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Factors affecting the hatchability of snail eggs (*Archachatina marginata*) in the Western Highlands of Cameroon

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A study to investigate the factors affecting hatchability of *Archachatina marginata* eggs, had 600 eggs collected from snail pens and incubated in treated substrates; moist soil/sawdust mixture (MSD), moist sawdust (MD), moist soil (MSb), dry sawdust (DD) and dry soil (DS) as well as in rearing pens (1 m × 1 m × 0.8 m) containing moist soil (MSp). Results showed that snail egg weights at laying ranged from 250 to 1100 mg (mean of 670±247 mg). Egg lengths and widths ranged from 13.0 to 16.00 mm (mean of 14.88±0.94 mm) and 9.00 - 11.00 mm (mean of 10.80±1.02 mm) respectively. Snail egg hatchability in MSD and MD of 64.0±4.8 and 61.5±3.9%, respectively were better ($p<0.05$) than in MSp (50.5±4.4%) and MSb (45.5±3.5%) among media in which eggs hatched. Dry Incubation media (DS and DD) had 0% hatchability. Best hatchability was between 50 and 70% humidity. Embryonic mortality showed a reverse trend with MSD (36.0±4.8%) and MD (38.5±3.9%) recording significantly lower ($p<0.05$) values than MSp (49.5±4.4%) and MSb (54.5±3.5%). Incubation temperature ranged from 17 to 20°C and Incubation duration ranging from 22 to 35 days. Correlated analysis revealed a weak significantly ($p<0.05$) positive relationship ($r=0.097$) between egg weight and hatchability and a negative relationship ($r=-0.234$) between egg weight and embryonic mortality. Therefore, snail eggs could be incubated in moist sawdust and moist soil/sawdust mixture with humidity from 50 to 80%, at temperatures ranging from 17 to 20°C and that snail eggs with higher weights are most recommended for incubation.

Key words: Temperature, humidity, hatchability, embryonic mortality, *Archachatina marginata* eggs.

INTRODUCTION

Non-conventional and micro livestock have potentials as good sources of animal protein in the human diet (Merkramer, 1992). Snails have been known to be an important and valuable source of animal protein in many countries of the world (Akinnusi, 1998; Jennifer, 2009)

and many parts of West and Central Africa (Blay et al., 2004), due to good quality protein of up to 60.56% (Essien et al., 2016) and rich in potassium, phosphorus, essential amino acids, vitamins C and B complex (Baba and Adeleke, 2006, Okpeze et al., 2007). In some

countries like Ghana, Nigeria, Cote d'Ivoire, and Cameroon, snail meat is particularly popular and important due to its high iron content, thus important in treating anaemia (Fagbuaro et al., 2006). Also, it is rich in essential amino acids such as Lysine, Leucine, Isoleucine, and Phenylalanine (Imevbore, 1990; Stievenart, 1996; Ebenebe, 2000). Moreover, it is recommended for the treatment of ulcers, asthma, high blood pressure, and other related ailments due to its relatively low cholesterol levels (Awesu, 1980; Akinnusi, 2002).

Orthocalcium phosphate extracted from snails can cure kidney disease, tuberculosis, anaemia, diabetes, and asthma (Mead, 1961; Ademolu et al., 2006). The Giant African Land Snail (*Archachatina marginata*), is one of the micro livestock that could serve as a readily available and cheap source of animal proteins for the human populations where snails thrive widely (Ngoupayou, 1992). Nevertheless, snail farming has not kept pace with demand (Etchu et al., 2008) with different environmental and technical factors implicated like temperature, humidity, and incubation media. Duration of egg incubation of giant African land snail has been shown to range between 30 and 45 days (Segun, 1975; Ajayi et al., 1978).

The variation in the incubation period is attributed to the fact that eggs of snails are laid with embryos at different stages of development. Plummer (1975) cited by Awesu (1980), however, opines that egg incubation duration in these species might be related to soil temperatures. It was observed that the incubation period was between 35 and 41 days at a temperature of 17 to 19°C. Moreover, at a temperature of 22.5 to 23°C, the incubation period was between 29 and 35 days (Awesu, 1980). However, there is a paucity of information on these in Cameroon mainly in the Western Highlands.

This study, therefore, aimed to evaluate how climatic factors, egg weight, incubation media influence the hatching efficiency of giant African land snails.

MATERIALS AND METHODS

Study site, housing, and feeding of experimental animals

This study was carried out at the snailery unit of Helvy Farms Upskill Research at station Bamenda of the Western Highlands of Cameroon. The methodology used in snail management, egg collection, and incubation was adapted from Agbelusi and Adeparusi (1999).

Two hundred (200) mature *A. marginata* from the snail unit were housed in built-up pens and breeding (natural mating) patterns were as prescribed by Ibom et al. (2012).

The study was conducted under a shade made of aluminium

roofing sheets in an open space with adequate cross ventilation. Twelve (12) snail pens (1 m of length by 0.5 m of width by 0.5 m of depth) were built with blocks and rough plastered with cement/sand mixture. The doors were hinged with movable wire-mesh framed to avoid predation and/or escape of the snails. The floor of each pen was covered with humus soil to serve as bedding material and food. Dry banana leaves (*Musa paradisiaca*) were placed in each pen to serve for beddings, food and to make this habitat as natural as possible. The snails were fed fresh vegetable leaves (cabbages) as well as fruits (bananas, watermelons). This house design is typical of snail rearing facilities used by most small-scale snail farmers in the area. Feeding and watering trays were provided in each snail pen and the snails were fed a basal diet. The composition of the basal diet used is presented in Table 1.

Trial management

This study lasted for 16 weeks from July to October 2020. The already mature snails started laying eggs after a week of acclimatization and adaptation in the rearing pens. Eggs incubated in the trials were collected from clutches in pens daily (early morning). Characteristics of eggs collected were registered. Length and width of eggs were taken with the aid of a 150 mm length stainless steel "eisco" Verniercaliper with an accuracy of ± 0.2 mm before incubation. The weight of the eggs was taken with a sensitive precision mini LCD digital electronic balance with an accuracy of ± 0.01 g.

Experiment 1

Wooden incubation boxes (50 cm × 30 cm × 30 cm) were constructed for the study. Each box was filled up with a different incubation substrate, dry sawdust (DD) and moist sawdust (MD), dry soil (DS) and moist soil (MSb) as well as a 1:1 soil/sawdust mixture (MSD), and one of the rearing pens with moist soil (MSp) was also used for incubation. The substrates were heat-treated before introducing snail eggs for incubation, to destroy any potential microbes in the soil. Eggs were embedded in each medium to a depth of 3 to 5 cm and lightly covered with the incubation substrate. 100 eggs, that is, 10 batches of 10 eggs each secured in mesh nets were randomly distributed in five (5) boxes each with a different substrate and one of the snail pens making a total of six (6) incubation media. A total of six (600) eggs were incubated for experiment one. DD and DS incubation media were left without water for the duration of the experiment. MSb, MD, and MSD were watered regularly and maintained at a humidity level of 50 to 80%. The box for the moist soil inbox (MSb) was lined with an impermeable plastic film before filling with treated soil. This box was watered twice every week and the humidity level was maintained at 80 to 90%. Temperature readings were taken using a liquid-in-glass thermometer (0.1°C sensitivity) and humidity of the incubation substrates was determined with an analog three-way (soil moisture, pH, and light) soil tester. Measurement of humidity level of each incubation medium continued throughout the incubation period. The temperature of each medium was recorded at 09:00 am and 04:00 pm daily with the help of a liquid-in-glass thermometer throughout the incubation period. Eggs in all boxes were checked every 3 to 5 days for any hatchlings. After 45 days

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Table 1. Feed formula of basal diet fed to snails

| Nutrient | Proportion (%) |
|----------------------------------|----------------|
| Maize | 49.08 |
| Groundnut cake | 24.3 |
| Wheat bran | 14.4 |
| Fish meal | 4.22 |
| Concentrate | 6.75 |
| Premix | 1.25 |
| Total | 100 |
| Crude Protein (%DM) | 16.0 |
| Metabolizable energy (kcal/kgDM) | 2439 |

Sources: Authors

unhatched eggs were broken for examination of the content.

The snails started laying eggs after a week of acclimatization and adaptation in the rearing pens. Eggs were collected from clutches in pens daily (early morning). Characteristics of eggs collected were registered. Length and width of eggs were taken with the aid of a verniercaliper (150±0.2 mm) before incubation. Eggs were weighed on a digital electronic balance (accuracy of g±0.01 g). Fresh eggs with recorded characteristics were incubated in experiments 1 and 2.

Experiment 1: Hatchability and embryonic mortality of *A. marginata* eggs incubated in different incubation media with varied environmental characteristics

Treatment 1: 10 batches of 10 eggs each were tied in a net and incubated in dry soil (DS).

Treatment 2: 10 batches of 10 eggs each were tied in a net and incubated in dry sawdust (DD).

Treatment 3: 10 batches of 10 eggs each were tied in a net and incubated in Moist Sawdust (MD).

Treatment 4: 10 batches of 10 eggs each were tied in a net and incubated in moist soil/sawdust (MSD) mixture.

Treatment 5: 10 batches of 10 eggs each were tied in a net and incubated in moist soil in rearing pens (MSP).

Treatment 6: 10 batches of 10 eggs each were tied in a net and incubated in moist soil in box (MSb).

The dry incubation media (DD and DS) had humidity values less than 20%, the range of humidity in the moist incubation media (MD, MSD and MSP) was from 50 to 80% and the humidity range of the moist soil in box (MSb) was from 80 to 90%. Table 2 shows the experimental layout for egg incubation.

Experiment 2: Effect of egg weight on hatchability and embryonic mortality

The second experiment consisted of verifying the relationship between egg weight and hatchability, egg weight, and embryonic mortality. Snail eggs were collected and their lengths and widths were taken using a Vernier Calliper.

The eggs were weighed using an electronic balance and categorized into three (3) groups. Each group was made up of 100 eggs with a total of 300 eggs incubated for the trial. Eggs for this experiment were incubated in moist soil in rearing pens (MSP) as

the MSP had proven in the previous experiment to be a better incubation medium.

Group 1: Average egg weight 250 - 500 mg

Group 2: Average egg weight 500 - 750 mg

Group 3: Average egg weight < 750 mg

Reproductive parameters such as fertility, hatchability, and embryonic mortality were evaluated in the two experiments.

Reproductive parameters measured

Data collected included the egg characteristics (egg weight, egg length, and width) before incubation. The eggs were observed and checked for hatchlings and turned every 3 to 5 days from day 15 in incubation. The first hatchlings emerged after the 22nd day of incubation. The hatchlings were removed and transferred to a growing pen to prevent them from consuming the other eggs still in incubation. Unhatched eggs were allowed in incubation for up to 45 days, after which the eggs were gently cracked and their contents examined.

The number of eggs incubated, the number of hatchlings, the number of unhatched eggs with and without embryos, and the number of empty shells were counted and the following parameters were evaluated:

Fertility: Percentage of fertile eggs (No. of eggs that hatched + number of dead-in-shell embryos) to the total number of eggs incubated:

$$Fertility (\%) = \frac{No\ of\ fertile\ eggs\ (w)}{Total\ No\ of\ eggs\ incubated\ (x)} \times \frac{100}{1}$$

where w=No. of eggs that hatched + number of dead-in-shell.

Hatchability: Percentage number of hatchlings to the number of fertile eggs.

$$Hatchability (\%) = \frac{No\ of\ eggs\ that\ hatched\ (z)}{No\ of\ fertile\ eggs\ (w)} \times \frac{100}{1}$$

Embryo mortality: Percentage number of eggs with dead-in-shell embryos to the number of fertile eggs.

$$Embryo\ mortality (\%) = \frac{No\ of\ dead\ -\ in\ -\ shell\ eggs\ (y)}{No\ of\ fertile\ eggs\ (w)} \times \frac{100}{1}$$

Table 2. Egg characteristics of *A. marginata*.

| Egg characteristics | Range | Mean \pm STDEV* |
|---|--------------|-------------------------------------|
| Egg Lengths (mm) | 13.0 - 16.0 | 14.88 \pm 0.94 |
| Egg widths (mm) | 9.0 - 11.0 | 10.80 \pm 1.02 |
| Egg weights (mg) | 250 - 1100 | 670 \pm 247 |
| Fertility rate of <i>A. marginata</i> eggs incubated in the study | 91.25% | - |
| Egg Colour | Lemon Yellow | - |

*STDEV: Standard Deviation.
Sources: Authors

Data analyses

Data collected were subjected to descriptive statistics (means, percentages) and analysis following statistical procedures described by Steel and Torrie (1980). Data on hatchability and embryo mortality resulting from the various treatments were equally subjected to one-way ANOVA to test significant differences in the hatchability and embryo mortality means. Means that showed significant differences were separated using the Duncan Multiple Range Test. Correlated analyses were equally done to check the effect of egg weights on hatchability and embryo mortality. A linear regression analysis was done to determine the degree to which the predictor variable (egg weight) influenced each of the dependent variables, hatchability, and embryonic mortality. Statistical Analyses were done using MS Excel 2010 and SPSS 18.

RESULTS AND DISCUSSION

Egg characteristics of *A. marginata*

Egg characteristics recorded in this study included egg length, egg width and egg weight, and egg colour. Fertility was determined from the number of hatchlings and dead-in-shell embryos to the total number of eggs incubated.

The mean egg weight of *A. marginata* at lay was 670 mg and the corresponding mean egg length and width were 14.88 and 10.80 mm, respectively. The overall fertility rate of the eggs incubated in this study was 91.25%. The eggs had a lemon-yellow colour at lay which however became whitish in incubation (Table 2). The number of unfertilized eggs constituted less than 9% of the total number of eggs incubated (Total number of eggs incubated – (No. of eggs that hatched + number of dead-in-shell)).

Effect of incubation medium on hatchability, embryo mortality, and incubation duration of *A. marginata* eggs

Effect of incubation medium on hatchability of *A. marginata* eggs

Egg hatch rate varied from one incubation medium to another with the highest obtained in the MSD incubation

medium.

Moist soil/sawdust mixture (MSD) showed the highest hatchability (64%) but not significantly different ($p > 0.05$) from moist sawdust (MD; 61.5%). Hatchability of both MSD and MD was significantly higher ($p < 0.05$) from that of MSp and MSb. However, hatching rate values for MSp and MSb were not significantly different ($p > 0.05$). Dry sawdust and dry soil had 0.0% hatchability (Table 3).

Effect of incubation medium on embryo mortality of *A. marginata* eggs

Dry sawdust (DD) and dry soil (DS) had 100% embryo mortality. In incubation media where hatchability was above 0%, embryonic mortality was the highest in moist soil in the box (MSb; 54.5%) followed by moist soil in pens (MSp; 49.6%). Moist soil/sawdust mixture (MSD) showed embryo mortality of 36% (lowest) not significantly different ($p > 0.05$) from moist sawdust (MD; 38.5%). Likewise, embryonic mortalities in MSp and MSb were not significantly different ($p > 0.05$). However, embryonic mortality in MSD and MD was significantly different ($p < 0.05$) from those of MSb and MSp (Table 4).

Incubation medium and duration of incubation of *A. marginata* eggs

The incubation period ranged from 22 to 35 days in all incubation media, with rearing pens (MSp) recording the shortest average incubation duration of 26 days and moist soil in box (MSb) recording the highest average incubation duration of 29.5 days. The eggs incubated in dry soil and dry Sawdust were incubated for up to 45 days but no hatchling was obtained from them.

Effect of environmental factors on hatchability and embryo mortality of *A. marginata* eggs

Effect of humidity on hatchability and embryo mortality of snail eggs

Moist sawdust (MD), moist soil/sawdust mixture (MDS)

Table 3. Effect of incubation medium on hatchability of snail eggs.

| Incubation medium | Mean \pm SEM* (%) |
|----------------------------------|-----------------------------|
| Moist soil in pens (MSp) | 50.4 \pm 4.4 ^a |
| Moist soil in box (MSb) | 45.5 \pm 3.5 ^a |
| Moist sawdust (MD) | 61.5 \pm 3.9 ^b |
| Moist soil/sawdust mixture (MSD) | 64.0 \pm 4.8 ^b |
| Dry sawdust (DD) | 00.0 \pm 00 |
| Dry soil (DS) | 00.0 \pm 00 |

*Means denoted with different superscript letters are statistically significantly different at $p < 0.05$. SEM: Standard error of the mean.

Sources: Authors

Table 4. Effect of incubation temperature on hatchability, embryonic mortality, and incubation duration of snail eggs.

| Incubation Media | Mean incubation temperature (°C) | Humidity (%) | Hatchability (%) | Embryonic mortality (%) | Incubation duration (days) |
|------------------|----------------------------------|--------------|-----------------------------|-------------------------------|----------------------------|
| DS | 18.9 \pm 1.2* | 0 - 10 | 00.0 \pm 00 ^c | 100.0 \pm 00 ^c | - |
| DD | 18.5 \pm 1.3 | 10 - 20 | 00.0 \pm 00 ^c | 100.0 \pm 00 ^c | - |
| MD | 19.4 \pm 1.5 | 50 - 80 | 61.5 \pm 3.9 ^b | 38.5 \pm 3.9 ^a | 27.5 |
| MSD | 18.3 \pm 1.1 | 50 - 80 | 64.0 \pm 4.8 ^b | 36.0 \pm 4.8 ^a | 28.5 |
| MSp | 18.6 \pm 1.2 | 50 - 80 | 50.4 \pm 4.4 ^a | 49.6 \pm 4.4 ^{a,b} | 26.0 |
| MSb | 19.2 \pm 1.3 | 80 - 90 | 45.5 \pm 3.5 ^a | 54.5 \pm 3.5 ^b | 29.5 |

*An ANOVA for temperature data showed F_{cal} (1.43) to be less than F_{crit} (2.35) hence revealing no significant difference in the temperature means of the various incubation media. *Means in the same column denoted with different superscript letters are statistically significantly different at $p < 0.05$.

Sources: Authors

with humidity of 50 to 80% showed hatchability values of 61.5 and 64%, respectively. Hatchability of 50.4% was obtained for moist soil (MSp) with humidity between 50 and 80%. Moist soil in the box (MSb) with humidity of 80 to 90% showed the lowest hatchability (45.5%) among the media in which eggs hatched. Dry media (DD and DS) with humidity values less than 20% had 0% hatchability and the highest embryonic mortality. Duration of incubation in days averaged at 27.33 for humidity range between 50 and 80% and 29.5 at humidity levels above 80%.

Hatchability and embryonic mortality showed an inverse relation in dry media. When the moisture content of the incubation medium was low, embryonic mortality was at its highest while hatchability was zero. Hatchability and embryonic mortality equate to each other at about 40 and 85% humidity levels.

Hatchability and embryonic mortality attained their peak (64%) and minimum (36.0%) values at about 70% humidity. Hatchability was inversely related to the moisture content of incubation media at humidity values below 50% and directly related to the moisture content of the incubation media up to 80% humidity and started decreasing. An identical reverse trend was observed for

embryonic mortality (Table 4).

Effect of temperature on hatchability, embryo mortality, and incubation length of *A. marginata* eggs

The incubation temperature in all incubation media ranged between 17 and 21°C. The temperature did not vary much throughout incubation (Table 5). There was no significant effect ($p > 0.05$) of temperature variation on hatchability and embryonic mortality as the variation in temperature from one medium to another was not statistically significant.

Out of the 600 eggs incubated, 217 hatched giving an overall hatchability of 36.2% from all incubation media. Of the 383 unhatched eggs, 148 died as embryos, 197 undeveloped and 38 shells were empty. The highest number of undeveloped eggs, as well as empty shells, was recorded in the dry soil and dry sawdust incubation substrates while those incubated in moist soil/sawdust mixture and moist soil in the box had the least. Embryonic mortality was highest in moist soil in boxes, moist sawdust, moist soil/sawdust mixture, and the least in rearing pens. Eggs only hatched in the range of

Table 5. Effect of egg weight on hatchability, embryonic mortality, incubation length, and the number of undeveloped snail eggs.

| Egg weight range (mg) | Hatchability (%) | Embryonic Mortality (%) | Undeveloped eggs (%) | Incubation length |
|-----------------------|-------------------|-------------------------|----------------------|-------------------|
| 250 - 500 | 28.6 ^a | 71.4 ^a | 29.4 ^a | 27.5 |
| 500 - 750 | 33.8 ^a | 66.2 ^a | 25.6 ^a | 28.5 |
| > 750 | 65.4 ^b | 36.6 ^b | 8.2 ^b | 26.0 |

*Means in the same column denoted with different superscript letters are statistically significantly different at $p < 0.05$.

Sources: Authors

Table 6. Correlation of weight, hatchability, and embryonic mortality.

| Correlation | Egg weight | Hatchability (%) | Embryonic mortality (%) |
|-------------------------|------------|------------------|-------------------------|
| Egg weight | - | 0.097 | -0.234* |
| Hatchability (%) | 0.097 | - | -1.000** |
| Embryonic mortality (%) | -0.234* | -1.000** | - |

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Sources: Authors

humidity from 50 to 90% and an incubation temperature range ranged from 17 to 21 °C. The overall hatching percentage from all the incubation media that had hatchlings stood at 54.25%. Fertility was not 100% in the population. Considering the number of eggs that hatched and the number of dead-in-shells fertility rate in the population of snails used for this study was estimated at 60.8%.

Effect of egg weight on hatchability and embryo mortality

Mean hatchability showed significant increase ($p < 0.05$) with increasing egg weight. Mean hatchability of 65% was obtained with the egg weight group >750 mg compared to 28 and 33%, obtained with 250-500 mg and 500-570 mg egg weight, respectively. A reverse trend was observed with embryonic mortality and undeveloped eggs. A significant drop ($p < 0.05$) in embryonic mortality and undeveloped eggs was observed as egg weight increased.

Correlated analysis of weight, hatchability and embryonic mortality

Correlated analysis revealed a significantly ($p < 0.05$) weak positive relationship ($r = 0.097$) between hatchability and egg weight, a very significant ($p < 0.01$) inverse relationship ($r = -0.234$) between egg weight and embryonic mortality, and a perfect inverse relationship ($r = -1$) between hatchability and embryonic mortality (Table 6).

Regression analysis of egg weight on hatchability and embryonic mortality

Regression analysis of egg weight on embryonic mortality revealed that an increase in egg weight will result in a corresponding significant drop in embryonic mortality ($r^2 = 0.55$). Regression equation: Embryonic mortality = $0.779 + (-0.026)(\text{Weight})$. Scatterplots of the linear regression analysis are as shown in Figures 1 and 2.

Regression analysis of weight on hatchability showed that there exists a positive relationship between weight and hatchability of snail eggs with a regression coefficient of $r^2 = 0.09$ indicating the statistical significance of the regression model that was run. This shows that the egg weight of a snail predicts hatchability. The hatchability equation shows that an increase in egg weight leads to a significant positive increase in the hatchability of snail eggs (Figure 1).

From the regression equation, hatchability = $0.521 + 0.005(\text{Weight})$, if the average egg weight increase by 80 units, *ceteris paribus*, hatchability values of 0.92 (92%) could be achieved.

From The regression equation, embryonic mortality = $0.779 - 0.026(\text{Weight})$, if the average egg weight increase by 25 units, *ceteris paribus*, embryonic mortality values could drop to as low as 0.129 (12.9%).

DISCUSSION

Egg characteristics

Egg characteristics of lengths and widths observed in this study are similar to the egg length range of 13.00 to

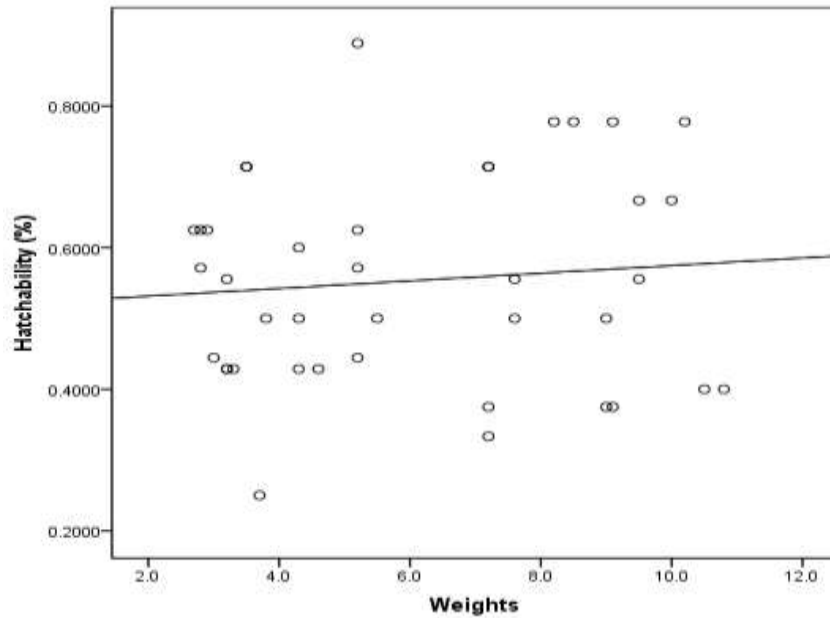


Figure 1. Scattergram for the regression analysis of snail egg weight on hatchability. Regression equation: Hatchability = 0.521 + 0.005(Weight). Sources: Authors

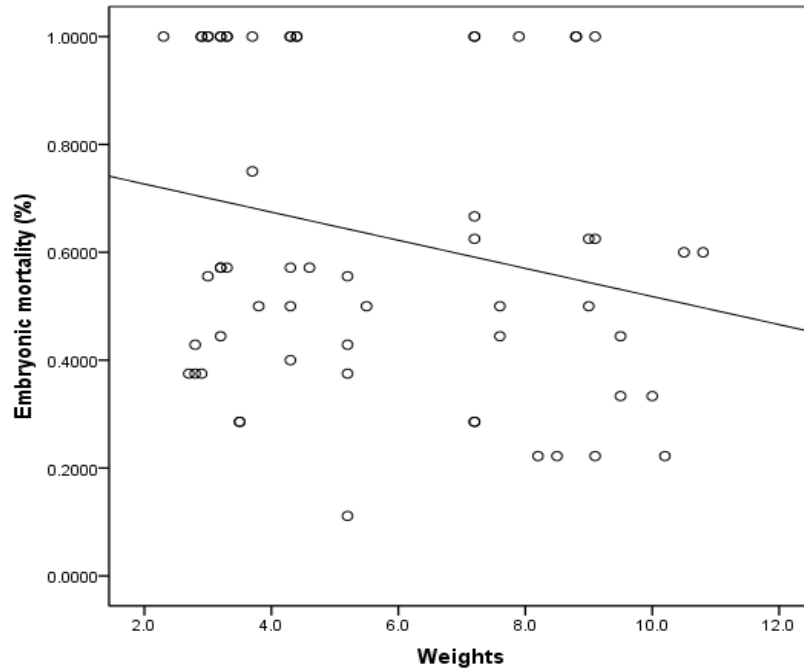


Figure 2. Scattergram for the regression analysis of snail egg weight on hatchability. Regression equation: Embryonic mortality = 0.779 - 0.026(Weight). Sources: Authors

16.00 mm (mean of 14.88 mm) and widths from 9.00 to 11.00 mm (mean of 10.80 mm) reported by Okon et al.

(2013).

The weights recorded in this research, however, fall

below the range recorded by Okon et al. (2013) who showed that snail egg weights at laying ranged from 1.54 to 2.45 g (mean of 2.00 g). Ibom et al. (2008) gave the average egg weights of the black and white-skinned ecotypes of *A. marginata* to be 1.80 and 1.05 g, respectively. Other authors reported that giant African land snail egg weight range from 2.3 to 5.1 g with a range of 2.1 to 3.2 and 1.5 - 2.6 cm in length and width, respectively (Plummer, 1975; Awesu, 1980; Stievenart, 1996; Ejidike, 2009; Ibom et al., 2008). The huge variation in the average egg weight ranges could be linked to the species, ages, and nutrition of the giant African land snail.

Effect of incubation medium on hatchability, embryo mortality, and incubation duration of *A. marginata* eggs

No hatchlings were recorded in dry soil or sawdust. However, the embryos were still at very early stages of development not beyond day 4 of incubation compared to plates developed by Okon et al. (2013). This could be because egg fluid diffused from the eggs into the very dry surrounding substrate. Hatching successes of 61.5, 64, and 45.5% were recorded from eggs in MD, MSD, and MSb, respectively. Eggs incubated in rearing pens showed a hatching success of 50.5%. These hatching rates are quite low compared to those earlier reported by Agbelusi and Adeparusi (1999) and more recently by Chika et al. (2018) and Oyeagu et al. (2018). The low hatching rates could be linked to the lower egg weights of the eggs used in this study. Eggs with higher weights incubated under similar conditions and substrates could result in higher hatching rates. The embryos in dry soil (DS) and dry sawdust (DD) got dehydrated and dried up in the shells in less than one week after incubation. Water probably diffused through the eggshells into the very dry milieu. The range of incubation duration in days was much wider (22 – 35 days for all media, table 10) than that reported by Okon et al. (2013) who stated that snails hatched between 28 and 30 days with a mean incubation period of 29 days. Omole and Kehinde (2005) reported a similar incubation period range of 25 to 32 days. Okon et al. (2013) opined that the disparity in incubation period may be attributed to variation in genetic factors like breed, strain, age, and size of the snail, egg size, and environmental factors like temperature and relative humidity. Besides, Ibom et al. (2012) observed that exposure of eggs to fluctuating environmental conditions which differed from their near-constant uterine environment may influence or increase the incubation period. In addition, Ibom et al. (2012) noted that incubation conditions such as uptake and loss of moisture and increased transpirational water loss resulting from increased heat produced by the developing

embryo can also cause this variation in the incubation period. The dead embryos from dry media were probably between days 2 to 8 of incubation compared to photographic plates reported by Okon et al. (2013). Our results are in line with those obtained by Agbelusi and Adeparusi (1999), who reported no hatching of *A. marginata* eggs incubated in dry soil and dry sawdust.

Effect of environmental factors on hatchability and embryo mortality of *A. marginata* eggs

Results of this study showed that snail eggs hatch well at a humidity range from 50 to 80% and temperature range of 17 to 22°C in the Western Highlands of Cameroon. Embryonic mortality decreases with increasing humidity and only starts increasing when the humidity of the incubation substrate becomes too high (>80%). This study revealed that the optimum humidity for incubation of snail eggs lies between 50 and 80%. This corroborates the work of Agbelusi and Adeparusi (1999), who demonstrated that egg hatchability in *A. marginata* was environmentally induced and highly dependent on the moisture content of incubation media. The hatchability of values obtained is lower than those reported by Ukpong et al. (2013) of 67.86 and 82% reported by Agbelusi and Adeparusi (1999) in a moist soil medium. Agbelusi and Adeparusi (1999) however reported a lower hatching rate (30%) in moist soil with humidity above 80% compared to the 45.5% obtained in this study. Variation in incubation duration could equally be linked to these environmental factors of humidity and temperature. When these factors are strained the embryos find it difficult to develop and in case they don't die their development is retarded, extending the incubation period by some more days, and in extreme cases, there are morphological deformations of the shells of the embryo observed when shells are cracked. An observation similar to that reported by Agbelusi and Adeparusi (1999), of freshly laid eggs making a cracking sound when exposed to room temperature 23 to 25°C was made. Agbelusi and Adeparusi (1999), posited that cracks in eggs could lead to leakage of egg content when incubated in dry media, leading to a high number of empty shells recorded. Not all eggs in a batch hatched on the same day probably because GALS eggs are laid with embryos at different stages of development. This same observation was made by Plummer (1975), Segun (1975), Awesu (1980), and Agbelusi and Adeparusi (1999).

The highest embryonic mortality was observed in batches of eggs incubated in MSb. This shows that too little or too much moisture in incubation media has adverse effects on egg hatchability. The embryos in MSb died between days 18 and 25th of incubation as the embryo closely resembles those revealed by Okon et al. (2013) in their study of developmental stages of *A.*

marginata eggs in incubation. This conforms with the observations of Agbelusi and Adeparusi (1999). Though no regression analysis of humidity or temperature on undeveloped eggs was done in this study, the highest number of undeveloped eggs were observed in dry media (DS and DD) which is in line with the linear regression carried out by Agbelusi and Adeparusi (1999), revealing that there exists an inverse relationship between the moisture content of the soil and the number of undeveloped eggs and a direct relationship between temperature and number of undeveloped eggs. The few (less than 20) undeveloped eggs in MSb, MSp, MSD, and MD remain clear as at lay similar to that at laying (day one) as reported by Okon et al. (2013) who observed that the egg content was translucent or clear (blank) when observed with light from a powered microscope. This gives reason to posit that some clutches or eggs in a clutch are laid without having been fertilized especially if lay continues for a long time after copulation with the stored sperm exhausted from the spermathecal.

Effect of egg weights on hatchability and embryo mortality

A regression analysis of egg weight on hatchability reveals that hatchability could be improved with an increase in egg weight. Also, testing the effect of egg weight on embryonic mortality showed an indirect relationship. This gives reason to think that an increase in egg weights to above the mean egg weight obtained in this research (0.675 g), say the mean weight (2.00 g) reported by Okon et al. (2013) could lead to a remarkable decrease in embryonic mortality and an increase in hatchability. As embryonic mortality drops, there is reason to believe that hatchability values will be improved. Abiona et al. (2012) observed that egg weight had no meaningful effect on incubation length in days but rather on hatchling weights.

Conclusion

It can be concluded from this study that substrates like moist sawdust, moist soil-sawdust mixture, and moist soil make up appropriate incubation media for *A. marginata* eggs. Environmental factors such as temperature and humidity affect the embryonic development and hatchability of *A. marginata* eggs. Snail eggs can be hatched at temperatures between 17 and 20°C though with a hatching rate of less than 65%. Too wet (>80% humidity) or too dry (<20% humidity) incubation substrate will lead to either embryonic mortality or undeveloped eggs and hence low hatching rates. The most suitable humidity range for incubation should be between 50 and 80%. Egg weight is proportionally related to Hatchability

and embryonic mortality. The relationship that exists between weights and hatching rates is mutually inclusive whilst the relationship between egg weights and embryonic mortality of *A. marginata* eggs is mutually exclusive. Lower snail egg weights will favor embryonic mortality and higher egg weights will increase hatching rates.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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