THE EFFECTS OF DIFFERENT DIETS ON THE GROWTH, SEXUAL MATURATION AND REPRODUCTIVE PERFORMANCE OF *ACHATINA FULICA*

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Summary

The effects of different diets on the live growth weight, reproductive maturity and egg laying of a giant African snail, *Achatina fulica* was investigated. When maintained by labour intensive method with elimination of aestivation, growth is continuous. Snails grew fastest and reached earliest reproductive maturity when fed on a diet of mixed vegetables, fruits and a formulated compound feed. It is suggested that the greater range of nutrients available in the different diets resulted in the observed differences in growth rates and reproductive performance. In all groups of snail, the primary reproductive organ, the acini of the ovotestis differentiate as buds which push through the digestive gland follicles. Glands along the reproductive tract accumulate during the formation of gametes.

Introduction

Laboratory investigations concerning growth of African snails have usually concentrated on growth in live weight. The main criteria for assessing growth as affected by different variables, e.g., food, have included measurements of fresh weight and dimensions of shell. While most of these investigations have demonstrated the effectiveness of such criteria (Esobe, 1986; Cobbinah & Osei Nkrumah, 1988), it is also evident that the results are short term.

With the establishment of commercial farming of *"achatina"* snails (Runham, 1989), it is imperative that long term experiments are designed to study the problem. In the case of rearing snails for commercial purposes, the diet which gives the fastest growth to the size at which the consumer prefers the snails and/or the food regime which results in a high reproductive performance in the snail (e.g. laying a large number of viable eggs) may be considered by the snail farmer.

This study has therefore dealt with the effect of different diets on the growth and reproductive performance of *Achatina fulica* (Bowdich). Apart from changes in dimensions of the snail, e.g., shell diameter, changes in the reproductive tract have also been followed. This is because it is contended in this study that time taken to reach a specified readily recognizable reproductive maturity must be coupled with the usual method of dimensional changes, e.g., weight which might have limitations due to fluctuations in hydration levels of the snails.

Experimental

The investigations were carried out in an environmental steel cabinet (8x3x3m) set aside as a snail house for rearing and breeding the snails. Temperature was maintained at 25 ± 1^{0}C and relative humidity in excess 80%. The two factors were monitored on a thermohygrograph. Sixteen hour light/day was obtained from overhead fluorescent lighting.

Stocks of young *A. fulica* were obtained from some snails farms in the UK and a colony of adult snails from the same sources laid eggs which were incubated and used. Snails from the same clutch were weighed, and shell dimensions measured at the age of 1 month; then randomly allocated to 3 different groups of 15 each. These were then maintained in 26 x 12 x 8cm transparent perspex containers with holes drilled in the lid for ventilation. Each container was provided with moist sterilized peat to about 1cm depth with the moisture maintained by sprinkling on distilled water every 4 days. The soil was renewed every other week.

At the age of 10 weeks, the snails were transferred to 44x26x24cm glass aquarium tanks or 48 x 34 x 16 polystyrene boxes. Powdered calcium carbonate was occasionally sprinkled on the soil. Containers were cleared of excreta every day with a clean water-soaked foam sponge.

The three groups of snails were provided with different diets as follows:

Group A: Fed on vegetables and fruits, viz: carrots *(Daucus carota)*, cucumber *(Cucumis edulis)*, lettuce *(Lactuca sativa)* and apple *(Malus pumila)*. These were provided together every day.

Group B: Fed on a formulated compound feed marketed by Wynnstay Farmers Ltd. Llansantriad, Powys, Wales as exclusive snail feed.

Group C: Fed on both Group A and B diets. In all cases, food was supplied in excess and as the snails grew older, the quantities of food were progressively increased to compensate for increased consumption.

The following trials were conducted:

i. *Growth of Snails on different diets*: Fresh weight, shell length and shell diameter of 10 randomly sampled snails from each group were measured and the average computed. The test was replicated 6 times each over one year period and the results were pooled.

ii. *Egg production*: Four (4) snails from each of the 3 different groups were used for the production of eggs.

iii. *Maturation and histology of the reproductive system*: In another set-up as in (i) above, a snail from each of the 3 groups was dissected every month and the reproductive systems were separated into individual components and weighed.

This served as a check on the replicability of the measurements made on the total weight. The gonad is embedded in the digestive gland and so it can not be dissected with the accessory reproductive system. However, it was kept for histological studies. The different portions were fixed in Susa and cleared in cellsolve and later embedded in hitoresin. Five (5) μm sections were stained in toluidine blue, or Heidenhain's haematoxylin or Mallory's triple stain.

Results

(a) *Growth*

Growth in live weight, shell length and shell diameter showed a similar pattern. For example growth in diameter of the shell was highest in Group C (Fig.1) for most of the experimental period.

Between the 4th and 12th week, growth in shell diameter was highest in Group A snails although it was almost the same in all groups thereafter, growth in shell diameter in group A snails fell below that of Group C, but was higher than Group B from 12th - 16th week. From the 16th week till the 48th week, the highest growth in shell diameter was in Group C and the lowest in Group A. Growth in diameter of shell had ceased by the 44th week and 48th week in Group C and B, respectively. Table 1 show inter-group comparisons at 6 and 12 months of age.

(b) *Maturation of the reproductive system*:

At the age of 10 weeks, the sexual organs in the Group C snails could be dissected out and separated into their component parts but could be done in all groups at 12 weeks of age. At this age also (12 weeks), the genital opening can be observed as a round pore with a white tissue around it. Before the time the sexual organs can be separated into their components parts, they can be identified in young snails as a thin strip of white tissue descending from the ovotestis.

Although an attempt was made to correlate diet and age of a snail with the stages of gametogenesis, there was too much overlap between different groups. However, young snails - usually below three months of age yielded the following information: Differentiation of the gonad commences at certain points along the upper part of the thin white reproductive tract embedded in the digestive gland. The walls of the tract swell out into a mass of buds. It is the mass of buds at a particular point which will later constitute an ovotestis lobe composed of acini (Fig.2).

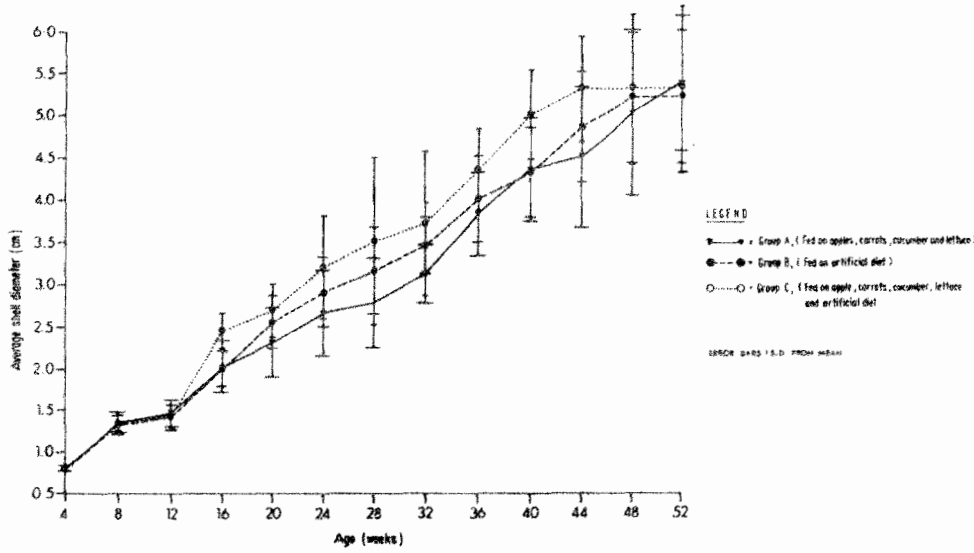

Fig.1: *Shell Diameter Growth In A. fulica*

TABLE 1

Inter-group comparisons of growth in A. fulica on different diets by Student's (t-test at 5%)

6 Months	Group A x Group B	Group A x Group C	Group B x Group C
Live weight	NS	S	NS
Shell weight	S	S	S
Shell diameter	NS	S	NS
Average live weigh	S	S	S
Average shell length	S	S	S
Average shell diameter growth rate	S	S	S
12 Months			
Live weight	NS	NS	NS
Shell length	NS	S	NS
Shell diameter	NS	S	NS
Average live weight growth rate	NS	NS	NS
Average shell length growth rate	NS	NS	NS
Average shell diameter growth rate	NS	NS	NS

NS Non-significant
S Significant

Thus, the acini arise between the diverticulae of the digestive gland and group together to form ovotestis lobes. On examination under a stereo microscope, it was observed that 2 or 3 ovotestis lobes started developing synchronously. Concomitant with the increase in the number of acini in a lobe is the growth of the previously differentiated acini. Therefore, a section through a lobe at this stage shows various stages of gametogenesis in the different acini (Fig.3). Progressive stages of gametogenesis were difficult to follow but of particular significance is the occurrence of what may be termed undifferentiated cells in the acini of young animals (2 months old or below) irrespective of diet supplied. The acinus wall at this stage is thick and composed of connective tissue and muscle. Located at the inner side of the acinar wall are a group of cells, the cavity of the acinus is hollow (Fig.4). These cells may correspond to gametogonia of Parivar (1978). However, the term gametogonia is avoided here because not all the cells differentiate into gametes. Some may differentiate later into Sertoli cells and perhaps follicle cells (Fig.5, 6, 7, 8, 9).

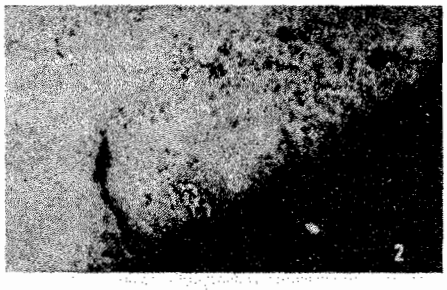

Fig.2. *Acinus developing in the vicinity of digestive gland follicles x 50. (a: acinus)*

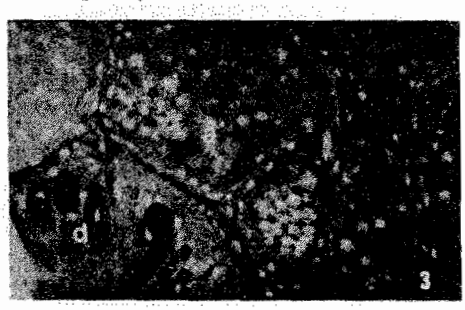

Fig.3 *Acini at different stages of maturation x 150. (a: acinus; d: digestive gland follicle)*

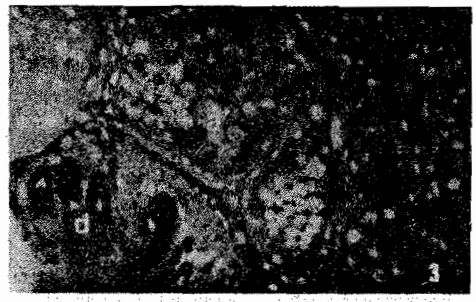

Fig.4 *Newly-differentiated acinar cells x 200. (a: acinus; V: vesicular connective tissue cells)*

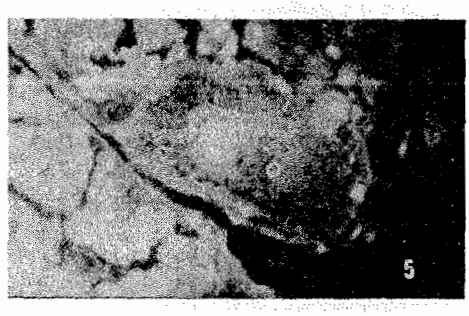

Fig. 5 *Oocyte with follicle cells (arrowhead) x 250. (O: oocyte)*

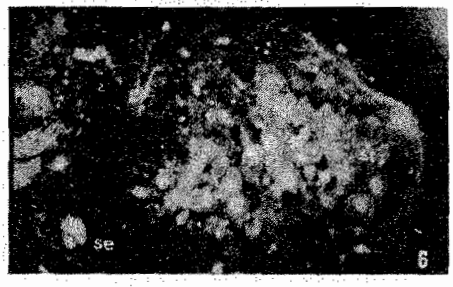

Fig.6 *Some different cells in the acinus.* x 100. *(se: Sertoli cell; st: spermatocyte)*

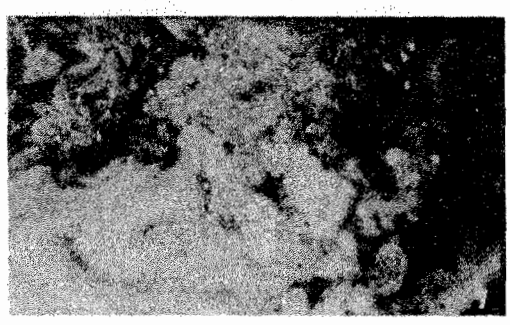

Fig.7 *Acinus showing spermatids x 100. (sp: spermatid)*

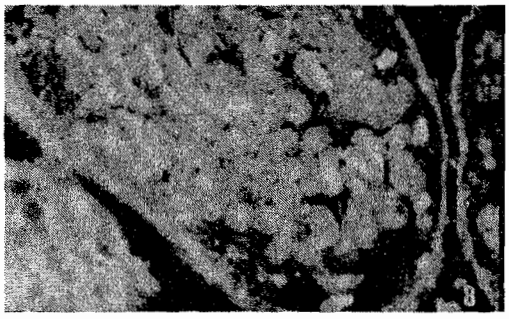

Fig. 8 *Some male cells (arrowed) showing different types of cell x 100.*

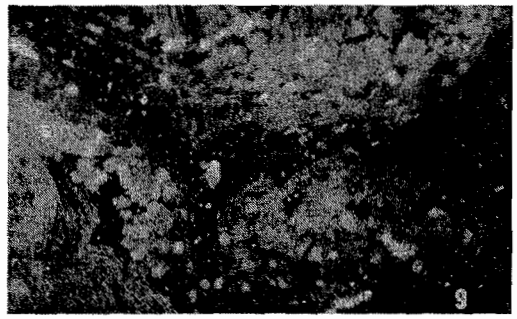

Fig.9 *Fully mature sperm in acinus x 150. (sm: spermatozoon)*

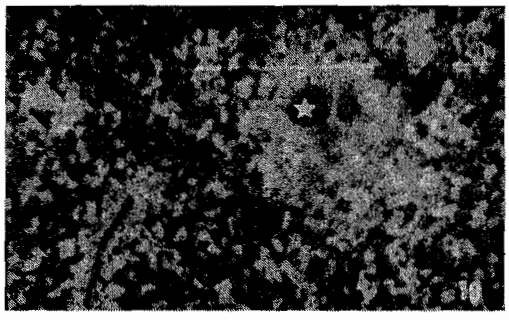

Fig.10 *Albumen gland follicle. Star indicates region of accommodation of secretary material x 50.*

So far as the accessory reproductive organs are concerned, those of interest are the albumen, prostrate and oviductal glands. Again, their histological development could not be correlated with diet but they could all be divided into the synthesis/accumulation phase and the secretion phase. At the synthesis/accumulation phase, secretory products e.g., the galactogen of the albumen gland are synthesized and packed into granules which accumulate in the cytoplasm. At the secretory phase, the secretory products are released (Fig.10).

Table 2 shows the inter-group comparisons of the weights of the accessory reproductive organs.

TABLE 2

Inter-group comparison of the accessory reproductive system by Student's (t-test)

6 Months	**Group A x Group B**	**Group A x Group C**	**Group B x Group C**
Ovotestis duct	S	S	NS
Albumen gland	S	S	S
Common duct	S	S	NS
Vas deferens and penis	S	S	NS
Oviduct, vagina, gametolyric gland	NS	NS	NS
12 MONTHS			
Ovotestis duct	NS	NS	NS
Albumen gland	S	S	NS
Common duct	NS	S	S
Vas deferens and penis	NS	S	S
Oviduct, vagina, gametolyric gland	NS	NS	NS

NS Non-significant at 5%
S Significant at 5%

TABLE 3
Egg production in different groups of snail

Age (Months)	Group A	Group B	Group C
5	0	0	35,45
6	0	28,31	103,52
7	32	155,162	113,110
8	62	100	198,27
9	45	98,212	0
10	201	48,53,112	0
11	100,105	0	145
12	83,81	78,73	109,68
13	0	43,48	45
14	0	51	53
15**	49	33,113	50,52,54
Total No. Eggs	58	1491	1338
Eggs/Clutch	84	83	84
Eggs/Month	69	136	122

**2 snails from Group B died and 1 from Group C also died
Data collection was therefore discontinued Eggs/clutch and Eggs/month are averages

Egg production: The age at which a snail laid its first clutch of eggs was taken as the onset of sexual maturity. This varied for the different groups fed on the different diets. In the 21st week, Group C snails laid their first batch of eggs while Group B (those fed on the artificial diet) laid their first batch in the 25th week. Group A snails laid their first batch in the 27th week. Table 3 shows the results of egg laying for 4 specimens in each of the 3 groups. No significant difference was found between the average clutch of eggs but there was significant difference in the clutch size per month between Group A and B and then A and C (One way analysis of variance p = 5%). This seems to suggest that physiological factors controlling clutch size may be different from monthly egg laying rate. Monthly egg laying rate may be sensitive to the diet supplied.

Discussion

Most reports of the factors affecting growth and reproduction of terrestrial pulmonates concern temperate species such as *Helix*; for instance the importance of photoperiod (Stephens and Stephens, 1966); density (Dan and Bailey, 1982): calcium (Crowell, 1973), soil (Gomot *et al.*, 1989) and temperature (Gomot, 1990). But for the work of Hodasi (1982) corresponding report in the Achatindae are lacking.

The effect of only one factor (i.e., food) was investigated in this study and it was realised that it has profound effects. Studies on nutrition in Achatinidae have included among others; feeding in the wild (Ajayi *et al.*, 1978: *Archachatina achatina*), olfactory orientation to food (Croll and Chase, 1980: *Achatina fulica*) and food selection in the laboratory (Egonmwan, 1991: *Limicolaria flammea*).

A. fulica accepts a wide range of food including horse meat (Weel, 1959). Diet has an influence on the growth and sexual maturation of *A. fulica* and the earliest age that a snail could lay eggs was at 5 months.

The results of the present study, though inferential, suggests *A. fulica* may require a wide range of nutrients for optimal growth. It may be that the nutrient composition of diets complement one another in supplying the nutritional requirements of the snails. Group C snails showed the highest growth and the diet was vegetable, fruit and artificial diet which contain more nutrients compared with vegetable-fruit diet (Group A) or artificial diet (Group B). The ability of an organism to eat two or more foods in the proportions which results in an intake of nutrients which is better balanced than that gained by eating any one of these foods alone has been reported in *Limicolaria flammea* (Egonmwan, 1991). It appears also that complex interactions between variable range of nutrients

and assimilation efficiency may be operating to effect the different growth rates observed in the snails. However, until a more systematic approach has been employed to elucidate the nutrient requirements of giant African snails, statements about dietary requirements of giant African Snails must be interpreted with caution.

The investigations have also demonstrated that differences in diet can be reflected in the gross morphological changes in the reproductive system. Not much is known about the environmental factors that may influence the development of the reproductive system in hermaphrodites. When *Limax maximus* was subjected to artificial photoperiod consisting of long or short days, female phase maturation was not found to be significantly affected by photoperiod. In contrast, male phase maturation appeared to be induced by a short-day to a long day transition. (Sokolove &McCrone, 1978).

In this study, oocytes were generally seen in far fewer numbers than the male cells. There was no clear-cut correlation between any of the reproductive structures and the diet supplied.

For the time being, it may just be enough to state that the differences in diet were rather manifested on the gross fresh weight of the reproductive organs.

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