Global Veterinaria 4 (4): 400-408, 2010
ISSN 1992-6197

Some Haemolymph Biophysical Parameters in the Giant African Land Snail *Archachatina marginata* During a Six-Week Aestivation Period

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Abstract: Two experiments were conducted to determine changes in some biophysical parameters of the haemolymph in two liveweight groups of the Giant African Land Snail *Archachatina marginata* during and after aestivation. Experiment 1 was carried out on haemolymph biophysical changes during aestivation, while experiment 2 was carried out on changes post-aestivation. Both experiments were of 2 x 4 x 2 factorial design, with two liveweight groups (smaller snails-100 to 200 g vs. larger snails-201 to 300 g), four periods (0, 2, 4 and 6 weeks) of aestivation (Experiment 1) or post-aestivation after six weeks aestivation (Experiment 2) and two types of haemolymph (oxygenated and deoxygenated). The experiments were replicated five times. Each experiment involved 40 snails. Samples of haemolymph were obtained from the mantle (oxygenated) and pedal sinus (deoxygenated). The results showed that haemolymph volume during the sixth week of aestivation declined significantly ($P<0.05$) by 73.3% of the haemolymph volume (8.91±0.702 ml) in the control snails. Similarly, haemolymph weight in aestivating snails declined significantly ($P<0.05$) by 82.6% of the haemolymph weight (9.02±0.723 g) in non-aestivating control snails at the end of the six-week aestivation period. As the haemolymph volume in aestivating snails declined, its specific gravity increased significantly ($P<0.05$) by 3.9% of the specific gravity (1.03±0.003) in the non-aestivating control group. During post-aestivation, haemolymph volume increased significantly ($P<0.05$) at the end of six-week post-aestivation period by 6.53 times the corresponding value (1.65±1.269 ml) in six-week aestivated snails, while haemolymph weight increased by 5.95 times the corresponding volume (1.84±1.279 ml) in six-week aestivated snails. However, haemolymph specific gravity decreased significantly ($P<0.05$) by 2.8% of the haemolymph specific gravity (1.06±0.002) in six-week aestivated control. Larger aestivating snails had a significantly ($P<0.05$) higher haemolymph volume and weight than smaller aestivating snails. The mantle had significantly ($P<0.05$) higher haemolymph volume than the pedal sinus during aestivation and post-aestivation. In conclusion, this study showed that haemolymph volume and weight declined during aestivation but increased post-aestivation due to rehydration. Haemolymph specific gravity on the other hand, increased during aestivation due to haemoconcentration, but decreased during post-aestivation due to rehydration. The compensatory phenomenon seen implied that the Giant African Snail *Archachatina marginata* can withstand six-week aestivation period with no adverse effect to its physiology.

Key words: Aestivation · Post-aestivation · *Archachatina marginata* · Haemolymph

INTRODUCTION

Haemolymph is the blood analogue found in all arthropods and most molluscs which have an open circulatory system [1]. It is composed of water, inorganic salts (mostly Na, Cl, K, Mg and Ca) and organic compounds (mostly carbohydrates, proteins and lipids). Muscular movements by the animal during locomotion can facilitate haemolymph movement, but diverting flow from one area to another is limited [2]. Responses to high or low temperatures in the snail include aestivation and hibernation [3, 4].

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Snails are terrestrial and marine shell-bearing animals of approximately 80,000-100,000 species of the phylum *Mollusca* [5]. They are the second largest phylum in the animal kingdom. Europe and Africa are two important regions notable for snail production and consumption involving mainly the snail families *Helicidae* and *Achatinidae,* respectively. However, the giant African land snails *Achatina achatina* and *Archachatina marginata* are the most popular edible snails in the West African high forest zones [6, 7].

During dry spells free-living snails withdraw into their shells and conserve water by sealing the shell opening with an epiphragm and aestivate by reducing mobility, reproductive behaviour and growth. Water loss is further retarded by the use of discontinuous breathing pattern; the pneumostome opens intermittently to allow a rapid exchange of CO_2 and O_2 [8]. When humidity falls below 75% (as witnessed during the dry season: October to Mid-March in West Africa), *A. achatina* becomes inactive and seals itself into its shell with a white calcareous layer and aestivates in order to prevent loss of water from the body [9]. *A. marginata* were observed to form epiphragm more readily and replace them more frequently than *A. achatina* [10]. Snails are said to survive many months without food and water under aestivation [11]. The aestivated snails draw on their reserve of fat and glycogen at much reduced rate, which implies an imminent reduction in weight and loss of valuable growing time as growth during aestivation is said to reduce.

The success of gastropod molluscs in terrestrial habitats has been due to various structural, physiological and behavioural specializations [12]. One specialization that is well developed among the pulmonate land snails is the capacity to enter the dormant state of aestivation during periods of hot and dry environmental conditions [13].

The haemolymph is an important medium for the transport of nutrients to and wastes from various organs of the snail. Aestivation, being a structural, physiological and behavioural response to desiccation, probably plays a role in the dynamics of haemolymph changes in land snails as haemolymph ionic concentration is said to show seasonal fluctuations, strongly influenced by hydration, feeding and acid/base balance [14, 15]. There are limits to the duration of aestivation that can be tolerated by land snails and mortality eventually increases as aestivation is prolonged [13]. Could haemolymph characteristics be a valuable predictor of aestivation tolerance in land snails?

Comprehensive haemolymph biophysical values for the giant African land snails are scarcely reported in the literature. Reference range values for haemolymph biophysical parameters from non-aestivating, aestivating and post-aestivating *Archachatina marginata* could therefore be useful for assessment of physiologic and pathologic alterations in wild as well as captive snails and establish their possible application in the evaluation of health and disease status. The information may also be useful in domestication, management and bio-conservation initiatives involving this species.

This study, therefore, investigated the effect of six-week aestivation period on haemolymph volume, weight and specific gravity in the Giant African Land Snail *Archachatina marginata.*

MATERIALS AND METHODS

Experimental Site: The research work was carried out at the Snail Research Unit of the College of Animal Science and Livestock Production (COLANIM), University of Agriculture, Abeokuta, Nigeria. Abeokuta lies within the Rain forest vegetation zone of Western Nigeria on latitude 7° 10'N, longitude 3° 2'E 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).

To meet the objectives of the research, two experiments were carried out concurrently.

Experiment 1: Aestivation Experiment

Materials: Two groups of 20 snails each weighing 100-200 g and 201-300 g, 40 well ventilated plastic basket cages of 40 X 25 X 20 cm with cover, 40 each of shallow containers for feed, water and humus soil, a sensitive electronic weighing scale, marker and masking tape for proper identification, pawpaw leaf meal, layer's mash and water were used.

Experimental Design: The experiment was laid out in a 2 x 4 x 2 factorial design with 5 replicates, using a total of 40 snails. The snails were assigned as follows:

Factors:

A. Liveweight group: I (100-200 g) vs. II (201-300 g)
B. Duration of aestivation: 0 vs. 2 vs. 4 vs. 6 weeks
C. Type of haemolymph: I (Oxygenated) vs. II (Deoxygenated) per snail
D. Replicate: 5 snails

Experimental Procedure: The cages were prepared and to each cage were assigned a plastic trough containing top humus soil, a drinker and a feeder. The weight of the snails (initial weight) was measured in grams using an electronic weighing scale (Mettler, Type BD 6000, Mettler-Toledo AG, Switzerland). The snails were allocated to the treatments based on liveweight group with individual snail in a basket. Feed (fresh pawpaw leaf) and water was provided in the first week of acclimatization and then a meal of layer mash + dried milled pawpaw leaves (1:1; w/w) and water *ad libitum* in the second week of acclimatization to normalize all previous treatments. Top humus soil was withdrawn three days to the end of the acclimatization period. At the end of the two-week acclimatization period, the liveweights of the snails (fed weight) in all treatment groups were taken; feed and water were withdrawn from all the treatment snails to induce aestivation. Also, the control group was sacrificed immediately. Liveweight of aestivating snails (aestivation weight) was taken weekly, while the treatment groups were sacrificed after 2, 4 and 6 weeks of aestivation. Prior to sacrifice, the final liveweight of each snail was determined after which the shell was broken according to the procedure of Segun [16] and haemolymph was obtained separately from two regions, namely the mantle cavity (lung) and the pedal sinus. Haemolymph volume was determined using a measuring cylinder, while the weight was measured using a sensitive weighing scale (Mettler PM 4000 Sensitive Scale, Switzerland). The specific gravity of the haemolymph was determined using a hand refractometer (Sugar Refractometer, Bellingham and Stanley and Co., UK) by the method of Egan *et al*. [17].

Statistical Analyses: Parameters were subjected to analysis of variance (ANOVA) in 2 x 4 x 2 factorial design with 5 replicates using the Systat Analytical Computer Package, Version 5.02 [18]. Tukey's honest significant difference (HSD) was used to separate the means where significant differences exist.

Statistical Model: The full model that was used for the analysis is as follows:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + ACi_k + BC_{jk} + ABC_{ijk} + e_{ijkl}$$

Where,

Y_{ijkl} = Parameter of interest
μ = Population mean
A_i = Effect due to i^{th} liveweight group, where i = 1-2
B_j = Effect due to j^{th} duration of aestivation, where j = 0, 2, 4, 6 Weeks
C_k = Effect due to k^{th} haemolymph type, where k = 1-2
AB_{ij} = Effect due to interaction between liveweight group and duration of aestivation
AC_{ik} = Effect due to interaction between liveweight group and type of haemolymph
BC_{jk} = Effect due to interaction between duration of aestivation and type of haemolymph
ABC_{ijk} = Effect due to interaction between liveweight group, duration of aestivation and type of haemolymph
e_{ijkl} = Residual error

Experiment 2: Post-aestivation Experiment

Materials: Same as in Experiment 1 above.

Experimental Design: The experiment was carried out in a 2 x 4 x 2 factorial design with 5 replicates. The snails were assigned as follows:

Factors:

A. Liveweight group:	I (100-200 g) vs. II (201-300 g)
B. Length of post-aestivation:	0 vs. 2 vs. 4 vs. 6 weeks
C. Type of haemolymph:	I (Oxygenated) vs. II (Deoxygenated) per snail
D. Replicate:	5 snails

Experimental Procedure: The cages were prepared and each assigned a plastic trough containing top humus soil, a drinker and a feeder. Feed (fresh pawpaw leaf) and water were provided *ad libitum* for the first week of acclimatization and then a meal of layer mash + dried milled pawpaw leaves (1:1; w/w) and water were also provided *ad libitum* for the second week of acclimatization to normalize all previous treatments. Top humus soil was withdrawn three days to the end of the acclimatization period. At the end of the two-week acclimatization period, the liveweight of the snails (fed weight) in all treatment groups were taken. Feed and water were withdrawn from all the treatment snails to induce and sustain aestivation for 6 weeks. Animals were weighed weekly during the aestivation period. At the end of the aestivation period, the control group were weighed and slaughtered. Humus soil, feed and water were provided to awake and activate the other groups out of aestivation. Weekly weight was recorded, while snails in the respective treatments were slaughtered 2, 4 and 6 weeks post-aestivation. Top humus soil was withdrawn three days before determination of weekly weight gain.

Prior to sacrifice, the final liveweight of each snail was determined after which the shell was broken according to the procedure of Segun [16] and haemolymph was obtained separately from two regions, namely the mantle cavity (lung) and the pedal sinus. Haemolymph volume was determined using a measuring cylinder, while the weight was measured using a sensitive weighing scale (Mettler PM 4000 Sensitive Scale, Switzerland). The specific gravity of the haemolymph was determined using a hand refractometer (Sugar Refractometer, Bellingham and Stanley and Co., UK) by the method of Egan *et al*. [17].

Statistical Analyses: Parameters were subjected to analysis of variance (ANOVA) in a 2 x 4 x 2 factorial design with 5 replicates using the Systat Analytical Computer Package, Version 5.02 [18]. Tukey's honest significant difference (HSD) was used to separate the means where significant differences occurred.

Statistical Model: The full model that was used for the analysis is as follows:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + AB_{ij} + ACi_k + BC_{jk} + ABC_{ijk} + e_{ijkl}$$

Where,

Y_{ijkl} = Parameter of interest
μ = Population mean
A_i = Effect due to i^{th} liveweight group, where i = 1-2
B_j = Effect due to j^{th} length of post-aestivation, where j = 0, 2, 4, 6 Weeks
C_k = Effect due to k^{th} haemolymph type, where k = 1-2
AB_{ij} = Effect due to interaction between liveweight group and length of post-aestivation
AC_{ik} = Effect due to interaction between liveweight group and type of haemolymph
BC_{jk} = Effect due to interaction between length of post-aestivation and type of haemolymph
ABC_{ijk} = Effect due to interaction between liveweight group, length of post-aestivation and type of haemolymph
e_{ijkl} = Residual error

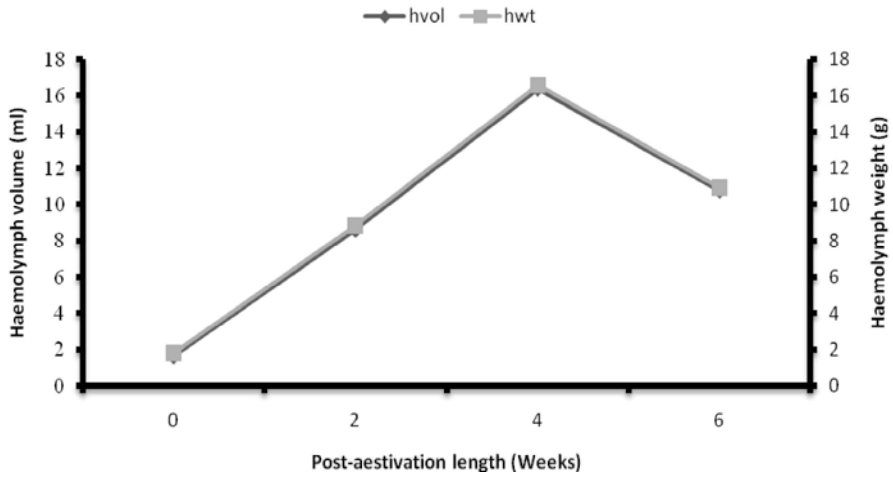
RESULTS

The summary of least squares analysis of variance showing the effects of liveweight group, duration of aestivation and type of haemolymph on haemolymph biophysical parameters (haemolymph volume, weight and specific gravity) of *A. marginata* is highlighted in Table 1. There was a highly significant (P<0.001) effect of liveweight group on haemolymph volume and weight in aestivating *A. marginata*. Duration of aestivation had a highly significant (P<0.001) effect on haemolymph volume, weight and specific gravity. Type of haemolymph had a highly significant (P<0.001) effect on haemolymph volume and weight and a significant effect (P<0.05) on haemolymph specific gravity. The interaction between liveweight group and duration of aestivation; liveweight group and type of haemolymph; duration of aestivation and type of haemolymph and liveweight group, duration of aestivation and type of haemolymph, on haemolymph volume and weight were highly significant (P<0.001).

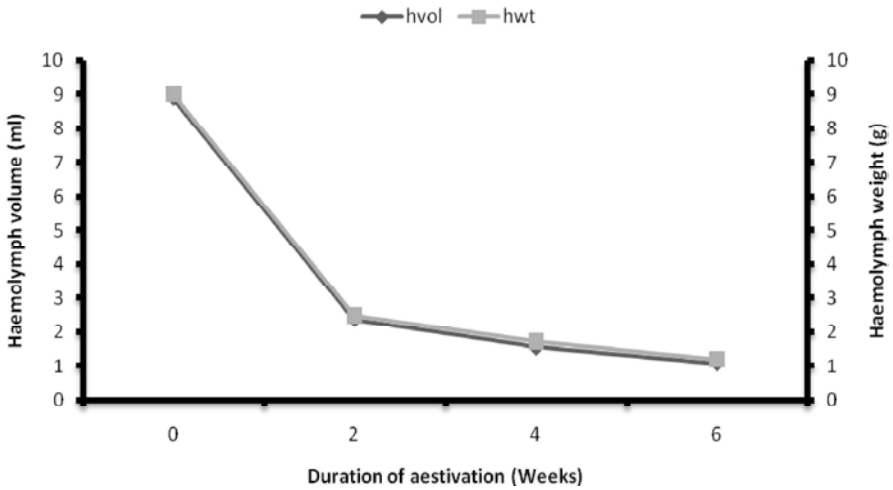
Table 1: Summary of least squares analysis of variance showing the effects of liveweight group, duration of aestivation and type of haemolymph on haemolymph biophysical parameters in *A. marginata*

		Mean-square		
Source of variation	df	Haemolymph volume	Haemolymph weight	Haemolymph specific gravity
Liveweight group (L)	1	291.193***	293.669***	0.00026
Aestivation-week (A)	3	263.814***	261.899***	0.00469***
Type of haemolymph (T)	1	634.012***	653.411***	0.00145*
L x A	3	97.619***	93.672***	0.00021
L x T	1	226.193***	222.540***	0.00003
A x T	3	270.959***	266.315***	0.00007
L x A x T	3	94.984***	89.642***	0.00006
Error	62	9.864	10.459	0.00021[1]

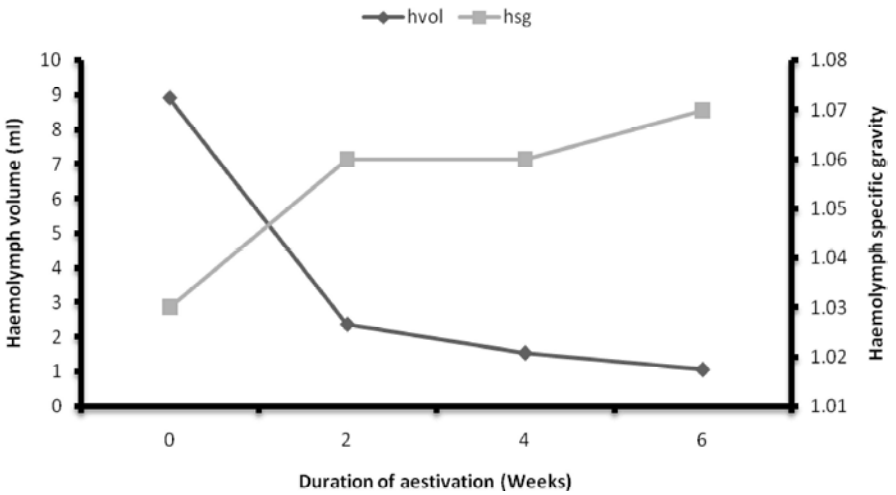
*P<0.05, ***P<0.001, [1]df of Error = 56



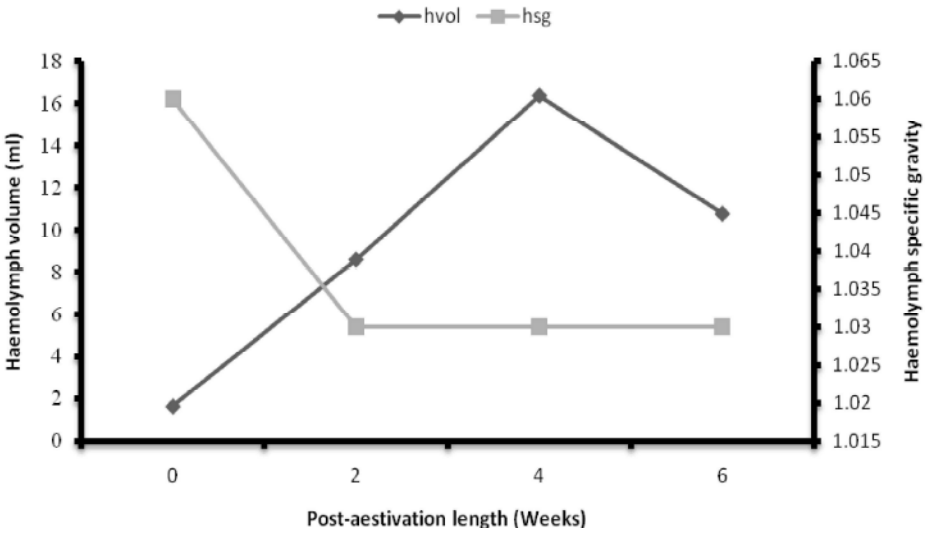
a: Changes in haemolymph volume and weight in *A. marginata* during aestivation



b: Changes in haemolymph volume and specific gravity in *A. marginata* during aestivation



c: Changes in haemolymph volume and weight in *A. marginata* during post-aestivation



d: Changes in haemolymph volume and specific gravity in *A. marginata* during post-aestivation

Fig. 1: Effects of aestivation and post-aestivation on haemolymph volume, weight and specific gravity in *A. marginata*

Table 2: Summary of least squares analysis of variance showing the effects of liveweight group, post-aestivation length and type of haemolymph on haemolymph biophysical parameters in *A. marginata*

		Mean-square		
Source of variation	df	Haemolymph volume	Haemolymph weight	Haemolymph specific gravity
Liveweight group (L)	1	75.173	81.476	0.00000
Post aestivation-week (P)	3	603.538***	606.926***	0.00402***
Type of haemolymph (T)	1	4122.980***	4171.741***	0.00053*
L x P	3	12.483	13.114	0.00027*
L x T	1	36.431	37.301	0.00000
P x T	3	572.264***	568.461***	0.00011
L x P x T	3	7.751	8.561	0.00002
Error	50	28.622	29.085	0.00004[1]

*P<0.05, ***P<0.001, [1]df of Error = 49

The summary of analysis of variance showing the effects of liveweight group, post-aestivation length and type of haemolymph on haemolymph biophysical parameters of *A. marginata* is presented in Table 2. Post-aestivation length had a highly significant (P<0.001) effect on haemolymph volume, weight and specific gravity. There was a highly significant (P<0.001) effect of haemolymph type on haemolymph volume and weight and a significant (P<0.05) effect on haemolymph specific gravity. The interaction between liveweight group and post-aestivation length on haemolymph specific gravity was significant (P<0.05). There was a highly significant (P<0.001) interaction between post-aestivation length and type of haemolymph on haemolymph volume and weight.

The effects of aestivation and post-aestivation on haemolymph volume, weight and specific gravity in *A. marginata* are shown in Figure 1. Haemolymph volume declined significantly (P<0.05) in aestivating *A. marginata* with weeks of aestivation, falling by 73.3, 82.6 and 88.0% of the haemolymph volume (8.91±0.702 ml) in the pre-aestivation control snails at the end of six weeks aestivation (Figure 1a). Haemolymph weight in aestivating snails declined significantly (P<0.05) with weeks of aestivation, falling by 72.5, 80.9 and 86.8% of the weight of the non-aestivating control (9.02±0.723 g) at the end of the six weeks aestivation period (Figure 1a). As the haemolymph volume in aestivating snails declined significantly (P<0.05), haemolymph specific gravity increased significantly (P<0.05) with weeks of aestivation by 3.9% of the specific gravity in non-aestivating control group (1.03±0.003) (Figure 1b).

Haemolymph volume increased significantly ($P<0.05$) post-aestivation, rising to 6.53 times the haemolymph volume of the six weeks aestivating control snails (1.65±1.269 ml) at the end of the six weeks rehydration period (Figure 1c). Haemolymph weight increased significantly ($P<0.05$) post-aestivation, rising by 5.95 times the haemolymph weight of the control snails (1.84±1.279 g) at the end of six weeks post-aestivation (Figure 1c). As haemolymph volume increases post-aestivation, haemolymph specific gravity decreases with weeks of post-aestivation by 2.8% of the specific gravity in control snails (1.06±0.002) at the end of six weeks post-aestivation period (Figure 1d).

DISCUSSION

In this study, haemolymph volume declined significantly with weeks of aestivation. Haemolymph weight followed a similar pattern while haemolymph specific gravity increased significantly. These findings could be due to dehydration, decreased heart rate, decreased venous return, increased haemolymph osmoconcentration and accumulation of end products of metabolism. Withers *et al*. [19] reported that evaporative water loss in the Australian land snail *Rhagada tescorum* declined by 95% to less than 1 mg/g/h when aestivating, from more than 10 mg/g/h when awake. They explained this marked reduction in evaporative water loss as possibly due to low loss of water across the shell and retardation of water loss through the shell aperture in part by epiphragms, but mainly by a reduced water permeability of the mantle itself. However, in this study, it could be postulated that the ability of *A. marginata* to readily form and replace epiphragms provides an avenue for increased evaporative water loss through the shell aperture as aestivation progressed, hence the decrease in haemolymph volume and weight observed in this study. Rizzatti and Romero [20] revealed that dormancy is associated with a parallel decrease in heart rate and total body weight. They found that the decrease in heart rate of dormant snails was significantly greater than that of actively feeding snails, indicating that in addition to temperature, other factors such as starvation and dehydration may be responsible for the decrease in heart rate during dormancy. It could be possible that this decrease in heart rate coupled with the decrease in venous return are responsible for the decrease in haemolymph volume and weight observed in this study. This was corroborated by Rizzatti and Romero [20] in their study on the effects of hydration and feeding on heart rate of dormant snails. They reported that rehydration of dormant snail induced significant increases in body weight and heart rate. They further explained that the weight gain reflected the increase in water volume, probably haemolymph volume. The increased haemolymph volume may have led to an increase in heart rate, probably due to an increase in venous return [21]. This could be a possible explanation for the increase in liveweight, haemolymph volume and weight observed in *A. marginata* subjected to six weeks hydration in this study.

The increase in haemolymph specific gravity with weeks of aestivation could be due to increased haemolymph osmolarity as opposed to the decreased haemolymph specific gravity observed in post-aestivating *A. marginata*, which could be due to a decrease in haemolymph osmolarity as a consequence of rehydration. Rizzatti and Romero [20] postulated that a probable decrease in haemolymph osmolarity in hydrated dormant snails could have resulted from the increase in water volume, consequently contributing to the increase in heart rate seen post-rehydration. Neuckel [22] studied the uptake of water in 35 central European terrestrial pulmonate snail species by giving the inactive, withdrawn snail dyed water and observing its bulk flow microscopically. He revealed that during emergence 32 of the species studied take up water through the pneumostome by a rectal pump which rapidly conveys this water through the anus into the gut and stomach. He further quantified anal water uptake during emergence to be equivalent to 20-40% of the total body water of the inactive snails before water uptake and concluded that the anal mechanism permits rapid uptake of large quantities of water into the extrasomal compartment, which helps terrestrial pulmonate snails to exploit short wet spells and presumably helps to stabilize blood concentration during activity under dry conditions. These findings when extrapolated to the present study could possibly relate the likely absence of a functional anal mechanism in aestivating *A. marginata* to the increase in haemolymph specific gravity probably due to haemoconcentration as a consequence of the hypovolaemia seen with weeks of aestivation. Also, the possible presence of a functional anal mechanism in *A. marginata* post-rehydration could be related to the decrease in haemolymph specific gravity due to haemodilution as a consequence of the hypervolaemia seen post-aestivation.

This study showed that haemolymph volume and weight declined during aestivation due to dehydration, but increased post-aestivation following rehydration. Haemolymph specific gravity on the other hand increased during aestivation due to haemoconcentration but decreased during post-aestivation due to rehydration. The compensatory phenomenon recorded, therefore, implied that the Giant African Snail *Archachatina marginata* can withstand six-week aestivation period with no adverse effect to its physiology.

REFERENCES

1. Ebroadcast. "Hemolymph," Website:http://www.ebroadcast.com.au/lookup/encyclopedia/he/Hemolymph.html (Accessed February 5, 2007).
2. Burton, R.F., 1965. Na, K, Mg in the blood of the snail *Helix pomatia.* Physiol. Zool., 38: 335-342. [cited by Langley, C.K., 1979. Thermal acclimation of a central neurone of *Helix aspersa*. I. Effects of temperature on electrolyte levels in the haemolymph. J. Exp. Biol., 78: 181-186].
3. Burton, R.F., 1968a. Ionic regulation in the snail *Helix aspersa.* Comp. Biochem. Physiol., 25: 501-508. [Cited by Langley, C.K., 1979. Thermal acclimation of a central neurone of *Helix aspersa*. I. Effects of temperature on electrolyte levels in the haemolymph. J. Exp. Biol., 78: 181-186].
4. Burton, R.F., 1968b. Ionic balance in the blood of *Pulmonata.* Comp. Biochem. Physiol., 25: 509-516. [cited by Langley, C.K., 1979. Thermal acclimation of a central neurone of *Helix aspersa*. I. Effects of temperature on electrolyte levels in the haemolymph. J. Exp. Biol., 78: 181-186].
5. Cooper, J.E. and C. Knowler, 1991. Snails and snail farming. An introduction for the veterinary profession. Vet. Rec., 129(25-26): 541-549.
6. Ajayi, S.S., O.O. Tewe, C. Moriaty and M.O. Awesu, 1978. Observations on the biology and nutritive value of the African giant snail (*Achatina achatina*). East Afr. Wildl. J., 16: 85-95. [Cited by Ogunsanmi, A.O., V.O. Taiwo and T.O. Akintomide, 2003. Haemolymph biochemical parameters of the African giant snail (*Achatina achatina*) and the big black snail (*Archachatina marginata*). Trop. Vet., 21(2): 43-48].
7. Cobbinah, J.R. 1997a. Cited by Ogunsanmi, A.O., V.O. Taiwo and T.O. Akintomide, 2003. Haemolymph biochemical parameters of the African giant snail (*Achatina achatina*) and the big black snail (*Archachatina marginata*). *Tropical Veterinarian* 21(2): 43-48.
8. Hermes-Lima, M., J.M. Storey and K.B. Storey, 1998. Antioxidant defenses and metabolic depression. The hypothesis of preparation for oxidative stress in land snails. Comp. Biochem. Physiol. B. Comp. Biochem., 120: 437-448.
9. Cobbinah, J.R., 1992. Cited by Omoyakhi, J.M., 2007. Effects of aestivation on growth, body composition and reproductive performance of Giant African Land Snails, *Archachatina marginata* and *Achatina achatina,* Ph.D thesis, University of Agriculture, Abeokuta, Nigeria.
10. Omoyakhi, J.M., 2007. Effects of aestivation on growth, body composition and reproductive performance of Giant African Land Snails, *Archachatina marginata* and *Achatina achatina,* Ph.D thesis, University of Agriculture, Abeokuta, Nigeria.
11. Akinnusi, O., 1998. A practical approach to backyard snail farming. Nig. J. Anim. Prod., 25: 193-197.
12. Riddle, W.A., 1983. Cited by Rees, B.B. and S.C. Hand, 1993. Biochemical correlates of aestivation tolerance in the Mountain snail *Oreohelix* (Pulmonata: Oreohelicidae). Biol. Bull. 184: 230-242.
13. Rees, B.B. and S.C. Hand, 1993. Biochemical correlates of aestivation tolerance in the Mountain snail *Oreohelix* (Pulmonata: Oreohelicidae). Biol. Bull., 184: 230-242.
14. Burton, R.F., 1971. Natural variation in cation levels in the blood of 3 species of land snail (*Pulmonata, Helicidae*). Comp. Biochem. Physiol., 39A: 267-275. [cited by Langley, C. K., 1979. Thermal acclimation of a central neurone of *Helix aspersa*. I. Effects of temperature on electrolyte levels in the haemolymph. J. Exp. Biol., 78: 181-186].
15. Burton, R.F., 1971b. Concentration of cations in the blood of some terrestrial snails. Comp. Biochem. Physiol., 39A: 875-878. [cited by Langley, C. K., 1979. Thermal acclimation of a central neurone of *Helix aspersa*. I. Effects of temperature on electrolyte levels in the haemolymph. J. Exp. Biol., 78: 181-186].

16. Segun, A.O., 1975. The giant land snail *Archachatina* (*Calachatina*) *marginata* Swainson: dissection guides of common tropical animals. Ethiope Publishing House, Benin City, Nigeria, pp: 254.
17. Egan, H., R.S. Kirk and R. Sawyer, 1981. Pearson's Chemical Analysis of Foods. 8th Edition. Churchill Livingstone/Longman Group Limited, Edingburgh, pp: 591.
18. Systat, 1992. Systat computer package, version 5.02. Systat, Inc., 1800, Sherman Ave., Evanston, IL, USA.
19. Withers, P., S. Pedler and M. Guppy, 1997. Physiological adjustments during aestivation by the Australian land snail *Rhagada tescorum* (Mollusca: Pulmonata: Camaeniidae). Aust. J. Zool., 45: 599-611.
20. Rizzatti, A.C.S. and S.M.B. Romero, 2001. Heart rate and body weight alterations in juvenile specimens of the tropical land snail *Megalobulimus sanctipauli* during dormancy. Braz. J. Med. Biol. Res., 34: 959-967.
21. Dieringer, N., J. Koester and K.R. Weiss, 1978. Adaptive changes in heart rate of *Aplysia californica*. J. Comp. Physiol., 123: 11-21. [cited by Romero, S.M.B. and A. Hoffmann, 2006. Role of the cardiac nerve in the effect of a novel innocuous stimulus on the heart rate of *Megalobulimus mogianensis*. Braz. J. Med. Biol. Res., 39(6): 833-837].
22. Neuckel, W., 1985. Anal uptake of water in terrestrial pulmonate snails. J. Comp. Physiol. B: Biochemical, Systemic and Environmental Physiology, 156(2): 291-296(Abstr.).