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Original Research Article

Study of Sexual Maturation in Snail *Achatina Fulica* in Breeding Environment

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Abstract

Achatina fulica snails from two environments (natural and breeding) were subjected to two diets, one based on green fodder and the other in the form of flour concentrate, respectively, in order to assess their impacts on the development of gametes. 50 snails were dissected, with 25 from the natural environment and 25 from the breeding environment. Subsequently, the extraction of gonad (ovotestis) was performed on each of the selected animals. After inclusion, histological sections were made to assess the degree of evolution of gonad according to development scales. This allowed us to identify the different stages of sexual development, sexual maturation per size class according to diets.

Keywords: Gametes, snails, breeding, growth, molluscs, ovotestis, gonad.

INTRODUCTION

In Ivory Coast, studies on snails relate to research works on the characterization of the flesh of the *Limicolaria flammea snail* [1], the breeding of edible giant snails from Africa. Otchoumou *et al*., [2] the influence of animal density on the growth and reproduction of the *Limicolaria flammea* snail [3] and the impact of dietary calcium content on the growth performance of *Achatina Achatina* [4, 5].

With a view to carry out successfull breeding of snails, it is therefore necessary to intensify this research on the influence of other parameters on the performance of snails.

It is within this framework that we decided to conduct a study aimed at studying the influence of diet (living environment) on the growth and the development of gamete for *Achatina fulica* snail [16].

The general objective of this study is the fight for food security and the promotion of mini-livestock farming. Specifically, it aims to study the influence of leaves and concentrate food but also certain factors (biotic and abiotic) on the growth and the development of gametes in the *Achatina fulica* snail [16].

Study Area

The study took place in the area of Abidjan (Ivory Coast) precisely in the district of Abobo. This work began in early June and ended in November 2015.

This center includes a shelter-based building where breeding is under shelter and an outdoor experimentation area. The average monthly relative temperature and humidity in the livestock building were 26.7 ± 1.4 ° C and 82.6 ± 1.4%, respectively. The photoperiod was 12 hours of light and 12 hours of darkness (Figure-1).

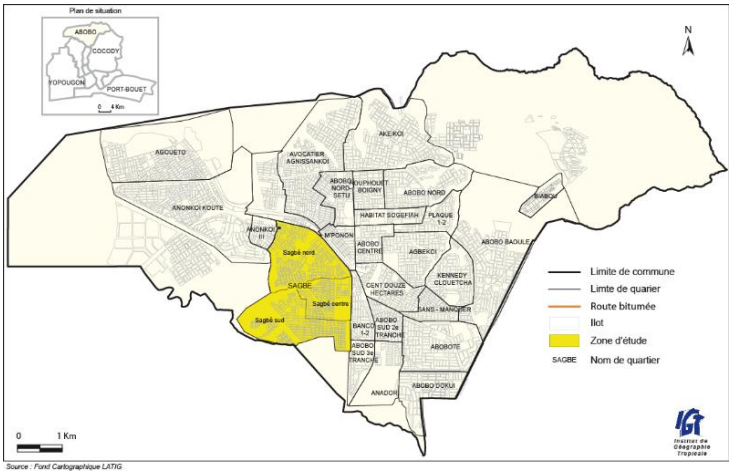

Fig-1: Study area

Animals

The animals used in this work are Molluscs, Gastropods, Pulmonates. They belong to the order of Stylommatophores, the Super family of Achatinaceae, the family of Achatinidae, the genus Achatina and the *Achatina Fulica* species Bowdich, 1720.

Fig-2: *Achatina Fulica* species

Breeding Enclosures

The snails were raised in plastic bins of 0.66 m long, 0.6 cm wide and 0.2 m high, giving a base area of about 0.4 m2 and a volume of 0.08 m3. The enclosures are equipped with a mosquito net lid type making a leak-proof facility. Their bottom is covered with potting soil at a height of 4 cm thickness.

Fig-3: Breeding Enclosures

METHODS

240 snails, of which 120 from the natural environment and 120 from the breeding environment were used in this study. Snails from the natural environment (fed with green fodder) were picked up in the scrub around housings and garbage dumps. As for the snails from the breeding environment, they come from a breeding farm and are fed with a concentrate food formulated under the basis of the work of [6].

These animals from the natural environment and the breeding environment were subsequently broken down in 5 repetitions per diet depending on the length of their shell: [3-4 cm]; [4.1-5 cm]; [5.1 - 6 cm]; [6.1 - 8 cm]; [8.1 - 10 cm]. And afterwards, we carried out the weighting of the snails in each class of length according to each environment (natural and breeding) in order to compare the evolution of various parameters.

The green forage plant diet was made up of *lactuca sativa* (Asteraceae), *carica papaya* (Caricacea), *Brassica oleracea* (Brassicaceae), *Cecropia peltata* (Moracae), *Laportea aestuans* (Urticaceae) and *Phaulopsis falcisepala* (Acanthaceae) leaves [7, 8].

Food is weighed before being served to the animals every two day. At the end of the two days, the feed refusals are weighed and the feeders cleanly washed before being reused.

For each food served, a 100 g control is placed under the same experimental conditions in bins containing no animals. The weighing of these control feeds at the same time as the food refusals, makes it possible to correct the weight due to the desiccation for the green fodder plant diet and to the hydration for the flour concentrate diets.

Table-1: Percentage composition (g/100g) of flour concentrate diet

Corn	Soybean Meal	Soja bean	Dicalcium phosphate	Vitamin	Calcium Carbonate	NaCl	Trace elements	Remolding soft wheat
19,3	16	16	4	0.5	28.7	0.4	0.10	15

Table-2: Characteristics (in% MS) of the various ingredients of compound feed

Gross Energy cal/g	Nitrogenous material total	Calcium total	Phosphate total	Fat	Amidon	Free sugar	Free cellulose	Ash	Total
2785	17.48	12.02	1.2	4.71	12.56	03.10	4.76	33.43	100

Table-3: Plant-based diet components

Components (g)	Diets
Carica papaya	16.66
Lactuca sativa	16.66
Brassica oleracea	16.66
Cecropia peltata	16.66
Phaulopsis falcisepala	16.66
Laportea aestuans	16.66
Total (g)	**100**

Table-4: Composition of four plants determined by chemical analysis

	Sample Weight	Dry	Protein matter	Lipids Total	Mineral	Gross energy substances in cal/g
Lactuca sativa	45.12	88.42	22.28	1.26	1.1	4.195
Brassica oleracea	34.01	91.5	26.36	2.11	10.8	3.91
Laportea aestuans	48.84	94.13	32.22	4.37	23.71	3.644
Phaulopsis falcisepala	28.20	93.47	23.52	3.51	21.85	3.329
Xanthosoma mafafa	36.57	90.24	28.24	5.06	18.36	4.254
Carica papaya	56.47	92.48	27.69	4.08	13.58	3.588
Cecropia Feltata	38.18	85.25	21.46	3.19	13.58	1.048

Histology of the gonads

The technique used falls within classical histology according to [9].

Dissection, Extraction and Fixation of Gonads

It should be noted that snails from the natural and breeding environment were used for this histological study. In this regard, 5 individuals from different classes of shell length are randomly selected and dissected. A total of 50 snails were dissected, with 25 from the natural environment and 25 from the breeding environment. Subsequently, the extraction of the gonad (ovotestis) was performed on each of the selected animals. Only a small fragment of about 0.1 to 0.4 g of this organ was extracted followed by fixation in 5% formalin for 3 to 5 days.

Inclusion

The principle of inclusion consists of treating the parts in an order determined by different solvents so as to penetrate into a tissue originally hydrated, a substance that is often hydrophobic which will keep its elements in place during the section.

Indeed, before inclusion, the part is perfectly dehydrated and this step consists of immersing the part in two baths of 95° alcohol and 3 baths in 100 ° alcohol for 30 minutes each.

Then, the organs are immersed in three successive 15 min baths of toluene. Finally, make paraffin baths that are done in the oven between 56 and 58 ° C. To do this, three paraffin baths are performed. The first two have a duration of one hour each, and the last one lasts all night. Finally, do the blocking. Thus, the organs are taken out from the last bath of paraffin and transported in a mold made up of a capsule filled with new melted and previously filtered paraffin

Sections and Coloring

The 5 µm sections are made with a microtome. After having removed the paraffin in the 100 °, 95 ° and 75 ° alcohol baths and rinsed with distilled water, the actual coloration with the hemalum-Eosin is carried out. The blades are immersed in the hemalum for one minute, rinsed with water for 2 minutes and immersed in eosin for one minute. The sections are then dehydrated by two alcohol baths of 70 °, 95 ° and 100 ° and the marks of alcohol are then removed by two baths of toluene.

Assembly and Observation

Once the blade is removed from the last toluene bath, a drop of canada balm is added to the preparation. A slice is then deposited and the whole is put in the oven for 48 hours and the observations are subsequently made by light microscopy.

Microscopic Analysis

Depending on the degree of evolution in Achatina fulica, development scales have been identified to determine the different stages of gametogenesis. The scale used in this study corresponds to that adopted by [10]**.**

Indeed, *Achatina fulica* being a protandrous hermaphrodite animal, the better it was possible to first detect male gametes in the ovotestis at early stages and then female gametes. This is why, during our study, depending on the environment, we were determined to observe all the stages of these evolution of gametes in ovotestis sections.

Sexual Cycle

After the identification of the different sexual stages of development, sexual maturation by size class will be established for the animals fed on concentrates and those fed on leaves. A class-by-class comparison of the two groups of individuals fed differently will be carried out Thus, the classification of gonad is as follows:

A→ Beginning of maturation: phase of sexual rest, acini are therefore reduced to islets of quiescent gonies.
B→ Maturation: the gonies multiply by successive mitosis. Acini are poorly developed in number and volume in the connective tissue. The side walls of the tubules are lined with primary cells which have a large nucleus and have a scanty cytoplasm.
C→ Advanced maturation: the cells remain adherent to the side wall of the acini but enter into growth, this corresponds to the phase of vitellogenesis. As a result of the increase in size of the acini, the connective tissue has almost disappeared.
D→ Maturity: the acini are then completely filled with mature oocytes (with a relatively homogeneous size), which have a distinct nucleus and sometimes even the very visible nucleolus. The sperms abound and form packets in the light of the follicles.

RESULTS

Histology in *Achatina Fulica*

DESCRIPTION OF THE STAGE OF DEVELOPMENT OF GONAD

To better monitor the evolution of gametogenesis, we carried out a histological study of the gonads. Thus, according to the different length classes established as well as the original environment, we proposed that the cycle of gametogenesis could be divided into 4 stages in the male and in the female as well:

Stage 1: Beginning of maturation: phase of sexual rest, acini are therefore reduced to islets of quiescent gonies.

Stage 2: Maturation: the gonies multiply by successive mitosis. Acini are poorly developed in number and volume in the connective tissue. The side walls of the tubules are lined with primary cells which have a large nucleus and have a scanty cytoplasm.

Stage 3: Advanced maturation: the cells remain adherent to the side wall of the acini but enter into growth, this corresponds to the phase of vitellogenesis. As a result of the increase in size of the acini, the connective tissue has almost disappeared.

Stage 4: Maturity: the acini are then completely filled with mature oocytes (with a relatively homogeneous size), which present a very distinct nucleus and sometimes even the very visible nucleolus. The sperms abound and form packets in the light of the follicles.

HISTOLOGY OF SNAILS FROM THE NATUREL ENVIRONMENT

C1 (class 1): corresponds to the first class whose shell lengths are between 3-4 cm. Thus, the observation of the section reveals acini (ac) reduced in quiescent islets, then demonstrating the beginning of maturation or phase of sexual immaturation.

C2 (class 2): at the level of this second class whose lengths are between 4.1-5 cm, we observe a phase of gonies development to give spermatogonia (Sg) and some clearly visible spermacytes (Sc). This is the maturation phase.

C3 (class 3): this class is composed of individuals whose length is between 5.1-6 cm and observations show that these individuals are therefore in an advanced maturation phase with the development of spermatids (St) and ovogonies (Og) which are grouped on the side walls.

C4-C5 (class 4 and 5): individuals with a length between 6.1-8 cm and 8.1-10 are therefore mature. Spermatozoa (**Sz**) can be seen all around the mature oocytes (Ov mat) and then ready to fertilize them (Figure-4).

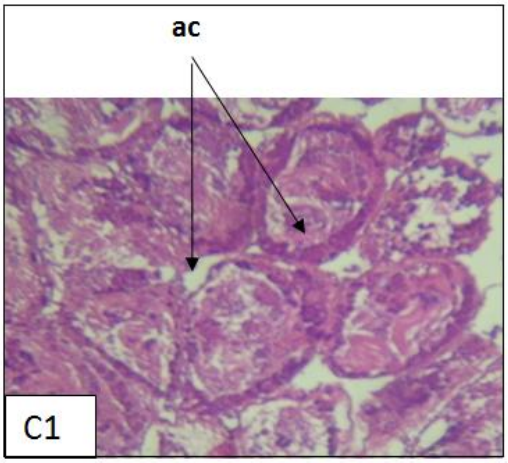

Fig-4a: Stage1: early maturation or phase of sexual
ac: acini
G × 40

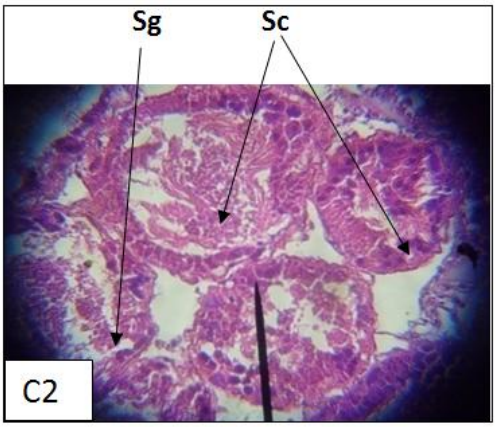

Fig-4b: Stage 2: maturation phase, development gonia
Sg: spermatogonia**; Sc:** spermatocyte
G × 40

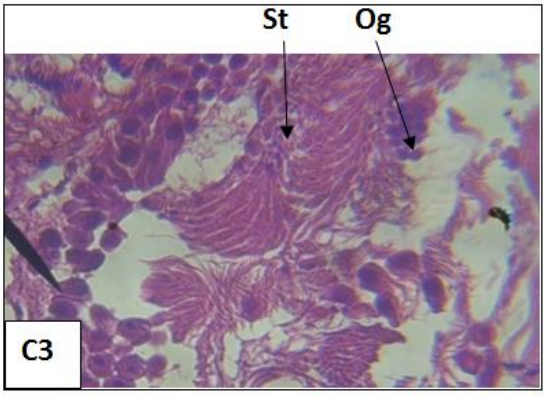

Fig-4c: Stage 3: advanced maturation phase with development of spermatids
St: spermatide**; Og:** ovogony
G × 40

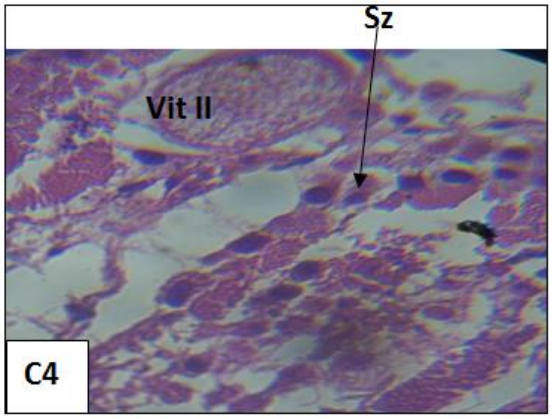

Fig-4d: Stage 4: mature spermatozoa and oocytes
Sz: spermatozoon**, Vit:** vitellogenesis
G × 40

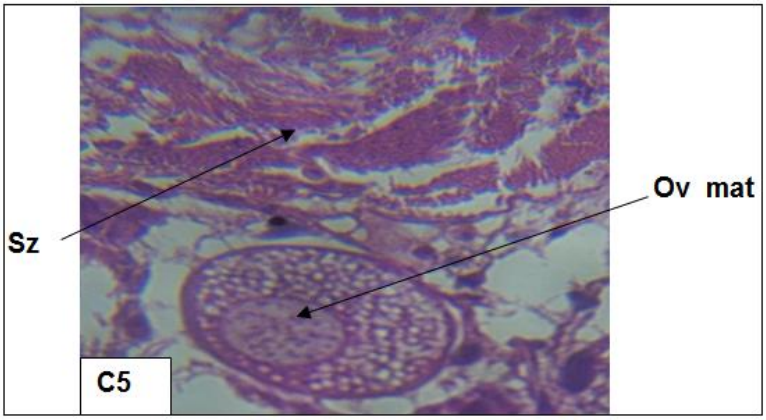

Fig-4d: Stage 5: mature spermatozoa and oocytes
Sz: spermatozoon**; Ov mat:** mature oocyte
G × 40

Figure-4 Histological sections showing gametogenesis according to classes in natural environment.

HISTOLOGY OF SNAILS FROM THE BREEDING ENVIRONMENT

C1 (class 1): in this class (3-4cm), the sections show us a stage of early maturation of male gametes. We then see the Spermatogonia, which are well differentiated.

C2 (class 2): the observations show us here that the individuals of this class (4.1-5cm) are at a more advanced stage in gametogenesis. In fact, we observe

both the development of spermatocytes and spermatids as well as that of the ovogonies that begin their growth.

C3-C4 (class 3 and 4): in these two classes (5.1-6cm and 6.1-8cm), gametogenesis is at an advanced stage of maturity with spermatozoa and oocytes II which continue their growth.

C5 (class 5): this class (8.1-10 cm) is characterized by a phase of the maturity of gametes. Thus, the oocytes mature and the spermatozoa are distributed at the level of the light (Figure-5).

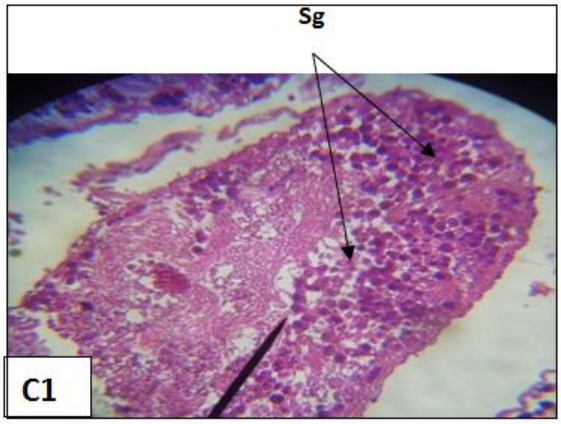

Fig-5a: Stage 1: mature spermatozoa and oocytes
Sg: spermatogonia
G × 40

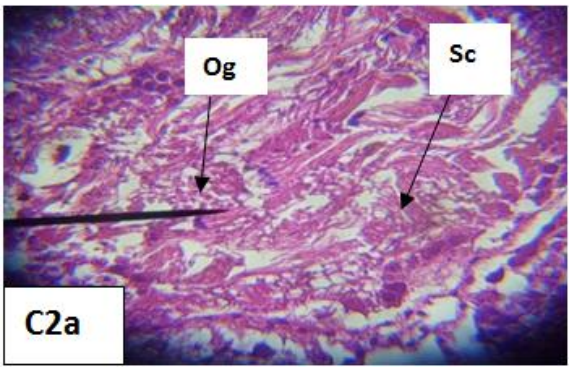

Fig-5b: Stage 2: development of spermatocytes, spermatids and ovogonies
Sc: spermatocyte **Og**: ovogony
G × 40

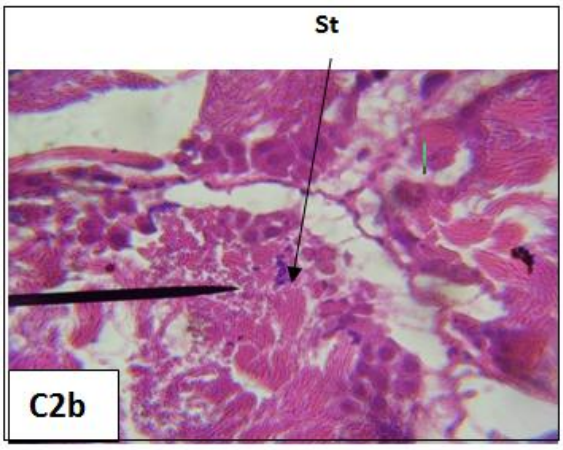

Fig-5c: Stage 3: development of spermatocytes, spermatids and ovogonies
St: spermatide
G × 40

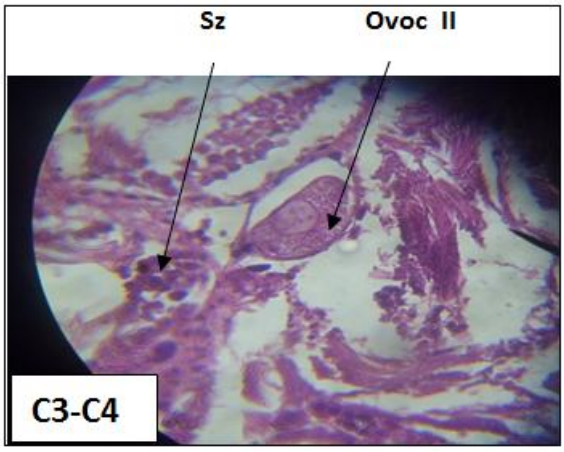

Fig-5d: Stage 4: advanced stage of maturite
Sz: spermatozoon; **Ovo**: oocyte
G × 40

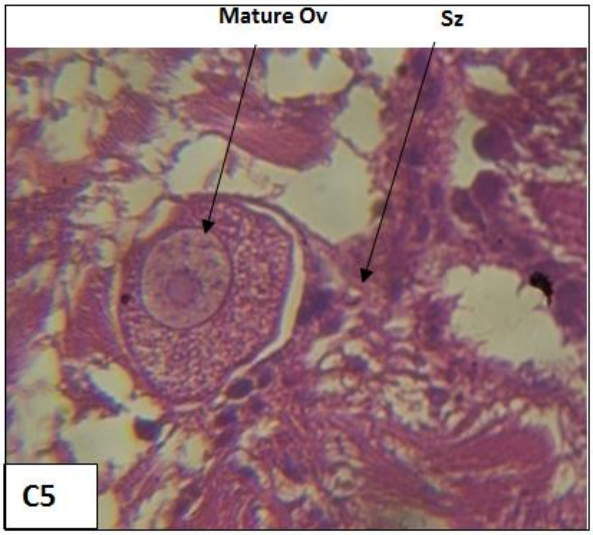

Fig-5e: Stage 5: maturity phase of the gametes
Sz: spermatozoon; **Ov mat**: mature oocyte

Figure-5 Histological sections showing gametogenesis according to classes in breeding environment.

DISCUSSION

Our results show that gametogenesis depends on the physiological stage, the living environment and also the diet. In fact, during this gametogenesis four stages of development were observed in *Achatina fulica* from both environments. However, for *Achatina fulica* from the natural environment (fed on the leaves), we notice that the maturation of gametes is less rapid than that of *Achatina fulica* from the breeding environment (fed on concentrate).

Our results are therefore not in line with those of [11] who found after their research that Achatina fulica had a good yield at the level of the maturation of gametes when this species was feeds on leaves and on fruits in the breeding environment. Thus the mismatch of the results was thought to be due to the natural environment in which lived our snails got from collection. Because, several factors (biotic and abiotic) can indeed influence the development of gamete in the snails. Among these factors include the substrate and the food. The substrate is for the snail a place of life, supply in nutrients and make it possible for a good growth.

It may also contain factors stimulating growth and the development of gamete [12]. Our snails from the natural environment have been collected on sandy soils and in areas devoid of herbs, and indeed justify the weak development of gametes. As for the speed of the maturation of gamete in Achatina fulica from the breeding environment, the early maturation would probably be due to a good breeding environment and the food. As factors such as air humidity, temperature and photoperiod are not to be neglected for a good management of a snail breeding. A low relative humidity causes estivation in the snail, a phenomenon that causes growth disorders [13]

Also according to [14], a too high temperature would lead to conditions that are not in favour of the growth and reproduction of snails. However, the most important factor for good growth is food. All this better support our results, as the food is balanced and the other factors are well monitered. In fact, in achatiniculture, the formation of the egg and its envelopes, the weight and shell growths are not only closely related to the content of certain nutrients such as proteins and calcium, but also to the type and shape of the food [15]. Thus, the poor maturation of gamete in Achatina fulica from the natural environment can be justified by the poor minerals in soil, but also by the absence of plants more appetizing for snails.

CONCLUSION

At the end of our study, we notice that Achatina fulica puts on weight less than it grows in length, whether in the natural environment or in the breeding environment where snails are fed on concentrate.

Also, the study of gametogenesis revealed four stages of development that are influenced by various biotic and abiotic factors as well. However, we acknowledge that sexual maturity is early in the breeding environment where Achatina fulica is fed on concentrate compared to that of Achatina fulica from the natural environment, where the animals consume only leaves in a vegetation where soils are often very poor.

Thus, given the importance and high nutritional value of snails, particularly for the Achatina fulica species, it is therefore essential to develop the "Achatiniculture" in Côte d'Ivoire but also elsewhere in Africa. It is suitable to sensitized the famers on feed formulation more rich in nutrients for good productivity and, therefore, for good quality meat. All this would contribute to food security among people in sub-Saharan Africa. This study is indeed a contribution to the study of Achatinidae. However, for more knowledge on the growth and maturation of these snails, we propose for future research to:

- Conduct a comparative study of the growth of Achatina fulica with other species of Achatinidae and if possible with European species;
- Study the impact of a coexistence in Achatina fulica breeding with other Achatinidae on the growth and on the development of gamete in Achatina fulica;
- Study the impact of other food concentrates on the growth and the development of gamete in Achatina fulica.

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