Water Relations, Weight Loss and Nitrogenous Waste Products in the Giant African Land Snail *Archachatina Marginata* (Swainson) – Pulmonata: Achatinidae

Abiodun B. Onadeko* and Rosemary I. Egonmwan
Department of Zoology, University of Lagos, Akoka-Yaba, Lagos, Nigeria
*****aonadeko@unilag.edu.ng

Abstract
Variations in the water content, water loss and nitrogenous waste products of the edible giant land snail *Archachatina marginata* were investigated. The water content of the food component of the snails was also determined. The percentage water content of the active snails at 48.37%–51.74% was higher than that of the inactive snails at 30.31%–39.74%. The water loss in inactive snails was between 3.05%–15.35%, which was lower than that of the active snails between 6.32%–27.02%. There was no ammonia in the haemolymph of *A. marginata*, however, traces of urea and a large quantity of uric acid were observed. Analysis of the kidney contents revealed only the presence of urea. Qualitative analysis of the urine sample showed that only uric acid was present. It was observed that tomatoes had the highest water content while pawpaw leaves had the lowest. The highest organic matter was obtained from orange and the lowest was from the corn shaft. A positive correlation existed between the length and weight relationship of active ($R^2 = 0.708$) and inactive ($R^2 = 0.698$) snails. However a negative correlation existed between the weight and percentage water content of the snails; with similar results observed between the weight and percentage water loss. In relation to previous findings on the life-cycle strategies as well as nutritional and reproductive physiology of *A. marginata*, the results are discussed in the context of the physical adaptations of this species for survival in dry conditions.

Keywords: *Archachatina marginata*, haemolymph, nutritional and reproductive physiology, snails

Introduction

Snails have moist permeable integument through which water is lost by evaporation and as mucus. Mucus is an important component of water transported to the surface of the integument preventing surface drying (Machin, 1972; Lyth, 1983). Therefore terrestrial snails face severe problems of water conservation in dry places due to the nature of their skin and the use of mucus for locomotion (Cameron, 1970). The substrate moisture is also a major influence on growth and egg production in terrestrial snails (Plummer, 1975). One important criterion for successful egg development is a permanent moist site as drought is the most common mortality factor for eggs (Hodasi, 1979).

The importance of water in the lives of terrestrial gastropods has been extensively studied (Machin, 1975; Prior, 1985; South, 1992; Luchtel and Deyrup-Olsen, 2001). The availability of adequate supplies of environmental water is a major factor in determining the distribution and behaviour of gastropoda (Luchtel and Deyrup-Olsen, 2001). Although their survival depends, in part, on their behaviour, different species of snails have different adaptations for water conservation (Machin, 1967; Cameron, 1970; Luchtel and Deyrup-Olsen, 2001). The terrestrial pulmonates particularly survive only by regulating their activities according to the availability of water (Mead, 1952). The physical adaptations for survival under dry conditions include: variations in shell thickness and epiphragm, shape and size of the shell aperture (Machin, 1967; Cameron, 1970; Solem, 1985) and, probably, the burning of food stores to improve their water balance during aestivation (Yom-Tov, 1971). The shell, which is impermeable to water is used by the snail as a means of protection from water loss and when faced with dry conditions, the snail retracts into the shell and covers the opening with one or two layers of calcium carbonate epiphragm (Cameron, 1970; Segun, 1975) or operculum, which is present in the terrestrial prosobranch but absent in the stylommatophora (Luchtel and Deyrup-Olsen, 2001).

Behavioural responses to environmental factors, such as humidity and dampness, to replenish its reserve have been used by snails living in habitats subjected to long dry spells to prevent excessive water loss. On the other hand, snails living in damp habitats where prolonged dry conditions are rare can afford to ignore fluctuations in humidity since water loss is not experienced for a long time (Cameron, 1970). Snails and slugs also take advantage of cooler night-time temperatures and the presence of dew by being active at night, especially between sunset and sunrise, and

during frequent feeding (Cameron, 1970). Slugs, which have no protective shells, are limited in their distribution to places with seasonal moisture content and rely on their ability to tolerate extreme loss of body water and on behaviours that minimise water loss (Luchtel and Deyrup-Olsen, 2001). Active terrestrial snails maintain a thick water-rich layer of mucus on their external surface (Machin, 1975) and the rate of evaporation of water from this layer is high. However, in an inactive snail, which has withdrawn into its shell, water loss from the exposed surfaces, such as the mantle collar, decreases rapidly.

Snails obtain water from their food and directly from the atmosphere through their skin when humidity is high. Water is lost by evaporation from all exposed surfaces of the body once the ambient humidity falls below the blood equilibrium humidity. Water is also lost from the skin through the secretion of mucus and during locomotion. Dainton (1954) attempted to separate water loss during locomotion from that lost during evaporation and found that the pedal losses showed a great variability and were more frequent than evaporation.

Another important factor, which affects the body water content of an animal, is the need to remove exogenous nitrogenous waste products resulting mainly from the metabolism of ingested proteins (Odiete, 1999). The three major types of excretory products are ammonia, urea and uric acid. Ureotelic animals that secrete urea need considerable amounts of water but not as much as ammonotelic animals, which secrete ammonia. Uricotelic animals secrete uric acid, which does not require water for its removal. This enhances water conservation for the animals that are in short supply of water. Therefore, the secretions of these excretory products depend on the availability of accessible environmental water (Odiete, 1999). Blood, as the transporting medium, plays a major role in the physiology of aestivation in terrestrial snails. An accumulation of nitrogenous wastes in the blood of *Pila globosa* during aestivation has been previously reported by Brahmanandam and Krishnamoorthy (1973).

Achatinids are native to tropical and subtropical Africa, south of the Sahara (Pilsbry, 1904). The giant African land snail, *Archachatina marginata*, is endemic to West Africa (Bequaert, 1950). The species is restricted to an area from Benin Republic to Zaire (Bequaert, 1950) and attains an average adult weight of 700 g. In many parts of Nigeria, especially in the rural communities of the Niger Delta, *A. marginata* is a delicacy (Ebenso, 2002; 2004). Okun *et al.* (2011) also reported that *A. marginata* was one of the two most popular breeds of snails in Nigeria. However, the production of snails has not kept pace with demand (Etchu *et al*., 2008); with different environmental and technical factors implicated. Environmental manipulations, such as: urbanisation, deforestation, burning of biomass and the use of harmful chemicals, and climate change as well as the lack of training on intensive snail-rearing have been identified as impediments to increased snail supply from the wild and in captivity (Ngenwi *et al.,* 2010).

The aim of this study is to determine the water content of *A. marginata* under natural and laboratory conditions and evaluate some environmental factors that operate in modifying water loss in the species.

Materials and Methods

Maintenance of the Snails

Specimens of *A. marginata* weighing 20–45 g were purchased at the Yaba and Bariga markets in Lagos, Nigeria. The snails were acclimatised for one week in a large terrarium in the University of Lagos' Biological Sciences Garden. The terrarium, which had a layer of moist humus soil at the bottom, was placed in a cool shaded area of the garden. The terrarium was regularly cleaned with 40% isopropyl alcohol to prevent fungal and bacterial growths, which may cause infection to the snails. The activities of the snails were observed in the terrarium. The snails were considered to be active when their tentacles were fully everted and moving and inactive when they were stationary with their tentacles non-everted after a 1-hour observation. The snails were also considered inactive when their entire soft body was withdrawn into their shells. Active and inactive snails were used in this experiment.

Determination of the Total Body Water Content in Active and Inactive Snails

10 active and inactive snails were each removed from the stock colony and brought to the laboratory. The snails were weighed with an electric balance to the nearest 0.01 mg and their shell lengths were measured with a Vernier calliper (Model TCM D-22290) to the nearest 0.1 mm. The snails were de-shelled by breaking the shells around the penultimate whorl with the aid of a hammer. Care was taken not to break the columella muscle so that the intestine would not rip open. For the inactive snails with calcium carbonate epiphragm, a sharp knife was used to remove the membrane covering the opening of the shell. The de-shelled snails were then dried in an oven at 70 °C until a constant weight was obtained.

The subtraction of the dry body weight from the initial wet weight yielded the total water weight of

each specimen; allowing the body water content to be expressed as a percentage of the body weight.

Calculations:

Weight of the animal with shell	=	a (g)
Weight of the animal only	=	b (g)
Weight of the shell	=	(a – b) (g)
	=	c (g)
Weight of dried body	=	d (g)
Water content from the body only	=	(b – d) (g)
	=	e (g)
Percentage water content from the body	=	(e/b x 100)%

Determination of Water Loss in Active and Inactive Snails

10 active and inactive snails were obtained from the stock colony, weighed, measured and placed in a plastic container (60 x 60 x 60 cm). The container was covered with a net of mesh size (0.5 x 0.5 cm) to allow for the circulation of water vapour and air. Soil, water and food materials were absent in the container. The snails were again reweighed after 5 days and the amount of water loss was computed.

Estimation of Water Content in Food

7 food plants were fed to the active snails, namely: water leaf (*Talinum triangulare*), corn (*Zea mays*), cassava leaves (*Manihot esculentum*), pawpaw leaves (*Carica papaya*), banana (*Musa sapientum*), oranges (*Citrus sinensis*) and tomato (*Lycopersicon esculentum*). Each was weighed and dried in an oven at 70 °C until a constant weight was obtained. The subtraction of the dry weight from the wet weight yielded the water content of each food item, which was then expressed as a percentage. The organic matter of each food was taken as the dry weight of the food after the removal of water.

Calculations:

Weight of food	=	a (g) (W_1)
Weight of dried food	=	b (g) (W_2)
Water content from food	=	(a – b) (g)
	=	c (g)
Percentage water loss from food	=	(c/a x 100)%
Weight of re-dried food	=	d (g) (W_3)
Organic matter content	=	(b – d) (g)
	=	e (g)
Percentage organic matter	=	(e/b x 100)%

Effect of Soil Moisture Content on Incubation and Hatching of Eggs

30 eggs from 3 clutches were collected from the soil 1 day after they were laid. The eggs were divided into 3 groups; with 10 eggs in each group. 3 excavations of about 25 mm in length were made in the soil and each excavation was filled with coarse sandy soil, dry humus soil and moist humus soil, respectively. Group I eggs were incubated in the loose coarse sandy soil whereas Groups II and III eggs were incubated in the dry and moist humus soils, respectively. The holes were covered throughout the experiment for 1 month.

Collection of Haemolymph, Kidney and Urine Samples

6 large snails were used for easy collection of the desired samples. To collect haemolymph from the active snails, a small window was cut in the shell with a handsaw close to the pericardial area of the mantle. A hypodermic needle was used to draw the blood directly from the heart or the efferent branchial vein. The snails were then dissected to remove the kidneys, which were cleaned and ground in mortar with 10 mL of de-ionised water. It was left to settle before the supernatant was separated and tested for nitrogenous waste products. For the urine collection, a hole was made in the shell approximately 1 cm from the shell aperture. This revealed the surface of the mantle and the ureter. Urine was collected carefully and gradually with the hypodermic needle from the ureter.

Determination of Nitrogenous Waste Products in the Samples

Ammonia, urea and uric acid were qualitatively analysed in the haemolymph, kidney and urine samples. To detect ammonia, 2 drops of Nessler's reagent were added to each sample (2 mL). Ammonia reacts with Nessler's reagent to produce a brownish compound. Urea was determined by the action of urease on the samples. A small amount of powdered urease (2–3 g) was added to 2 mL of each sample, which degrades urea into ammonia and carbon dioxide. Benedict's uric acid reagent and anhydrous sodium carbonate (Na_2CO_3) powder were added to samples (2 mL) to test for uric acid. A blue colouration indicated the presence of uric acid (Odiete, 1999).

Statistical Analysis

The length–weight relationships of the snails were analysed and the existence of a positive or negative correlation was determined. Correlation analysis was determined between the weight and % water content and also between the weight and % water loss of the snails. The mean values of the % water content and % water loss were computed.

Results

Body Water Content

Tables 1 and 2 show the results of the body water content determination in active and inactive *A. marginata*, respectively. The % water content of the

active snails at 48.37%–51.74% (mean: 50.30%) was higher than that of the inactive snails at 30.31%–39.74% (mean: 35.03%). Figure 1 shows their body water contents and percentages.

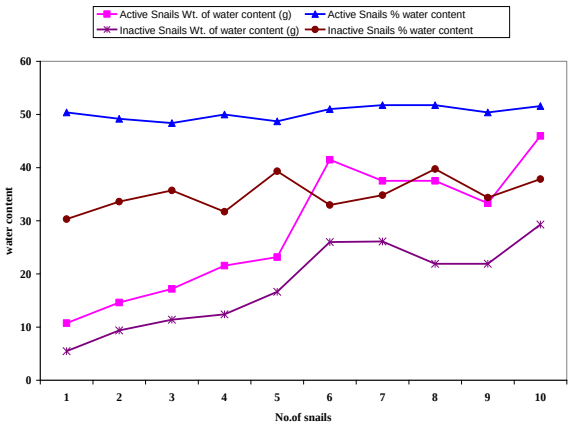


Figure 1: Body water contents and percentages of active and inactive snails

Loss of Water

The rates of water loss in inactive and active snails are shown in Tables 3 and 4, respectively. The results show that the water loss in inactive snails was 3.05%–15.35% (mean: 8.68%), which was lower than that of the active snails between 6.32% and 27.02% (mean: 12.84%).

Water Content of Food

Figure 2 shows that of the 7 food items fed to *A. marginata*, tomatoes had the highest water content while pawpaw leaves had the lowest. The highest and lowest organic matters were obtained from oranges and corn shafts, respectively.

Effects of Soil Moisture on Incubation and Hatching

As shown in Table 5, the eggs that were incubated in moist humus soil in a shaded area hatched after 21 days. The other two groups placed in loose coarse sandy soil and dry humus soil did not hatch.

Nitrogenous Waste in Active and Inactive Snails

As shown in Table 6, there was no ammonia in the haemolymph of *A. marginata*, however, there were traces of urea and a large quantity of uric acid was observed. Analysis of the kidney content revealed only the presence of urea. Qualitative analysis of the urine sample showed that only uric acid was present.

Table 1: Percentage Body Water Content in Active Snails

Length (mm)	Breadth (mm)	Weight of snail (g)	Weight of deshelled snail (g)	Weight of shell (g)	Weight of dried body (g)	Weight of water content (g)	Percentage water content (%)
58	37	31.43	21.30	10.13	10.57	10.73	50.37
66	40	46.00	29.69	16.31	15.09	14.60	49.17
70	40	54.00	35.53	18.47	18.35	17.18	48.37
78	45	67.90	43.11	24.79	21.56	21.55	49.98
81	40	75.04	47.56	27.48	23.40	23.16	48.70
85	50	111.50	81.33	30.17	39.85	41.48	51.00
88	53	106.36	72.61	33.31	34.99	37.52	51.74
90	54	105.82	72.51	33.31	34.99	37.52	51.74
100	66	101.50	66.09	35.41	32.80	33.29	50.37
120	75	141.50	89.37	52.37	43.17	45.96	51.57

Table 2: Percentage Body Water Content in Inactive Snails

Length (mm)	Breadth (mm)	Weight of snail (g)	Weight of deshelled snail (g)	Weight of shell (g)	Weight of dried body (g)	Weight of water content (g)	Percentage water content (%)
58	37	29.31	18.03	11.28	12.57	5.46	30.31
66	40	44.39	27.88	16.51	18.52	9.36	33.59
70	40	51.29	30.90	19.39	20.51	11.39	35.70
78	45	65.90	40.80	25.10	27.87	12.39	31.69
81	40	71.81	42.31	29.50	25.68	16.63	39.31
85	50	111.35	78.85	32.50	52.86	25.99	32.97
88	53	100.41	65.69	34.75	42.80	26.10	34.81
90	54	100.39	65.66	34.70	39.59	21.91	39.74
100	66	99.71	63.80	35.91	41.89	21.91	34.35
120	75	131.50	77.13	54.37	47.85	29.28	37.84

Table 3: Rate of Water Loss in Inactive Snails

Initial weight of snail (g)	Final weight of snail (g)	Weight of water loss (g)	Percentage water loss (%)
21.30	18.03	3.27	15.35
29.69	27.88	1.87	6.00
35.53	31.90	3.63	10.21
43.11	40.80	2.31	5.35
47.56	42.31	5.25	11.0
81.33	78.85	2.48	3.05
72.61	65.66	6.95	9.57
72.57	65.69	6.82	9.40
66.09	68.80	2.29	3.46
89.13	77.13	12.0	13.4

Table 4: Rate of Water Loss in Active Snails

Initial weight of snail (g)	Final weight of snail (g)	Weight of water loss (g)	Percentage water loss (%)
24.61	19.59	5.02	20.03
29.05	21.20	7.85	27.02
34.29	29.16	5.13	14.96
41.62	36.70	4.95	11.89
56.38	50.16	6.22	11.03
63.42	55.28	8.14	12.83
68.80	63.70	5.10	7.41
70.16	65.12	5.04	7.18
79.23	74.22	5.01	6.32
96.20	86.82	9.40	9.77

Correlation Analysis

A positive correlation existed between the length and weight relationships of the active (R^2 = 0.708) and inactive (R^2 = 0.698) snails (see Figure 3) but in Figure 4, a negative correlation resulted between the weight and % water content of active (R^2 = –220.06) and inactive (R^2 = –14.44) snails. Similarly, a negative correlation was observed between the weight and % water loss of the active (R^2 = –1.632) and inactive (R^2 = –0.984) snails (see Figure 5).

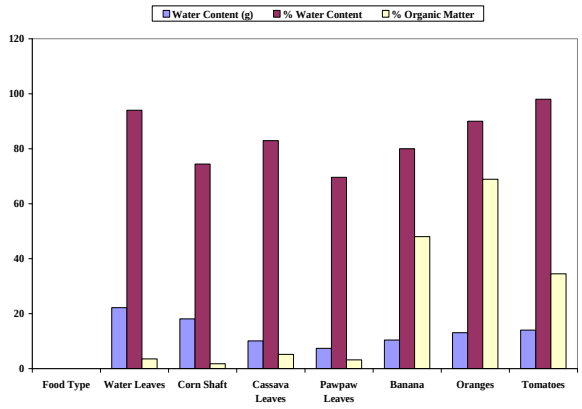


Figure 2: % water content, dry weight and % organic matter of the food fed to *A. marginata*

Discusssion

A positive correlation was observed on the length–weight relationship of the snails, which indicated the morphological wellbeing of the snails. As the length of the snail increased, the weight increased. However the body water content and the rate of water loss were negatively correlated. This indicated that these aspects of the snails did not increase or decrease uniformly with increasing or decreasing weights, respectively. A smaller snail could, therefore, have a greater % of water content or loss than a larger snail.

During this study, a large fluctuation in the body weights of active and inactive *A. marginata* snails was observed. This can be attributed to water loss. It may be an indication that *A. marginata* has a strong behavioural reaction to low humidity as reported in *Cepaea nemoralis* where inactivity and the formation of a thin epiphragm tend to occur with low weight and humidity. It failed to come out of its aestivation phase until humidity increased (Cameron, 1970).

Table 5: Effects of Soil Moisture on Incubation and Hatching in *A. marginata*

Type of Soil	Days of Hatching	Total number of eggs hatched
Moist humus	21–30	9
Loose coarse sandy	–	0
Dry humus	–	0

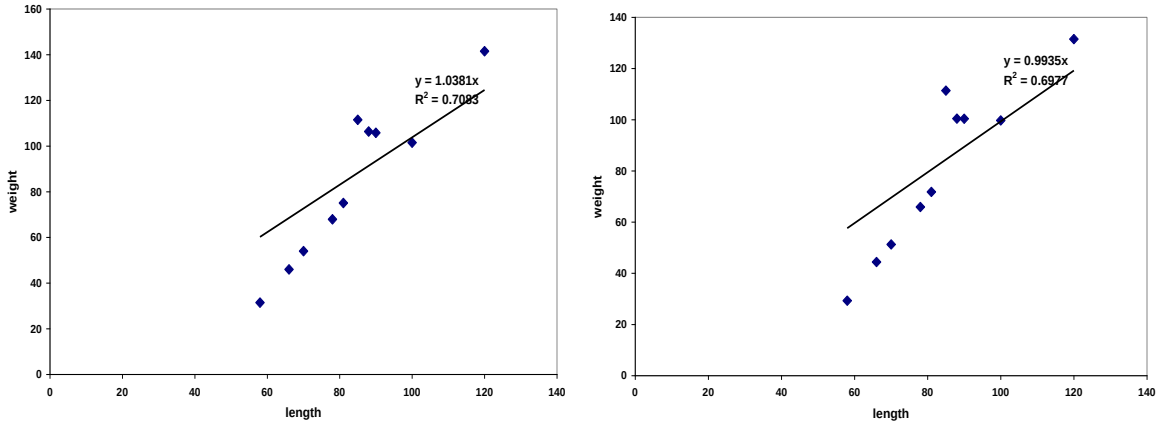
The problems facing terrestrial snails include: the evaporation of water through the body and shell and the loss of water in urine and faecal matter. The crucial elements for survival during aestivation for land snails are water retention and sufficient energy reserves (Giokas *et al.*, 2005). Snails obtain water from succulent fruits and vegetables and by diffusion through the integument of their soles or bodies (Oyenusi, 1992; Luchtel and Deyrup-Olsen, 2001).

Table 6: Ammonia, urea and uric acid content in kidney, urine and haemolymph of *A. marginata*

	Ammonia	Urea	Uric acid
Kidney	–	++	–
Urine	–	–	+++
Haemolymph	–	+	+++

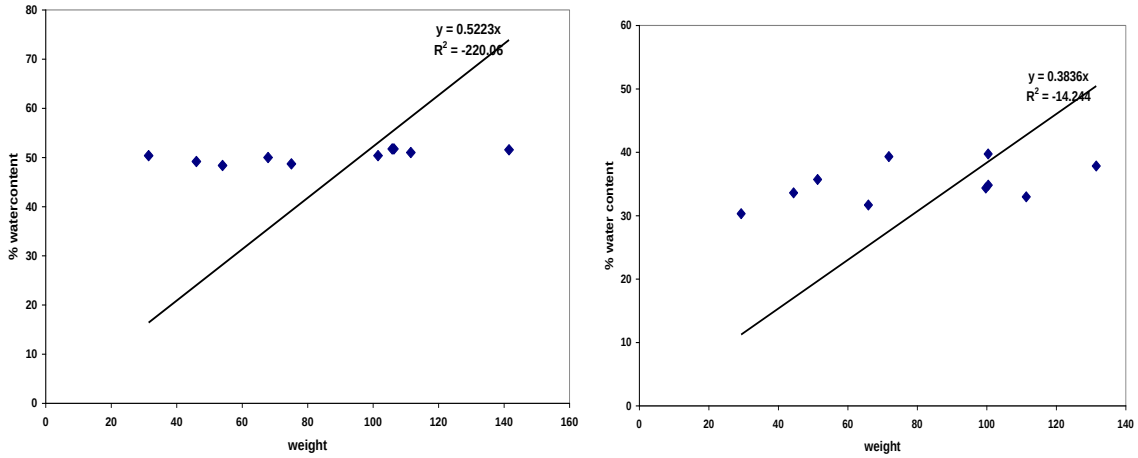
– not present, + trace present, ++ present, +++ highly present

The water contents of the plant foods provided to the snails in this study varied between 70–90%, with the highest been from tomatoes. Morphologically, shell and aperture modifications and epiphragm secretions are of great importance during aestivation in land snails and are related to water losses, which are reduced by sealing the shell's aperture directly to the



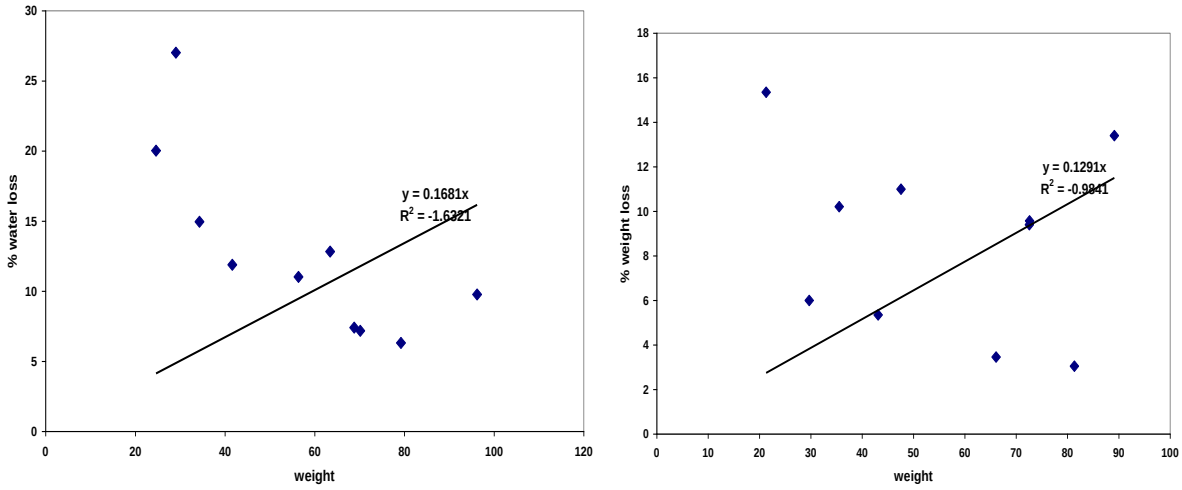
A **B**

Figure 3: Length-weight relationship of active (A) and inactive (B) snails



A **B**

Figure 4: Weight and percentage water content relationship of active (A) and inactive (B) snails



A **B**

Figure 5: Weight and percentage water loss relationship of active (A) and inactive (B) snails

substratum or the shell of another animal. If the evaporation of water continues, continued activity will deprive the animal of water and this could result in temporary or permanent aestivation depending on the availability of water in its environment or the snails could move in search of moist spots. Snails usually seek sheltered locations during aestivation in order to conserve body water, minimise exposure and avoid predation (Giokas *et al.*, 2005).

The body water content of the active snails was higher than that of the inactive snails. The former were able to move to moist areas of the terrarium and feed on high-water content foods. Also, water can be obtained from metabolised food (oxidative water), which helps to cover losses from evaporation (Hoar, 1975). Ejidike (2002) reported that on/off aestivation was observed in *A. marginata*; the yellowish mucus that covered the surface of some of the snails broke a few minutes after wetting the enclosure with well water. The on and off aestivation occurred during the dry season, which corresponded with the period snails normally aestivate in the wild or under poor captivity.

Snails also take-up water through the skin in high humidity. The active snails took-up water through their skins; with increasing body water content but the inactive snails remained stationary without eating and lost water from their bodies through evaporation despite their withdrawal into their shells. According to Rees (1975) and Oyenusi (1992), the activities of snails are inhibited by water loss if the water lost is not replaced immediately. In the land snail, *Albinaria caeridea*, mortality was not high during aestivation (Giokas *et al.*, 2005). This indicated that there were morphological, behavioural and physiological adaptations to dry conditions in the snail population and that snails burn their food stores to improve their water balance during aestivation (Yom-Tov, 1971).

The eggs placed in the dry humus and loose coarse sandy soils failed to hatch probably due to insufficient water whereas the eggs placed in the moist humus soil hatched in 21 days. These results confirmed the observation of Egonmwan (1988) that the relative humidity of soils affected the viability and hatching of eggs in *A. marginata*. Ghose (1960) reported that the eggs of *Macrochalmys indica* did not develop but became desiccated when removed from their damp surroundings. Ebenso (2004) also observed that the eggs of *Limicolaria aurora*, buried in a 15% water incubation chamber hatched while those in lower percentages of water did not and, therefore, concluded that higher hatchability was assured with increased water uptake after 15 days of incubation.

The results herein indicate that only uric acid was present in the urine of *A. marginata*. The nature of the nitrogenous compounds excreted by an animal correlates with the availability of water in its natural environment (Odiete, 1999). Ammonia is excreted by animals with adequate water supply e.g., fresh water animals whereas urea is excreted by animals with reduced water supply e.g., man. Animals with very little water supply produce uric acid, which can be excreted in crystalline form in some animals to conserve water.

The accumulation of nitrogenous wastes in tissues during aestivation is due to the lack of a medium for excretion. An increase in the total blood amino acids during aestivation may suggest a higher degradation of tissue proteins or blood globulins and the disintegration of smaller red cells (Brahmanandam and Krishnamoorthy, 1973). According to Newmann *et al.* (1975), ammonia decreased significantly while urea and uric acid increased in *Bakerilymnaea cockerelli* during aestivation. It was also observed that uric acid predominated after 97 days of aestivation. *A. marginata* may experience more water loss through evaporation between October and March (dry season in Nigeria); mainly through their mucus, which is produced during locomotion. Therefore, the urine will contain more uric acid during the dry season in order to conserve water.

Conclusion

This study demonstrates some of the physiological and behavioural adaptations of *A. marginata* to water availability in the environment. Animal life requires a steady supply of water to fulfil its vital functions: transportation, lubrication and temperature regulation, etc. Physiologically, in the dry season, the snails respond by aestivating and secreting a membrane of dry mucus (epiphragm). Aestivation is a survival strategy used by these snails to endure arid environmental conditions; to conserve body water, deal with water restrictions and, sometimes, used to handle nitrogenous end-products. This is an important phenomenon in the survival of these animals as hot temperatures and arid conditions may last for months and water loss can lead to death.

References

Bequaert, J. C. (1950). Studies in Achatinae, a group of African Land Snail. *Bulletin of Museum of Comparative Zoology at Harvard College,* **105**: 1–216.

Brahmanandam, V., Krishnamoorthy, R. V. (1973). Hemochemistry and hematology of the aestivating pond snail *Pila globosa*

(Gastropoda: Ampullaridae). *The Veliger*, **16**: 176–180.

Cameron, R. A. D. (1970). The survival, weight loss and behaviour of three species of land snails in conditions of low humidity, *Journal of Zoology*, **160**: 143–147.

Dainton, B. H. (1954). The acidity and induction of activity by changing temperatures in slugs, *Journal of Experimental: Biology*, **31:** 165–197.

Ebenso, I. E. (2002). Consumption and sale of domesticated snails *Archachatina marginata* in rural southern Nigeria, *Tropical Science (England)*, **42**: 185–187.

Ebenso, I. E. (2004). Molluscicidal effect of neem *Azadirachta indica* extracts on edible tropical land snails, *Pest Management Science (England)*, **60**: 178–182.

Egonmwan, R. I. (1988). Reproductive biology and growth of the land snails: *Archachatina marginata ovum* and *Limicolaria flammea*, Ph.D. Thesis, University of Oxford, London, 186pp.

Etchu, K. A., Mafeni, J. M., Ngenwi, A. A. (2008). Comparative performance of three edible snail species using intensive cage housing system in Cameroon, *Bull. Anim. Health Prod. Afr*. **56**: 345–352.

Ghose, K. C. (1960). Observation on the mating and oviposition of two land pulmonates *Achaatinafalica Bowdich* and *Macrochiamys indica Godwin Austen*, *Journal of the Bombay Natural History Society*, **56**: 63–127.

Giokas, S., Pafilis, P., Valakos, E. (2005). Ecological and physiological adaptations of the land snail *Albinaria caerulea* (Pulmonata: Clausiliidae). *Journal of Molluscan Studies*, **71**: 15–23.

Hoar, W. S. (1975). *General and Comparative Physiology*, New York Academic Press, 1039pp.

Hodasi, J. K. (1979). Preliminary studies on the feeding and burrowing habits of *Achatina achatina*, *Ghana Journal of Science* **15**: 193–199

Luchtel, D. L., Deyrup-Olsen, I. (2001). Body Wall: Forms and Function. In: *The Biology of Terrestrial Molluscs*, G. M. Barker, ed., CAB International, Oxford, p. 147–178.

Lyth, M. (1983). Water content of slugs (Gastropoda: Pulmonata) in natural habitats, and the influence of culture conditions on water content stability in *Anon ater* (Linné), *Journal of Molluscan Studies*, **49**: 179–184.

Machin, J. (1967). Structural adaptation for reducing water-loss in three species of terrestrial snails, *Journal of Zoology*, **151**: 55–65.

Machin, J. (1972). Water exchange in the mantle of a terrestrial snail during periods of reduced evaporative loss, *Journal of Experimental Biology*, **57**: 103–111.

Machin, J. (1975). Water relationships. In: Pulmonates, Vol. 1., Fretter, V., Peake, J., eds., Academic Press, London, p. 105–163.

Mead, A. R. (1952). *The Giant West African Snails*: *A Problem in Economic Malacology*, Chicago, IIIinois University of Chicago press, l28pp.

Ngenwi, A. A., Mafeni, J. M., Etchu, K. A., Oben, F. T. (2010). Characteristics of snail farmers and constraints to increased production in West and Central Africa. *Afr. J. Environ. Sci. Tech*., **4**(5): 274–278.

Newmann, K. C., Thomas, R. E. (1975). Ammonia, urea and uric acid levels in active and aestivating snails *Bakerilymnaea cockerelli*, *Comparative Biochemistry and Physiology* **50**(1): 109–112

Odiete, W. O. (1999). *Environmental Physiology of Animals and Pollution.* Diversified Resources Ltd., Lagos, 261pp.

Okon, B., Ibom, L. A. (2011). Phenotypic correlations and body weights prediction using morphometric traits of snails in Calabar, Nigeria, *Ethiopian Journal of Environmental Studies and Management*, **4**(3): 7–11.

Oyenusi, O. (1992). *Effect of Environmental Factors on Snails*, Graduate Work Project, 4lpp.

Pilsbry, H. A. (1904) Family *Aschatinidae*. Manual of Conchology, Edinburgh, 1986, 488pp.

Plummer, J. M. (1975). Observation on the reproduction, growth and longevity of a laboratory colony of *Archachatina (Calachatina) marginata ovum* (Swainson). *Proc. Mal. Soc. London* **41**: 395–413

Prior, D. J. (1985). Water regulatory behaviour in terrestrial gastropods, *Biological Reviews*, **60**: 403–424.

Rees, W. J. (1975). The Giant African Snails. *Proceeding of the Zoological Society of London*, **120**: 577–598.

Segun, A. O. (1975). The Giant Land Snails 2 *Archachatina (Calachatina) marginata (Swainson)*, Ethiope Publishing House, Benin City, Nigeria. 25pp.

Solem, A. (1985). Origin and diversification of pulmonate land snails. In: *The Mollusca, Vol. 10, Evolution*., Trueman, E. R., Clarke, M. R., eds., Academic Press, New York, p. 269–293.

South, A. (1992). *Terrestrial Slugs*. Chapman and Hall, London, 428pp.

Yom-Tov, Y. (1971). Annual fluctuation in the water content of desert snails, *Malacological Review*, **4:** 121–126.