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Study of the reproduction of *Archachatina ventricosa* in the National Center of Floristic (NCF) of the Félix Houphouët-Boigny University of Abidjan (Côte d'Ivoire)

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Abstract

The reproduction study of the snail *Archachatina ventricosa* took place at the National Center of Floristic (NCF) from January 2017 to December 2018 for two years. All the different plots of the NCF were searched monthly according to a chronogram to look for snails in mating. In addition, dissections were carried out each month on 15 snails to detect ovigerous snails and the average number of eggs per snail. Then histological sections were made on 180 individuals of this same species for the study of gametic maturation. The results of this study made it possible to locate the reproduction period of this snail in a natural environment. This period extends from March to December with high mating frequencies between the months of July and August. a total of five ovigerous snails were harvested with 33 eggs, an average of 6.6 ± 1.52 eggs per snail. Mating is for individuals larger than 6 cm. The study of gametic maturation revealed that gametogenesis begins at 6 cm by spermatogenesis and has five stages of maturation. Sexual maturity is reached from 7 cm in length, which corresponds to 22 months of age.From the results of this study, it can be suggested that the collect of these snails when they measure more than 9 cm.

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Introduction

In sub-Saharan Africa hunting or gathering of animals in their natural habitat continues to be an important source of animal protein for consumption. Among the most consumed animals are some Achatinidae commonly known as African giant snails (Ebenso, 2002). In Côte d'Ivoire, for example, the snail Archachatina ventricosa and Achatina achatina are the most widely consumed snail varieties (Kouassi *et al.,* 2008; Amani *et al.,* 2016). The consequence of this important gathering of these animals is the drastic reduction of their natural stock causing an imbalance in the forest ecosystems because these animals play several important roles in several food webs. In addition, the degradation of ecosystems, the expansion of cultivated lands and the reduction of forest areas pose a threat to the survival and sustainability of these snails in their natural habitat. Thus, for better stock management of these animals and to ensure their conservation, it is necessary to popularize their breeding. In this sense, several studies concerning their growth (Bouye *et al.,* 2013; Karamoko *et al.,* 2015; Bouye *et al.,* 2017); their nutrition (Otchoumou *et al.,* 2004) and their density (Karamoko *et al.,* 2011) were carried out in breeding. However, knowledge of the biology of these species in their natural environment seems to be a prerequisite for their domestication.

The National Center of Floristic at Félix Houphouët-Boigny University, the second largest urban forest in Abidjan after the Banco National Park forest, hosts to several species of land snails including *Archachatina ventricosa* (N'dri *etal.*, 2019). These molluscs help to strengthen the center's role in biodiversity conservation.

The objective of this work is to study the reproduction of the snail *Archachatina ventricosa* living in a natural area (the national center of floristry of the University Felix Houphouet-Boigny of Abidjan). It will allow specifically to determine the reproductive period of this snail in the wild, to determine the size of maturity and to follow the evolution of gametic maturation to determine the different stages of gametic maturation and the age of maturity of this snail in the natural area.

Material and methods

The National Center of Floristics (NCF) is located in the University Felix Houphouet Boigny (UFHB) of Abidjan. It is located between 05 ° 19'N and 04 ° 01'W. It is traversed internal and peripheral alleys delimiting several thematic plots. (Kouamé, 2013) (Fig.1).

The climate of the NCF is that of Abidjan which Guinean type is. Indeed, it is characterized by four seasons differentiated by their rainfall regime: the great rainy season which starts from March to July with a maximum of precipitation in June and the small rainy season which covers the months of September, October, November and December with a high of precipitation in October. These two wet seasons are separated by a short dry season that covers only the month of August and a long dry season that runs from January to February. The temperatures of the city of Abidjan are relatively mild. They vary from 24.54 ± 0.3 ° C for the month of August considered the coldest month to 28.11 ± 0.52 ° C for the month of April considered as the hottest month.

The maximum value of the recorded temperature is 35.5 ° C. As for precipitation, they give an average of 126.9 ± 55.76 mm of rain per month. The wettest month is June with an average of 415.1 ± 22.8 mm. The month of January remains generally the driest with an average of 16.8 ± 4.49 mm. The relative humidity of the ambient air remains high throughout the year because of the distribution of rainfall over the months. It ranges from 82 ± 0.84% (January) to 91.7 ± 2.19% (August) with an average of 87.2 ± 2.75%.
www.tutiempo.net/en/climate/Abidjan/655780.htm)

The NCF's vegetation is dominated by dense moist evergreen forest. It is characterized by the presence of two species: *Turraeanthus africanus* (Meliaceae) and *Heisteria parvifoliaSm* of the family Olacaceae.

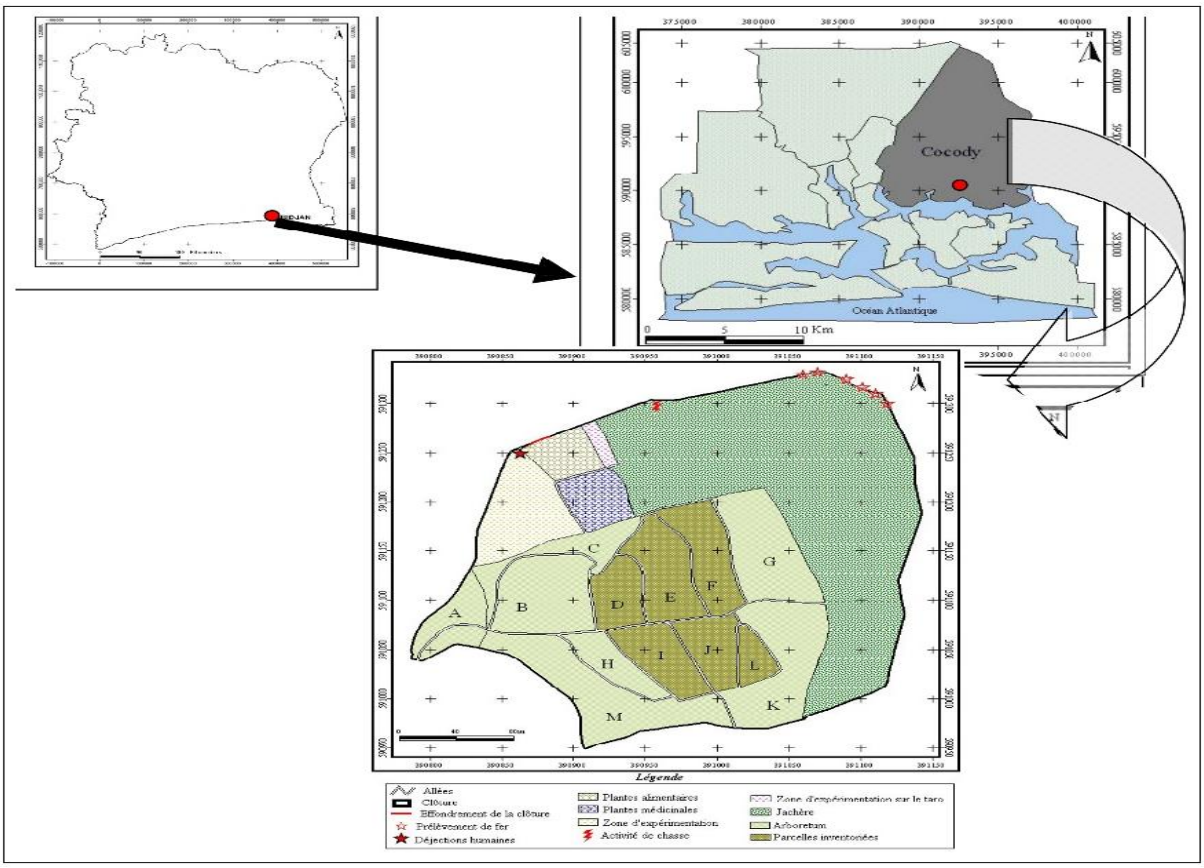


Fig. 1. Location of the National Center of Floristic (NCF) within the Félix Houphouët-Boigny University (Kpangui, 2009).

The NCF is also populated with savanna species and species from other ecological zones that are more discreet. The arboretum of the botanical garden houses a large collection of plants estimated at 750 species belonging to the Ivorian flora, as well as that of the subregion. In addition, the NCF has a herbarium that contains about 19,500 samples of Ivorian and West African flora species.

This study took place for (02) years (from January 2017 to December 2018). The NCF forest has two parts: the arboretum and fallow. The arboretum is divided into 11 plots by alleys serving as botanical trails. As for the fallow land, it is divided into four parcels marked J1, J2, J3 and J4.

Within each plot, two quadras whose total area is equal to 5% of the area of the parcel on which they are located have been delimited monthly using a dekameter. So every month, 30 quadras have been delimited and sampled. This rotation of the quadras made it possible to sample not only the totality of the area of the NCF but also the different strata that compose the NCF. These quadrats were searched by a team of four researchers to identify mating specimens. When specimens have been identified as mating, their shell length is measured using an electronic vernier caliper. The period (months) is also noted on cards provided for this purpose. Each month during the first 12 months (from January 2017 to December 2017), 15 potentially mature snails (shell length ≥ 6 cm) were captured and returned to the laboratory where they were washed and cleaned. Then they were sacrificed, dissected and the uteri examined to detect and count any eggs. This made it possible to determine ovigerous snails and the average number of eggs per uterus. Mating, potential fecundity, which is the presence or absence of eggs in the uterus and the number of eggs per uterus, were used to estimate "macro reproduction" (macroscopic scale of sexual maturity). As part of the study of gametic maturation (microscopic scale of sexual

maturity), 120 specimens of *Archachatina ventricosa* were collected. Then they were grouped into six size classes according to their shell length (Table I). Each class has 20 individuals. Each specimen is weighed using an electronic scale. These snails were subsequently transported to the laboratory where they are washed, cleaned, dissected. The ovotestis were collected and then classified according to the established order. They are then stored in cassettes, then preserved in 70 degrees formaldehyde before being transported to the laboratory for the realization of histological sections. The technique used is classic histology according to Martoja and Martoja-Pearson (1967). The observation of the sections was made using a sound microscope equipped with a video camera.

Results

Study of Archachatina ventricosa reproduction on a macroscopic scale

The graph in Fig. 2 shows the evolution of mating between specimens of *Archachatina ventricosa*during the two years of sampling. The evolution of mating is similar in both years. The curve indicates that mating starts between February and March and runs until December of the same year. The analysis of this curve makes it possible to distinguish three periods.

Table 1. Characteristics of snails used for gametic maturation.

Species	*Archachatina ventricosa*					
Size class of snails (cm)	[5 – 5.9]	[6 – 6.9]	[7 – 7.9]	[8 – 8.9]	[9 – 9.9]	[10 – 10.9]
Number of snail	20	20	20	20	20	20
Mean of seize (cm)	5.45 ± 0.10	6.49 ± 0.24	7.31 ± 0.21	8.30 ± 0.11	9.28 ± 0.18	10.13 ± 0.08
Mean of weight with shell (g)	27.25 ± 1.83	41.63 ± 4.25	64.31 ± 4.76	76.63 ± 8.88	112.83 ± 11.13	142.27 ± 16.26
Mean of weight without shell (g)	16.33 ± 0.73	27.51 ± 3.36	40.53 ± 3.73	49.80 ± 5.40	68.33 ± 6.32	77.32 ± 7.02

The first concerns, the months of February, March, April, May and June during which the number of matings changes slightly. The second concerns, the period from July to the end of December. This period is characterized by a sharp increase in breeding to reach peaks from August to October before falling sharply from the end of October. The third concerns,the months of January and February during which no mating is observed.

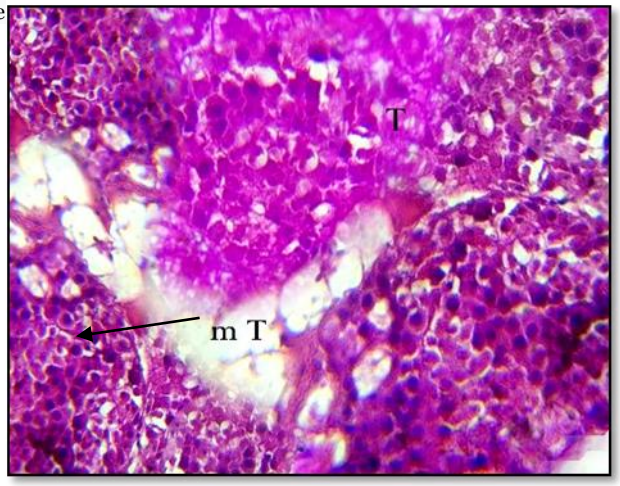
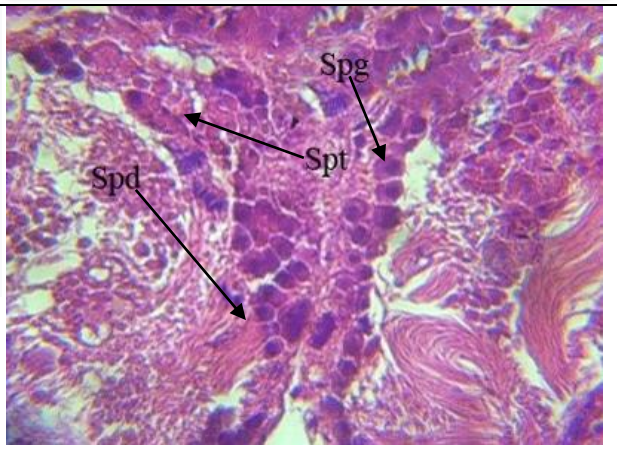
Table 2. Size classes of ovigerous snails.

Classes (cm)	[6 - 6,9]	[7 - 7,9]	[8 - 8,9]	[9 - 9,9]	[10 - 10,9]	Total
Nomber of ovigerous snails	0	1	3	1	0	5
Number of eggs collected	0	4	23	6	0	33
Mean of eggs collected	0	4	7,66 ± 1,11	6		6,6 ± 1,52

As for Fig. 3, it presents the proportions of the different size classes involved in matings in *Archachatina ventricosa* in the NCF. It appears that *Archachatina ventricosa* mates from 6 cm. However, mating occurs most often when the size of the specimens is between 8 and 10 cm. During the year 2017, 5 ovigerous snails were recorded. 1 snail in June, 2 in September and 2 others in December.

In total, 33 eggs of very heterogeneous sizes were collected. The average number of eggs per snail is 6.6 ± 1.52 eggs. Table 2 shows the different size classes of ovigerous snails. Snails with a size between 8 and 9 cm are the most ovigerous.

Table 3. Stage of gamete evolution of *Archachatina ventricosa*.

Stage of gamete development	Charateristics	Illustrations
The undifferentiated stage or stage 1	- connective tissue abundance undifferentiated germ cells - cell proliferation in the tubules - indeterminate sex (mT: microtubule)	
Stage early maturation or stage 2	Beginning of spermatogenesis with evolution of spermatogonia into spermatocytes and spermatids - No ovogony - Well developed tube with visibility of light (Spg: spermatogonia, Spt: spermatocyte, Spd: spermatide)	

Study of gametic maturation

The dissections performed in the different constituted classes made it possible to distinguish five stages in the gametic maturation of*Archachatina ventricosa*.The different stages of gametic maturation of*Archachatina ventricosa* with their characteristics and illustrations are presented in Tables 3, 4 and 5 below.

The undifferentiated stage or stage 1 or sexual rest phase

At this stage, there may be an abundance of connective tissue with an absence of tubules. This finding was made in individuals of the class [5cm - 5.9cm]. This stage is also observed in some specimens of the class [6cm - 6.9cm] which have a smaller amount of connective tissue compared to the previous class. In the latter, the presence of a large number of small tubules (microtubules) is observed with the appearance of a few cell proliferations in the tubules.

Stage early maturation or stage 2

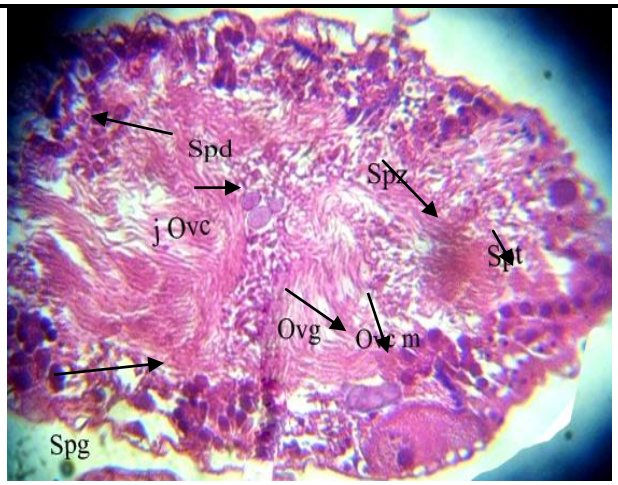
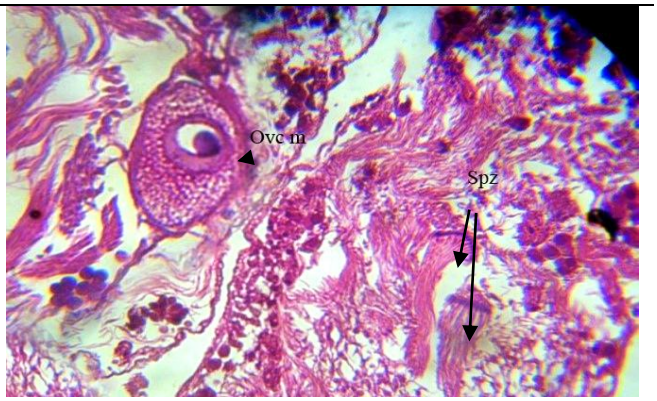
This stage is characterized by sections with spermatogonia (sperm stem cells), spermatocytes and spermatids with connective tissue regression. At this stage there is still no spermatozoa. Spermatogenesis or sperm formation process begins at this stage. Moreover, no ovogony is observable at this stage. The sections that showed this stage were obtained exclusively in individuals belonging to the class [6cm - 6.9cm].

Advanced ripening stage or stage 3

The sections made present on the one hand the presence of a large number of well-developed tubules, spermatogonia, spermatocytes I, spermatocytes II,

spermatids, and finally spermatozoid. Indeed, spermatogonia, stem cells of spermatozoid undergo several successive divisions to give spermatocytes I. These evolve to give Spermatocytes II, then spermatids and finally spermatozoid. Other sections have very abundant spermatozoa invading the tube lumen. For these cuts, spermatogenesis was completed. In addition, several immature oocytes and a few mature oocytes with a distinctive nucleus and a somewhat visible nucleolus can be observed. Some individuals of the class [6cm - 6.9cm] are at this stage of ripening, but most of the cuts with these characteristics come from the class [7cm - 7.9cm].

Table 4. Stage of gamete evolution of*Archachatina ventricosa* (continuation).

Stage of gamete development	Charateristics	Illustrations
Advanced ripening stage or stage 3	- Presence of all phases of spermatogenesis - Appearance of ovogonies that evolve to give oocytes with undifferentiated nucleus (Ovc: young oocyte, Ovc m: mature oocyte, Spz: spermatozoid)	
Stage of maturity or stage 4	- Several spermatozoa invading the tube lumen - Several mature oocytes (De Graaf's follicle) with clearly visible nucleolus -	

Stage of maturity or stage 4

This stage also presents all stages of spermatogenesis as the previous stage but with a larger number of spermatozoa present in the tube lumen. On the other hand, the end of ovogenesis is observed with several mature oocytes with a clearly visible nucleus and nucleolus. This stage is observed in specimens belonging to the class [7cm - 7.9cm].

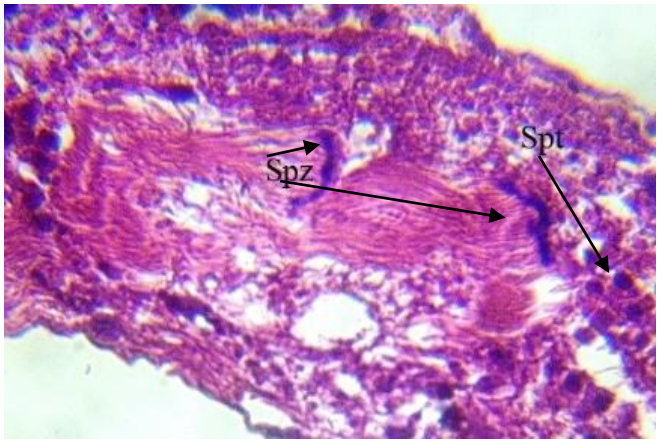
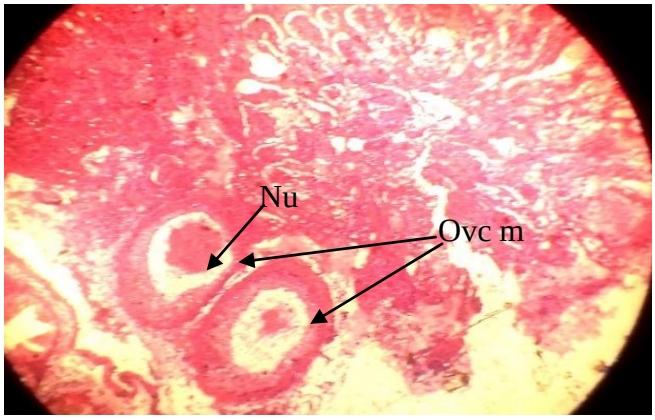
Stage of final maturity or stage 5

This stage is reached by individuals belonging to the size classes [8cm - 8.9cm], [9cm - 9.9cm] and [10cm - 10.9cm]. For the first two classes, the sections have acini which are completely filled with mature oocytes (with a relatively homogeneous size), which have a distinctive nucleus and a clearly visible nucleolus. Spermatozoa abound and form packets in the light of acini. We can also notice a regression of the feeder cells. For the last class, the sections show only a few mature oocytes and less abundant spermatozoa than previously. We are witnessing a total disappearance of the feeder cells.

Discussion

The study of the reproduction of *Archachatina ventricosa* in the National Center of Floristic (NCF) of Felix Houphouet-Boigny University in Abidjan was done in two phases. The first phase concerns macro reproduction, that is to say the study of the visible (macroscopic) aspect of the reproductive activity through mating and the detection of eggs in the reproductive system by dissection. In the NCF, mating takes place from March to December.

Table 5. Stage of gamete evolution of*Archachatina ventricosa* (continuation and end).

Stage of gamete development	Characteristics	Illustrations
Stage of final maturity or stage 5	- Spermatozoid grouped in clusters in the tube lumen and waiting for emission - Regression of feeder cells - All mature oocytes with clearly differentiated nucleus visible (Nu: Nucleolus)	 

This period includes the long rainy season, the short dry season and the short rainy season. Couplings are therefore preferably conducted during rainy seasons. Moreover, no mating is observed during the months of January and February which correspond to the long dry season. Several authors have reported similar results (Zongo, 1994 ; Kouassi *et al.,* 2008). For these authors, the rainy seasons are used by the snail to feed, move and, especially to reproduce. In addition, the number of matings is low during the first three months of the main rainy season (March, April and May). This could be explained by the fact that the snail comes out of an estivation period during which it stops feeding. When conditions become favorable, the snail would use the energy provided by the diet primarily to compensate for the energy lost during the estivation period and the development of its organism. Reproduction would therefore be in the background. Moreover, the analysis of the length of mating individuals indicates that *Archachatina ventricosa* begins to mate from 6 cm. This suggests that *Archachatina ventricosa* would be able to reproduce when it reaches 6 cm. However, the size classes [8cm - 8.9cm] cm and [9cm - 9.9cm] cm have the largest numbers, respectively 30.77% and 33.98% of the mating individuals. This would simply mean

that at these lengths, *Archachatina ventricosa* would be in full reproduction activity. In this case, avoid picking them. Similar results were obtained by N'da *et al.* (2004). Indeed, these authors have shown that with the exclusively vegetarian food, the sexual maturity of *Archachatina ventricosa* is between 8 and 10 cm in length. On the other hand, Amani *et al*., (2016) having obtained identical results, advocated that the collection of *Archachatina ventricosa* should be from 9 cm.

Moreover, the dissections made it possible to establish that in the NCF, from June to December *Archachatina ventricosa* is succeptible to carry eggs. This delay observed in our work goes beyond that proposed by Otchoumou *et al.* (1997). For these authors, laying on *Archachatina ventricosa* takes place from April to July. The extension of this period until December in our work would be related to the short duration of the short dry season (only the month of August). Clutches of *Archachatina ventricosa* would be related to climatic conditions. In other words, this snail could spawn in all seasons if the environmental conditions are mastered.

Analysis of ovigerous individuals indicates that the size class [8cm - 8.9cm] cm has the highest number of ovigerous individuals, with the highest average number of eggs. These results corroborate those previously expressed. This would mean that most of the reproduction of *Archachatina ventricosa* occurs when the individual has a size between 8cm and 9 cm. Individuals in the 9cm-9.9 cm size class have the eggs with the largest size. In fact, the average number of eggs per snail would decrease when the length of the snail increases but the size of the egg evolves in the same order as the size of the snail.

Examination of the histological sections in the different size classes, allowed us to discern five stages of maturity of the gonad in individuals of *Archachatina ventricosa* from the NCF. These are the stage of sexual resting or stage 1, the stage of early maturation or stage 2, the stage of advanced maturation or stage 3, the stage of maturity or stage 4 and the stage of final maturity or stage 5. Similar observations have been made byKouato *et al*., 2015in the bivalve mollusk *Cardium costatum*. Indeed, this author identified five stages during gamma maturation. However, the analysis of the different sections indicates that the gametic maturation in *Archachatina ventricosa* does not necessarily take into account the size of the specimens. Indeed, different stages of gonad maturation have been observed in the same size classes.

This could be because maturation would depend on age, not length. Indeed, in the natural environment, growth in length can be negatively influenced by certain factors such as stress, shell fractures, light (sunshine), the availability of food, the quality of the food. Under these conditions, older specimens could end up in a size class smaller than the size they should belong to. In addition, gametic maturation may be related to genetic predispositions. Indeed, some authors have noted that within the same population of individuals of the same age, some individuals may have early maturities. This finding was made in the rough triggerfish *Canthidermis maculata* by N'guessan *et al.* (2017); in Red mullet (*Mullus surmuletus* L., 1758) (Teleosts, Mullidae) by El Bakali *et al.* (2010). This could be the case with *Archachatina ventricosa*. In addition, histological sections reveal that sexual maturity is reached from 7 cm in length. This length corresponds after resolution of the Von Bertalanffy linear growth equation at 22 months of age. This age of sexual maturity is longer than that proposed by Kouassi *et al.* (2007). This author estimated the age of maturity of this snail at 18 months.

The delay of the maturity noted in our work could be related to the numerous disturbances undergone by Archachatina ventricosa in the NCF. Indeed, the stress caused by the many anthropic activities of the NCF (research activities, sightseeing, cleaning, etc.) could delay the growth and physiological development of this snail. This same observation was made by Kouassi *et al.* (2015).

Conclusion

The reproduction of the snail *Archachatina ventricosa* was used to determine the reproduction period and the maturity sizes of the species in the wild. It also identified five (5) stages of gametic maturation. This reproduction is relatively late in comparison with that observed by some authors. Moreover, this study has revealed that in the natural environment, different individuals do not reach maturity at the same size.

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