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Effects of pesticides on reproductive parameters in *hoplobatrachus occipitalis* (günther, 1858), an edible frog living in the drainage furrows of a banana plantation in the locality of dabou

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Abstract

Sexual maturity size, gonado-somatic index, sex ratio and fecundity were studied during four seasons, from January to December 2020, in *Hoplobatrachus occipitalis* which lives in the water of drainage furrows of banana plantations treated with phytosanitary products in the locality of Dabou. Our results reveal that the sizes of first sexual maturity in females and males of this species are 91.39 mm and 85.62 mm, respectively. Females reproduce during the main rainy season. The sex ratio is in favor of females (1:1.18) except during the main rainy season. Regarding fecundity, female gonads of this frog contain 2413 to 151 eggs with an average of 642 ± 541 and an oocyte diameter of 0.20 ± 0.03 mm. In this habitat, this frog has single oviposition (The coefficient of variation is 17.29).

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Introduction

Amphibians are poikilothermic animals with both lung and skin respiration (Morin, 2008) whose growth is directly related to environmental temperature. They represent the most threatened group of vertebrates on a global scale (Stuart *et al.*, 2008). They are recognized as very sensitive to any modification of their habitat. However, they are subject to many disturbing factors such as the outright destruction of water bodies, landscape fragmentation and various pollution (Blaustein and Kiesecker, 2002; Pellet, 2005).

In addition, Tavera-Mendoza *et al.* (2002a; 2002b), Orton *et al.* (2006) and Hayes *et al.* (2006a) report that atrazine, a herbicide used in various plantations, causes a decrease in gonad volume and hermaphroditism in *Xenopus leavis* larvae. Similarly, Qin *et al.* (2007) and Hayes *et al.* (2011) report that in the same species, feminization phenomena, sometimes complete, have been observed in the laboratory following exposure to herbicides or polychlorinated biphenyls. Therefore, we found it appropriate to study the effect of the use of herbicides and insecticides by the banana cultivation company (SCB) on the reproductive parameters of *Hoplobatrachus occipitalis,* a frog species of economic interest (Garner *et al.*, 2009; Mohneke *et al.*, 2011; Onadeko *et al.*, 2011; Akinyemi and Efenakpo, 2015; Tohe *et al.*, 2014; 2015) that lives in the water of banana plantation drainage furrows.

Materials and methods

Study area

The present work was carried out in the locality of Dabou in the south of Côte d'Ivoire, between 05°28'14"N latitude and 04°17'53"W longitude (Fig. 1).

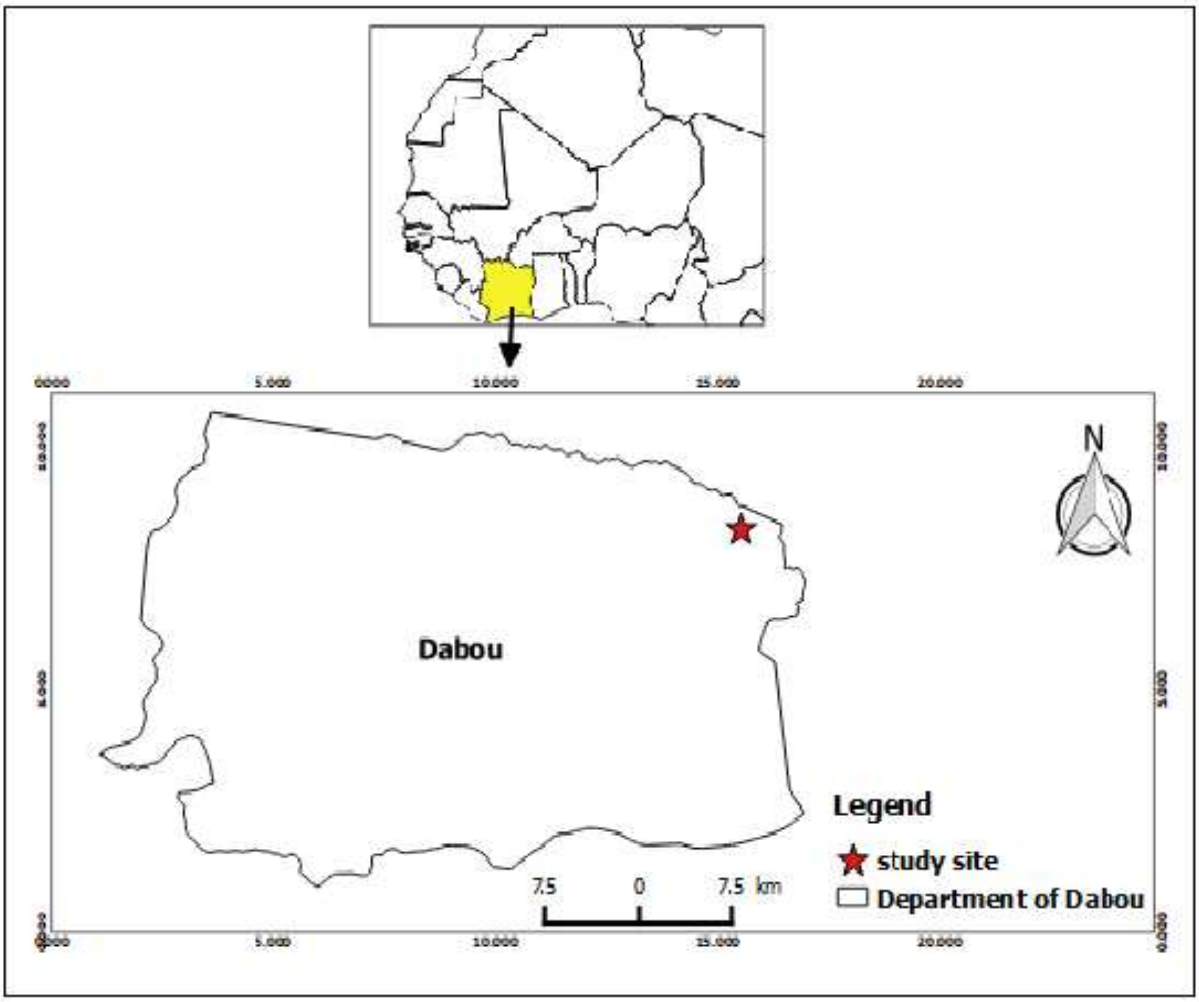

Fig. 1. Location of the sampling area in the locality of Dabou.

The vegetation is composed of banana plantations of « the Banana Culture Society (SCB) », rubber trees and oil palms. For the maintenance of these banana plantations, the SCB (Fig. 2) uses phytosanitary products (insecticides, herbicides). We accompany the hunters on the field and once the specimens of *Hoplobatrachus occipitalis* are captured with the forks, they are sold to us immediately. The dead specimens were immediately transported to the laboratory in aquariums.

Sampling

Sampling was carried out from January to December 2020 with two trips per season. In the laboratory, the length of the specimens was measured to the nearest millimeter; its total weight was determined to the nearest gram. After dissection using a dissection kit, the collected gonad was weighed to the nearest milligram along with the eviscerated weight of each specimen.

The gonad is then removed from its envelope and the oocytes are separated from each other with forceps and counted. The measurement of the diameter of the oocytes is done with a micrometer mounted on a binocular magnifying glass. After calibration, one end of the oocyte is made to coincide with the chosen mark of the micrometer and the whole surface of the egg is scanned. When this operation is well done, one millimeter on the field of the magnifying glass corresponds to a scan of one millimeter on the reading dial.

Size at first sexual maturity

The size of first sexual maturity (L50) is defined as the average standard length at which 50% of the individuals are capable of reproduction (Lévêque and Herbinet, 1980; Albaret, 1994; Légendre and Ecoutin, 1996). The formula for the size range is as follows:

$$Class\ interval = \frac{Maximum\ size - Minimum\ size}{Number\ of\ class} \quad (1)$$

Three categories of individuals can be distinguished according to size (Moreau, 1979) :

- L0 : size below which no individuals are mature;
- L50: size from which there are as many mature individuals as immature individuals;
- L100: size at which all individuals are mature.

Gonado-Somatic Index (GSI)

The Gonado-Somatic Index (GSI), which represents the gonad weight expressed as a percentage of the wet body mass, was estimated according to the method of west (1990) and Vazzoler (1996) as follows:

$$GSI = \frac{Gonad\ weight\ (g)}{Eviscerate\ weight\ (g)} \times 100 \quad (2)$$

Sex-ratio

The sex ratio (SR) refers to the proportion of the sexes (males and females) of a species in the given population. It can be expressed as a percentage of the number of males or females in the sampled population (Kartas et Quignard, 1984). Its formula is as follows :

$$SR = \frac{M}{M+F} \times 100 \quad (3)$$

M : number of males;

F : number of females

Fecundity and oocyte diameter of the specimens

The measurement was made on 30 selected oocytes per gonad. Absolute fecundity is obtained by counting all the oocytes actually present in the ovary with a manual particle counter (Stéquert and Ramcharrun, 1996).

Coefficient of variation

The coefficient of variation (CV) was used as à test of homogeneity. Its formula is the following:

$$CV = \frac{Standard\ deviation}{Mean} \times 100 \quad (4)$$

CV = Coefficient of variation

If CV < 2%, the structure is said to be very homogeneous,

If 2% < CV < 30%, the structure is homogeneous,

If CV > 30%, the structure is heterogeneous.

Statistical analysis

Statistica 7.1 software and R version 4.0.2 software were used for this work. The Kruskal-wallis and Mann-whitney tests, they allowed us to determine the significant differences between the treatment groups.

Results

Size of first sexual maturity

Fig. 3 shows the first sexual maturity sizes studied in female and male specimens of *Hoplobatrachus occipitalis*. The L50 is 91.39 mm in females (Fig. 3A) versus 85.62 mm in males (Fig. 3B). The smallest mature individuals in females and males are 85 and 60 mm, respectively. All specimens are mature at 116.46 mm in females and 123.12 mm in males.

Gonado-somatic index

Variations in gonado-somatic index (GSI) in males and females of *H. occipitalis* are shown in Fig. 4. In females of this frog (Fig. 4A), variations in GSI noted during the main rainy season are highest (7-13%). The lowest (1-3%) are observed during the short dry season. The difference between these seasonal values is significant ($p < 0.05$; Kruskal-Wallis test). As for the males of this species (Fig. 4B), the variations in GSI during the short rainy season (0.4 to 0.8%) are the highest.

The low variations are observed during the long rainy season (0.1 to 0.2%). The is no significant ($p > 0.05$; Kruskal-Wallis test) difference.

Table 1. Characteristics of the parameters of fecundity and oocyte diameter in females of *H. occipitalis* in the locality of Dabou.

Characteristics	*H. occipitalis*
Total number of eggs	151 - 2413
Egg average	642 ± 541
Average oocyte diameter	0,20 ± 0,03
Coefficient of variation	17,29

Sex-ratio

The monthly variations of the proportions of both sexes in *H. occipitalis* studied are represented in Fig. 5. The overall sex ratio evaluated is in favor of females with values of 1: 1.18. In this frog, with the exception of the long rainy season (LRS) where the sex ratio is dominated by males (1: 0.90), females appear more abundant the other three seasons of the year.

Fig. 2. A view of the sample site.

Fecundity and oocyte diameter

Absolute fertility is related to 42 females of sizes between 98 and 122,90 mm and mass ranging from 46,99 to 206,07g. It varies from 151 to 2413 oocytes with an average of 642 ± 541 oocytes (Table1). The average oocyte diameter varies between 0,20 ± 0,03 mm.

Discussion

The results obtained in the present study show that in *Hoplobatrachus occipitalis*, the size of first sexual maturity (L50) and the size of the smallest mature individual caught are higher in females than in males. Thus, males are more precocious than females. However, our values are lower than those obtained in Banco National Park by Tohé *et al.* (2016) in the same species. These authors reported this frog's first sexual maturity sizes of 124.5 mm and 95 mm in females and males, respectively. Regarding the evolution of the seasonal gonado-somatic index, our results indicate that *H. occipitalis* females breed during the major rainy season. Thus, the breeding period of this study species is during the rainy season. These results can be explained by the egg-laying closely related to water. Thus our results corroborate that of Tohé (2009) who also discovered the breeding period of this species in the Banco forest during the rainy season.

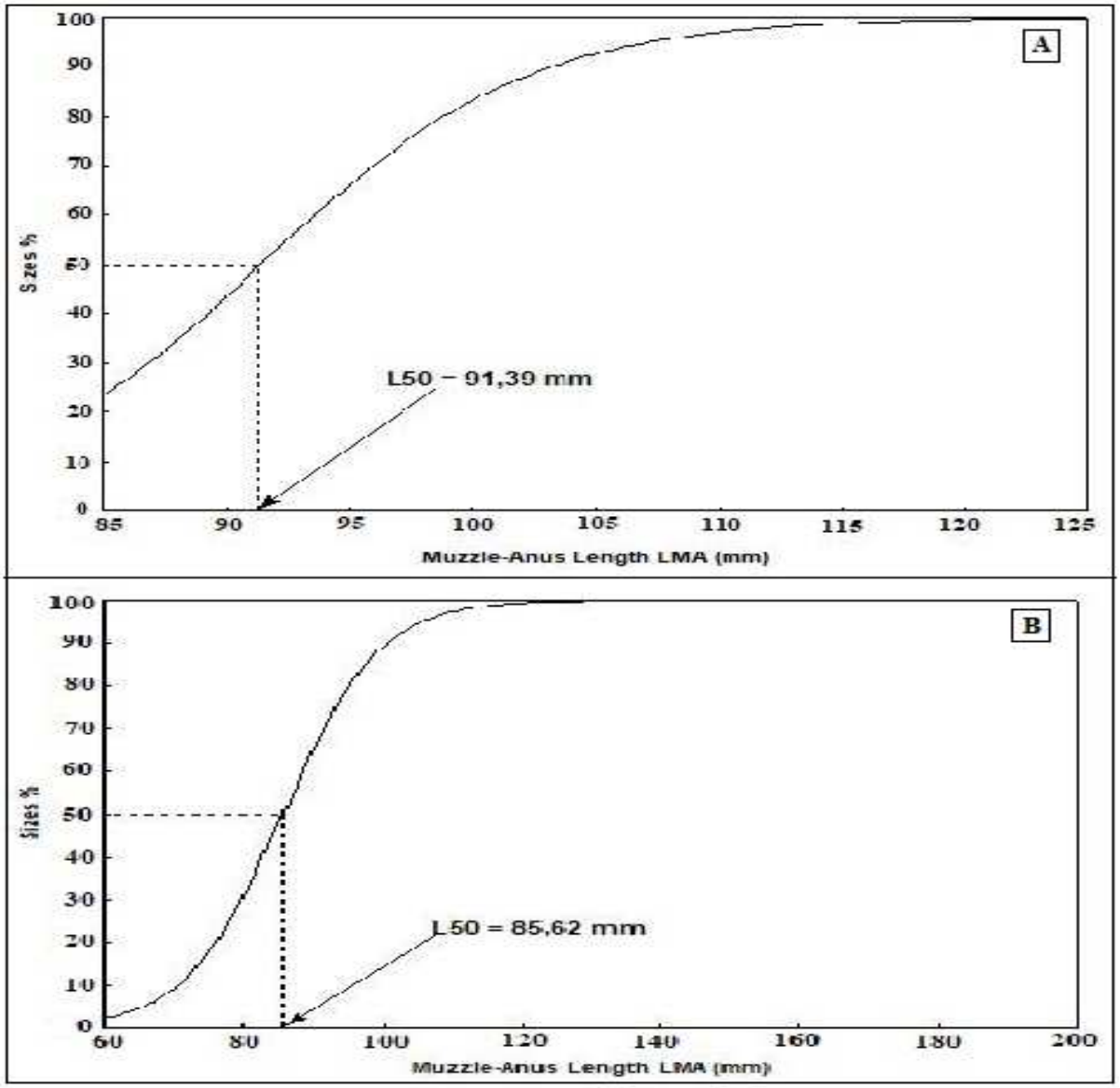

Fig. 3. Variation in size at first maturity in females (A) and males (B) of *Hoplobatrachus occipitalis* from the Dabou site.

Our values for absolute fecundity (2413 eggs) and mean egg diameter value (0.2 mm) in *H. occipitalis* are lower than the results obtained by Tohe, 2015 in Banco National Park. The latter report an absolute fecundity equal to 3286 oocytes with an egg diameter of approximately 2.74 mm. On the other hand, in the Comoé National Park (Ivory Coast), Rödel (2000) reports an even larger value of oocyte diameter approaching 3 mm.

The sex ratio is globally in favor of females. Our results corroborate those of Qin *et al.* (2007) and Hayes *et al.* (2011) who observed sometimes complete feminization phenomena in the laboratory following exposure of *Xenopus leavis* to herbicides or polychlorobiphenyls. Indeed, the specimens used in this work were sampled in drainage furrows of the banana plantation that regularly uses herbicides (glyphosate) and insecticides for maintenance.

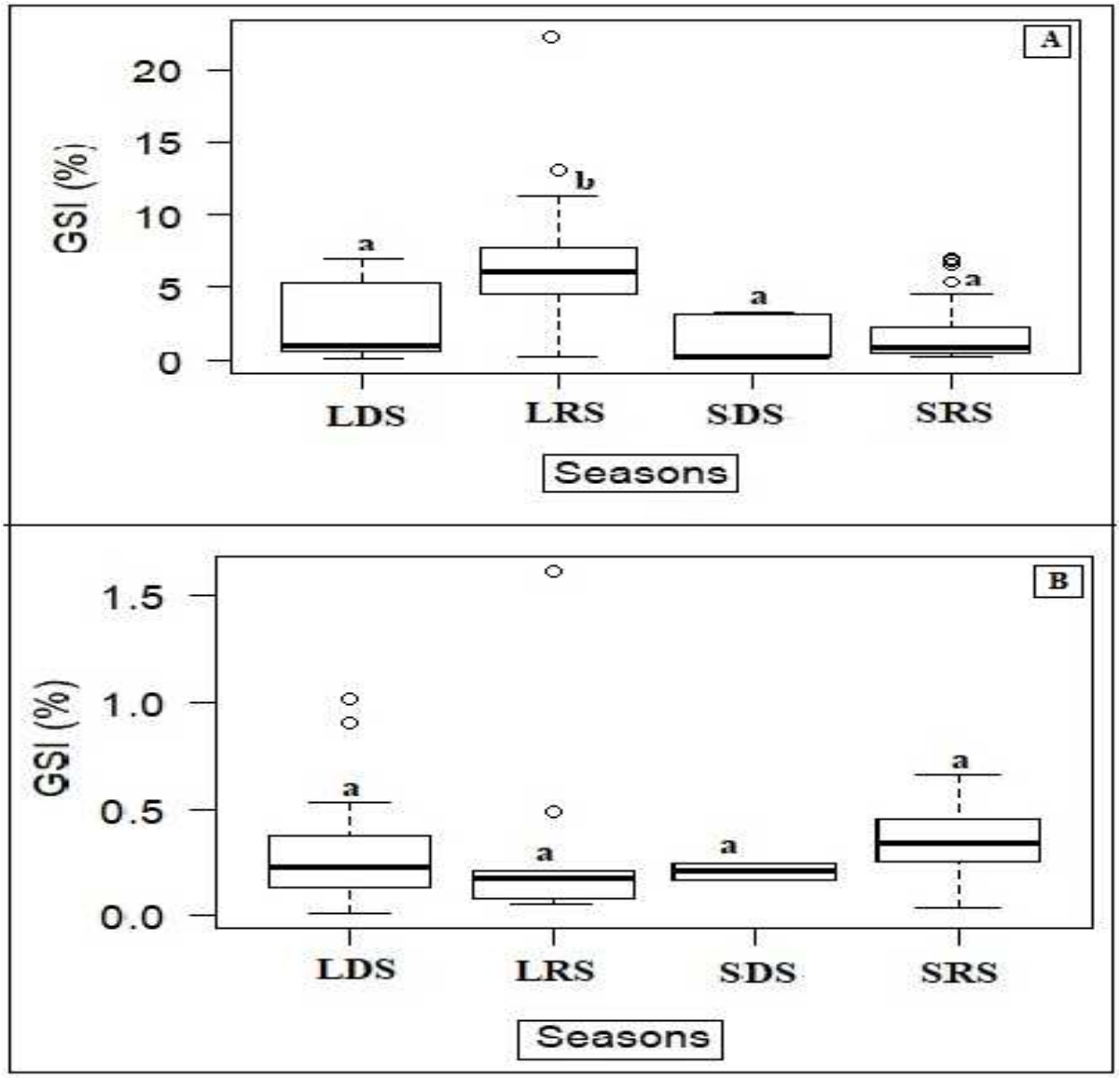

Fig. 4. Monthly variations in GSI of female (A) and male (B) *Hoplobatrachus occipitalis* from the Dabou site: LDS = long dry season; LRS = long rainy season; SDS = short dry season; SRS = short rainy season; Median values sharing the same letter (a and b) do not differ significantly (Kruskal-Wallis test ; $p > 0.05$).

These products drained by erosion mix with the water of the lowlands and they could affect the parameters of reproduction. This result requires a little more in-depth study. It should be remembered, however, that Tavera-mendoza *et al.* (2002a; 2002b), Orton *et al.* (2006) and Hayes *et al.* (2006a) state that atrazine, a

herbicide used in various plantations, causes a decrease in gonad volume and hermaphroditism in exposed *Xenopus leavis* larvae. This decrease in the volume of the gonad could explain the reductions in diameter and number of oocytes observed in this work at the level of this species of frog.

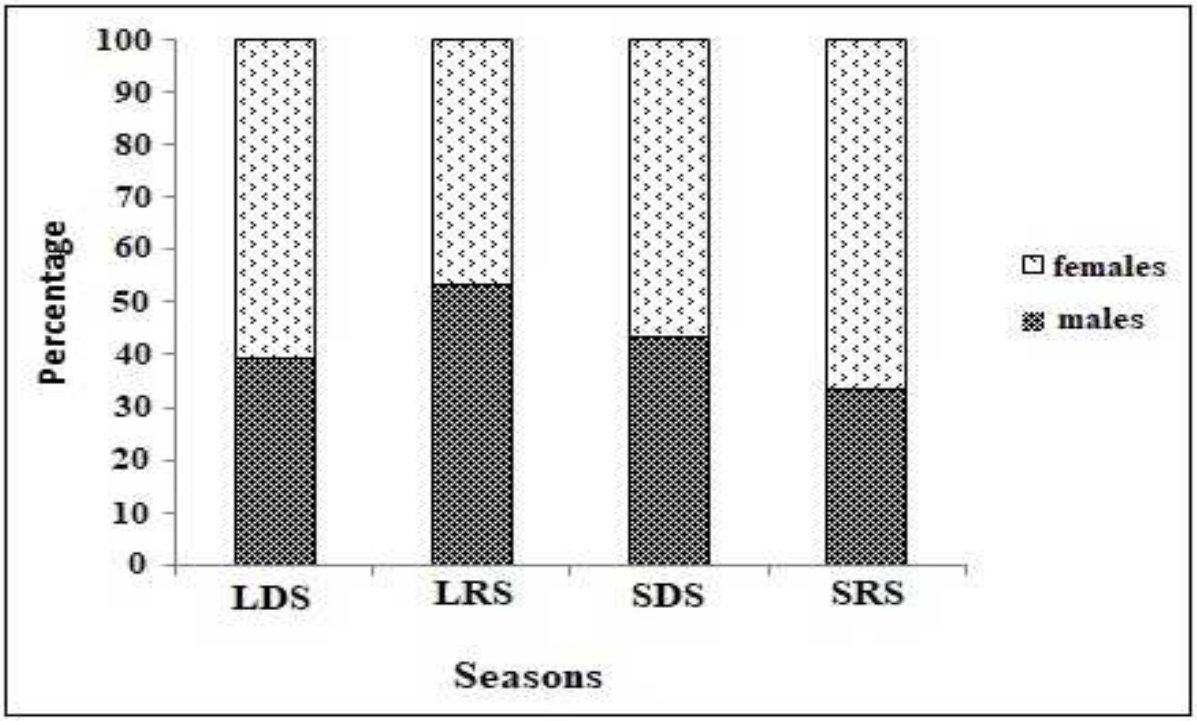

Fig. 5. Seasonal variation in sex ratio in *Hoplobatrachus occipitalis* from the Dabou site.

Conclusion

It can be noted from this study that in the drainage furrows of the Dabou banana plantations, the females of *Hoplobatrachus occipitalis* reproduce during the long rainy season in single laying with small eggs. The number and size of its eggs are influenced by the maintenance products.

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