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Study Compared by the Embryonic Metamorphosis of *Hoplobatrachus occipitalis* (Günther on 1858) in Checked (Controlled) and Natural Environment in the District of the Lagoons of Ivory Coast

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Abstract

A study of the larval development of *Hoplobatrachus occipitalis* was carried out in a pond as well as a laboratory from June to September 2016. From a total of 60 larvae that hatched from eggs, the growth of 30 was followed from an aquarium in the laboratory while the development of the remaining 30 larvae was observed in the pond. From both sites, tadpoles were fed with the same artificial food throughout their growth. Comparisons made at measurement level showed that tadpoles growth was lower in laboratory (tail length: $\bar{x}$ = 4.90 ± 0.07 cm; total length: $\bar{x}$ = 7.25 ± 0.07 cm), than in the pond (tail length: $\bar{x}$ = 5.70 ± 0.04 cm; total length: $\bar{x}$ = 8.12 ± 0.01 cm). During their development, the eyes and hind legs were firstly observed and were thereafter followed by nostrils and forelegs. Whereas the mean daily of eyes and hind legs development in the pond and laboratory was respectively 29.5 and 32.5, these of nostrils and hind legs reached each 52.5 and 55.5 days. The mortality rate of tadpoles in the pond was lower (20%) than this in the laboratory (33.33%).

Keywords: amphibian; *Hoplobatrachus occipitalis*; larval breeding; metamorphosis; laboratory; pond.

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1. Introduction

In Côte d'Ivoire, frogs are not only an important source of animal protein in the feeding habits of humans, but their trade can also generate considerable financial income particularly for local populations from the western part of the country [1]. Anuran species from natural stocks are usually harvested in habitats such as ponds, flooded savannahs, degraded forests, river borders and lowlands. The consumption of *Hoplobatrachus occipitalis* has recently increased to a considerable extent in the country where this species is sold at a local scale at different markets of the towns of Biankouma, Daloa, Duékoué, Issia and Man [1, 2]. However, the frog now seems to be more and more desirable in the feeding habits of people from other regions such as the centre, south and east where it is preyed there as well (KAL, pers. obs.). Usually, adult and sub-adult frog meat are sold fresh, dried of fried [2].

Although *H. occipitalis* is an important species of economic interest, its population dynamics are still poorly known in Côte d'Ivoire. This is worrying because as the amount of natural stocks of harvested frogs are unknown so far, any overexploitation may lead the species to irreversible extinction. The animal is threatened in its natural habitats by abusive sampling techniques such as artisanal catches, harpoons, darts [3]. Furthermore, anthropogenic activities such as bushfires, deforestation, chemical pollution [4] and industrial waste are responsible to a rapid decline of the natural stock of frogs [5]. *H. occipitalis* is a species whose reproduction is periodic; however, it is regularly preyed during the year. The lack of regulations may have drastic effects on the protection of the species.

To face these threats, the survival and preservation of *H. occipitalis* is a challenge. Ranching is thus an essential solution for the protection of existing species [6]. It is within this framework that our study, which had the objective of acquiring reliable data on the dynamics of the larval stage of the edible frog *H. occipitalis*, focuses on the species' breeding.

2. Material and methods

2.1. Study Area

A survey in breeding ponds was carried out in Yakassé-Attobrou (5°11' − 6°35' W; 4°29'−6°32' W), a locality 120 km from Abidjan.

Additional data from larval development were conducted in the Laboratory of Biology and Animal Cytology at the Nangui Abrogoua University, Abidjan (6°10' − 7°45' N; 3°39' − 6°22' W). Yakassé-Attobrou is characterized by the presence of many wetlands. According to [7], these habitat types generally represent amphibian breeding sites (Figure 1).

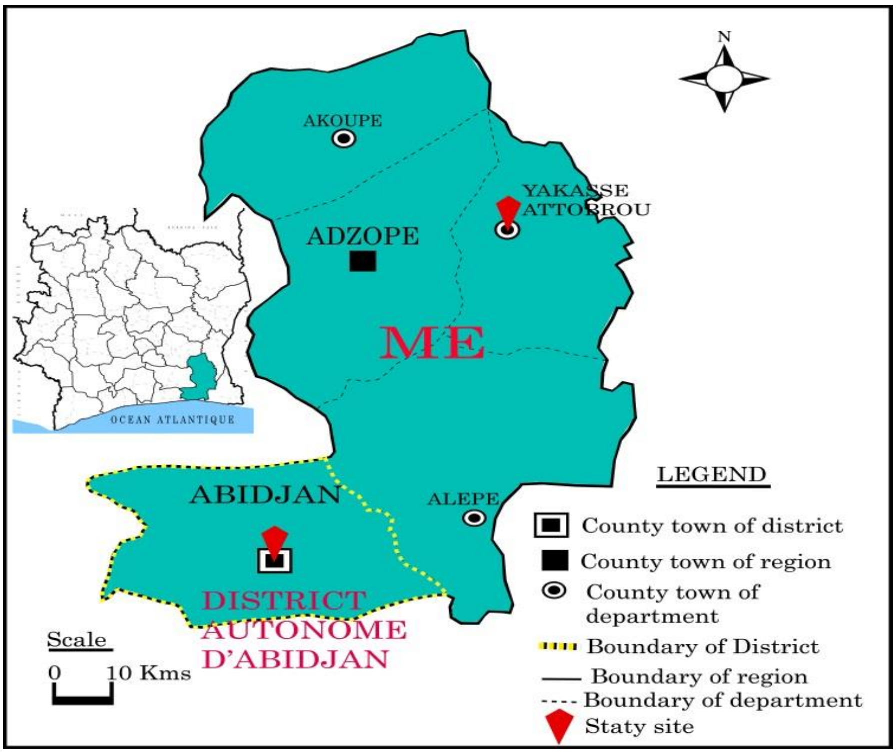

Figure 1: Geographical location of the study area and location of *Hoplobatrachus occipitalis* larval breeding sites (source: Open Map Street by Kohou, 2018)

2.2. Biological material

The biological material consisted of larvae of *Hoplobatrachus occipitalis* that hatched from eggs that were deposited in ponds by females in Yakassé-Attobrou. A total of 60 tadpoles, were fed with wheat flour and egg yolk.

2.3. Material for recording physic-chemical parameters of water

At the two breeding sites, physic-chemical water parameters were measured using a BANTE 900P multiparameter

2.4. Larval rearing equipment Hoplobatrachus occipitalis in the laboratory

The breeding took place in the laboratory in a rectangular aquarium with a capacity of 90 dm3. A cylindrical net 7 cm high, 5 cm in diameter was used to collect tadpoles. A SAMSUNG air conditioner was used to regulate the temperature of the laboratory. A plastic bowl with a capacity of 40 liters was needed to store and renew the chlorinated water. A manual magnifier, model T A S was used for observations.

2.5. Larval rearing equipment in ponds

A 90 dm3 (90 cm long, 40 cm wide and 25 cm high) net and 4 mm mesh set was installed in a 20 m2 pond supported by 4 7 m high wooden stakes at the facility.

2.6. Measurement and weighing equipment for Hoplobatrachus occipitalis larvae

Biometric measurements of the larvae were carried out using a 0.5 mm precision sliding foot. Weighing was done with an electronic scale (type ANG 200A1; accuracy 0.01 g). Finally, measurements of the physic-chemical parameters of the water were made with a BANTE 900P millimeter.

3. Methods

3.1. Laboratory breeding of Hoplobatrachus occipitalis larvae

In the laboratory, thirty (30) *Hoplobatrachus occipitalis* larvae were reared in a 90 dm3 aquarium containing water up to mid-height. These larvae were fed artificial food consisting of wheat flour and egg yolk. 400g of this food is distributed every day early in the morning between 6am and 7am and in the afternoon between 5pm and 6pm.

3.2. Pond rearing of Hoplobatrachus occipitalis larvae

In pond, thirty (30) larvae were reared in a 90 dm3 capacity appas installed inside a 20 m2 pond. The distribution of 400g of the artificial food (wheat flour + egg yolk) took place early in the morning between 6am and 7am and in the afternoon between 5pm and 6pm.

3.3. Measures

3.4. Measurement of physical-chemical parameters of water and the environment

Parameters such as temperature T (water), pH, dissolved oxygen (O2D) and transparency (Trp) of the rearing water as well as air humidity (Hyg) and air temperature T (air) were measured using the meter probe.

3.5. Measurements and observations of Hoplobatrachus occipitalis larvae in media

Daily measurements were made on each larva placed on a transparent glass plate in a small amount of water. The tail (Lq) and the total length of the animal (Lt) were measured using the sliding foot. Then, the various changes observed, using a manual magnifier, on the animal's body were noted. These include the appearance and evolution of organs such as hind legs (Pp), forelegs (Pa), eyes (Ye) and nostrils (Na) on each tadpole. The numbers of tadpoles undergoing transformation, as well as the number of deaths and survivors are recorded.

3.6. Statistical Processing of Data

Metric characters collected from tadpole batches, in water and in the breeding environment were subjected to Students' test to compare the averages of these traits. In addition, the Khi-2 test compared mortality rates of tadpoles observed at study sites. These processes were performed using Statistica 7.01 software. (Statsoft 2005) at the threshold of α = 0.05.

4. Results

4.1. Changes in site water and environmental parameters

The results of values of the physical-chemical parameters of water and air in ponds and aquaria are summarized in Table I. In ponds, the mean values of air temperature (T air), dissolved oxygen (O2D), and water temperature (T water) are high compared to those of the laboratory. The averages obtained were 27.56 ± 0.04 °C; 7.25 ± 0.42 mgl-1 and 24 ± 0.5 °C in ponds compared to 26.5 ± 0.04 °C; 6.06 ± 0.5 mgl-1 and 23.9 ± 0.06 °C recorded in the laboratory. The pH, transparency (Trp) and hygrometry (Hygro) are low in ponds but high in the laboratory. The measured averages are 6.7 ± 0.05; 36.93 ± 3.4 mm and 81.3 ± 0.05 % in ponds compared to 7.1 ± 0.04; 56.93 ± 1.3 mm and 88.3 ± 0.75 % recorded in the laboratory.

Table I: Means and standard deviations ($\overline{x}$ ± sd) of the physico-chemical parameters of the water of the pond at Yakassé-Attobrou and the water of aquaria (laboratory)

Physical-chemical parameters	Water Temperature $\overline{x}$ ± sd	pH $\overline{x}$ ± sd	O_2D $\overline{x}$ ± sd	Trp $\overline{x}$ ± sd	Air Temperature $\overline{x}$ ± sd	Hygrometry $\overline{x}$ ± sd
Pond	24,56 ± 0,5	6,7 ± 0,05	7,25 ± 0,42	36,93 ± 3,4	27,56 ± 0,04	81,3 ± 0,05
Aquaria	23,9 ± 0,06	7,1 ± 0,04	6,06 ± 0,5	56,93 ± 1,3	25,9 ± 0,06	88,3 ± 0,75

Air temperature: air temperature (°C); Water temperature: water temperature (°C); O2D: dissolved oxygen (mgl-1); Trp: transparency; Hygrometry (%)

4.2. Evolution of the tail of Hoplobatrachus occipitalis tadpoles reared in sites

The blue and red curves show variations of tail length of *Hoplobatrachus occipitalis* larvae in ponds as well as in the laboratory. The length of the tadpole tails showed a significant difference on both sites ($p < 0.05$; Student t-test). The development of the tail length of tadpoles took place in five phases, three of which were growth (phases A, B, C) and two of regression (phases D and E). The averages recorded during the different phases are respectively 2 ± 0.15 cm phase (A), 4.42 ± 0.14 cm phase (B) and 5.7 ± 0.16 cm phase (C) in pond against 1.98 ± 0.01 cm in (A), 3.84 ± 0.17 cm in (B) and 4.9 ± 0.07 cm in (C) to the lab. The phases (D) and (E) lasted six weeks, and the averages obtained are 3.32 ± 0.17 cm phase (D) and 0.43 ± 0.01 cm phase (E) in pond compared to 2.99 ± 0.05 in (D) and 0.34 ± 0.03 cm in (E) in laboratory (Figure 2).

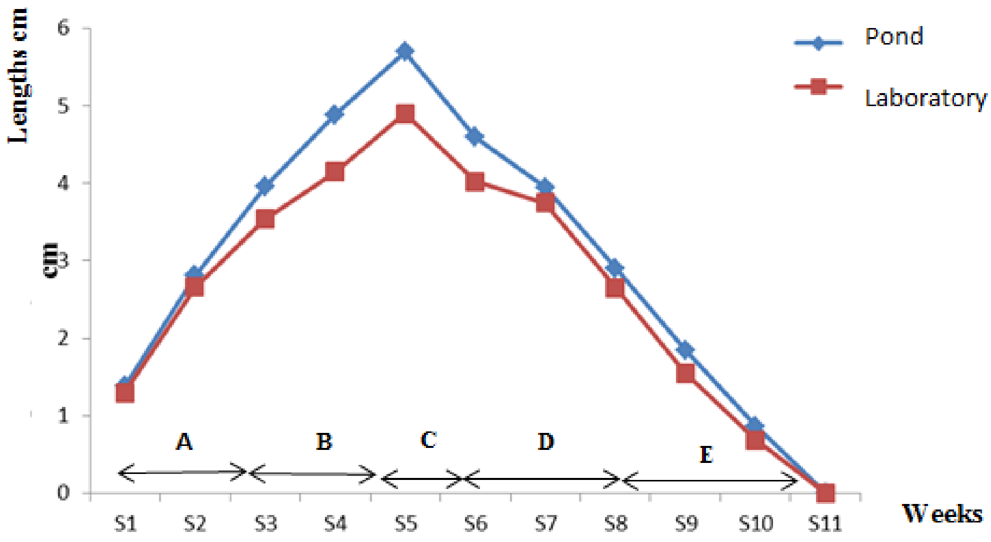

Figure 2: Evolution of mean tail size measured in *Hoplobatrachus occipitalis* larvae as a function of pond and laboratory rearing weeks

4.3. Changes in the size of Hoplobatrachus occipitalis larvae reared at sites

The evolution of the total larval length was carried out in two major stages (Figure 3; Figures A, B, C, D, E). The first concerns the growth phase. It reached its maximum after six weeks and was subdivided into three stages of duration of (A) 2.89 ± 0.19 cm, (B) 6.41 ± 0.09 cm and (C) 8.12 ± 0.07 cm in pond against (A) 2.39 ± 0.03 cm (B) 5.05 ± 0.01 cm and (C) 7.25 ± 0.07 cm in the laboratory. The second phase concerns face regression. It consists of two stages rated D, E; the respective durations were three and two weeks. Means obtained are 6.73± 0.05 cm and 4.21± 0.04 cm respectively in ponds compared to 5.70 ± 0.05; 0.13 ± 0.04 cm in the laboratory. The differences observed in the change in size are significant ($p < 0.05$; Student t-test) (Figure 4).

Figure A: Slow growth phase

Figure B: Accelerator growth phase

Figure C: Maximum growth phase or peak growth

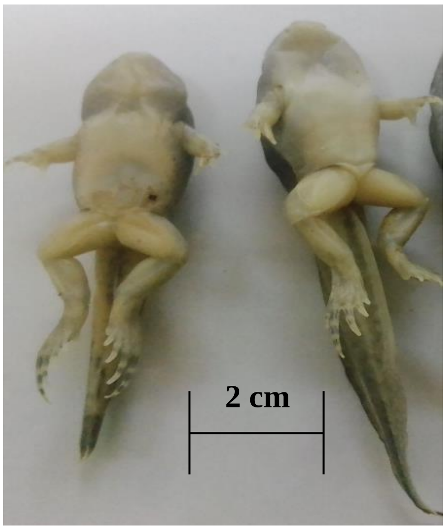

Figure D: Accelerator regression phase

Figure E: Slow regression phase

Figure 3: Various stages of development of the larva *Hoplobatrachus occipitalis*

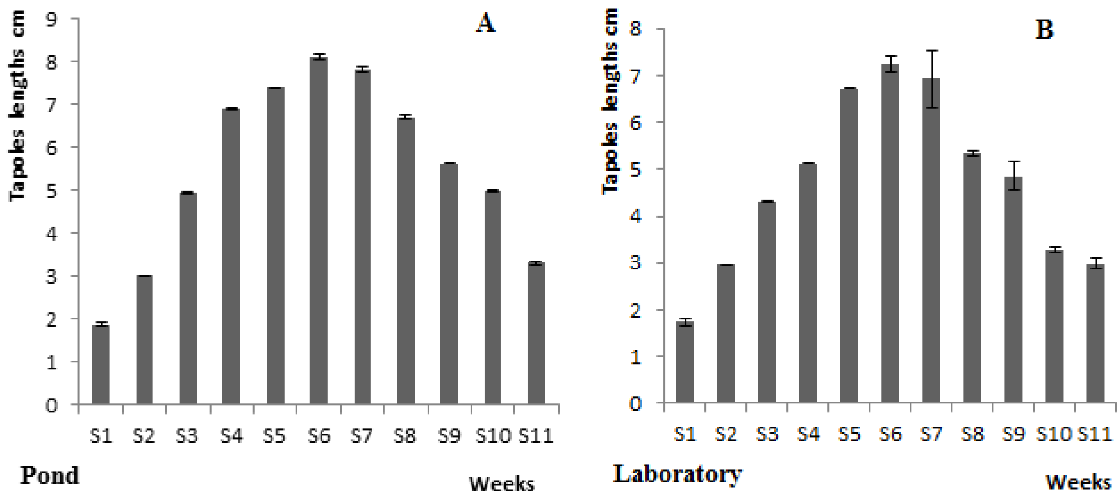

Figure 4: Comparison of average size of *Hoplobatrachus occipitalis* larvae reared in pond (A) and laboratory (B) according to breeding weeks

4.4. Acquisition of eyes and hind legs of Hoplobatrachus occipitalis larvae

The Figure F illustrates the eyes and hind legs whose appearance was observed from the 28th to the 31st day in the pond and from the 31st to the 34th day in the laboratory. The proportion of tadpoles recorded was 21 %, 29 %, 40 % and 10 % respectively for the 28, 29, 30 and 31 the days in ponds; compared to 11 %, 23 %, 31 % and 35 % respectively for the 31, 32, 33 and 34 days in the laboratory. There is a difference in the appearance of these larvae in both media ($p< 0.05$; Chi-2 test) (Figure 5).

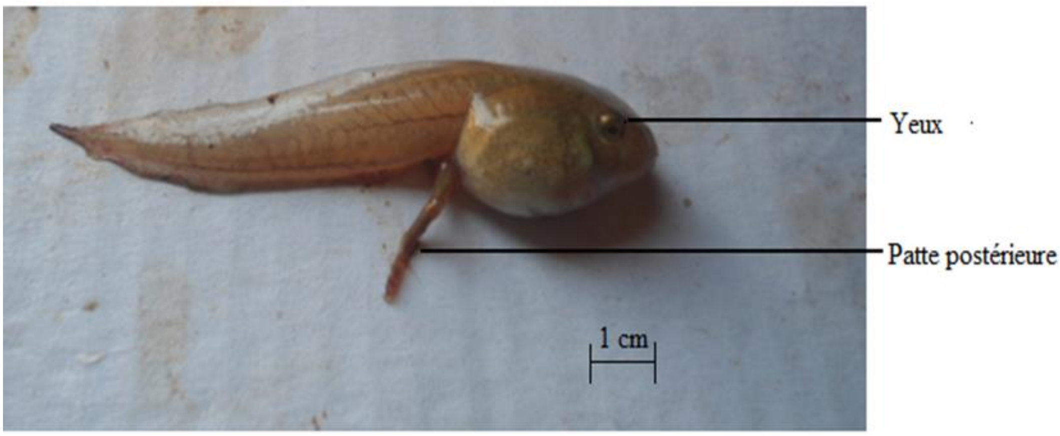

Figure F: Profile view of a *Hoplobatrachus occipitalis* larva with eyes and hind legs

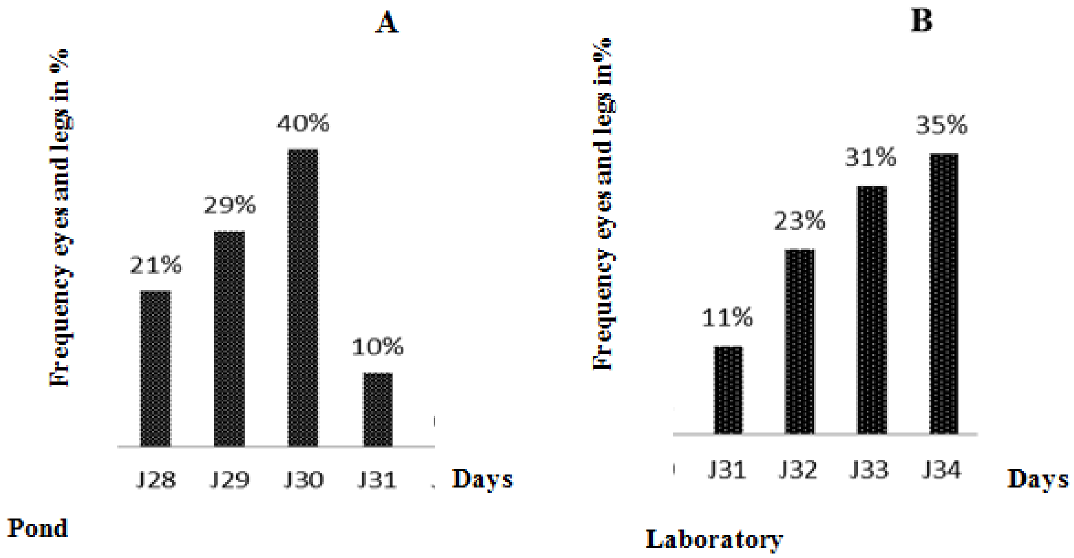

Figure 5: Frequencies of *Hoplobatrachus occipitalis* tadpoles with eyes and hind legs in pond (A) and laboratory (B) depending on the days of rearing

4.5. Acquisition of nostrils and forelegs of Hoplobatrachus occipitalis larvae at study sites

Figure G shows the nostrils and forelegs of the *Hoplobatrachus occipitalis* larvae from the 51st to the 54th days of pond rearing and from the 54th to the 57th days of laboratory culture. The proportion of tadpoles recorded was 20 %, 30 %, 42 % and 18 % in ponds on the 51, 52, 53 and 54th days respectively, compared with 9 %, 20 %, 30 % and 41 % in the laboratory during the 54, There is a difference in the appearance of these larvae in both sites ($p < 0.05$; Chi-2 test) (Figure 6).

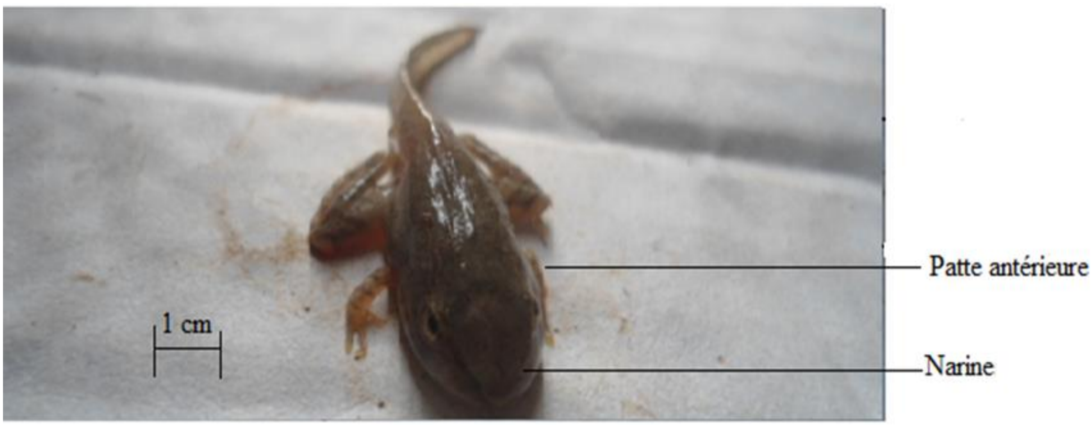

Figure G: Partial view of *Hoplobatrachus occipitalis* larvae with appearance of nostrils and forelegs in the ponds

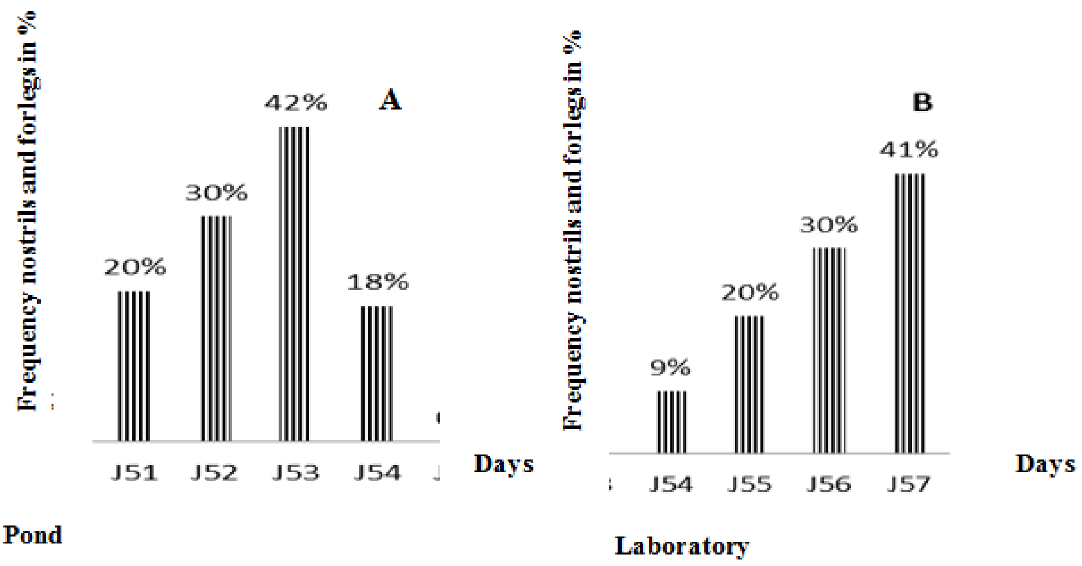

Figure 6: Proportions of *Hoplobatrachus occipitalis* larvae with nostrils and forelegs in pond (A) and laboratory (B) depending on the duration of rearing

4.6. Mortality rate of Hoplobatrachus occipitalis larvae at both sites

In the pond, 6 larvae died on the 30 animals raised and the mortality rate was 20 %. In the laboratory, however, 10 tadpoles died on 30 larvae raised and the mortality rate was 33, 33 %. The difference in deaths recorded in these two batches of tadpoles is significant ($p < 0.05$; Chi-2 t-test) (Figure 7A and 7B).

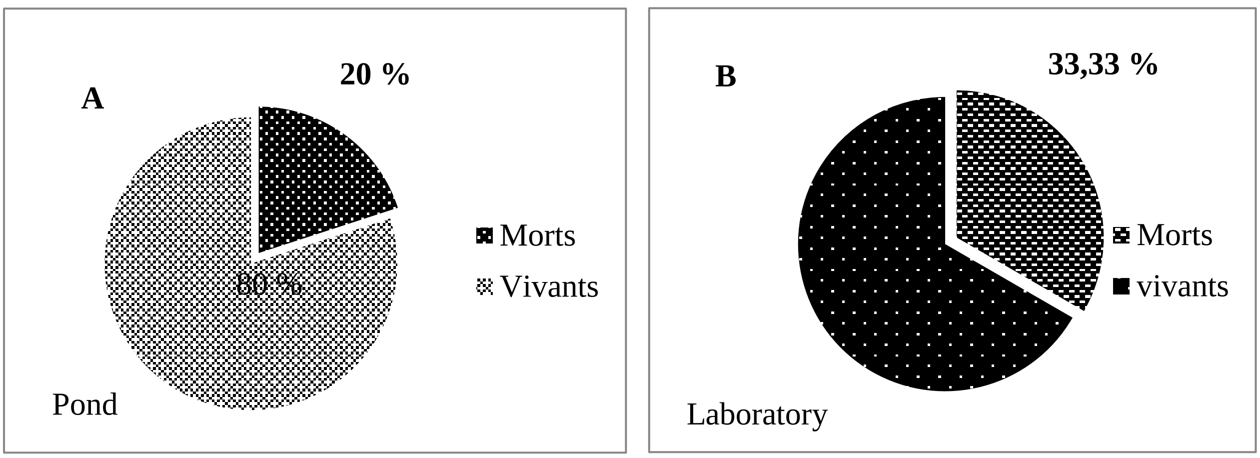

Figure 7: Mortality rate of *Hoplobatrachus occipitalis* larvae high in natural pond (A) and laboratory (B).

5. Discussion

Changes in physical-chemical water parameters measured at the two study sites (natural pond and laboratory) showed that the high temperature (27.56°C and 25.9°C) did not change significantly from site to site. The high temperatures recorded in the natural pond could be justified by the exposure of this site to solar radiation. The low temperature values obtained from the laboratory can be explained by the fact that this site is located in a closed concrete enclosure and receives little solar radiation. However, low temperature changes would be related to solar influence [8]. For the relative humidity of the air, the values obtained in the laboratory are higher than those of the pond. This wetter atmosphere of the laboratory is favored by the permanent presence of two air conditioners that produce moisture in the room. The low humidity values of the pond air could be justified by warming phenomena due to solar radiation and water evaporation [9]. The water temperature values obtained in the laboratory and in the natural pond are similar. The slightly elevated pond is favored by direct exposure of the pond to sunlight. The low temperature values of the laboratory water would be due to the continuous operation of the air conditioners. Regarding the pH of the water, the recorded values of the laboratory tap water and the natural pond water did not change significantly. Tap water has a pH slightly higher than that of the natural pond (7.1 versus 6.7). This increase in the pH of the laboratory tap water is favored by the presence of chemical elements from the water treatments at the level of the castles made of sodium hypochlorite (bleach) by the water distribution company. The low pH value obtained in the natural pond is favored by a slight increase in chemical elements (hydronium ion H3o+) from the amounts of rain that fall directly into the pond. O2D values recorded in the natural pond are lower than in the laboratory and this difference in oxygenation is significant. The lower oxygenation of the water observed in ponds, comes from the decomposition activities of phytoplankton present in the environment and this activity increases during the day and decreases at night. Our results are higher than those obtained by [10] on green frogs in Canada. The required value in aquarium larval rearing by this author is (> 4 mg/l). As regards tail growth in these larvae, the lengths obtained are greater in ponds than those recorded in the laboratory. This difference in tail size could be explained by the fact that tadpoles have reached their threshold size beyond which tail growth is slowed down [11]. Similar studies have been done on Rana pipiens and Rana catesbeiana by some authors [12]. The parameters measured lq and Lt respectively 5.7 cm for tadpoles in ponds and 4.9 cm for tadpoles in the laboratory are higher than the data estimated by [13]. These authors obtained 4.8 cm for pond larvae and 4.3 cm for tadpoles in the laboratory. In terms of larval size, the total lengths measured in ponds were greater than those recorded in the laboratory. These values are in the same order as those obtained by [12, 13] for larvae in ponds (7.8 cm) and laboratories (6.88 cm). This difference in size observed in these animals could translate nutritional additive intakes in the natural environment in addition to the food provided during the experiment [14]. The best growth performance is related to the quality of the food, the substances ingested and the enhancement of the proteins contained therein [15]. Although the metamorphosis took place continuously in both media, the time of appearance of the eyes, nostrils, hind legs and forelegs in the larvae were shorter in ponds than in the laboratory [16]. These observed day lags could be due to the different environmental conditions prevailing in these two environments [17]. In fact, the noises from the operation of air conditioners, student entrances and exits and the almost permanent presence of light in the laboratory have contributed to the development of stress in the larvae. According to [18], noise, pollution, excessive handling promotes the installation of stress in farm animals leading to the development of pathologies that cause stunting in some cases and digestive disorders in others [15]. Finally, the various mortalities observed could be explained by the daily manipulations of the larvae during measurements and observations. The stress caused by the manipulations may have been exacerbated by the stress caused by the environmental conditions prevailing in the laboratory.

6. Conclusion

Overall, the results obtained indicate that the morphometric method used to compare larvae in each medium provided reliable results for the development of the two populations of *Hoplobatrachus occipitalis* studied. The mean tail lengths recorded in ponds were significantly higher than those obtained in the laboratory. In pond, where larvae had a longer tail mean was 5.70 ± 0.169 cm and these seem to show a faster loss of tails compared to those raised in laboratory. The total length of larvae in ponds was on average significantly higher than in their laboratory-bred congeners. In addition, the time of appearance of the eyes, nostrils and paws was shorter, indicating a faster larval development in the natural environment.

Acknowledgements

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7. Recommendations

Hunting of *Hoplobatrachus occipitalis* should be regulated during rainy seasons to allow the species to renew its populations

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