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Formation and composition of epiphragm in three giant African land snails (*Archachatina marginata, Achatina fulica* and *Achatina achatina*)

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ABSTRACT. Aestivation is the state of dormancy that occurs in snails when the weather condition is harsh, either dry season or warm season. During this period, the giant African land snails form epiphragm, sealing the aperture of the shell which serves as water preservative strategies, preventing mechanical damage of the inner soft tissues. A study on the formation and mineral composition of the epiphragm in three species of giant African land snails namely: *Achatina achatina, Achatina fulica* and *Archachatina marginata* was carried out. Twenty matured snails were used for this experiment. They were induced into aestivation by withdrawing food and water. The snail weight, shell length, aperture length, and epiphragm weight were measured during aestivation as well as duration of epiphragm formation. The ionic concentration (calcium, iron and phosphorus) of the epiphragm was also analyzed. The morphometric analysis showed that *A. marginata* formed the heaviest epiphragm while the least was formed by *A. fulica* during aestivation. Analysis of the ions revealed that calcium was the most abundant ion in the epiphragm of the snails. Comparison of mean values reveals that *A. achatina* has the highest ionic composition in the epiphragm when compared to other species. Regression analysis (r^2) revealed that a positive relationship existed between the aperture length and days for epiphragm formation (+0.62) on one hand and between weight of epiphragm formed and the days of its formation on the other hand (+0.77).

Introduction

The giant Africa land snails are distributed all over the humid tropical zones of Africa and according to Akinnusi [2014] species that are common in tropical Africa include *Achatina achatina* (Linnaeus,1758), *Achatina fulica* (Férussac,1821) and *Archachatina marginata* (Swainson, 1821).

The success of land snails in terrestrial habitat has been attributed to various structural, physiological and behavioral specializations [Riddle, 1983]. One specialization of pulmonate land snails is the capacity to enter the dormant state or aestivation during periods of dry and hot environmental conditions [Rees, Hand, 1993]. Aestivation is a natural phenomenon known to interrupt the normal physiological process of snails leading to the slow growth rate. Under dry condition, land snails withdraw into their shells closing the aperture with a calcified mucus membrane, epiphragm, to minimize water loss due to evaporation [Omoyahki *et al*., 2008]. The aestivated snail draw on the reserve of fat and glycogen at much reduced rate, which implies the imminent reduction in weight, and loss of valuable growing time during aestivation is reduced [Ademolu *et al*., 2009, Abdulssamad *et al*., 2010].

Aestivation in land snails is recognized as a typical state of inactivity that allows them to survive dryness, high ambient temperatures and a shortage of food [Storey, Storey,1990]. Some species can aestivate for as long as many months without food and water under aestivation [Akinnusi, 2014]. Aestivation plays a critical role in dynamics of haemolymph which is an important medium for the transport of nutrients and waste to various organs of land snail. Ademolu *et al.* [2004] reported that the flesh and haemolymph of snails are rich in Ca^{2+}, Mg^{+}, Na^{+}, Zn^{+}, Fe^{2+}, and Cl^{-}. The ionic and organic composition of snails shell and epiphragm increased during aestivation while that of the haemolymph were decreasing and the physiological basis of epiphragm formation in snails is mobilization of calcium from the haemolymph [Ajayi *et al.*, 2012].

Epiphram is a calcified mucus membrane that snails use to cover the shell aperture during aestivation to minimize water loss [Omoyakhi *et al*., 2008]. The mucus epiphragm formation during aestivation in snails is an important behavioral adaptation to withstand stress. The secretion of mucus epiphragm is one of the several water preserving strategies accounting for up to 20% water saving during aestivation in snails [Arad, 2001]. Epiphragm also

Table 1: Morphometrics of experimental snails (n=20)*.

Табл. 1. Морфометрия экспериментальных особей моллюсков (n=20)*.

Species	Average weight of snail(g)	Shell length (cm)	Aperture size (cm)
Achachatina marginata	146.78 ± 1.3 [a]	5.20 ±0.10	3.50 ±0.01 [a]
Achatina fulica	77.78±0.01 [c]	5.0 ±0.02	2.10 ±0.10 [b]
Achatina achatina	80.93±0.15 [b]	4.8 ±0.13	2.72 ± 0.30 [b]

*Mean values in the same column having different superscript are significantly different ($p< 0.05$).

Table 2: Days of formation and weight of epiphragm by three snail species*.

Табл. 2. Длительность формирования и вес эпифрагмы у трех видов.

Species	Days for epiphragm formation	Weight of epiphragm (g)
Archachatina marginata	16±2.00[a]	0.32±0.10[a]
Achatina fulica	14±1.20[b]	0.09±0.13[b]
Achatina achatina	13±0.30[b]	0.09±0.26[b]

*Mean values in the same column having different superscript are significantly difference ($p< 0.05$).

serves as a deterrent to predators, pathogens, preventing mechanical damage of soft tissue and also acts as holdfast organ. It also permits gaseous exchange and is sensitive to external environmental changes [Struthers, 2002]. However, there is lack of information in literature on the duration of formation of the epiphragm in different snail species as well as its composition. Thus, the thrust of this study is to investigate the time taken to form epiphragm and its composition in three snail species commonly encountered in Abeokuta, Nigeria.

Materials and methods

Experimental site

The experiment was conducted at the Snailery of the Department of Pure and Applied Zoology, Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

Experimental snails

The snails were obtained from Kuto market (7°2´N, 3°4´E) Abeokuta, Ogun state, Nigeria. Twenty matured snails of *Achatina fulica*, *Achatina achatina* and *Archachatina marginata* each were used for the experiment.

Experimental procedures

The cages (1.0 m x 1.0 x 0.42 m) were filled with humus soil up to a depth of 6cm and moistened with water. Each cage was supplied with feeding and drinking troughs. The snails were feed with pawpaw leaves for one week in order to acclimatize. After acclimatization, the snails were induced to enter aestivation by withdrawing food and water and discontinuing moistening of the soil.

Data collection

The body weight, shell weight and the weight of epiphragm formed by the snails were measured using sensitive weighing scale (Mettler PM 11-K). The size of the shell aperture was measured with a vernier caliper.

Preparation of samples for analysis

A week after the epiphragms formation in each snail species, they were carefully removed from the snails with the aid of forceps into clean petri dishes. After weighing, the epiphragm was ground into powder inside mortar and pestle. 5g of the powdered epiphragm was used for chemical analysis. Ca^{2+}, P and Fe^{2+} were determined using AAS (Atomic Absorption Spectrophotometry).

Statistical analysis

The data obtained from this study were subjected to one-way analysis of variance (ANOVA) and where there were significant differences, means separation was done by Duncan Multiple Range Test (DMRT) and regression analysis was used to determine the relationship between body measurements.

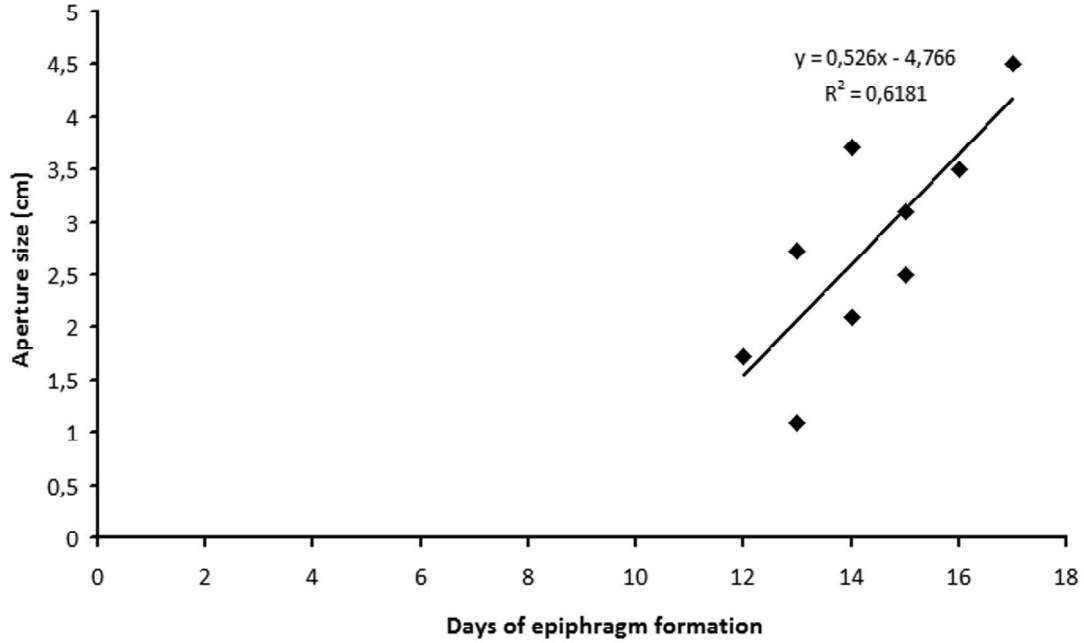

FIG. 1. Relationship between aperture size and days of epiphragm formation (based on the combined results for three species).

РИС. 1. Корреляция между размером устья и временем формирования эпифрагмы (обобщенный результат для трех видов).

Results

The morphometrics analysis of the experimental snails (snail weight, shell length and aperture size) is presented in Table 1. It was observed that *A. marginata* had the highest body weight, shell length and aperture size.

Table 2 shows the duration of epiphragm formation. *A.achatina* was the first species to form epiphragm followed by *A. fulica* while *A. marginata* was the last species to form epiphragm. Also, *A. marginata* had the highest weight of the epiphragm formed followed by *A. fulica* and *A. achatina.*

The relationship between the aperture size and days of epiphragm formation is shown in Fig 1. There was a positive correlation (+0.62) between these two parameters. Also, there was a strong positive correlation (+0.77) between the weight of epiphragm formed and days of epiphragm formation (Fig. 2).

The mineral composition of the epiphragm formed by three snail species during aestivation is shown in Table 3. *A. achatina* had the highest mineral composition while *A. fulica* had the least concentration. Calcium is the most abundant mineral followed by iron while the least found was phosphorus.

Discussion

Archachatina marginata is the biggest snail species in the tropical West Africa. It has the highest body weight and shell parameters than other snail species [Idowu *et al.*, 2008; Okon, Ibom, 2012]. This study agrees with this report as *A. marginata* recorded significantly higher average body weight and shell length than other snail species. Similarly, the aperture of *A. marginata* was longer/wider than those of *A. fulica* and *A. achatina*.

There was a significant difference in the days taken for epiphragm to be formed in the three species. The days ranged between 13-16 days with *A. achatina* forming epiphragm earlier than other snails and *A. marginata* forming last. This duration was longer than 7 and 9 days earlier reported by Abiona *et al.* [2013], Abdussamad *et al.* [2010] for epiphragm formation in *A.marginata*. The delay of epiphragm formation noticed in this study might be due to the coldness of the environment during the experiment. The study was carried out in August which was characterized with high humidity and coldness. Abdussamad *et al.* [2010] had earlier observed that cold wet season delayed the onset of aestivation.

There was a positive relationship between the aperture size and duration for epiphragm formation, that is, the wider the aperture, the longer the days for formation of epiphragm. Similarly, a strong positive relationship existed between weight of epiphragm and days of epiphragm formation. During aestivation the aperture of the snail is covered with epiphragm [Yoloye, 1994] hence, the wider the aperture, the more the epiphragm needed to cover

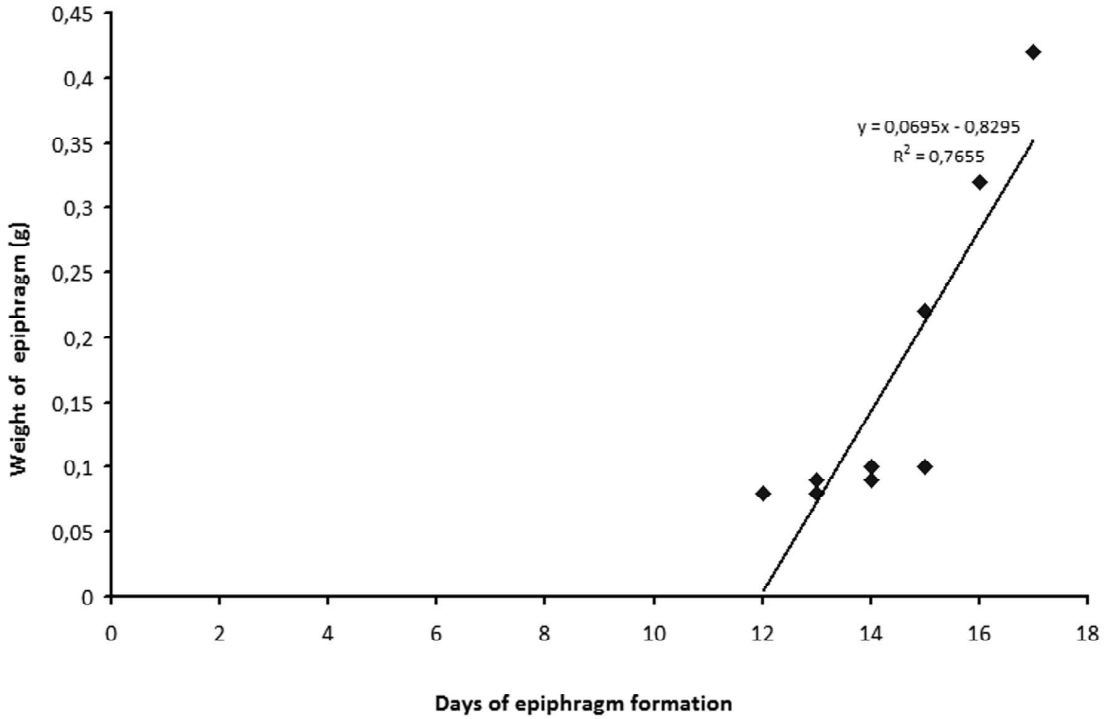

FIG. 2. Relationship between weight of epiphragm and days for formation (based on the combined results for three species).

РИС, 2. Зависимость между весом эпифрагмы и количеством дней ее формирования (обобщенный результат для трех видов).

its aperture and the longer the time required for the formation. Therefore, bigger (older) snails are expected to form epiphragm later than lighter (younger) snails. Abdussamad *et al.* [2010] similarly observed that bigger snails (200-300 g) loss weight faster than smaller snails (100-200 g) at the end of 6-week aestivation period which might likely be responsible for higher mortality rate in larger snails.

The epiphragm of the three snail species contained Ca^{2+}, P^{2+} and Fe^{2+} in varying concentrations. This runs parallel with the findings of Ajayi *et al.* [2012] that during aestivation, the epiphragm of *A.marginata* had more P^{2+} and Ca^{2+} than other tissues (haemolymph, flesh and shell) examined. These three elements found in epiphragm are highly required for the rigidity, strength and toughness necessary for epiphragm function. The higher concentration of these ions in the epiphragm is not unexpected as they are similarly present in the shell of snails at high concentration [Ademolu *et al*., 2015]. Also, Omoyakhi and Osinowo [2010] observed that there was mobilization of Ca^{2+}, P^{2+} in and out of the shell of *A.marginata* due to epiphragm dynamics.

Comparisons of means revealed that *A.achatina* had the highest mineral composition in epiphragm relative to other species. Likewise, Idowu *et al.* [2008] reported that *A.marginata* had the highest body ionic composition among snails found in Abeokuta, Nigeria. Presented here result might likely be responsible for the observation made by Omoyakhi, Osinowo [2010] that *A. achatina* lost less weight than *A. marginata* during aestivation. The

Table 3: Mineral composition of epiphragm formed by three snail species (mg/g)*.

Табл. 3. Минеральный состав эпифрагмы, сформированной тремя видами моллюсков (mg/g)*.

Species/Minerals	*A.achatina*	*A.marginata*	*A.fulica*
Calcium	40.68 ± 1.20^{a}	10.12 ± 0.32^{b}	10.04 ± 0.25^{b}
Phosphorus	4.26±0.13	4.10±1.01	3.82±0.22
Iron	21.67 ± 0.11^{a}	20.81 ± 0.32^{a}	15.72 ± 0.10^{b}

*Mean values in the same row having different superscript are significantly difference ($p< 0.05$).

higher concentration of minerals in the epiphragm of *A. achatina* makes it thicker and more resistant to water loss through dehydration.

It can thus be concluded that smaller snails form epiphragm faster than bigger ones due to their size and *A. achatina* has higher epiphragm mineral composition than other snail species.

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Формирование и состав эпифрагмы у трёх гигантских африканских улиток (*Archachatina marginata*, *Achatina fulica* и *Achatina achatina*)

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РЕЗЮМЕ. Эстивация есть состояние спячки, в которую впадают животные при неблагоприятных условиях – во время сухого или жаркого сезона. В течение такого периода улитки образуют эпифрагму, способствующую сохранению воды и предотвращающую повреждения мягких тканей. Проведено изучение формирования эпифрагмы и её минерального состава у трёх видов: *Archachatina marginata*, *Achatina fulica* и *Achatina achatina*. В экспериментах использовано по 20 особей каждого вида. Улитки побуждались к эстивации путём лишения их пищи и воды. В течение эстивации измеряли вес моллюска, высоту раковины и устья, а также вес эпифрагмы; анализировали концентрацию в эпифрагме ионов кальция, железа и фосфора. Показано, что *A. marginata* образует самую массивную эпифрагму, тогда как *A. fulica* – самую лёгкую. Содержание ионов кальция в эпифрагме наиболее высокое по сравнению с ионами железа и фосфора. Регрессионный анализ показал существование положительной зависимости между, с одной стороны, размером устья и временем, необходимым для формирования эпифрагмы (+0,62), и между весом эпифрагмы и временем её образования – с другой (+0,77).