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Constitutive and adaptive changes of giant african land snails, *Archachatina marginata* and *Achatina achatina*, to support transition from the aestivating state to arousal

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Abstract

The morphological alterations through aestivation to arousal and subsequent changes were determined in giant African land snails, A. marginata and A. achatina. The snails were induced to enter into and sustained in aestivating state for 6 weeks. The control group was sacrificed while others were aroused and fed for 2, 4 and 6 weeks before sacrificed. During the 6 weeks of aestivation preceding the post-aestivation (arousal), there was a mean liveweight reduction of 59.7 % of initial liveweight of A. marginata and 60.4 % of A. achatina. Hydration and feeding provoked a new increase in liveweight by 192.6 % and 160.3 % of the liveweight within two weeks for A. marginata and A. achatina respectively. While A. marginata regain its lost weight within 2 weeks, A. achatina recovered only about 76.13 % which was significantly lower (P < 0.01) than the 72.67 \pm 7.41 g weight loss over the 6 weeks aestivation period. In both species, shell weight markedly increased during the first 2 weeks of arousal, tending to stabilize thereafter. The haemolymph significantly increased (P < 0.001) with weeks of post-aestivation rising to 384.8, 427.1 and 335.1 % of aestivated haemolymph for A. marginata and to 180.7, 223.1 and 221.9 % for A. achatina by 2, 4 and 6 weeks respectively. The study concludes that the growth changes during and after aestivation are basically the result of the dynamics of the haemolymph and body moisture.

Keywords: Morphology, alteration, aestivation, hydration, transition, snail CONSTITUTIVE, ADAPTIVE CHANGES, GIANT AFRICAN LAND SNAILS, TRANSITION, AESTIVATION, AROUSAL

INTRODUCTION

Many tropical zone species show seasonal changes in physiology and morphology that are directly related to changes in environment. In response to an unfavourable environmental conditions such as occur during the dry season, tropical zone animals show a variety of responses including changes in behaviour [1], biochemistry [2] and physiology [3]. These changes may maximize the individuals' chances of successfully going through the harsh environment. The fact that changes in environmental and internal factors can change the behaviour, biochemistry and physiology, allowing the animal to develop a suitable performance in a complex situation, represents a highly adaptive phenomenon.

Our previous study [4] determined the adaptive changes in growth and morphological composition of aestivating snails, as well as to establish the relative contribution of intrinsic control. This investigation has revealed significant decrease in body weight, haemolymph and tissue fluid associated with long aestivation period.

Aestivation (dry season fasting) is a natural programmed phenomenon in snails [1]. It may be classified as an important phase of the snail's life cycle. It may be likened to 'fasting' common among the animal kingdom. That some animals fast during the mating season, after birth, when angry or excited, in captivity, when wounded or in disease, food scarcity and to keep fit are well known facts. The benefits range from agility, fertility, guts clearance, healing and rejuvenation. Thus, the objective of the present study was to extend our previous studies by evaluating the effects of arousal from aestivating state on the growth changes and body composition and establishing some mechanisms contributing to such transition.

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, Nigeria. Materials used included a total of 40 apparently healthy snails (20 *A. marginata* and 20 *A. achatina*) of 150-200 g liveweight. The snails were obtained from the Wildlife Domesticated Unit of the Department of Forestry and Wildlife Management of the University and the surrounding suburban communities. 40 well ventilated plastic basket cages of 40 cm by 25 cm by 20 cm with covers and 40 each of shallow feeders and drinkers were used.

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. The snails were randomly allocated to the treatments with one snail per basket or cage. Feed and water were provided *ad libitum* and the soil was kept constantly moist. The snails were maintained on artificial diet for 2 weeks of acclimatization before being placed into aestivation. At the end of the 2 weeks adjustment period, the liveweight of the snails in all treatment groups were taken.

Snails were induced to enter aestivation by withdrawing feed and water and discontinued moistening of the soil. The snails were also left undisturbed while they aestivated for 6 weeks.

At the end of the aestivation, the control group (6 weeks aestivation) was slaughtered. The soils of others were moistened to arouse out of aestivation while snails in the respective treatments were slaughtered 2, 4 and 6 weeks post-aestivation.

The effect of duration of post-aestivation on liveweight changes were 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 post-aestivation) in a completely randomized design of 5 replicates using the Systat Analytical Computer Package, Version 5.0 [5]. Tukey's highest significant difference (HSD) was used to separate the means where significant differences existed.

RESULTS

During the 6 weeks of aestivation preceding the post-aestivation, there was a mean liveweight reduction of 59.7% of initial liveweight of A. marginata and 60.4% of A. achatina. Hydration and feeding provoked a new increase in liveweight by 192.6% and 160.3% of the aestivated liveweight within two weeks for A. marginata and A. achatina respectively. Analysis of variance showed that the liveweight associated with aestivating snails differed significantly (P < 0.001 and P < 0.01) from the liveweight associated with 2 weeks post-aestivating in both A. marginata and A. achatina respectively. There was further increases in liveweight to 200.5% and then dropped to 167.0% of the aestivated liveweight in A. marginata and 191.2% and 180.0% in A. achatina for 4 and 6 weeks of post-aestivation respectively. While the increase due to hydration and feeding were significantly higher (P < 0.001) than the aestivated snails in both species, liveweight returned to pre-dormancy values and shot above them 2 weeks of post-aestivation for A. marginata and 4 weeks for A. achatina (Figure 1).

The prediction equations below show that post-dormancy liveweight changes are asymptotic indicating an exponential increase to a maximum and then through a gradual change.

$$\begin{split} PWTM &= 93.264 + 105.168 \; (1 - e^{-0.107tp}); \\ PWTA &= 96.152 + 125.608 \; (1 - e^{-0.146tp}) \; ; \end{split} \qquad \begin{matrix} (R^2 = 67.1 \; \%) \\ (R^2 = 71.6 \; \%) \end{matrix}$$

Where PWTM and PWTA are post-aestivation liveweight of *A. marginata* and *A achatina* at t_p weeks of rehydration.

Table 1 shows the weight gain over 2, 4 and 6 weeks of hydration and feeding for *A. marginata* and *A. achatina*. *A. marginata* had lost between 68.80 ± 5.75 g and 80.33 ± 7.77 g while *A. achatina* lost between 65.00 ± 5.75 and 72.67 ± 7.41 g over the 6 week dominancy period. While *A. marginata* regained its lost weight within 2 weeks, *A. achatina* recovered only about 76.13% which was significantly lower (P < 0.01) than the 72.67 ± 7.41 g weight loss over the 6 weeks aestivation period. The result also showed that weight gain both peaked at the 4th week of hydration and feeding which were higher than the amount of weight lost during aestivation in both species.

The table also presents the least square means of the effect of duration of post-aestivation on the shell weight (SW) of A. marginata and A. achatina. The overall average SW after six weeks of

post-aestivation was not significantly higher (P > 0.05) for *A. marginata* (24.17 ± 1.31 g) than for *A. achatina* (22.71 ± 1.39 g). In both species, SW significantly (P < 0.05) incressed by 40.6% for *A. marginata* within 2 weeks of post-aestivation and 56.8% for *A. achatina* (P < 0.01) within the same experimental period. Further changes due to continuous hydration and feeding were not significantly (P > 0.05) different from the SW recorded at 2 weeks post-aestivation. In both species hydrated shells gradually became thick, opaque and hard.

The effect of length of post-aestivation on soft body weight (SB) of *A. marginata* and *A. achatina* is also presented in the table. *A. marginata* had significantly higher (P < 0.05) overall average SB (95.79 \pm 4.67 g) than for *A. achatina* (82.60 \pm 4.78 g) over the 6 weeks of hydration and feeding. However, both species showed an increase in SB associated with an increasing length of post-aestivation. The aestivated SB (control) of *A. marginata* was significantly higher (P < 0.05) than *A. achatina*. In both species, SB increased significantly (P < 0.001) when rehydrated, rising to 195.7, 179.3 and 159.0% of aestivated weight for *A. marginata* and to 211.7, 266.1 and 250.9% for *A. achatina* by 2, 4 and 6 weeks respectively. There was however, no significant (P > 0.05) interaction between the species.

Both species showed a consistent increase in the dry weight with increasing length of post-aestivation. A. marginata built a dry mass of 18.9% of the 25.68 \pm 2.97 g of the control snails and 16.9% in A. achatina over a 6 week post-aestivation period. These increases were however not large enough to result to any significant (P > 0.05) change in the DW of the dormant and the active feeding snails in both species within the experimental period.

The overall average % DW of A. marginata $(28.30 \pm 1.37\%)$ was significantly (P < 0.001) lower than for A. achatina over the 6 weeks post-aestivation. In both species, the % DW significantly declined (P < 0.001) with continuous rehydration and feeding from the aestivated snails to the 6^{th} week post-dormancy. There was also significant interaction (P < 0.01) between the % DW of the two species.

The summary of the effect of post-aestivation on the haemolymph (HL) of *A. marginata* and *A. achatina* is presented as least squares means in Figure 2. The HL content of *A. marginata* (34.76 \pm 2.91 g) was significantly lower (P < 0.001) than that of *A. achatina* (55.31 \pm 2.98 g). The HL significantly increased (P < 0.001) with weeks of post-aestivation rising to 384.8, 427.1 and 335.1% of aestivated HL for *A. marginata* and to 180.7, 223.1 and 221.9% for *A. achatina* by 2, 4 and 6 weeks respectively. Like in most other parameters, the increases were relatively sharp for the first 2 weeks, reached a peak and then stabilized with continuous hydration and feeding.

Unlike the HL, aestivated A. marginata significantly (P < 0.01) retained more fluid in the tissue than A. achatina at the end of the 6th week of dormancy (Figure 3). When hydrated and fed, the rate of accumulation of body moisture (BM) was high during the first 2 weeks. In A. marginata, the tissues seemed to be saturated first which significantly (P < 0.001) increased by 248.1% in the second week of post-aestivation and stabilized to 238.3 and 218.4% of the aestivated values at the 4th and 6th weeks of arousal. In A. achatina, rehydration and feeding significantly (P < 0.001) triggered 376.5% increase of BM of the aestivated snails at 2 weeks of post-aestivation. Unlike A. marginata, A. achatina took about 4 weeks of active rehydration and feeding to saturate the tissue with the fluid rising up to 520.9% of the aestivated values which subsequently

stabilized to 435.9% at the 6^{th} week of post-aestivation. Summarily, the soft body seems to be the sink where fluid is actively collected soon after arousal.

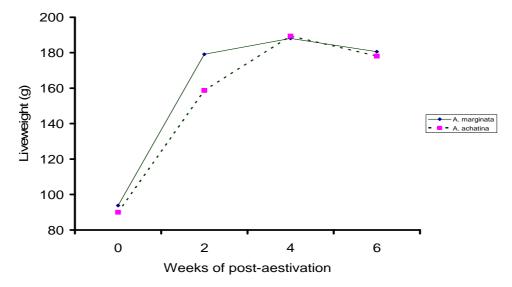


Figure 1 . Effects of duration of post-aestivation on the liveweight of the giant African land snails

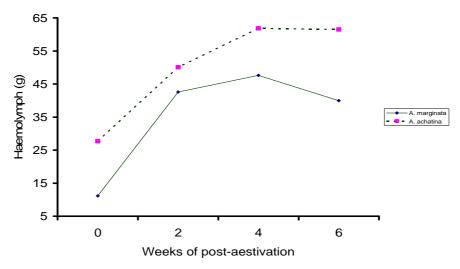


Figure 2 Effects of duration of post-aestivation on the haemolymph of the giant African land snails

Table 1. Effects of length of post-aestivation on morphological composition of A. marginata and A. achatina

Parameter	Species	Length of post-aestivation (Weeks)			
	-	0	2	4	6
Weight gain (g)	A. marginata	0.00 ± 7.53^{d}	76.50 ± 8.43^{bc}	87.00 ± 9.75^{a}	69.40 ± 7.54^{bc}
· ·	A. achatina	$0.00 \pm 7.54^{\rm d}$	$55.33 \pm 9.73^{\circ}$	94.00 ± 8.43^{a}	83.00 ± 8.43^{a}
Shell weight (g)	A. marginata	17.04 ± 2.28^{bc}	23.98 ± 2.54^{a}	27.43 ± 2.94^{a}	22.20 ± 2.28^{ab}
	A. achatina	15.01 ± 2.28^{c}	24.16 ± 2.94^{a}	26.56 ± 2.54^a	24.69 ± 2.54^a
Soft body weight (g)	A. marginata	60.24 ± 8.42^{d}	117.86 ± 9.41 ^a	108.02 ± 10.90^{ab}	97.03 ± 8.42^{bc}
	A. achatina	$39.87 \pm 8.42^{\rm e}$	84.39 ± 10.87^{c}	106.09 ± 9.41^{ab}	100.04 ± 9.41^{bc}
Dry weight (g)	A. marginata	25.68 ± 2.97	25.06 ± 3.32	24.00 ± 3.83	30.53 ± 2.97
, , ,	A. achatina	24.42 ± 2.97	25.69 ± 3.83	27.34 ± 3.32	28.56 ± 3.32
Dry weight (%) (as	A. marginata	42.42 ± 2.47^{b}	26.82 ± 2.76^{c}	22.15 ± 3.19^{c}	21.83 ± 2.47^{c}
% SB)	A. achatina	61.61 ± 2.47^{a}	31.02 ± 3.19^{bc}	$23.45 \pm 2.76^{\circ}$	32.09 ± 2.76^{bc}
Body moisture (%)	A. marginata	$57.59 \pm 2.54^{\text{b}}$	72.81 ± 2.83^{a}	76.15 ± 3.28^{a}	78.11 ± 2.54^{a}
(as % SB)	A. achatina	$35.60 \pm 2.54^{\circ}$	68.98 ± 2.83^{a}	76.55 ± 2.89^{a}	67.91 ± 2.84^{a}

Values are least square means (\pm sem), n = 5 abcde Means with different superscripts within the same parametric row differ significantly (P < 0.05)

When the BM was expressed as a percent of the SB (shown in the table) there was significant (P < 0.001) difference in the overall average % BM between A. marginata (71.18 \pm 1.41%) and A. achatina (62.26 \pm 1.44%) over the 6 weeks post-aestivation. There was a significant rise (P < 0.001) in the % BM with increasing length of post-aestivation in both species with significant interaction (P < 0.01) between the species and weeks of post-aestivation.

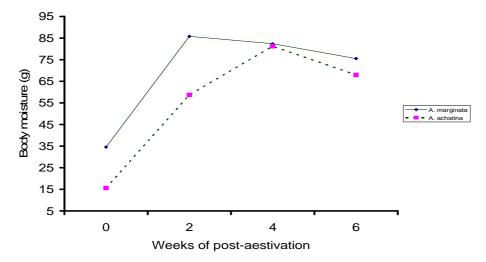


Figure 3. Effects of duration of post-aestivation on the body moisture of the giant African land snails

DISCUSSION

The results from this research agrees with our earlier findings [4,6] that both species can survive aestivation for 6 weeks when induced under atmospheric conditions that do not favour natural aestivation. When the soil was moistened and feed and water supplied, arousal was initiated and snails emerged from their shells within 6 hours. Mechanical activities with increase feed and water intake resumed immediately.

The results of investigation on the effects of rehydration and feeding on the liveweight change of dormant snails are consistent with the observations made by Cobbinah [7]; Hermes-Lima *et al*. [2] and Rizzatti and Romero [8]. As shown, hydration of dormant snails induced significant increase in body weight. The increase in liveweight was sharp within the first week of hydration and then maintained a constant rate which however decreased to a stable weight at the 5th and 6th weeks.

Within the first 2 weeks, post-aestivation liveweight almost doubled the liveweight at dormancy in both species. At the 4th week, post-aestivation liveweight shot above the pre-dormancy liveweight indicating a compensation of weight loss during dormancy. The weight increase observed within the first two days seems to reflect the increase in water or fluid volume, probably haemolymph volume. Feed in turn may have also promoted the compensatory increase in body weight but may not be enough to contribute such significant changes. Feeding also promoted an increase in the mechanical work.

Significant changes observed in the shell weight at hydration suggest that the shell may be involved in the adjustments that accommodate physiological balances during aestivation [4]. In view of the above consideration, it could be suggested here that the decrease in shell weight during aestivation and the reversed increase at post-aestivation in both *A. marginata* and *A. achatina* may be due to at least two factors. One is the loss [4] and gain of water. Another is the mobilization of calcium and phosphorus in and out of the shell due to epiphragm dynamics. The analysis of snail shell by Mead [9] indicated that only 0.2 % was water thereby eliminating the possibility of shell rehydration as the cause of significant change in the shell weight. Mead [9] also identified that the extremely low percentage of calcium in the flesh suggests that this important constituent is not accumulated to any great extent in the mantle or elsewhere in the body, but is speedily deposited in the shell. In any case, it is likely that the changes in the shell weight may result from the movement of calcium among the shell, flesh and epiphragm during aestivation and the replenishment of the shell at post-dormancy.

Hydration and feeding triggered an aggressive increase in the soft body weight that peaked at the second week in *A. marginata* and the fourth week in *A. achatina*. To a large extent, these effects were a direct result of the rehydration of the tissue. This confirmed the report of Rizzatti & Romero [8], who noted that hydration induced significant increases in weight of the flesh of surviving dormant tropical land snail, *Megalobulimus santipauli*. As usual, these increases occurred more quickly at the onset of hydraton and feeding, reached a peak and then dropped to a slower steady rate. The rate of hydration of the flesh at each stage mentioned above was much slower in *A. achatina* than *A. marginata*. This may probably be responsible for the lower mechanical activities observed in *A. achatina* long after arousal.

The results of the study revealed that, although dormant snails showed parallel decreases in dry tissue mass during aestivation, hydration and feeding did not significantly stimulate dry tissue growth within the period of study. The slow increase in the dry weight of the flesh may imply that a significant compensation may be considered over an extrapolated period.

In our previous research [4], we partition the fluid into two for proper investigation. These included haemolymph which was simply drained immediately at sacrifice. It forms partly the blood component. The second involves the fluid trapped in the tissues which was only removed after oven drying. The partitioning of the body fluid here again demonstrates the constitutive or adaptive changes in the fluid to support transitions to and from the aestivating state. Arousal from dormancy in A. marginata resulted in a 4-fold increase in haemolymph within the first 2 weeks of hydration while A. achatina was still below a 2 fold rise. This agreed with earlier observation by Dieringer, Koester and Weiss [10] who noted that the weight increase of Aplysia corlifornica at arousal reflected the increase in water volume, probably haemolymph volume. Omoyakhi, et al. [4] however noted that 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. Rizzatti and Romero [8] however emphasized the importance of this change as the increase in the volume obviously decreases the haemolymph osmolarity which promotes a new significant increase in heart rate and consequently, induces mechanical work and feeding that would eventually increase the haemolymph nutrient levels. This may partly explain why A. marginata were more active and feeding immediately after arousal. It was also observed that the A. achatina with excessive weight increase at rehydration,

probably from high haemolymph volume, died within the first 2 weeks of arousal. According to Odiete [1], if the external medium is hyposmotic as in the case of haemodilution, the cells gain water, become turgid and swell and may burst. Again, the animal dies as the metabolites and enzyme concentration become so dilute that the metabolic rates are too low for life processes to continue. Hermes-Lima, Storey and Storey [2] however noted that the rise in oxygen tension and consumption in snail organs during arousal could result in an elevated production of oxyradicals which may cause oxidative injuries. They suggested that there is always a proportional increase in the activity of key antioxidant enzymes for defense against peroxide and O₂ mediated oxidative injury. It is speculated therefore in this study that the specific pattern and rate of haemolymph and body moisture reconstitution in the species help to regulate these enzymes to avoid the injurious levels.

While the interpretation of our data for the transition in and out of aestivation is still tentative, our study represents real progress in the research of mechanisms for aestivation in the giant African land snails. The study presents the useful observation that the liveweight of the snails is depressed by aestivation but there is a compensatory recovery soon after arousal. Moreover, growth changes during and after aestivation are basically the result of the dynamics of the haemolymph and body moisture. Shell weight changes, metabolism of the body reserves and dry mass have minimal effects.

The study also concludes that one of the major adaptive mechanisms of *A. marginata* over *A. achatina* is its ability to effectively regulate the body moisture while changes are dramatic in both the haemolymph body moisture of *A. achatina*

REFERENCES

- [1] Odiete W.D. **1999**. *Environmental physiology of animals and pollution*. Diversified Resources Ltd. Surulere, Lagos. 95-97p.
- [2] Hermes-Lima M., Storey J.M. and Storey K.B. **1998**. *Comp. Biochem. and Physiol*. (B) 120: 437 448.
- [3] Storey K.B. **2001**. *Molecular mechanisms of metabolic arrest: life in limbo*. BIO Scientifc publishers Ltd. Oxford UK, 216pp
- [4] Omoyakhi J.M., Osinowo O.A., Onadeko S.A. and Ozoje M.O. **2008a**. *African Journal of General Agriculture*. (4) 4: 241-249
- [5] SYSTAT, 1992. Systat computer package, version 5.02. Systat, Inc. **1800** Sherman Ave, Evanstor IL USA, 6021, 708, 864, 5670.
- [6] Omoyakhi J.M., Osinowo O.A., Onadeko S.A. and Ozoje M.O. **2008b**. *African Journal of General Agriculture*. (4) 4: 251-258
- [7] Cobbinah J.R. 1992. Snail farming in West Africa. A practical guide, CTA, UK, p 1 15.
- [8] 8. Rizzatti A.C.S. and Romero S.M.B. **2001**. *Braz. J. Med. Biol. Res.* 34 (7):959 967.
- [9] Mead A.R. 1961. *The Giant African Snail: A problem in economic malacology*. The University of Chicago Press, USA. 146-171p.
- [10] Dieringer N., Koester J.E. and Weiss, K.R. 1978. J. Comp. Physiol. 123: 11-21.