Table 1). The snails upon activation were found crawling on leaves, humid cement walls and often aggregated with neighbours. Homing is a characteristic attributable to *A. achatina* in the present study as an individual snail homes as night draws near. Mortality was low although many empty shells were found in the field but these may be attributed to predation of the snail species.

Changes in water content A. achatina

The mean monthly record of changes in the water content of *A. achatina* is presented in Fig. 1. The present data showed no significant difference ($p > 0.05$) in the water content of *A. achatina* in the first three months of study (December–February) with a slight fluctuation. Further increase was recorded from March and April, and a subsequent non-significant decrease in May ($p > 0.05$). However, the water content recorded in July was statistically significant when compared with the rest of the months but not significant to water content recorded in June.

Changes in biochemical fuel reserves A. achatina

Table 2 shows the mean monthly changes in tissue biochemical fuel reserves (lactate, lipid, glycogen, protein and activity of LDH) of *A. achatina*. The lipid content was found to be in December followed by a decrease in January (mean = 0.188, $F = 8.343$, $P = 0.000$). Subsequently, lipid content increased significantly ($p < 0.05$) in February, declined steeply till April, and then recorded another phase of accumulation till July (active month). Glycogen was found to be low throughout the aestivation months (December–March) followed by a significant increase in May ($p < 0.05$) and then a decrease in June. Further increase was recorded in July though no significant difference was found between the two months ($p > 0.05$). Protein recorded a definite pattern throughout the study duration. Its content recorded an increase in the first three months (December–February) and then an abrupt decline in March. A significant increase was recorded from April to June ($p < 0.05$) and it decreased swiftly in July.

Lactate content decreased from December to March (aestivation months), remained steady for the following two months and decreased slowly during the active months (May–July). The changes in lactate content of *A. achatina* from January to May were not significant ($p > 0.05$). The activity of LDH recorded a non-significant decrease ($p > 0.05$) from December to April. It increased significantly in May (176.48 ± 29.09) and decreased in June. The changes in LDH activity between May and June were significant ($p < 0.05$). The LDH activity decreased further in July but no significant difference was found between June and July ($p > 0.05$).

Table 2 Monthly changes in biochemical fuel reserves and activity of LDH of *A. achatina*.

	December	January	February	March	April	May	June	July
Lactate (mg/dl)	16.16 ± 0.75^{c}	9.13 ± 0.35^{b}	7.10 ± 0.28^{b}	6.97 ± 0.43^{b}	7.87 ± 3.01^{b}	7.06 ± 0.45^{b}	3.11 ± 0.45^{a}	3.03 ± 0.33^{a}
Lipids (g/l)	0.351 ± 0.03^{a}	0.188 ± 0.11^{a}	$0.952 \pm 0.41^{c,d}$	0.864 ± 0.68^{bc}	$0.380 \pm 0.45^{a,b}$	$1.244 \pm 0.10^{c,d}$	1.422 ± 0.36^{d}	$1.288 \pm 0.19^{c,d}$
Glycogen (μg/l)	8.67 ± 6.34^{a}	12.33 ± 7.63^{a}	25.33 ± 8.63^{a}	14.07 ± 2.21^{a}	7.00 ± 5.66^{a}	141.73 ± 104.02^{b}	166.47 ± 5.80^{a}	173.03 ± 4.88^{a}
Protein (g/dl)	11.87 ± 0.17^{a}	$12.21 \pm .78^{a,b}$	$12.89 \pm 0.30^{b,cd}$	11.87 ± 0.15^{a}	$12.27 \pm 0.43^{a,bc}$	$13.07 \pm 0.62^{c,d}$	13.18 ± 0.26^{d}	$12.27 \pm 0.43^{a,b,c}$
Activity of LDH (U/L)	70.90 ± 25.85^{a}	70.36 ± 3.54^{a}	48.80 ± 12.14^{a}	46.60 ± 10.43^{a}	47.34 ± 17.05^{a}	176.48 ± 29.09^{c}	140.43 ± 21.78^{b}	123.87 ± 11.86^{b}

Mean values with different letters as superscripts in a row are significantly different ($p < 0.05$). Values are given as Mean ± SE.

Table 3 Monthly changes in biochemical enzyme concentrations of *A achatina*.

	December	January	February	March	April	May	June	July
ALP (U/L)	48.00 ± 8.19^{c}	39.00 ± 2.00^{b,c}	32.00 ± 1.00^{a,b}	27.00 ± 5.00^{a}	24.67 ± 2.31^{a}	26.00 ± 3.46^{a}	30.67 ± 7.51^{ab}	31.33 ± 7.09^{ab}
AST (U/L)	151.33 ± 26.10^{c}	144.00 ± 24.33^{c}	158.00 ± 5.29^{c}	163.33 ± 8.08^{c}	163.33 ± 6.11^{c}	54.66 ± 12.22^{a}	96.00 ± 2.00^{b}	96.00 ± 5.29^{b}
ALT (U/L)	157.33 ± 31.07^{c}	156.00 ± 6.93^{c}	116.67 ± 16.65^{b}	108.33 ± 9.50^{b}	105.33 ± 8.33^{b}	50.00 ± 14.00^{a}	125.33 ± 6.43^{b}	123.00 ± 5.57^{b}

Mean values with different letters as superscripts in a row are significantly different ($p < 0.05$). Values are given as Mean ± SE.

Table 4 Effects of sampling periods on water content, biochemical fuel reserves and activity of LDH; and biochemical enzyme concentrations of *A. achatina*.

	Aestivation period (dry season)	Active period (wet season)	*t*-Value	*P*-value
Water content	53.47 ± 1.41	72.25 ± 2.17	3.13	0.006*
Biochemical fuel reserves and activity of LDH				
Lactate (mg/dl)	9.84 ± 1.34	5.51 ± 0.71	3.22	0.005*
Lipids (g/l)	0.57 ± 0.12	1.08 ± 0.15	2.73	0.012*
Glycogen (μg/l)	15.01 ± 2.49	50.23 ± 20.65	1.69	0.106^{ns}
Protein (g/dl)	12.21 ± 0.16	12.69 ± 0.17	2.04	0.054^{ns}
Activity of LDH (U/L)	59.17 ± 5.12	122.03 ± 15.03	3.96	0.001*
Biochemical enzyme concentrations				
ALP (U/L)	36.50 ± 2.67	28.17 ± 1.62	2.67	0.0016*
AST (U/L)	154.17 ± 5.05	102.5 ± 11.89	4.00	0.001*

ns = not significant.
* Significant at $p < 0.05$.

Changes in biochemical enzymes of A. achatina

Table 3 shows that the ALP value decreased from December to April (aestivation months) followed by an increase from May till July (active months). The changes recorded between these months were statistically not significant ($p > 0.05$). The data of AST level of *A. achatina* were statistically the same from December to April. A significant decrease ($p < 0.05$) was observed in May (54.66 ± 12.22) and a subsequent significant increase in July (96.00 ± 5.29). The lowest level of ALT was recorded in May, and the highest level was recorded in December and January. In June and July, the level of ALT was significantly higher than that of February, March, April and May, whereas it was significantly lower than that of December and January.

Effects of active and aestivation periods on the water content, biochemical fuel reserves and haemolymph enzymes of A. achatina

The water content exhibited a significant change (*t-value* = 3.13, $P = 0.006$), being higher during the active period (72.25 ± 2.17) than during the aestivation period (53.47 ± 1.41) (Table 4). The results recorded a significant

Table 5 Principal Component Analysis of the Biochemical variables of *A. achatina* (fuel reserves, activity of LDH and biochemical enzyme concentrations).

	Eigen values	Proportion	Cumulative proportion
a. Component matrix			
1	4.22	0.53	0.53
2	1.29	0.16	0.69
Variables	PC 1		PC 2
b. Eigen values, proportion and cumulative proportion.			
Glycogen	−0.69		0.42
Protein	−0.67		−0.01
Lipids	−0.74		−0.04
LDH	−0.81		0.36
ALT	0.77		0.17
AST	0.86		−0.40
ALP	0.57		0.71
Lactate	0.66		0.54

PC = principal component.

effect of both periods on all the biochemical fuel reserves and haemolymph enzymes ($p < 0.05$) except protein and glycogen. There is no significant effect ($p > 0.05$) of active and aestivation periods on the protein and glycogen although these values were higher during the active period than the aestivation period. The Lactate content, ALP, AST and ALT were found to be higher during the aestivation period than the active period.

Discrimination of aestivation and active periods of A. achatina

The stepwise discriminant analysis (SDA) of the *A. achatina* disclosed an explicit discrimination of aestivation and active periods. The results revealed that lactate and AST accounted for most of the variations between aestivation and active periods with 91.7% classified cases (Lactate and AST respectively, $F = 16.00$ and 13.31, Wilks lambda = 0.579 and 0.441, Canonical coefficient = 0.878 and 0.664, Eigenvalue = 1.268, $P = 0.001$). From the principal component analysis of the biochemical variables (Table 5), components 1 and 2 accounted for maximally 69% of the variation AST, LDH, ALT and lipids contributed most to component 1, ALP to component 2 (eigenvalue > 1). The biplot of the PC1 and PC2 showed the interrelationship among the sampled variables (Fig. 2).

Correlations of the biochemical variables of A. achatina with climatic data

A significant correlation (Table 6) was recorded in monthly biochemical variables for the following; LDH and AST ($r = 0.91$, $P = 0.01$), lactate and ALP ($r = 0.69$, $P = 0.01$), Lipids and ALT ($r = 0.61$, $P = 0.01$), glycogen and ALT ($r = 0.65$, $P = 0.01$), glycogen and AST ($r = 0.64$, $P = 0.01$). No significant correlation was found on the biochemical variables with climatic data (Table 7) except relative humidity and ALT, whereas lipids and ALP correlated positively with relative humidity though not statistically significant.

Discussion

The survival of organisms in a stressful environment depends on the potential to fully exploit their adaptive responses which include a series of morphological, behavioural, physiological and biochemical adaptations. According to Storey (2002), the indispensable elements for survival during physiologically critical conditions such as aestivation, among pulmonate land snails are water retention and sufficient energy reserves. The

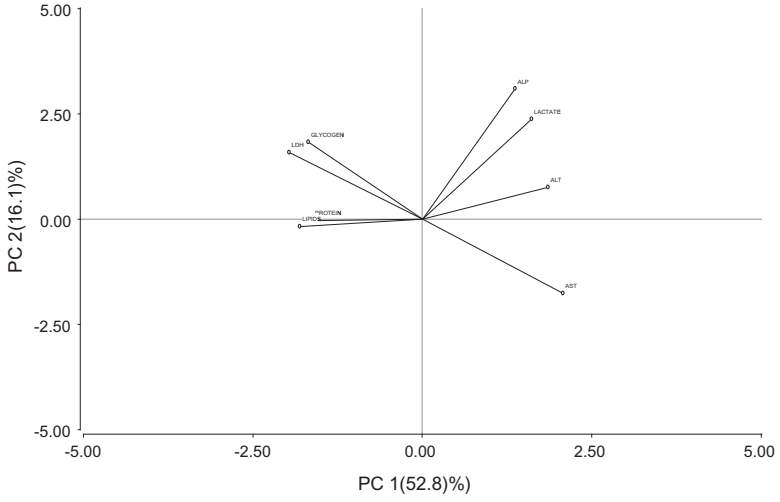

Fig. 2 Principal component analysis biplot of the biochemical variables of *A. achatina*. *NOTE:* LDH = Lactate dehydrogenase, AST = Aspartate amino-transferase, ALT = Alanine amino-transferase, ALP = Alkaline phosphatase, PC = principal component.

Table 6 Cross-correlation matrix of biochemical variables and water content of *A. achatina* in Nsukka area.

	Glycogen	Protein	Lipids	LDH	ALT	AST	ALP	Lactate	Water
Glycogen (µg/l)	1								
Protein (g/dl)	0.41*	1							
Lipids (g/l)	0.37	0.42*	1						
Activity of LDH (U/L)	0.55**	0.44*	0.57**	1					
ALT (U/L)	−0.65**	−0.40	−0.41*	−0.45*	1				
AST (U/L)	−0.64**	−0.51*	−0.61**	0.91**	0.52**	1			
ALP (U/L)	−0.17	−0.28	−0.33	−0.26	0.65**	0.20	1		
Lactate (mg/dl)	−0.16	−0.46*	−0.57**	−0.37	0.40	0.41*	0.60**	1	
Water (%)	0.17	0.35	0.34	0.34	−.098	−0.33	−0.44*	−0.50*	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 7 Correlation of biochemical variables of *A. achatina* and climatic data of Nsukka area.

	Mean rainfall (mm)	Mean solar radiation (kW/m^2)	Mean ambient temperature (°C)	Mean soil temperature (°C)	Relative humidity (mmHg)
Glycogen (μg/l)	−0.37	−0.32	0.12	0.35	−0.21
Protein (g/dl)	0.34	0.10	0.22	0.24	0.36
Lipids (g/l)	0.27	0.04	−0.11	0.32	0.63
LDH (U/L)	−0.21	−0.15	−0.24	0.19	−0.42
ALT (U/L)	−0.31	−0.36	−0.43	0.37	−0.72*
AST (U/L)	0.25	0.27	−0.17	0.31	0.12
ALP (U/L)	−0.40	−0.01	−0.04	0.27	−0.70
Lactate (mg/dl)	−0.38	0.02	−0.14	0.16	−0.48
Water (%)	−0.49	0.29	0.51	0.22	−0.39

* Correlation is significant at the 0.05 level (2-tailed).

results of the present study on behaviour, water content, biochemical fuel reserves and LDH activity; and biochemical enzyme concentrations of *A. achatina* in Nsukka area indicated that some adaptations are in effect during aestivation and active periods of these snail species.

Land snails burrow into the soil or seek sheltered locations during aestivation in order to conserve body water, and minimise their exposure to and avoid predators (Giokas et al., 2005). In addition, water loss is reduced by sealing the aperture directly to substratum or the shell of another animal by more aggregating behaviour or by aestivating just in the ground so as to encounter lower temperatures (Machin, 1975). Most of these behaviours were identified in the land snail, *A. achatina* studied. Majority of the *A. achatina* snails were found burrowed into the soil with a thick epiphragm to prevent water loss during aestivation. Some were found aggregated and burrowed under leaf litters. The present study showed that the aggregation was not high during the aestivation period, this result agrees with the finding of Giokas et al. (2005) on *Albinaria caeurulea* of the Mediterranean.

The degree of clustering was observed to be rather high during the active period than aestivation in the present study. This may be attributed to the unpredictable climate of Nsukka area that may have necessitated compulsory aestivation due to the irregular pattern of rainfall and humid condition in the study area. Aggregation may offer an effective isolation from a critical environmental condition since in this way the exposed total surface area is decreased and a more humid microenvironment attained, resulting in a lower water vapour pressure gradient to the environment and hence, a lower rate of water loss (Prior, 1985; Arad and Avivi, 1998). Arad and Avivi (1998), therefore, concluded that resistance to desiccation is closely related to the specific micro-habitat that each species or population inhabits and that the drier the habitat, the more resistant is the snail and the more effective are its water preservation mechanisms. In this regard, the rate of water loss of the Nsukka land snail in the present study conforms to these mechanisms. Although data are lacking on the resistance to water loss of Nigeria snails, the rate of water loss is similar to that of the northern populations of Mediterranean *Xeropicta vestalis* and *Theba pisana* (Arad et al., 1998). Furthermore, epiphragm secretion is related to the reduction of water loss in land snails (Barnhart, 1983; Machin, 1975; Giokas et al., 2000; Cook, 2001) including the tropical land snails of Nsukka origin. The snails were found with a hard multi-layered and thick epiphragm that are whitish in appearance during aestivating periods and were found burrowed into the lower layer of the soil to prevent water loss.

Land snails show an adaptation that retards water loss during dormancy. Because water is lost during breathing and also across the integument/epithelium, snails normally enter aestivation with larger reserves of body water that can be drawn upon to keep tissues hydrated (Storey, 2002). In Nsukka area, the period of aestivation starts by late November due to the absence of rain and the increase in the intensity of solar radiation. A net fluctuation in the water content was recorded in the present study and this result contradicts with that obtained for *A. caerulea* (Gioka et al., 2005), *T. Pisana* (Arad and Avivi, 1998), and Israeli *Clausillid* (Warburg, 1972) of the temperate region. This finding may be confusing but a closer look at the relative humidity of the environment during these periods may explain the mechanism behind the gain of water by these land snails during dormancy.

Several water regulatory mechanisms have been suggested in various studies. One of the most potent mechanisms is the regulation of water evaporation from the mantle (Machin, 1965), probably through the control of water loss by a water proofing layer close to the surface of the mantle tissue (Machin, 1974). Furthermore, the rate of change in water content during aestivation was low in the present study. According to Barnhart and McMahon (1987), evaporative water loss during breathing would also be minimised by apnoeic breathing patterns and the establishment of high concentration of solutes elevates the osmolality of body fluids and slows down water loss (Giokas et al., 2005). Yom-Tov (1971) suggested that through metabolic pathways by oxidation of storage substrate that contributes extra metabolic water, snails regulate their water budget.

The conservation of fuel reserves, resulting from metabolic rate depression, is essential for survival during aestivation (Giokas et al., 2005). Metabolic rate in land snail during aestivation is low, usually 10–30% of the metabolic rate in active individuals (Herreid, 1977; Bemis et al., 1987; Pinder et al., 1992; Pedler et al., 1996). Reduction in metabolic rate results in a fixed reserve of fuels that can sustain basal metabolism probably due to cessation in digestion and movement, and reduced rates of breathing and heartbeat. The reduction in metabolic rate attributable to reduced rate of breathing and heart beat results in pH imbalance and reduced supply of oxygen to cells in oxy-conforming species (Storey, 2002). The factors that contribute to intrinsic metabolic rate depression include decreased rates of fuel catabolism, ion channel arrest

and reduced rates of protein synthesis (Churchill and Storey, 1989; Rees and Hand, 1991; Guppy et al., 1994; Storey, 2002). To deal with metabolic fuel supply during dormancy, aestivation is preceded by an accumulation of large reserves of endogenous fuels.

The results of the present study portrayed a definite pattern of use of metabolic fuels typical in *A. achatina*. Glycogen content was high during the onset of the study when these land snails entered the dormant period. *A. achatina* were found to have accumulated a high glycogen content during aestivation (December), which depleted towards the end of the aestivation period. Lipid contents recorded were high in December and depleted in January and April. The observed variation in depletion of energy reserves and fluctuation in some of the biochemical content during aestivation may constitute a significant physiological advantage and peculiarity for survival for *A. achatina* in the tropics. The work of Giokas et al. (2005) on *A. caerulea*, corroborated with the above findings. They recorded the highest concentrations of lipids and sugars during the first month of aestivation.

Therefore, the decline in protein content began earlier during the study period (December) and was possibly associated with increased demand for urea synthesis due to decline in tissue water potentials. Protein contents recorded an increase in the aestivation months and wide fluctuation thereafter. The use of protein as energy source changes over time during the study period being high in December, decreased later and increased towards the termination of aestivation period, and remained high in the active period. In aestivating land snails, catabolism of carbohydrates seems to be of major importance (Livingstone and de Zwaan, 1983). Rees and Hand (1993) also found in *Oreohelix* that polysaccharides were the primary metabolic fuel for 2–4 months of a 7-month aestivation period studied, and when it depleted, net protein catabolism began and a low rate of lipid catabolism was maintained throughout. In our present study, the catabolism of carbohydrate (glycogen) began earlier during aestivation and depleted towards the end of aestivation.

Land snails utilise aerobic metabolism during aestivation with minimal employment of anaerobic pathways (Rees and Hand, 1990; Michaelidis, 2002). Usually, aestivating land snails do not show lactate accumulation and completely oxidised carbohydrates (Churchill and Storey, 1989; Brooks and Storey, 1997). However, in the present study, lactate and LDH accumulation were found in the first month of the study period which is the month of the onset of aestivation for Nsukka land snails, with subsequent depletion due to anaerobic metabolic processes. This result corroborates earlier works that shows that lactate and LDH accumulation occurred during the first months of aestivation as in *Albinaria caerulea* (Giokas et al., 2005), *Codringtonea helenae* (Giokas et al., 2007) and *Helix figulina* and *Helix aspersa* (Kotsakiozi et al., 2012). However, the result is at variance with that recorded for *Otala lactea* (Brooks and Storey, 1997). However, evidence indicates that during aestivation, energy production in land snails may also be based on anaerobic processes (Wieser and Wright, 1978; Michaelidis et al., 1999).

Additionally, anaerobic metabolism seems to occur in dormant pulmonates because the rates of oxygen consumption fall below measurable limits for hours or even days (Schmidt-Nielsen et al., 1971). Terrestrial snails sometimes may find themselves in oxygen free environment, e.g. when they burrow into the ground to escape from harsh climatic condition. According to the result of the present study, *A. achatina* species are capable of lactate oxidation when their metabolite concentration reaches a crucial level. Normally, LDH is partly inactivated by being bound to cellular structures. In this study, no cross correlation of LDH and lactate was established. This contradicts the work of Giokas et al. (2005, 2007) which recorded a significant correlation between the variables, LDH and lactate. This finding, according to Michaelidis et al. (1999) is controversial. However, critical changes in the intracellular environment result in the activation of LDH and thus accelerate the net removal of lactate from the tissues. The disappearance of lactate possibly introduces a second phase of anaerobic metabolism; involving the formation of succinates and perhaps other end products of metabolism (Wieser and Wright, 1978; Michaelidis et al., 1999). It is possible therefore, that other mechanisms are also involved in this process though lactate accumulation may be the factor that started the whole process.

Snails rapidly increase their oxygen uptake several folds as they awaken (Herreid, 1977) and during this short transition, there is an insufficient time to make major adjustments to metabolic enzymes to deal with any Hermes-Limaincrease in oxidative stress associated with arousal (Hermes-Lima et al., 1998). Hence, the need to produce enzymes of intermediary metabolism during aestivation when oxidative stress is not expected. In the present study, a decreasing value in activity of phosphatase enzyme (ALP) during aestivation and increased activity during the active period of these snails were recorded. The decreased activity of these enzymes observed in these snails during aestivation may have indicated a potential injury due to oxidative stress (Herme-Limas and Storey, 1995). The low hemolymph alkaline phosphatase and aspartate amino-transferase activity recorded during aestivation compared to the active period could be due to the important role the enzymes play in calcium and phosphate metabolism (Kaplan and Szabo, 1983). Similarly, Reddy et al. (1989) reported nearly a 12% increase in alkaline phosphatase activity in the tissue of aestivated *Pila globosa*. The reduction of AST, ALP and ALT enzymes in haemolymph of land snails could be partly due to cell injury of their different organs and this may have led to disturbances in their enzymatic systems (Mahmoud, 2006). The transferase, AST are not solely located in the hepatocytes but rather are also in many body organs. Additionally, the elevation in their activities could be due to a variety of conditions including muscle damage, hepatic injury and toxic hepatitis (Farkas et al., 2004).

The present results did not establish correlations of the biochemical variables of the species with the climatic data. Thus, the biochemical variables, in general, are adequate to differentiate the periods of aestivation and activity. It was observed that most snails have entered aestivation before the onset of the study. From field observations, the period of aestivation was marked by the formation of epiphragm during the first sampling month in December. It is therefore noted that the onset of aestivation is primarily controlled by an endogenous component. Changes in biochemical composition may be a by-product of this component and is being influenced by climatic or environmental factors such as temperature, light intensity, solar radiation etc. Likely, these modifications may be advantageous to *A. achatina* inhabiting an overlap habitat between the tropical rainforest and savannah belt of Nigeria.

Accordingly, Cook (2001) argued that pre-programmed elements coupled with physiological modifications which may be overridden by external cues control the onset and termination of dormancy in land snails. It is then of adaptive advantage for pulmonate snails not to become active in the dry season in response to rise in environmental temperature and absence of rainfall.

Conclusion

In conclusion, this study showed that the physiological adaptation of *A. achatina* is in effect to ensure adjustment under extreme condition such as intense heat and thermal death due to water loss, and to compensate for its metabolic regulation for adaptation in the tropics. Consequently, the survival of *A. achatina* is not environmentally predicted rather it depends on the species-specific inherent process for survival.

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