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Adaptive changes in growth and morphological composition of aestivating giant African land snails, *Archachatina marginata* and *Achatina achatina*

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ABSTRACT: The experiment investigated the changes in liveweight and body composition at 0 (control), 2, 4 and 6 weeks of aestivation using 40 snails. The experiment was conducted in a completely randomized design laid out in a species x weeks factorial arrangement. The results showed that though both species had identical initial liveweights, the overall average weight after 6 weeks of aestivation was significantly higher ($P < 0.05$) for *A. marginata* (138.31 ± 5.47 g) than for *A. achatina* (118.81 ± 6.61 g). In both species, liveweight declined significantly ($P < 0.001$) with weeks of aestivation to 88.7, 83.1 and 52.4% of initial liveweight for *A. marginata* and 82.1, 62.8 and 35.0% for *A. achatina* during 2, 4 and 6 weeks respectively. There were declines in shell weight ($P < 0.05$), soft body weight ($P < 0.05$), dry weight ($P < 0.01$), haemolymph ($P < 0.01$) and body moisture ($P < 0.001$) for both species over the 6 weeks of aestivation. The species effect was more pronounced in *A. achatina* than in *A. marginata*.

Keywords: Adaptation, aestivation, growth, body, composition, giant African land snails

Introduction

The dry season of the tropics spells a difficult period for many animals. With its long, hot, dry season, when food is scarce and vegetation is taking a rest, all animals adapt themselves in some manner to the dry season (Odiete, 1999). Migration especially in birds, is but one many ways to find solution to the problem. This is not so of animals that do not migrate.

Snails in particular adapt by aestivation. Aestivation is a dormant state of existence, accompanied by greatly diminished respiration, circulation and metabolism in which the animal spends the dry season (Anonymous, 2005). Aestivation, a normal phenomenon is known to interrupt the normal physiological processes of the snail (Odiete, 1999).

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During dormancy (aestivation) snails stop feeding, burrow in the ground or climb as high as possible on vegetation and withdraw deeply into their shells, greatly reducing many physiological functions (Russell-Hunter, 1969). These behavioural and physiological responses to dormancy reduce the impact of stressful environmental conditions on snails. The physiological information available on this dormancy is quite scanty. Moreover, a bulk of this information is the result of studies carried out on hibernation of snails in the temperate regions of the world. This study therefore seeks to establish the comparative growth changes, assesses the body energy reserve utilization and alterations of body composition of two species of the giant African land snails.

Materials and Methods

The experiment was carried out at the Snail Research Unit of the College of Animal Science and Livestock Production (COLANIM), University of Agriculture, Abeokuta. Abeokuta lies within the Rain Forest vegetation zone of Western Nigeria at latitude 7° 13' 49.46"N, longitude 3° 26' 11.98"E (Google Earth, 2006) and altitude 76 m above sea level. The climate is humid with a mean annual rainfall of 1,037 mm, an average temperature of 34.7°C and an average relative humidity of 82 % throughout the year (60 % in January and 94 % in July to September).

Materials used in this experiment included a total of 40 apparently healthy snails (20 *A. marginata* and 20 *A. achatina*) of 150 g average liveweight, 40 well ventilated plastic basket cages of 40 cm by 25 cm by 20 cm with covers, 40 each of shallow feeders and drinkers, humus soil, sensitive electronic weighing scale, oil paint to mark for proper identification, dried pawpaw leaf meal, layer's mash and water

The experiment was conducted between the months of January and March. The experiment was laid out in a 2 X 4 factorial (spp X duration of aestivation) in a completely randomized design in 5 replicates. The cages were prepared and filled with sun-dried humus soil up to a depth of 5 cm and moistened with 300 ml of water. Each cage was assigned a drinker and a feeder. The snails were weighed in grammes using a sensitive electronic balance. They were randomly allocated to the treatments with one snail per basket. The snails were balanced for liveweight. Feed (layers mash + dried milled pawpaw leaves; 1:1; w/w; Table 1) and water were provided *ad libitum*. At the end of a 2-week adjustment period, the liveweight of the snails in all treatment groups were taken, feed and water were withdrawn.

The control group was sacrificed immediately at the end of the 2-week adjustment period. Liveweight of aestivating snails were taken weekly, while the treatment groups were sacrificed after 2, 4 and 6 weeks of aestivation.

The effect of duration of aestivation on liveweight changes was subjected to linear and non-linear regression analyses to determine model of best fit. Other parameters were subjected to analyses of variance (ANOVA) in factorial arrangement (species X duration of aestivation) in a completely randomized design of 5 replicates using the Systat Analytical Computer Package, Version 5.0 (Systat Inc., 1992). Tukey's highest significant difference (HSD) was used to separate the means where significant differences existed.

Results

The results of the effects of duration of aestivation on the liveweight (LW), weight loss (WL), shell weight (SW), soft body weight (SB), percent dry matter (DM), dry weight (DW), body moisture (BM), haemolymph (HL) and epiphragm (EP) are presented. Though both species had identical initial weight, the overall average weight after six weeks of aestivation was significantly higher ($P < 0.05$) for *A. marginata* (138.31 ± 5.47 g) than for *A. achatina* (118.81 ± 6.61 g) in both species. LW declined significantly ($P < 0.01$) with weeks of aestivation dropping to 88.7, 83.1 and 52.4% of initial weight for *A. marginata* and 82.1, 62.8 and 35.0% for *A. achatina* by 2, 4 and 6 weeks respectively (Figure 1).

Table1: Effects of length of aestivation on the morphological compositions of *A. marginata* and *A. achatina*

Parameter	Species	Length of Aestivation (Weeks)			
		0	2	4	6
Shell weight (g)	<i>A. marginata</i>	47.03 ± 4.52 ^a	40.24 ± 4.52 ^{ab}	39.13 ± 4.52 ^{ab}	22.71 ± 7.15 ^b
	<i>A. achatina</i>	49.69 ± 4.52 ^a	46.30 ± 5.84 ^a	34.04 ± 5.84 ^{ab}	22.10 ± 7.15 ^b
Soft body weight (g)	<i>A. marginata</i>	98.67 ± 5.36 ^a	89.60 ± 5.36 ^a	79.68 ± 5.36 ^{ab}	66.61 ± 8.47 ^b
	<i>A. achatina</i>	77.66 ± 5.36 ^a	64.81 ± 6.91 ^{bcd}	59.79 ± 6.19 ^{cd}	44.59 ± 8.47 ^d
Dry weight (g)	<i>A. marginata</i>	35.36 ± 2.25 ^a	28.01 ± 2.25 ^{ab}	25.75 ± 2.25 ^{ab}	20.98 ± 3.56 ^{bc}
	<i>A. achatina</i>	19.31 ± 2.25 ^c	15.88 ± 2.91 ^{cd}	17.57 ± 2.91 ^{cd}	13.81 ± 3.56 ^d
Dry weight (%) (as % SB)	<i>A. marginata</i>	28.50 ± 1.61	31.24 ± 1.61	32.63 ± 1.61	33.63 ± 2.54
	<i>A. achatina</i>	24.14 ± 1.61	24.14 ± 2.07	29.03 ± 2.07	31.63 ± 2.54
Body moisture (%) (as % SB)	<i>A. marginata</i>	71.50 ± 1.86	68.76 ± 1.68	67.37 ± 1.86	66.37 ± 2.94
	<i>A. achatina</i>	75.86 ± 1.86	71.73 ± 2.40	70.97 ± 2.40	68.37 ± 2.94
Epiphragm (g)	<i>A. marginata</i>	0.00 ± 0.06 ^b	0.40 ± 0.06 ^a	0.42 ± 0.06 ^a	0.34 ± 0.11 ^a
	<i>A. achatina</i>	0.00 ± 0.06 ^b	0.26 ± 0.09 ^a	0.21 ± 0.09 ^a	0.39 ± 0.11 ^a

Values are least square means (± sem), n = 5.

^{abcd} Means with different superscripts within the same parametric row differ significantly (P < 0.05)

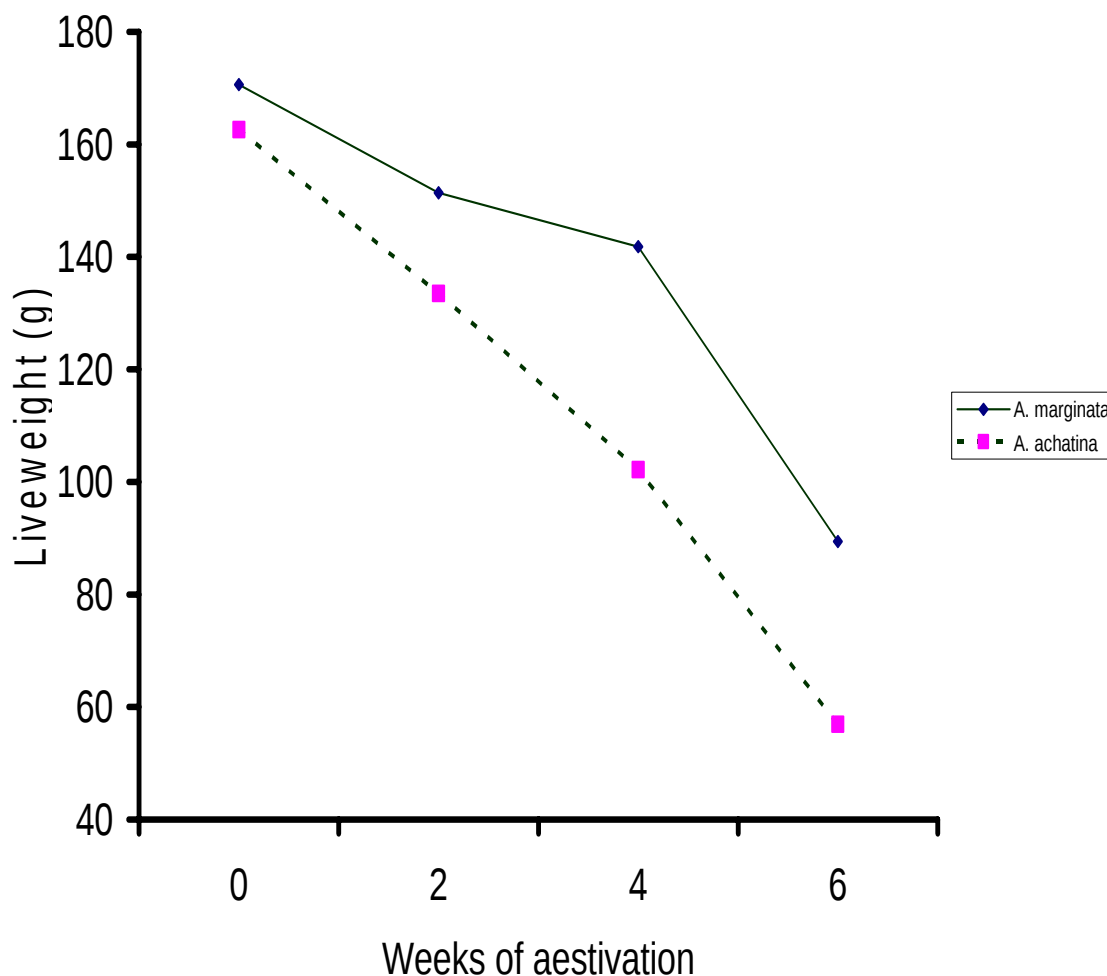


Figure 1. Effects of duration of aestivation on the liveweight of giant African land snails

Liveweight changes (decrease) were sharp at the first few days of onset of aestivation and decrease slightly or constantly after some weeks of dormancy in both snails though there were slight species differences. This is shown in the model of the prediction equations of the weekly liveweight changes of *A. marginata* and *A. achatina* as shown below:

$$\text{LWTA} = -46.504 + 213.850e^{-0.022t_a} \quad (R^2 = 86.3 \%)$$

$$\text{LWTM} = 103.337 + 72.899 (0.666)^{t_a} \quad (R^2 = 98.3 \%)$$

Where LWTM and LWTA are body weight decrease of *A. marginata* and *A. achatina* respectively for t_a week of aestivation

In the control snails, shell weight (SW) constituted 27.6% of the liveweight of *A. marginata* which was not significantly different from 30.6% SW component of *A. achatina*. However, SW progressively decreased with duration of aestivation. SW of *A. marginata* significantly reduced ($P < 0.05$) to 48.3% of the initial weight after 6 weeks of dormancy. *A. achatina* followed a similar trend with a significant decrease (P

< 0.05) to 44.47% after 6 weeks of aestivation. No significant difference ($P > 0.05$) was observed between the species SW in any case. In addition, shells became brittle as aestivation advanced.

The overall means of the soft body weight (SB) showed a very highly significant mean difference ($P < 0.001$) of 81.08 ± 2.30 g of *A. marginata* as compared to 61.71 ± 2.78 g of *A. achatina*. The SB of *A. marginata* decreased significantly ($P < 0.05$) with weeks of aestivation, dropping to 90.8, 80.5 and 67.5% of initial weight for *A. marginata* and to 83.5, 76.9 and 57.4% for *A. achatina* by 2, 4 and 6 weeks respectively.

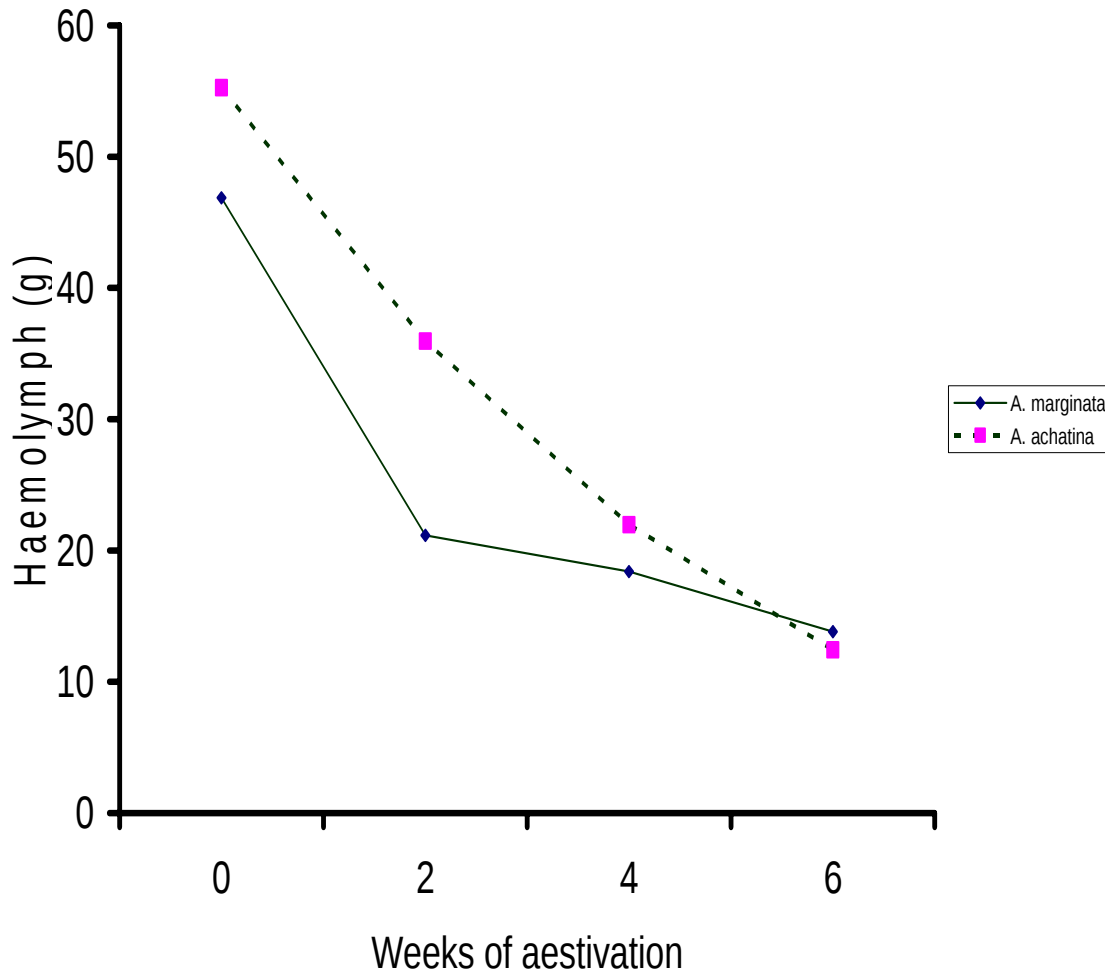


Figure 2. Effects of duration of aestivation on the haemolymph of the giant African land snails

The analysis of variance showed that there was a very high significant difference ($P < 0.001$) between the dry weights (DW) of both species. The overall average weight of DW after 6 weeks of aestivation was significantly higher ($P < 0.001$) for *A. marginata* (26.10 ± 1.32 g) than for *A. achatina* (16.64 ± 1.47 g). The least square means presented in the Table showed a consistent reduction of the DW in both species. *A. marginata* highly significantly decreased ($P < 0.01$) by 40.7% after 6 weeks in aestivation. *A. marginata* at 6 weeks of aestivation still had higher but not significant ($P > 0.05$) DW than the active feeding *A. achatina*. Similarly, the DW of aestivated *A. achatina* at 6 weeks was 28.4% lower ($P < 0.05$) than that of the active feeding snail (control). All mean values of *A. marginata* at their corresponding weeks of aestivation were significantly higher ($P < 0.001$, $P < 0.01$, $P < 0.05$ and $P < 0.05$) than those of *A. achatina* for 0, 2, 4 and 6 weeks respectively.

The summary of the least square means of the effects of duration of aestivation on the haemolymph (HL) of *A. marginata* and *A. achatina* is shown in Figure 2. Active feeding *A. marginata* had haemolymph consisting about 27.49% of liveweight which was not significantly lower than the 33.98% of the component of *A. achatina*. There were no significant differences between the species ($P > 0.05$) in the overall averages and in the interaction between species and weeks of aestivation ($P > 0.05$). HL significantly decreased ($P < 0.001$) to 45.11, 39.2 and 29.5% of the active feeding (control) value in *A. marginata* and to 65.1, 39.7 and 22.5% in *A. achatina* for the 2, 4 and 6 weeks aestivation period respectively.

Figure 3 presents the effects of duration on the body moisture (BM) of *A. marginata* and *A. achatina*. Unlike the haemolymph, the overall average quantity of the BM was significantly higher ($P < 0.001$) for *A. marginata* (57.11 ± 2.09 g) than for *A. achatina* (42.13 ± 2.32 g) after 6 weeks of aestivation. Across the weeks of aestivation, *A. marginata* maintained a relatively slow loss of BM. Of the 63.31 ± 2.91 g of BM of active feeding snails, 97.3, 84.8 and 78.72% were respectively retained at the second, fourth and sixth weeks of aestivation. There was only a significant decrease ($P < 0.05$) in BM of the control at the 6th week of dormancy. However, *A. achatina* showed a rapid and consistent loss of BM as in the HL. The BM reduced significantly ($P < 0.05$) to 72.2% of the control snails at 4 weeks of aestivation. Only about 37.3% of the total BM was retained at the 6th week of aestivation. This value was significantly ($P < 0.001$) lower than the value of the active feeding *A. achatina*.

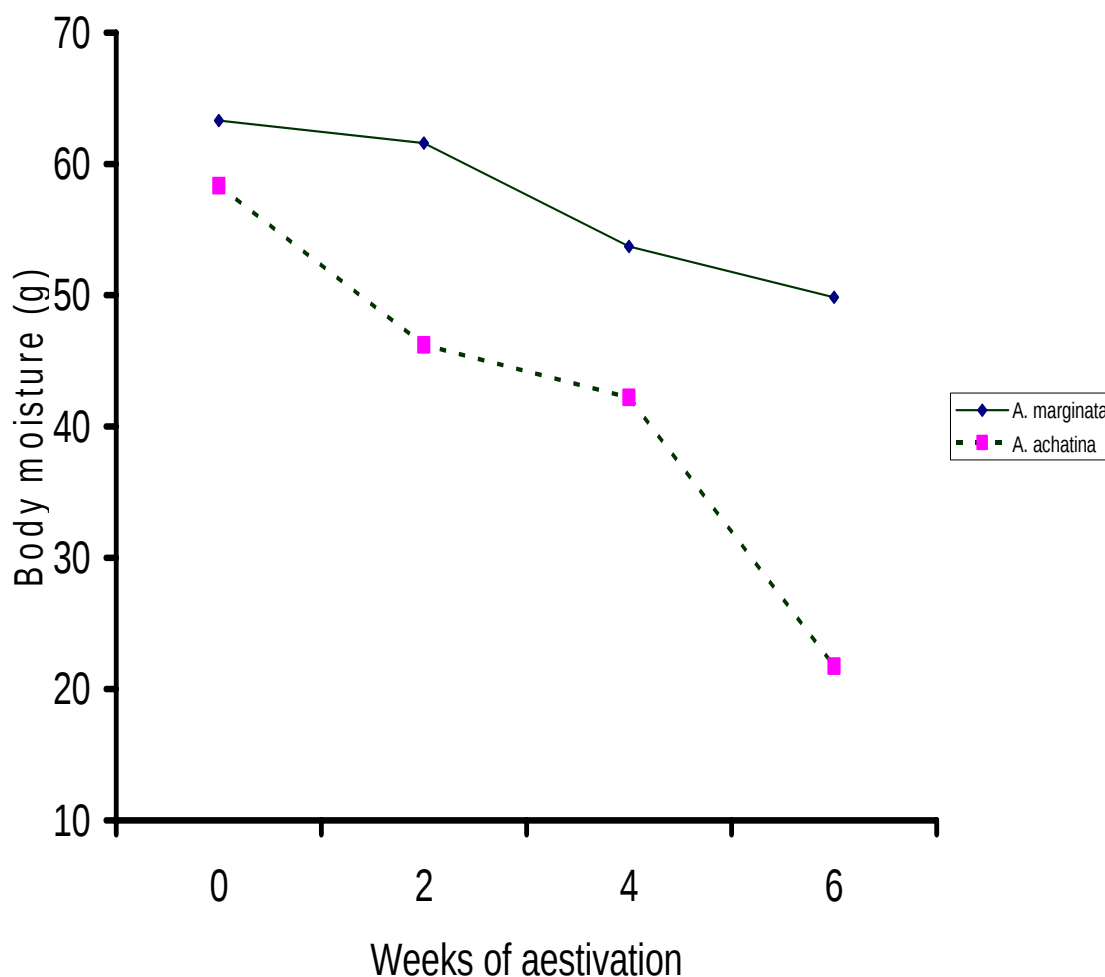


Figure 3. Effects of duration of aestivation on the body moisture of the giant African land snails

The least square means of the epiphragms (EP) formed during dormancy are presented in Table 8. The overall average weight indicated that there was no significant ($P > 0.05$) weight difference between EP formed by *A. marginata* (0.29 ± 0.03 g) and *A. achatina* (0.12 ± 0.04 g). Duration of aestivation significantly ($P < 0.001$) affected the weight of EP. There were significant weight differences ($P < 0.01$) between active feeding snails (control) and all dormant snails at 2, 4 and 6 weeks of aestivation. However, all EP formed by aestivating snails were relatively similar ($P > 0.05$) in weight under the various weeks of aestivation.

Discussion

The findings that food and water deprivation in snails resulted in aestivation agreed with several authors (Cobbinah, 1992; Odiete, 1999; Guppy et al., 2000; Storey, 2001). The studies also confirmed that snails respond to unfavourable conditions by withdrawal into their shells and cover the openings (aperture) with white, brittle, calcareous epiphragms. Epiphragms were formed as early as 5 days after withdrawal of feed and water. Snails which could not form epiphragms within 14 days of withdrawal of feed and water were prone to mortality. Formed epiphragm were continuously replaced at intervals.

The decrease in liveweight from the actively feeding snails from the start of the experiment until the 6th week of the aestivation for the two species has been postulated by Odiete (1999) and a similar trend observed in hibernating *Helix pomatia*, *H. lucorum* and *H. aspersa* (Radrizzani, 1990). The study revealed that the liveweight of snails at sacrifice were significantly different between the two species. This may partly be due to species weight differences resulting from higher growth rate of *A. marginata* as reported by Omole et al. (2000). Comparing the growth rates of the species, he observed that *A. marginata* grows almost twice the rate of *A. achatina* when subjected to the same feeding and management conditions. Moreover, *A. marginata* was found to form epiphragm more readily than *A. achatina*. Since snails lose water from their exposed body such as head, foot, collar and lung (Odieta, 1999), ability of *A. marginata* to form and replace epiphragm readily may partly account for reduced rate of liveweight change observed in the study.

However, where epiphragm was formed, individual observations showed that liveweight change was more stable in *A. achatina* than in *A. marginata*. In summary therefore, one of the defense mechanism against excessive loss in weight was by quick formation and constant replacement of epiphragm in *A. marginata* while *A. achatina* formed a more stable solid epiphragm with a longer life span.

The gradual reduction in the shell weight and brittleness observed with progressive length of aestivation in this study may have contributed to the loss of weight in both species. In a study of performance and carcass analysis of *A. marginata*, Adu et al. (2002) reported that the shell to liveweight of the snails range from 24.94 to 25.48 %. This is similar to the 27.56 % and 30.56% shell to liveweight of actively feeding *A. marginata* and *A. achatina* respectively observed in this study.

Mead (1961) in analyzing the composition of the shell of *A. fulica* put the water content to only account for 0.2 % of the shell. Reduction or loss of weight of the shell during aestivation therefore, may not have primarily resulted from the loss of shell water. A bulk of the shell component is known to contain calcium and phosphorus which coincidentally are also principal component of the epiphragms (Mead, 1961). It is possible therefore, that calcium and phosphorus were demobilized from the shell to form the epiphragm, hence the consistent reduction of shell weight with the length of aestivation since the exogenous sources such as feed and soil were not usable during dormancy. Osteoporosis which involves bone demineralization (calcium and phosphorus) and weakness (Olomu, 1995) may be compared to the brittleness of shell observed with length of aestivation.

The decrease in soft body weight shown by both species during dormancy was probably caused by the dehydration and consumption of endogenous food reserves which followed food deprivation. Rees and Hand (1993) demonstrated parallel decrease in dry tissue mass and tissue water in *Oreohelix* during aestivation. These losses occurred more quickly at the onset of aestivation as the snails entered aestivation and then reached a slower steady rate after the initial drop especially in *A. achatina*. Significant difference ($P < 0.05$) only existed between the active feeding group and those aestivated for 6 weeks in *A. marginata* showing a higher development of protective mechanisms over *A. achatina* which at the 4th week of aestivation had shown a significant difference when compared to their control groups. This may partly be due to the early formation of epiphragm by *A. marginata* and consequent preservation of the body fluid.

The decrease in total body weight shown by both species may not absolutely be caused by dehydration and consumption of endogenous food reserves. The parallel decrease in the flesh dry weight of both *A. marginata* and *A. achatina* as demonstrated previously by Rees and Hand (1993) in the snail *Oreohelix* confirmed the loss of the dry tissue mass as aestivation or dormancy lengthened. At the end of the 6th week of aestivation, *A. marginata* lost over 40 % of the dry tissue mass while *A. achatina* had almost half of its original dry weight lost. These losses may have resulted from the utilization of the body fuel reserve. Higher percent losses recorded in *A. achatina* suggests a possible poorer defense mechanism against unfavourable conditions. It may also be interesting to note that losses from dry tissue mass in the wild may not be as high as the ones recorded in this study as aestivation was induced against other natural contributors.

As stated earlier, the epiphragms were replaced at intervals. The inconsistencies in the weight therefore, indicate that sacrifices were done at various stages of epiphragm formation of the individual snails. Characteristically, *A. marginata* were observed to form epiphragm more readily and replace them more frequently than *A. achatina*. Nevertheless, when *A. achatina* formed an epiphragm, it was thicker and maintained a slower dynamic than *A. marginata*. Relatively, *A. marginata* formed heavier epiphragm than *A. achatina*. This may not be unconnected with the larger aperture of *A. marginata*.

From the results of the experiment under study, two different types of fluid were identified for proper investigation. These included haemolymph (body fluid) which was simply drained immediately at sacrifice. It forms partly the blood component of the snail (Odaibo, 1997). The second involved the fluid trapped in the tissues which was only removed after oven drying. In *A. marginata* and *A. achatina* the haemolymph component of the snails accounted for about 27.49 % and 33.98 % of the actively feeding snails respectively. In both species, losses were dramatic resulting in over 82 % and 87 % reduction of the total haemolymph of the active snails after 6 weeks of dormancy in *A. marginata* and *A. achatina* respectively. Guppy *et al.* (2000) noted that as snails enter aestivation, the cells immediately decrease their metabolic rate in response to a decrease in haemolymph and haemolymph oxygen tension, changes in pH and resulting in decrease in oxygen consumption of awake cells to 45 %. Here, the loss of 54.89 % and 42.06 % of the haemolymph of *A. marginata* and *A. achatina* respectively within the first 2 weeks of aestivation was large enough to possibly alter the pH, pO₂ and osmolarity of the aestivating snails which correspondingly altered other physiological processes. Peddler *et al.* (1996) demonstrated that when food and water were withdrawn without changes in atmospheric oxygen or ambient temperature, the land snail (*H. aspersa*) lost its haemolymph, with the pH decreased from 7.8 to 7.3 and its pO₂ decreases from 64 to 44 torr.

The separation of the fluid into the body moisture fraction here provided an understanding to the process of water loss and the adaptive defense mechanism against desiccation of the species. Unlike the haemolymph, result showed that *A. marginata* had higher body moisture than *A. achatina*. It follows therefore that about 57.45% and 51.36% of the total body fluid are found in the tissues for *A. marginata* and *A. achatina* respectively. In addition, *A. marginata* conservative system was capable of retaining large amount of fluid in the tissue with slow intrinsic losses in response to the environmental demand of the aestivating snails. This result presents useful observation that *A. marginata* and *A. achatina* may work through different intrinsic mechanism to accommodate the demands of water loss during aestivation. On the other hand, *A. marginata* may have a better mechanism that partitioned the different fractions of the fluid thereby preventing the rapid loss of body moisture. The possibility of losses of both the haemolymph and body moisture in *A. achatina* may provide additional explanation to the dramatic changes noticed in liveweight, soft body weight, dry weight and high mortality rate. From the foregoing therefore, a bulk of the weight loss experienced during aestivation can be traced to dehydration

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