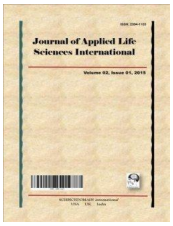

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Hormonal Concentration of Controlled and Naturally Induced Aestivated Snails (*Archachatina marginata*) at Different Reproductive Phases

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OEO and JMO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OET managed the analyses of the study. Author OTA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This research was carried out to examine changes in some hormones perceived to be implicated in the reproduction of giant African land snail (GALS) subjected to varied aestivation lengths and nature have driven natural cycle.
Study Design: Randomized complete block design (RCBD).
Place and Duration of Study: University of Benin Teaching and Research Farm, between November 2015 and October 2016.
Methodology: Apparently healthy *Archachatina marginata* species of snails were grouped into five. Group 0A was fed and watered throughout the experimental period. Group 6A and 12A were subjected to periods 6 - and 12 - week unbroken aestivation period while snails in group NA and WA were controlled by natural weather elements in captivity and the wild respectively. Hormonal

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concentrations were determined from each group in a year circle and grouped into reproductive phases.
Results: At dormancy phase, no significant differences were observed among treatments in all studied hormones except the progesterone and testosterone. The estradiol concentration in the pre-spawning phase was significantly higher (57.40 pg/mL) as compared with other treatment groups. During the post-spawning period, the NA and WA groups were statistically similar ($p>0.05$) but differed significantly from 12A in estradiol concentration. The highest FSH concentration (5.72 µ/L) was recorded in the 6A.
Conclusion: The mean concentrations of the hormones were found to be significantly influenced by the reproductive phases and aestivation treatments.

Keywords: Hormones; Archachatina marginata; aestivation; naturally controlled; reproductive phase.

1. INTRODUCTION

A thorough understanding of the physiological and environmental interaction of the land snail is vital for successful and sustained heliculture. All living organisms including snails exhibit specific biological cycle or the other. They possess "biological clock" that regulates the different phases of their life cycle. Period of rest or dormancy usually closes a cycle. The dormancy phase is expressed by different terms depending on the factor or group of elements that initiate it, and the organism is referred to. In the tropics land snails naturally recessed from reproductive activities and retarded other physiological processes within the dormancy phase referred to as aestivation. They resume afterwards to a pre-spawning phase and subsequently the spawning and post-spawning phase [1].

Evidence for an endocrine control of the reproductive system in gastropods has been produced [2]. The biosynthesis of steroids and their possible functions concerning reproduction has been studied in a number of gastropods. Steroids and steroid- synthesizing enzymes have also been demonstrated [3]. Casaaba and Birbaur [4] suggested that steroid hormones are essential in spermatocyte formation and testicular steroidogenesis as well as oogenesis in the snail. Moreover, testosterone rises with the onset of laying eggs in *Pomacea paludica* and at the beginning of the reproductive season in both male and female in *Ilayanassaobsoleta* [5]. However, there have been no studies on variation in hormone levels in the giant African land snail, *Archachatina marginata* which undergoes aestivation.

It is hypothesised that hormones in the snail will be different in concentrations in response to the length of aestivation, the culture environment and following physiological changes in the reproductive phases. Therefore, live *A. marginata* were collected during 12 months in which all ecophysiological-behaviour stages occurred, and the hormonal concentrations were determined.

2. MATERIALS AND METHODS

A total of 120 healthy snails of *Archachatina marginata* with a live weight of 150 to 280 g were used for the study. Other materials included; shallow containers for feed and water, sensitive electronic weighing scale, feed (concentrates, forages and fruits), water, dissecting sets and board.

2.1 Sources of Experimental Snails and Experimental Location

The snails in the continuous watered (0A) group were obtained from the Snail Unit of the Department of Forestry and Wildlife, Faculty of Agriculture, University of Benin, Benin City, Nigeria. These had been reared under continuous watering condition before the experiment. The others were obtained from the wild at Udo and Iguobazuwa communities both in Ovia South West LGA, Edo State, Nigeria. The research was carried out at the Snailry Unit of the University Teaching and Research Farm, University of Benin, Edo State, Nigeria. The farm is located within the tropical rainforest vegetation zone of Southern Nigeria lying between longitude 5°E and 6°42'E and latitude 5°45 and 7°34'N of the equator [6]. The climate of Edo is humid as inferred from the report of Ikhile and Aifeseshi [7].

2.2 Experimental Design and Procedure

Snails were collected from the wild and each group of the established heliculture bi-monthly from November, 2015 to October, 2016. Data obtained were thereafter grouped into the reproductive phases (Table 1). The experiment

was laid out as a 4x5 (Reproductive phase x aestivation treatment) factorial arrangement in a randomized complete block design (RCBD) with four (4) replications.

Snails in group 0A were fed, watered and soil moistened throughout the experimental period. Group 6A and 12A were allowed a 6 - and 12 - week unbroken aestivation period while snails in groups NA and WA were controlled by natural weather elements in captivity and in the wild respectively. Group 6A was induced 6 weeks after Group 12A had been induced by withdrawal of water and feed to allow for the formation of epiphragm. This enabled termination of aestivation of all the groups and simultaneous data collection. Watering was thereafter carried out as required in all the treatment groups except the NA group.

Samples of haemolymph were collected each from the five (5) treatment groups into plain reagent bottles for hormonal assay.

Table 1. Reproductive phases of GALS

Phases	Month
Pre Spawning	March April
Spawning	May June July
Post Spawning	August September October
Dormancy	November December January February

Source: Cobinnah [1]

2.3 Laboratory Analysis and Data Collection

ELISA procedure (an enzyme Immunoassay) for the quantitative *in-vitro* diagnostic measurement was utilized for the endocrinological parameters at the Chemical Pathology Department, University of Benin Teaching Hospital, Benin City. Kits were manufactured by Perfemed Group. The hormonal parameters examined basically comprised Follicle Stimulating Hormones (FSH), Luteinizing hormones (LH), prolactin, progesterone, esteradiol and testosterone.

2.4 Statistical Analysis

Dataobtained were subjected to two-way analysis of variance (ANOVA) using Genstat [8] and significant treatment means were separated by Duncan Multiple Range Test.

3. RESULTS

The results of the hormonal concentration of *A. marginata* m under controlled and natural aestivation at different reproductive phases are presented. Tables 2 – 7 shows the responses of the respective hormone to aestivation treatments and reproductive phases independently.

3.1 Haemolymph Estradiol Concentration (pg/mL) of *Archachatina marginata* under Natural and Varying Aestivation Lengths at Different Reproductive Phases

Table 2 showed the haemolymph estradiol concentration (pg/mL) of *Archachatina marginata* under the continuous watering regime (0A), 6- and 12 weeks aestivation treatments (6A and 12A) and captive and wild naturally controlled aestivation groups (NA and WA). The concentrations of the estradiol for specific aestivation treatment group at different reproductive phases are also presented in the table.

The concentration of estradiol (pg/mL) in the 0A group were significantly higher at the spawning (12.90 pg/mL) and post-spawning phase (14.18 pg/mL) as compared to the dormancy (4.80 pg/mL) and pre-spawning phases (1.50 pg/mL). The highest value in the 6A group was 27.57 pg/mL at the pre-spawning phase which did not differ significantly (P=0.06) from the dormancy (16.78 pg/mL) and post-spawning (16.92 pg/mL). The same finding was obtained in 12A group except that the concentration at the post-spawning phase was significantly different from the spawning phase in the 12A. The NA group recorded the least estradiol concentration at the pre-spawning phase where the WA had its highest value. In both groups (NA and WA), the concentrations at the dormancy, spawning and post-spawning phases were not significantly different from each other.

The aestivated groups recorded higher estradiol concentration (pg/mL) when compared with the 0A group at the dormancy phase. Continuous watering group differed significantly from the 6A

group and not from the others. At the pre-spawning phase, the 0A and NA groups recorded similar (P =0.07) lower concentration and WA gave a significantly highest value (57.40 pg/mL). The 6A and 12A did not differ significantly from each other. At the spawning phase, the 6A, 12A and WA were not significantly (*P* = 0.55) different from each other and the NA group neither differed from the 6A nor the WA group. The 12A group was significantly highest (23.75 pg/mL) at the post-spawning phase and closely followed by the 6A group (16.92 pg/mL) which did not differ statistically from the 0A (14.18 pg/mL). The naturally controlled and wild groups (NA and WA) recorded statistically similar values (10.22 and 8.55 pg/mL respectively).

The overall means indicated significant (*P* = 0.02) differences in the aestivation treatments and across the reproductive phases. The 6A, 12A and WA groups did not differ significantly (*P* = 0.05) but significantly (*P* = 0.004) differ from the 0A and the NA. *A. marginata* had an overall highest mean estradiol concentration at the pre-spawning phase and least at the spawning phase.

3.2 Haemolymph Follicle Stimulating Hormones (FSH) Concentration (u/L) of *Archachatina marginata* under Natural and Varying Aestivation Lengths at Different Reproductive Phases

The FSH was highest (*P* = 0.01) in all treatments during the post-spawning phase. *A. marginata* that naturally aestivated tended to exhibit a pattern of the steady drop in concentration across the reproductive phases with an upsurge at the post-spawning phase. The 6A group recorded the highest overall mean concentration (0.89 µ/L) although, this did not differ significantly from the WA group (0.44 µ/L). The latter also did not statistically (*P* = 0.95) differ significantly from the concentration of the 6A (1.30 µ/L) and 0A which recorded the least FSH concentration (0.27 µ/L).

Table 2. Haemolymph Estradiol concentration (pg/mL) of *Archachatina marginata* under natural and varying aestivation lengths at respective reproductive phases

Reproductive phases	Aestivation treatments					±SEM	Overall
	Continuous watering	6 weeks	12 weeks	Naturally controlled	Wild collection		
Dormancy	$4.80^{b}{}_{B}$	$16.78^{a}{}_{AB}$	$12.55^{ab}{}_{AB}$	$9.27^{ab}{}_{A}$	$11.25^{ab}{}_{B}$	2.48	10.93_{BC}
Pre Spawning	$1.50^{c}{}_{C}$	$27.57^{b}{}_{A}$	$22.33^{b}{}_{A}$	$1.57^{c}{}_{B}$	$57.40^{a}{}_{A}$	6.42	22.07_{A}
Spawning	$12.90^{a}{}_{A}$	$2.20^{c}{}_{B}$	$6.83^{bc}{}_{B}$	$10.53^{ab}{}_{A}$	$6.87^{bc}{}_{B}$	1.44	7.87_{C}
Post Spawning	$14.18^{b}{}_{A}$	$16.92^{b}{}_{AB}$	$23.75^{a}{}_{A}$	$10.22^{c}{}_{A}$	$8.55^{c}{}_{B}$	1.05	14.72_{B}
±SEM	0.77	5.99	3.72	1.46	3.30		1.59
Overall	8.35^{b}	15.87^{a}	16.37^{a}	7.90^{b}	21.02^{a}	1.78	

^{abc} Means with different superscripts within the same row differ significantly (P<0.05) with aestivation treatments
$_{ABC}$ Means with different subscripts within the same column differ significantly (P<0.05) with reproductive phases

Table 3. Haemolymph follicle stimulating hormones (FSH) concentration (µ/L) of *Archachatina marginata* under natural and varying aestivation lengths at respective reproductive phases

Reproductive phases	Aestivation treatments					±SEM	Overall
	Continuous watering	6 weeks	12 weeks	Naturally controlled	Wild collection		
Dormancy	$1.43^{a}{}_{B}$	$0.77^{a}{}_{B}$	$1.08^{a}{}_{B}$	$1.63^{a}{}_{AB}$	$1.40^{a}{}_{B}$	0.43	1.26_{B}
Pre Spawning	$0.50^{a}{}_{B}$	$0.67^{a}{}_{B}$	$0.93^{a}{}_{B}$	$0.57^{a}{}_{C}$	$0.73^{a}{}_{BC}$	0.23	0.68_{C}
Spawning	$0.50^{a}{}_{B}$	$0.40^{a}{}_{B}$	$0.60^{a}{}_{B}$	$0.73^{a}{}_{BC}$	$0.50^{a}{}_{C}$	0.13	0.55_{C}
Post Spawning	$2.65^{b}{}_{A}$	$5.72^{a}{}_{A}$	$2.58^{b}{}_{A}$	$1.77^{b}{}_{A}$	$3.13^{b}{}_{A}$	0.48	3.17_{A}
±SEM	0.31	0.53	0.30	0.30	0.22		0.16
Overall	1.27^{b}	1.89^{a}	1.30^{b}	1.18^{b}	1.44^{ab}	0.17	

^{abc} Means with different superscripts within the same row differ significantly (P<0.05) with aestivation treatments
$_{ABC}$ Means with different subscripts within the same column differ significantly (P<0.05) with reproductive phases

3.3 Haemolymphleutenizing Hormones (LH) Concentration (µ/L) of *Archachatina marginata* under Natural and Varying Aestivation Lengths at Different Reproductive Phases

There were no significant (P = 0.78) differences among the aestivation treatments at the dormancy and spawning phase (Table 4). Only the group aestivated for 6 weeks (6A) recorded significant differences in the reproductive phases. On the overall, no significant differences were recorded across the treatment groups and down the reproductive phases.

3.4 Haemolymph Progesterone Concentration (ng/mL) of *Archachatina marginata* under Natural and Varying Aestivation Lengths at Different Reproductive Phases

As shown in Table 5, significant (P = 0.03) differences in progesterone concentrations were observed among aestivation treatment groups in all reproductive phases. At the dormancy phase, the 12A group significantly differed from the other treatment groups, recording progesterone concentration of 1.18 ng/mL

On the overall, significant differences were observed with aestivation treatment and reproductive phases. The 12A recorded the highest progesterone concentration (0.82 ng/mL) when subjected to aestivation. Among the reproductive phases, the pre-spawning period had the highest concentration which statistically differs (P = 0.03) from the spawning phase.

3.5 Haemolymph Testosterone Concentration (ng/mL) of *Archachatina marginata* under Natural and Varying Aestivation Lengths at Different Reproductive Phases

In Table 6 significant differences among treatment groups were only observed at the

Table 4. Haemolymphleutenizing hormones (LH) concentration (µ/L) of *Archachatina marginata* under natural and varying aestivation lengths at different reproductive phases

Reproductive phases	Aestivation treatments					±SEM	Overall
	Continuous watering	6 weeks	12 weeks	Naturally controlled	Wild collection		
Dormancy	$0.45^{a}{}_{A}$	$0.22^{a}{}_{B}$	$0.32^{a}{}_{A}$	$0.23^{a}{}_{A}$	$0.33^{a}{}_{A}$	0.15	0.31_{A}
Pre Spawning	$0.67^{a}{}_{A}$	$0.40^{ab}{}_{A}$	$0.33^{ab}{}_{A}$	$0.23^{b}{}_{A}$	$0.23^{b}{}_{A}$	0.13	0.37_{A}
Spawning	$0.30^{a}{}_{A}$	$0.20^{a}{}_{B}$	$0.27^{a}{}_{A}$	$0.33^{a}{}_{A}$	$0.33^{a}{}_{A}$	0.06	0.29_{A}
Post Spawning	$0.23^{c}{}_{A}$	$0.37^{b}{}_{A}$	$0.52^{a}{}_{A}$	$0.38^{b}{}_{A}$	$0.40^{ab}{}_{A}$	0.04	0.38_{A}
±SEM	0.18	0.03	0.10	0.06	0.08		0.05
Overall	0.41^{a}	0.30^{a}	0.36^{a}	0.30^{a}	0.33^{a}	0.05	

^{abc} Means with different superscripts within the same row differ significantly (P<0.05) with aestivation treatments
$_{ABC}$ Means with different subscripts within the same column differ significantly (P<0.05) with reproductive phases

Table 5. Haemolymph progesterone concentration (ng/ml) of *Archachatina marginata* under natural and varying aestivation lengths at the respective reproductive phases

Reproductive phases	Aestivation treatments					±SEM	Overall
	Continuous watering	6 weeks	12 weeks	Naturally controlled	Wild collection		
Dormancy	$0.32^{b}{}_{C}$	$0.30^{b}{}_{A}$	$1.18^{a}{}_{A}$	$0.42^{b}{}_{B}$	$0.35^{b}{}_{B}$	0.10	0.51_{AB}
Pre Spawning	$0.70^{b}{}_{A}$	$0.43^{b}{}_{A}$	$0.57^{b}{}_{A}$	$0.23^{b}{}_{C}$	$1.90^{a}{}_{A}$	0.26	0.77_{A}
Spawning	$0.33^{ab}{}_{C}$	$0.20^{b}{}_{A}$	$0.37^{ab}{}_{A}$	$0.13^{b}{}_{C}$	$0.53^{a}{}_{B}$	0.07	0.31_{B}
Post Spawning	$0.52^{ab}{}_{B}$	$0.48^{ab}{}_{A}$	$1.17^{a}{}_{A}$	$0.80^{ab}{}_{A}$	$0.17^{b}{}_{B}$	0.29	0.63_{A}
±SEM	0.06	0.10	0.35	0.04	0.26		0.09
Overall	0.47^{bc}	0.35^{c}	0.82^{a}	0.40^{c}	0.74^{ab}	0.10	

^{abc} Means with different superscripts within the same row differ significantly (P<0.05) with aestivation treatments
$_{ABC}$ Means with different subscripts within the same column differ significantly (P<0.05) with reproductive phases

dormancy phase. The continuous watered group (0A) recorded the least testosterone concentration at this phase. This was however not significantly different from the concentrations of the NA, WA and 6A groups which in turn did not differ statistically from the concentration of the 12A group. There were also no significant (*P* = 0.05) differences in the concentration of testosterone down the reproductive phases in all the aestivation treatment groups.

3.6 Haemolymph Prolactin Concentration (ng/mL) of *Archachatina marginata* under Natural and Varying Aestivation Lengths at Respective Reproductive Phases

As presented in Table 7, the concentration of prolactin in the haemolymph of *A. marginata* under different aestivation treatments did not differ significantly at the dormancy and pre-spawning phases but statistical differences were observed at the spawning and post-spawning phases. Similarly, significant differences were observed at the 6A, 12A and NA down the reproductive phases but were not reflected in the 0A and WA groups.

At the spawning phase, the concentration of prolactin decreased with aestivation length with the highest recorded concentration at the 0A group (0.67 ng/mL) and the least at the 12A group (0.33 ng/mL). The NA (0.40 ng/mL) and WA (0.53 ng/mL) did not differ significantly from 6A and the 12A. The 12A experienced an exponential rise in concentration at the post-spawning phase which did not differ significantly from the NA group but from other treatment groups.

On the overall, no significant differences were observed among treatment groups. The concentration of prolactin decreased down the reproductive phases with significantly (*P* = 01) highest concentration at the post-spawning phase.

Table 6. Haemolymph testosterone concentration (ng/mL) of *Archachatina marginata* under natural and varying aestivation lengths at respective reproductive phases

Reproductive phases	Aestivation treatments					±SEM	Overall
	Continuous watering	6 weeks	12 weeks	Naturally controlled	Wild collection		
Dormancy	$0.10^{b}{}_{A}$	$0.15^{ab}{}_{A}$	$0.17^{a}{}_{A}$	$0.12^{ab}{}_{A}$	$0.12^{ab}{}_{A}$	0.02	0.13_{A}
Pre Spawning	$0.10^{a}{}_{A}$	$0.13^{a}{}_{A}$	$0.13^{a}{}_{A}$	$0.10^{a}{}_{A}$	$0.17^{a}{}_{A}$	0.03	0.13_{A}
Spawning	$0.10^{a}{}_{A}$	$0.10^{a}{}_{A}$	$0.10^{a}{}_{A}$	$0.10^{a}{}_{A}$	$0.10^{a}{}_{A}$	0.00	0.10_{B}
Post Spawning	$0.12^{a}{}_{A}$	$0.10^{a}{}_{A}$	$0.15^{a}{}_{A}$	$0.15^{a}{}_{A}$	$0.12^{a}{}_{A}$	0.02	0.13_{A}
±SEM	0.01	0.02	0.02	0.02	0.02		0.01
Overall	0.10^{b}	0.12^{ab}	0.14^{a}	0.12^{ab}	0.13^{ab}	0.01	

^{abc} Means with different superscripts within the same row differ significantly (P<0.05) with aestivation treatments
$_{ABC}$ Means with different subscripts within the same column differ significantly (P<0.05) with reproductive phases

Table 7. Haemolymph prolactin concentration (ng/mL) of *Archachatina marginata* under natural and varying aestivation lengths at respective reproductive phases

Reproductive phases	Aestivation treatments					±SEM	Overall
	Continuous watering	6 weeks	12 weeks	Naturally controlled	Wild collection		
Dormancy	$0.58^{a}{}_{A}$	$0.35^{a}{}_{B}$	$0.37^{a}{}_{B}$	$0.32^{a}{}_{B}$	$0.65^{a}{}_{A}$	0.18	0.45_{B}
Pre Spawning	$0.27^{a}{}_{A}$	$0.53^{a}{}_{AB}$	$0.47^{a}{}_{B}$	$0.33^{a}{}_{B}$	$0.53^{a}{}_{A}$	0.12	0.43_{B}
Spawning	$0.67^{a}{}_{A}$	$0.60^{ab}{}_{AB}$	$0.33^{c}{}_{B}$	$0.40^{bc}{}_{B}$	$0.53^{abc}{}_{A}$	0.08	0.51_{B}
Post Spawning	$0.72^{bc}{}_{A}$	$0.68^{bc}{}_{A}$	$1.07^{a}{}_{A}$	$0.93^{ab}{}_{A}$	$0.50^{c}{}_{A}$	0.09	0.78_{A}
±SEM	0.20	0.08	0.12	0.11	0.07		0.06
Overall	0.56^{a}	0.54^{a}	0.56^{a}	0.50^{a}	0.55^{a}	0.06	

^{abc} Means with different superscripts within the same row differ significantly (P<0.05) with aestivation treatments
$_{ABC}$ Means with different subscripts within the same column differ significantly (P<0.05) with reproductive phases

4. DISCUSSION

Underscoring the role of estradiol in stimulation of oestrous behaviour and facilitating mating, the exponential rise in the concentration at the pre-spawning phase is justified. The hormone also prepares the external genitalia for copulation and creates favourable conditions for the development of fertilised egg cells [9]. Moreso, estradiol is synthesised from cholesterol obtained from the relatively high feeding rate associated with this phase as reported by Cobbinah [1]. This was demonstrated in the aestivated groups. The 0A group differed in this observation with much lower haemolymph concentration of estradiol at the pre-spawning phase. Since feed and watering were continuously provided in the 0A group, compensatory feed consumption was not necessary. This may also depict physiological stress (since fasting imparts physiological rest) which tended to depress the overall concentration of the estradiol in the 0A group. According to Salam and Reetu [10], in stress, there is suppression of circulating gonadotropins and gonadal steroid hormones leading to disruption of the regular menstrual cycle. Risingestradiol concentration is understood to exert a positive feedback influence in the secretion of the gonadotropins; FSH and LH, which are essential for follicle maturation and egg release respectively.

(First you need to show what the role of FSH and LH in mollusk is as these are vertebrate hormones, you must evidence your suggestion with references.) Across the reproductive phases, the FSH was relatively unaffected by the aestivation treatments. However, there was a trend of higher concentration of FSH at the dormancy and a decline in the pre-spawning and spawning phase and an eventual upsurge at the post-spawning phase. FSH and LH are intimately involved in the control of the growth and reproductive activities of the gonadal tissues. The surge at the non or marginal reproductive phases (dormancy and post-spawning) of the FSH may, therefore, be suggestive of a non-reproductive function(s) of the FSH in the GALS which may be connected with gonadal tissue recovery, sustenance or both, post-spawning and at dormancy. Okhale et al. [11] though observed a decline in the gonadotropins while monitoring the concentration of reproductive hormones with 3 and 6 weeks aestivation lengths, but similarly observed an elevated concentration prior to aestivation (post-spawning) in the same species of GALS.

The continuous watering tends to promote the concentration of the LH pre-spawning which may have accounted for the foremost oviposition reported by Omoyakhi et al. [12]. Following the remarkable rupture of the follicles at the spawning phase as reported by Okhale [13], the elevated concentration of progesterone post spawning would be justified as suggestive of secretion from the corpus luteum typical of the luteal phase [14]. The peripheral and epithelial thickening of the tissues of some reproductive organs at dormancy [13] tends to parallel the concentration of the progesterone among treatment groups. This is therefore suggestive of a necessary mechanism for sustenance at dormancy and nonreproductive function of the progesterone in GALS as suggestive also of FSH. Tortora and Grabowski [15] stated that the epithelial forms a barrier between the body and the external environment and therefore play an important protective role to the organ against dehydration.

The presence of prolactin in GALS substantiates its multiple roles in reproduction in invertebrates. Marc et al. [16] also reported its multiple homeostatic roles in the organism. Indeed, the multiple roles and sources of prolactin had led Bern and Nicoll [17] to suggest renaming it “omnipotin” or “versatilin”. The reproductive and homeostatic roles of prolactin (this is a vertebrate hormone, what is the role of this hormone in mollusk, please give references show that) were evident in this study with variability in concentration with aestivation treatment and across reproductive phases. The mechanism of its function across aestivation treatment and phases would require further investigation. Prolactin level is said to be elevated in some situation of stress, exercise and hypoglycemia [18]. This would seem to infer that aestivation relieved the animal of certain stress culminating in a relative drop in concentration of the captive aestivated groups at dormancy. This was similarly observed by Okhale et al. [11] who reported a decline in prolactin concentration from 0.75 ng/mL at the pre aestivated state to 0.65 ng/mL at the 3rd week aestivation. Prolactin concentration was sustained through the 6th week of aestivation as similarly observed in this study with the only marginal rise in concentration between the 6weeks aestivated group and the 12 weeks. This may be suggestive of stable physiological status at the dormancy phase in the aestivated GALS. It can also be observed that the concentration of prolactin decreased with aestivation length at the spawning phase

showing tendency to halt active reproduction in the lower or non-aestivation lengths; probably at the long run since the continuously watered groups reproduced [12]. Prolactin is said to suppress gonadal function [19] which may as well explain the elevated concentration observed post spawning across aestivation treatment and overall.

5. CONCLUSION

The mean concentrations of the hormones were found to be significantly influenced by the reproductive phases and aestivation treatments. While hormones levels in the continuous watering group were similar, there were significant fluctuations in the standards of the hormones with distinct peaks in the groups that were allowed to aestivate either in captivity or in the wild among the reproductive phases. This implies the establishment of endocrine regulation of the biological cycle and reproductive activities of *A. marginata.*

The dynamics of the hormones with the reproductive phases of the GALS substantiates the peculiarity of respective phases and significance of the aestivation phenomenon which should not be undermined in captive rearing. Leaving stocks to natural weather elements throughout the cycle in captivity may not also be advocated.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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