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MORPHOMETRIC STUDY OF THE REPRODUCTIVE SYSTEM OF GIANT AFRICAN LAND SNAIL *Archachatina marginata ovum* (Pfeiffer) (Pulmonata: Achatinidae) FOUND IN ILE-IFE, SOUTHWESTERN NIGERIA.

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ABSTRACT

The morphometry of the reproductive organs of the giant African land snail, *Archachatina marginata ovum* from wild collection at Ile-Ife, southwest Nigeria, was assessed during March - May (early rainy season) and August – October (late rainy season) of 2010. A total 134 snails were dissected and classified into one of five different reproductive states (low mating readiness; high mating readiness; egg production; gravid and post reproductive). The five states were observed in snails collected during the early rainy season, whereas the gravid state was not observed in the collections during late rainy season. Albumen gland was considerably enlarged at egg production state forming about 60 % of the total weight of the reproductive system, while vas deferens was significantly longer in snails at high mating readiness state. The sizes of the reproductive organs were not substantially related to the size of the snail as the coefficient of determination (r^2) of the relationship were in most cases below 0.5. The overall results indicated that variations in the morphology of the reproductive organs of *A. marginata ovum* are mostly state dependent and less related to the size of the snail and thus could be used reliably to categorise the snails into reproductive states.

Keywords: *Archachatina marginata ovum,* reproductive system, reproductive states.

INTRODUCTION

The giant African land snail species, *Archachatina marginata* is the most consumed land snail in southern Nigeria where it constitute a very conspicuous portion of the terrestrial molluscan population (Oke *et al*., 2001). In the tropical humid southern Nigeria this snail breed and grow actively during the rainy season which last from March/ April to September/October and aestivate during the dry season, i.e. November – February (Ajayi *et al*., 1978; Hodasi, 1979; Egonmwan, 2004).

A. marginata belongs to the achatinidae family with a hermaphroditic reproductive system that is based on the Stylommatophoran plan (Segun, 1974; Egonmwan, 2007a). Like other pulmonates the reproductive system of *A. marginata* is made up of three groups of organs: common or hermaphroditic (comprising ovotestis or hermaphroditic gland and hermaphroditic duct); male (comprising prostate gland, vas deferens and,

penis) and; female (comprising the albumen gland, uterus, vagina, oviduct, and spermatheca) organs. The prostate gland and uterus are usually closely apposed and referred to as spermoviduct.

The reproductive anatomy of the achatinidae has been extensively studied (Mead, 1950; Kumprataung *et al*., 1989; Sirgel, 2000; Mead, 2004; Egonmwan, 2007a). Egonmwan (2007a) used relative sizes of the reproductive organs to categorise reproductive states in *A. marginata ovum* into the groupings proposed by Emberton (1985), i.e. low mating readiness (LMR); high mating readiness (HMR); egg production (EP) and post reproductive (PR) states. According to Egonmwan (2007a) observations, *A. marginata ovum* snails at low mating readiness state had small albumen gland, spermatheca, penis, hermaphroditic duct and spermoviduct while at high mating readiness state the spermatheca, penis, hermaphroditic duct and spermoviduct were described as large and albumen gland as medium. Snails at egg production state had large albumen gland and spermoviduct and medium spermatheca, penis and hermaphroditic duct whereas snails at post reproductive stage were said to possess medium sized reproductive organs. A fifth state that was subsumed in the egg production state is the gravid (GR) state in which the uterus contains shelled eggs ready to be laid.

It is important to note that Egonmwan (2007a) classification of the snail's into reproductive states were not supported with neither linear or weight measurement. In addition the report was silent on the influence that variations in snail body weights might have on the sizes of their reproductive organs. Thus, the aim of the present study is to provide quantitative morphometric information on the reproductive organs of *A. marginata ovum* found in Ile-Ife in southwestern Nigeria. This information will explain the morphologic differences in the reproductive system of the snail during different reproductive states and further assist in the understanding of the reproductive processes in the mollusks.

MATERIALS AND METHODS

Collection of snails

The study was conducted in the Department of Animal Sciences at Obafemi Awolowo University located in Ile-Ife, Nigeria. The snails used for this study were obtained within the months of March – May (early rainy period) and August – October (late rainy period) in Ile-Ife, a sub urban city in southwestern Nigeria. Ile-Ife lie around 7^{o} 28'N and 4^{o} 34'E at an altitude 286 m. It has a tropical warm humid climate and rain forest vegetation. The mean ambient temperature and relative humidity for Ile-Ife area for the months of March-May and August-October 2010 when the experiments were conducted were similar, 30 ^{o}C and 80 %, respectively, while precipitation data were given as 145 and 45 mm, respectively (Unpublished data from the weather observation station within the University). A total of 134 *A. marginata ovum* snails comprising of 68 (collected during early rainy period) and 66 (collected during late rainy period) were obtained in 3 batches from wild snail collectors. The snails weighing between 150 – 490 g with intact shell were briefly raised in the laboratory at room temperature of 25 – 30 ^{o}C and dissected within a week from time of acquisition.

Dissection and measurements of the reproductive organs

The snail shell was carefully broke open to avoid damage to the visceral mass. The freed

soft tissue was washed cleaned of mucus in alum water and then immersed in NaCl water for about 30 minutes. The immobilized snail was pinned to the dissecting table with the genital opening facing upward. With dissecting scissors the entire reproductive system was dissected out as described by Segun (1974). Using the morphological features pictured by Egonmwan (2007a), the dissected snails were categorized into one of the 5 reproductive states (low mating readiness, high mating readiness, egg production, gravid and post reproductive). The reproductive system was immediately weighed on a sensitive Mettler PB753 scale (max = 151 g. min =0.02 g). The organs were also weighed after separation into the different organs labeled in Figure 1. The lengths of the different organs were first taken with a thread which was then measured on a 30 cm ruler.

Statistical analysis of data

The mean weight and length of the reproductive organs were compared by one way analysis of variance and Duncan procedures between the reproductive states for each collection period using Gen Stat 3 statistical package. Pearson 2- tail correlation analyses were also run to determine the type of relationship between the reproductive organ weight/length and the snail's body measurements. For all procedures 5 % probability was set as the significance level.

RESULTS

Gross morphology of the reproductive system

Of the 134 snails, 22, 48, 26, 10 and 28 were categorized into LMR, HMR, EP, GR and PR states, respectively. The entire reproductive system as shown in Figure 1c and d from the albumen gland end to the penis end measured about 15 cm long. The characteristic and distinguishing features of each reproductive state as observed in the several samples examined are summarized in Table 1.

Snails at LMR state presented small and underdeveloped reproductive system. HMR and EP states showed similar features except for the spermatheca content which was fluidy in the former but jelly in the later. GR state contained shelled eggs in the apical part of the uterus (Fig. 1b). At the PR state the reproductive system appeared darkened and shrunken. The percentage occurrence of the 5 reproductive states varied from 14 – 31 % and 0 – 40 % in the collections for early and late rainy periods, respectively. As depicted in Fig. 2 the gravid state was not observed in the late rainy period but there were lesser number of snails in the LMR state and more in the HMR and EP states than in early rainy period .

Morphometry of the reproductive system

The organosystemic index (OSI = weight of organ/weight of reproductive system × 100) was calculated (Table 2) to balance for the weight difference among snails in the same reproductive state. Generally the reproductive system constituted between 5 - 12 % of the shelless body weight of the snail. The reproductive system weight constituted similar proportion of the shelless body weight at LMR, PR and GR states (during early rainy period only), and were lower than the proportions at HMR and EP states. The common hermaphroditic duct constituted only 1 – 3 % of the reproductive system weight and varied significantly between the reproductive states during the early rainy period. The snail's prostate gland and penis each constituted around 20 % of the reproductive system weight. Both organs were proportionately heavier in LMR and PR states, espe-

cially during the late rainy period. The vas deferens only constituted about 3 % of the reproductive system weight and was significantly bigger in the PR states during the late rainy period. In the snail's collected during both early and rainy periods, albumen gland constituted between 25 - 60 % of the reproductive system weight except in the LMR and PR states during the late rainy period when it was less than 20 %. Uterus constituted 5 – 10 % of the reproductive system weight while vagina-oviduct complex was slightly higher averaging about 12 %. The spermatheca constituted less than 4 % of the reproductive system weight.

Figure 1. The reproductive system of *A. marginata ovum* at high mating readiness (a) and gravid (b) stages. In diagram (c) the organs were stretched out to reveal their length. Diagram (d) is a schematic drawing of (c) to show the different organs that were measured. AG, albumen gland; GO, genital opening; HD, hermaphroditic duct; OV, oviduct; PG, prostate gland; PN, penis; PNS, penis sheath; PRM, penis retractor muscle; SP, spermatheca; SPd, spermatheca duct; UT, uterus; VD, vas deferens; VG, vagina

Table 1: Distinguishing features of the reproductive states of *Archachatina marginata ovum*

Reproductive State	Albumen gland	Uterus	Hermaphroditic duct	Spermatheca	Ovotestis
Low Mating Readiness	Small; creamy white	Basal: Light Yellowish Apical: Dry and whitish	Short and light coloured	Small and empty	Not or Slightly visible
High Mating Readiness	Medium -large; creamy pale yellow	Basal: Yellowish Apical: Creamy white	Long and whitish	Large; reddish; fluidy	Visible
Egg Production	Large; creamy, bright yellow	Basal: Dark yellow Apical: Big, creamy white	Whitish to dark brown or black	medium; dark red; gel	Visible
Gravid	Medium; dull yellow	Basal: thin, contain shelled eggs Apical: Creamy or dull white	Brown or black	Medium; reddish; gel	Visible
Post reproductive	Small-medium; dark/dirty yellowish/ white	Basal: Dark Apical: Dull, dry, flaccid	Light to dark coloured	Medium; reddish; gel or solid paste	Slightly visible

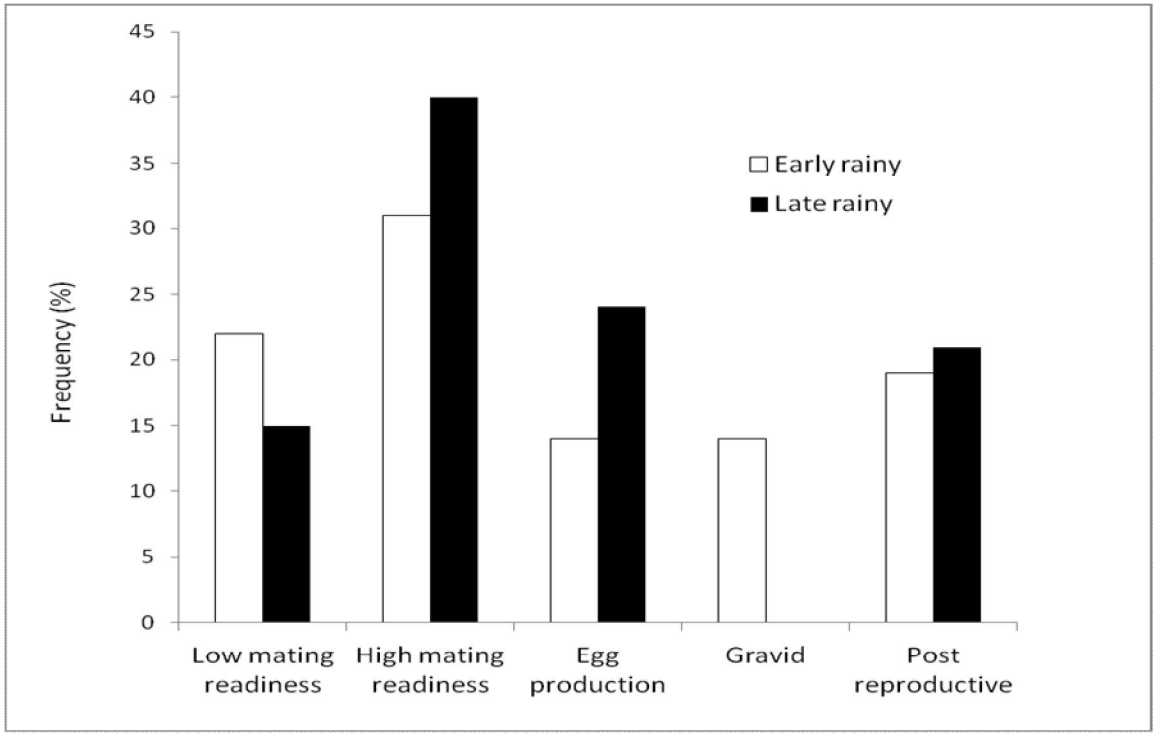

Figure 2: Frequency distribution of the reproductive stages in *A. marginata ovum* from wild collection during early and late rainy periods of 2010 in Ile-Ife, southwest Nigeria.

The lengths of the reproductive organs are shown in Table 3. Generally the reproductive organs were shorter in the LMR snails while variations among the other snails were not consistent. Of particular note however was the significantly longer vas deferens in the HMR snails during the early rainy period and also longer albumen gland in the EP snails during both early and rainy periods. Pearson correlation analyses (Table 4) revealed that the weights of the different reproductive organs except the spermatheca were all positively ($P<0.01$) correlated to the snail's body weights and length. However only the length of albumen gland, hermaphroditic duct, prostate gland, vas deferens, uterus and vagina-oviduct were significantly and positively correlated to all the body measurement parameters, although in many cases with correlation values less than 0.50.

Table 2: Organosystemic index (%) of the reproductive system of *Archachatina marginata ovum* at different reproductive states

Period	Reproductive stage	n	Snail weight	Reprod. System Weight	HD	Male organ			Female organ			
						PG	PN	VD	AG	UT	VO	SP
Early rainy	LMR	12	207.40a	5.51a	3.01c	17.85a	21.24c	1.85	33.46a	8.21b	10.56c	3.82b
	HMR	22	370.90b	10.46b	1.33ab	22.23b	14.87b	1.21	45.31b	6.37a	7.08b	1.61a
	EP	10	374.00b	12.48b	1.39ab	16.45a	9.42a	0.87	59.55c	5.78a	5.44ab	1.09a
	GR	10	373.40b	7.74a	0.98a	18.74ab	10.10a	0.83	25.68a	4.60a	3.94a	0.92a
	PR	14	343.90b	5.49a	2.24bc	27.44c	17.80bc	1.61	31.51a	5.90a	11.59c	1.90ab
		SD	98.18	3.44	1.33	5.86	5.06	0.55	13.16	1.70	3.06	2.09
Late rainy	LMR	10	196.20a	4.23a	2.60	22.67b	32.15c	2.27a	10.71a	10.04b	17.16b	2.41ab
	HMR	26	317.80b	7.66b	2.01	16.99a	17.31b	1.59a	42.17b	9.28b	8.20a	2.45ab
	EP	16	366.50b	10.98c	1.46	14.41a	11.78a	1.09a	56.72c	6.50a	6.36a	1.69a
	GR	0	-	-	-	-	-	-	-	-	-	-
	PR	14	379.60b	5.40a	2.50	23.67b	29.29c	3.61b	14.39a	8.69b	14.34b	3.51b
		SD	86.81	2.78	0.70	5.03	8.74	1.59	19.99	2.25	4.89	1.47

abc Values in the same column with different superscripts for each snail species and season are significantly different ($P<0.05$).

1 Reproductive system weight was expressed as percentage of shelless body weight.

HD, hermaphroditic duct; PG, prostate gland; PN, penis; VD, vas deferens; AG, albumen gland; UT, uterus; VO, vagina-oviduct; SP, spermatheca; LMR, low mating readiness; HMR, high mating readiness; EP, egg production; GR, gravid; PR, post reproductive.

Table 3: Length (cm) of reproductive organs of *Archachatina marginata ovum* at different reproductive states

Period	Reproduc - tive stage	n	Snail Shell length	Com-mon HD	Male organ			Female organ				
					PG	PN	VD	AG	UT	VO	SPd	SPb
Early rainy	LMR	12	13.36a	3.46a	2.75a	1.37a	7.90a	2.01a	2.75a	2.73a	1.95	1.18a
	HMR	22	16.37b	5.62b	7.91b	2.73b	18.36c	9.10c	7.91b	3.11ab	2.05	1.86b
	EP	10	18.50c	4.67a	7.48b	2.54b	11.60b	14.48d	7.48b	2.94ab	1.38	1.42ab
	GR	10	17.94c	7.12c	8.30b	1.25a	9.91b	6.59b	8.30b	3.52b	1.12	1.20a
	PR	14	16.63b	4.10a	6.55b	2.46b	9.96b	2.45a	6.55b	2.85a	1.64	1.39ab
		SD	2.06	1.72	2.61	0.76	2.00	5.00	2.61	0.51	0.93	0.55
Late rainy	LMR	10	13.02a	3.20a	5.50a	3.16a	8.74	2.36a	5.50b	2.44a	1.38a	1.12a
	HMR	26	14.97b	4.55b	7.02ab	4.23b	10.36	7.03b	6.95ab	3.01b	2.30b	1.43ab
	EP	16	15.15b	6.04c	7.86b	4.19b	11.00	10.48c	7.86b	3.35b	2.18b	1.88b
	GR	0	-	-	-	-	-	-	-	-	-	-
	PR	14	15.36b	5.11bc	7.03ab	4.36b	13.30	3.87a	7.03ab	3.50b	2.29b	1.74ab
		SD	1.23	1.28	1.26	0.67	3.16	3.13	1.32	0.56	0.81	0.66

Table 4: Correlation between the reproductive organs and body measurements

Body meas-urement	Reprod. organs	AG	HD	PG	SP	SPd	SPb	VD	PN	UT	VO	Re-prod. Sys-tem
Whole body weight	Weight	0.50a	0.57a	0.63a	0.29b	-	-	0.75a	0.77a	0.64a	0.80a	0.63a
Shelless body weight		0.53a	0.57a	0.65a	0.17	-	-	0.65a	0.68a	0.64a	0.75a	0.67a
Shell length		0.55a	0.55a	0.69a	0.18	-	-	0.59a	0.68a	0.65a	0.69a	0.70a
Whole body weight	Length	0.41a	0.62a	0.68a	-	0.03	0.33a	0.31a	0.24b	0.69a	0.46a	-
Shelless body weight		0.42a	0.56a	0.64a	-	-0.15	0.16	0.36a	0.07	0.64a	0.37a	-
Shell length		0.43a	0.53a	0.58a	-	-0.15	0.16	0.33a	-0.14	0.59a	0.29b	-

[a] $P < 0.01$; [b] $P < 0.05$ AG, albumen gland; HD, hermaphroditic duct; PG, prostate gland; SP, spermatheca; SPd, spermatheca duct; SPb, spermatheca bulb; VD, vas deferens; PN, penis; UT, uterus; VO, vagina-oviduct.

DISCUSSION

In support of the earlier description of the reproductive states in *A. marginata* proposed by Egonmwan (2007a), the current study have proposed additional criteria for the easy identification of the reproductive states in *A. marginata ovum*. Using just the absolute sizes of the reproductive organs to categorise the snails into reproductive states could be misleading, especially for snails in transition between two states and snails of different body sizes, as we have shown here that the sizes of the reproductive organs are positively related to the size of the snail. Thus a combination of the relative size, colour and appearance of the reproductive organs will give a better indication of the reproductive state of the snail.

The reproductive cycle of achatinid land snails is highly dependent on climatic variables, most especially, temperature and humidity (Ajayi *et al.,* 1978; Hodasi, 1979; Egonmwan, 2004; Albuquerque *et al*., 2009), as in other Stylommatophora pulmonates (Raut and Goshe, 1984; Albrecht *et al*., 1999; Horn *et al.,* 2005). The distribution pattern of the reproductive states in the population of snails for this study clearly indicated that *A. marginata ovum* breed and lay actively during the early rainy season in southwestern Nigeria, in agreement with many previous observations (Plummer, 1982; Awesu, 1988; Egonmwan, 2004). Egonmwan (2007a) also reported similar frequencies of 25 and 75 % of juvenile and matured *A. marginata ovum*, respectively, in the population collected during the breeding season in another region of southern Nigeria. The active reproductive stages are the HMR and EP (inclusive of gravid snails). During the early rainy period it was observed that similar proportions of the snails were in HMR (31 %) and EP/GR (28 %) compared to the wide disparity of 40 and 24 %, respectively, during the late rainy period. This could indicate the simultaneous copulation and egg laying that were going on in the early breeding season and a suggestion that matings had reduced in the late rainy period or were sterile and not followed with egg production. Although reports indicated *A. marginata* lay more than one clutch during the breeding season (Plummer, 1982; Awesu, 1988) it is not certain if the snail is multivoltine, but like many other pulmonates; *Radix auricularia* (Adam and Lewis, 1992) and *Megalobulimus abbreviates* (Horn *et al.,* 2005) it is certainly iteroparous breeding for more than 3years and living up to 8 years (Plummer, 1982; Awesu, 1988).

The organosystemic index (OSI) provided the relative weight of the reproductive organs in relation to the weight of the reproductive system. The weight of reproductive system of snails in this study reflected the interplay of reproductive state and weight of the snail. The reproductive system was significantly smaller in the LMR and PR states because these are the sexually non active states compared to HMR and EP states during which organs like albumen gland, prostate gland and uterus are actively secreting (Smith, 1965; Runham and Laryea, 1968; Emberton, 1985; Egonmwan, 2004). The largest differential in weight occurred in the albumen gland which was highly enlarged in the sexually active states forming between 40 - 60 % of the reproductive system weight. Horn *et al.* (2005) equally reported increase in OSI of reproductive organs of *Megalobulimus abbreviates* during the breeding season with the albumen gland and spermoviduct accounting for the bulk of the increased reproductive system weight. Just as the significantly enlarged albumen gland in the EP state seems to confirm its secretive activity at

this state, the highly elongated vas deferens in the HMR state also seem to confirm mating activities at this state. The albumen gland is the site for the production of periveliteline fluid (or albumen) which constitutes around 20 % of the egg and is the chief source of nutrient for the developing embryo (Emberton, 1985; Egonmwan, 2007b). The vas deferens on the other hand is a folded duct that merely serves as a conduit pipe for depositing spermatozoa and prostate secretions through the penis into the vagina-oviduct of the copulating partners (Duncan, 1975; Gomez, 2001). As the penis is stretched into the copulating partner so also is the vas deferens which may not retract back immediately to its former length when the penis is pulled back by its retractor muscle. The organs that were non or less secretory in function i.e. penis, vas deferens and vagina-oviduct appeared to contribute more to the weight of the reproductive system in LMR and PR states than HMR and EP states. These organs would therefore be more dependent on the size of the snail, an assumption that was supported by the higher correlation coefficient ($r \geq 0.75$) between them and the snail body weight. Thus, the penis of snails in LMR and PR states may look bigger since the albumen gland is not active.

The relationship of the reproductive organ weight or length to the snail body weight or shell length was weak, even though significant for many of the correlations. If we assume 50 % as the significant coefficient of determination, only the weights of vas deferens, penis and vagina-oviduct gave significant correlations with the snail body weight. As earlier said these organs are non secretory and likely to vary more with the snail's weight. Van Osselaer and Tursch (2000) equally reported no substantial relationship between the genital organ morphometry and the shell measurements in the hermaphroditic *Helix* species. Our result suggests that the correlation values could be higher if the analysis is performed within each reproductive states, meaning that within a reproductive state body weight could play a more significant influence. However, it is important to state that development of the reproductive system is not directly dependent on weight or age of the snail as asynchronous development of the reproductive tract is a common feature of land pulmonates (Bride and Gomo, 1991; Tomiyama, 1993).

In conclusion, our results have provided empirical information to support the observed cyclic morphological variation (Egonmwan, 2007a) in the reproductive system of *A. marginata ovum* snail. The differences in the size of the reproductive organs between the reproductive states lacked substantial relationship to the size of the snail and thus could be used reliably in conjunction with colour and appearance to determine the reproductive state of the snail.

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