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Research Article

OPTIMAL ENVIRONMENT FOR REARING *HOPLOBATRACHUS OCCIPITALIS* TADPOLES AND EVOLUTION OF THEIR MOUTH CAGE

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ABSTRACT

Tadpoles or frog larvae are the first stage of evolution. Control of their growth environment is therefore important for rearing. In this context, tadpole rearing trials for the frog *Hoplobatrachus occipitalis* were carried out in two environments, in concrete basins and in ponds, and the development of their mouth cage was monitored. To this end, 12 mosquito net enclosures were built in ponds and 12 concrete basins on land. To feed these two systems, seven waves of tadpoles were harvested progressively at different locations on the APDRACI fish farm at the start of the rainy season. The main results show that, in terms of physico-chemical parameters, the pH and temperature of the water were slightly higher in the concrete basins. Survival rates and morphological parameters are much higher on average in concrete ponds than in pond enclosures. As for the mouth cage, it begins with two rows of relatively harmless denticles and ends with sharp, pointed teeth. Ultimately, these trials will enable future raniculturists to be more efficient and, above all, to limit tadpole mortality rates.

Keywords: Tadpoles, *Hoplobatrachus occipitalis*, Structure, Mouth cage.

INTRODUCTION

Amphibians are poikilothermic animals (Morin, 2008). Their bare, moist skin and the gaseous exchanges that take place there make them very sensitive to variations in environmental conditions. Amphibians are therefore excellent indicators of stress in their living environments (Harwell & Olivier, 1998; Adams, 1999). Around fifty species of frog taken from natural stocks are consumed throughout the world (Neveu, 2004). Of these, the species *Hoplobatrachus occipitalis* is the most widely consumed in Africa, particularly in Côte d'Ivoire (Keita *et al*., 2022). Frogs also play an important role in the dynamics of food webs. In many stagnant aquatic ecosystems, their tadpoles are predators of insect larvae (Václav & Zbyšek, 1985). In this way, they help to control the vectors of malaria, an endemic disease in tropical regions. In addition, these tadpoles are excellent prey for aquatic insects, fish and turtles (Channing, 2001). Given the importance of adult frogs and tadpoles in terms of ecology, nutrition and health on the one hand, and the proven decline in stocks worldwide on the other, raniculture is proving to be an unavoidable alternative for reducing the pressure on wild populations (Neveu, 2004). In order to achieve this objective, and given the vulnerability of the tadpoles, it is essential to set up an environment adapted to their growth in the context of rearing the frog *Hoplobatrachus occipitalis*. It is important to note that the literature review did not find a study similar to that conducted in this essay. In parallel with these trials, the evolution of the tadpoles' mouth cages was also studied, given the lack of information in this area. All in all, this work has made it possible to optimise the most perilous stage in the rearing of the *H occipitalis* frog.

MATERIALS AND METHOD

Study site

This study was carried out in Daloa from march 2020, the capital of the Haut Sassandra region (Figure 1) in central western Côte d'Ivoire. The project was located on the fish farm of the Association for Fish Farming and Rural

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Development in Humid Tropical Africa (APDRACI), whose geographical coordinates are 6°51 latitude north and 6°27 longitude west. The farm is located just outside the town on the Daloa-Issia road, about 500 m from the old corridor. It has nineteen fish ponds, a large dam, three large concrete tanks, six covered hatcheries, a water tower and other facilities required for fish farming.

Setting up the infrastructure

Concrete basins

12 concrete basins with a capacity of 03 m^3 (02.5m x 01.2m x 01m) were built using a high proportion of cement for the bricks and interior plastering. Drainage and supply systems were installed. The concrete basins were divided into two environments (terrestrial and aquatic). The natural water in the aquatic part had a volume of 500 l. All the pools were enclosed with mosquito netting to protect the tadpoles from predators (Figure 2).

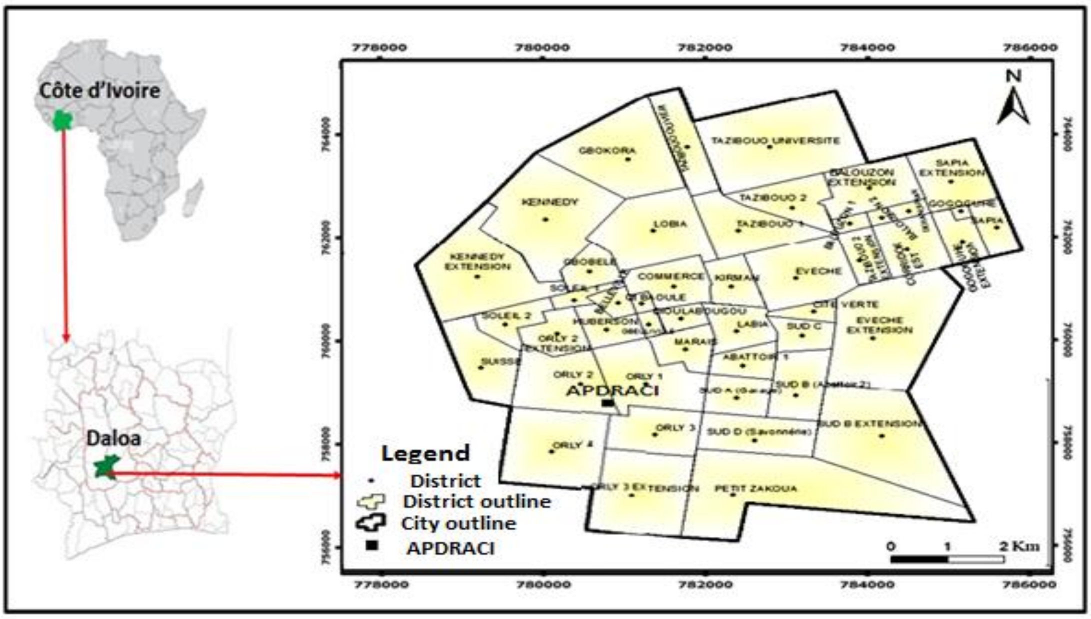


Figure 1. Geographical location of the research site (INS, 2014).

Figure 2. Experimental set-up of 12 concrete basins.

Figure 3. Experimental layout of the 12 net enclosures.

Mosquito net enclosures

12 mosquito net enclosures were set up with a capacity of $3m^3$ (2m x 1m x 1.5m). Each net was turned upside down and tied at three levels (base, middle, top) on all 4 sides of the net on the 4 rafters. The net was placed so that one part was in the water and the other part on the bank. A large hoe was used to cover the part of the net on the bank with local soil, in order to have two compartments (terrestrial and aquatic). We have managed to maintain a water volume of 500 l thanks to a monk system which allows us to regulate the quantity of water in the pond. Fishing net (10mm mesh diameter) was used to close the net pens to prevent predators (Figure 3).

Collection of tadpoles

H. occipitalis frog tadpoles were collected at the beginning of the rainy season in 2020. Two waves of tadpoles were collected at different locations on the APDRACI fish farm (Figure 4), according to the table below:

Table 1. Location of frog tadpole sampling at the Apdraci site.

	First wave	**Second wave**
Location	Dam canal Main canal Ponds C3 to C6	
Date	04-mars-20	10-apr-20

The tadpoles were caught using fine sieves or larger mesh sieves, to which pieces of mosquito netting were added in order to avoid losing tadpoles (Figure 5). The tadpoles were recovered and transferred to a bucket.

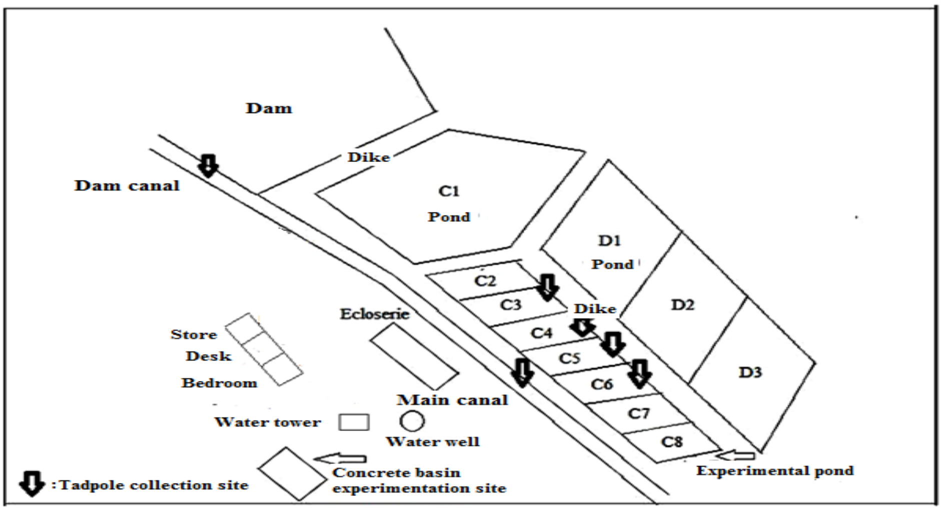


Figure 4. Layout of the rearing infrastructure at the APDRACI fish farm (Source APDRACI).

Figure 5. Harvesting of *H. occipitalis* tadpoles.

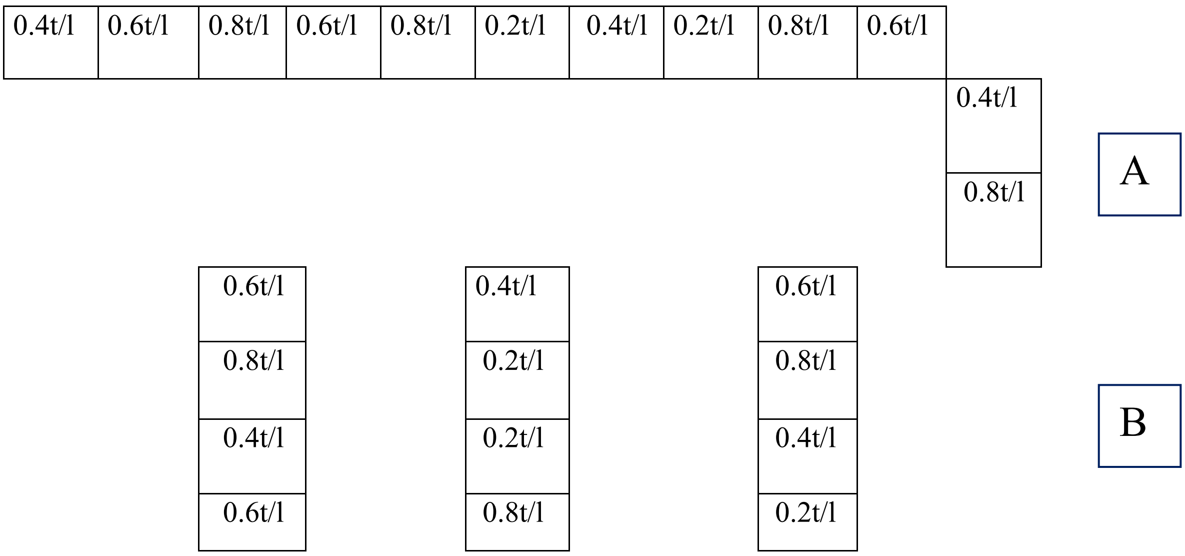


Figure 6. Breakdown of the different experimental units.
A: in ponds; B: in concrete basins; t: tadpoles; l: litre

Formation of experimental units

Four experimental units were formed, taking into account the quantity of water (0.2 tadpoles/l; 0.4 tadpoles/l; 0.6 tadpoles/l and 0.8 tadpoles/l). Each experimental unit (Figure 6) was repeated three times and randomly distributed. This was done in concrete basins and in pond enclosures for the 2 waves of tadpoles.

Feed

A feed formulated with 40.52% protein was prepared using local inputs. Pellets capable of floating for around ten minutes were produced (Figure 7). Given the small size of the tadpoles' mouths, the pellets were ground to a powder and sieved.

Figure 7. Electric baking of food formulated with 40.52% protein.

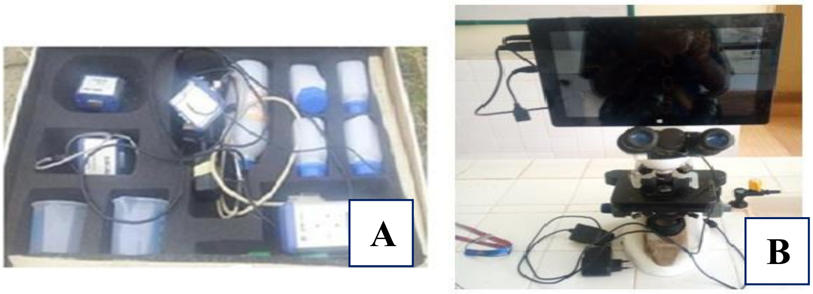


Figure 8. Multi-parameter (A) and microscope used for taking pictures of the buccal cages (B)

Monitoring the trial

After the tadpoles had been transferred to the experimental set-up, they were manually fed with the 40.52% protein powder every day for 20 days. This was done three times a day (08:00-09:00; 11:00-12:00 and 15:00-16:00) at 06% of their body weight according to Godomé *et al.* (2018). Mortalities were counted daily to determine survival rates at the end of the test for all replicates. Survival rates are determined by taking the ratio of the final number of individuals to the initial number of individuals multiplied by 100 (Castell & Tiews, 1980). The final body weights of 36 individuals per environment were taken at 10 and 20 days to determine average weights. Physico-chemical parameters (water and air temperature, relative air humidity and hydrogen potential) were also collected daily for each environment using a multi parameter cobra PHYWE (Figure 8A).

Evolution of the oral cage of *Hoplobatrachus occipitalis* tadpoles

In order to monitor the evolution of the buccal cage,1 tadpoles for each experimental living environment was collected (12 tadpoles/environment). The overall size as well as the length and width of the oral cleft of each tadpole were taken using a binocular magnifying glass and a calliper. These manipulations must be carried out quickly in order to reduce stress (Barnett et al. 2001) and to avoid damaging the antibacterial properties of the frogs' skin (Mattute *et al.*, 2000; Nasciemento *et al.*, 2003). We then proceeded to section the snout of 4 tadpoles at the same time and placed it between the slide and coverslip of an optical microscope equipped with a screen for taking photographs (Figure 8B). This operation was repeated every 5 days during the trial.

Statistical tests

The Kruskal-Wallis rank test is a non-parametric alternative to the one-factor analysis of variance between classes (ANOVA 1). The Mann-Whitney U test is a non-parametric alternative to the student t test for independent samples. These non-parametric methods do not involve estimating parameters such as the mean or standard deviation. These tests have been used for small samples. However, parametric tests were applied when the normality of the distribution was proven thanks to the Shapiro wilk (Kinnear & Gray, 2005).

RESULTS AND DISCUSSION

The pH of the aquatic environment was practically the same on average in the first and second waves, although it was significantly higher in the concrete basins (8.8 and 8.2) than in the net pens (6.7 and 7.4) (t-test; $p < 0.05$). In relation to water temperature, the mean values of this parameter evolved in much the same way as those of pH. In concrete basins, the water temperature rose from 29.5 to 29.9°C and in ponds from 27.2 to 28.1°C respectively from the first to the second wave. As for water temperature an relative air humidity, these two parameters moved in opposite directions on average. Thus, from the first to the second wave, the air temperature rose from 31.01 to 29.0°C in concrete basins and from 30.81 to 29.5°C in ponds. For relative air humidity in concrete basins, the average values rose from 40.83 to 50.10%, while in ponds from 49.00 to 50.25% (Table 2). Statistical tests show that there is no significant difference between the two environments for these last two parameters (t-test; $p>0.05$), with the exception of air humidity in the first wave.

Table 2. Average physico-chemical parameters recorded in the different environments.

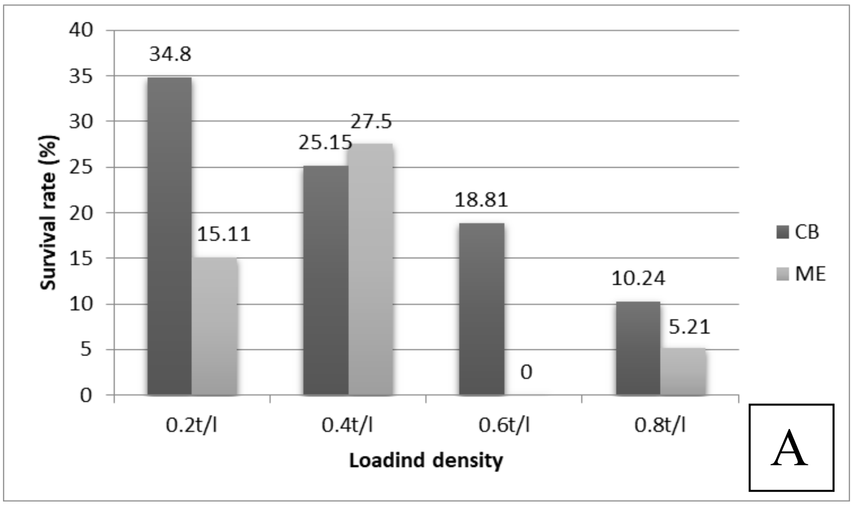
	Structure	First wave	Second wave
Hydrogen potentiel (pH)	CB	8.8 ± 0.60^a	8.2 ± 0.37^a
	ME	6.7 ± 0.21^b	7.4 ± 0.17^b
Water temperature (°C)	CB	29.5 ± 0.26^a	29.9 ± 0.31^a
	ME	27.20 ± 0.35^b	28.1 ± 0.19^b
Air temperature (°C)	CB	31.01 ± 0.55^a	29.0 ± 0.32^a
	ME	30.81 ± 0.81^a	29.5 ± 0.55^a
Relative humidity of air (%)	CB	40.83 ± 4.21^a	50.10 ± 2.12^a
	ME	49.0 ± 3.12^b	50.25 ± 5.25^a

^{abcd} mean values in the same column that are not assigned the same letter are significantly different ($p<0.05$). CB: concrete basin, ME: mosquito net enclosure.

Survival rates of *H. occipitalis* tadpoles in different environments

For the first wave, tadpole survival rates (SR) were higher in concrete basins (BS) than in mosquito netting ponds (EM), with the exception of the 0.4t/l density. The TS is zero in ponds at a density of 0.6t/l. For the second wave, there was no zero TS, but tadpole survival rates in ponds were well below those obtained in concrete ponds (Figure 9). Statistical tests showed a significant difference between the TS in the two environments (Mann-Whitney (MN); $p< 0.05$). Figure 10 shows the transfer of tadpoles in the two environments.

Table 3 shows a doubling of tadpole body weight in all environments from the first to the second wave. In the first wave, the average body weight of the tadpoles was higher in the net pens (0.7 to 01.42g) than in the concrete basins (0.65 to 1.39g). This ratio was the same on day 10 of the second wave trial. However, this trend reversed at the end of the trial, with a body weight of 01.65g in concrete basins and 01.55g in ponds. Overall, there was no significant difference between the mean weights (t-test; $p>0.05$) in the different environments, with the exception of the weights observed in the middle of the second wave trial. Figure 11 shows the different stages of development of *Hoplobatrachus occipitalis* tadpoles.



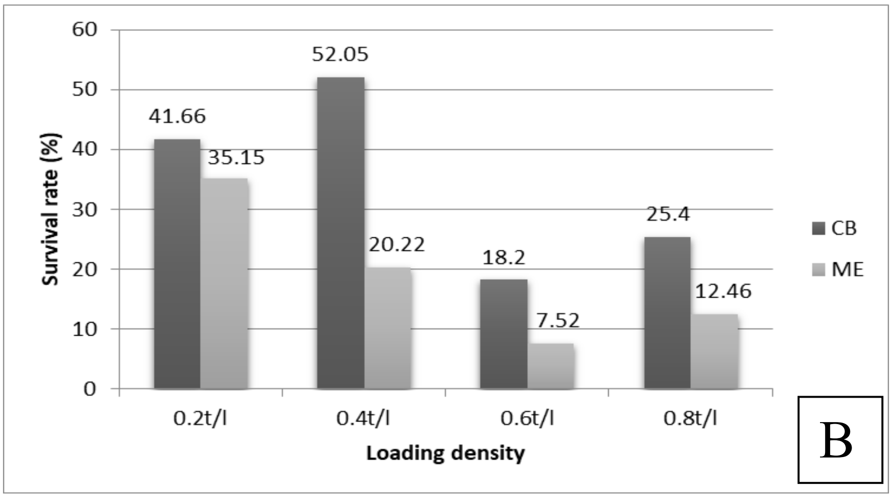


Figure 9. Survival rate of *H occipitalis* tadpoles.

A: First wave, B: Second wave, BS: concrete basins, EM: mosquito net enclosure t: tadpole, l: litre

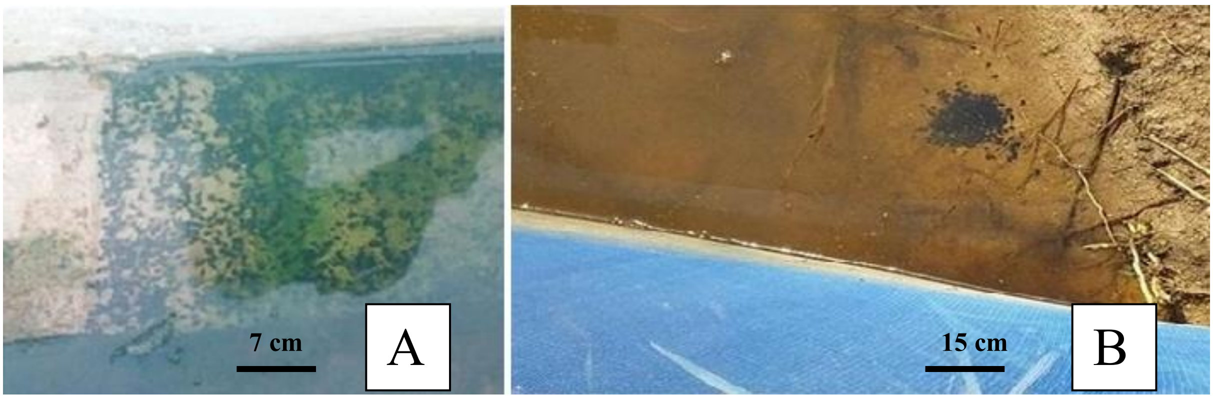


Figure 10. Growing *Hoplobatrachus occipitalis* tadpoles in different environments
A: concrete basins, B: mosquito net enclosure

Body weights of H. occipitalis tadpoles during and at the end of the trial

Table 3. Average body weights of *H. occipitalis* tadpoles in different environments.

		Middle test (10th day)	**End test (20th day)**
First wave	Weight in BS (g)	0.65 ± 0.005[a]	1.39 ± 0.024[a]
	Weight in EM (g)	0.7 ± 0.006[a]	1.42 ± 0.017[a]
Second wave	Weight in BS (g)	0.78 ± 0.010[a]	1.60 ± 0.031[a]
	Weight in EM (g)	0.84 ± 0.009[b]	1.55 ± 0.029[a]

[abcd] mean values in the same column that are not assigned the same letter are significantly different ($p<0.05$).
CB: concrete basin, ME: mosquito net enclosure.

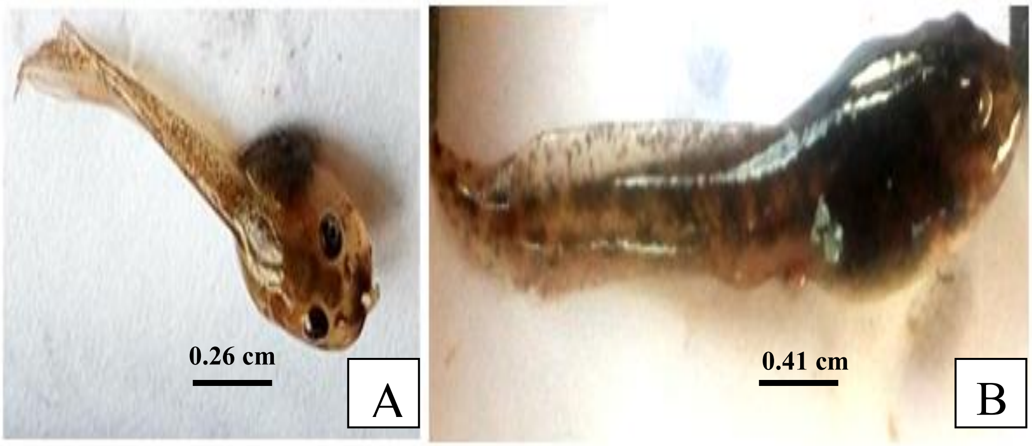


Figure 11. Different stages of development of *H. occipitalis* tadpoles during the trial.
A: 5-day-old tadpole, B: 21-day-old tadpole.

Evolution of the oral cage of *H. occipitalis* tadpoles before transformation

Photographs taken from sections of the buccal cage of *Hoplobatrachus occipitalis* tadpoles at different stages are shown in Figure 12. Analysis of the photographs shows that the buccal cage is made up of two parts (upper and lower). These parts are thin at the start (5 days), then gradually become very thick (20 days). As for the dentition, at 5 days it takes the form of two rows of vertical horny denticles. These then develop into several rows of sharp, claw-like teeth. Table 4 shows that the mean length and width of the buccal cage increased progressively from 1.04 ± 0.09 to 4.02 ± 0.15mm and from 0.34 ± 0.05 to 2.02 ± 0.08mm respectively from 05 to 20 days. The lengths and widths of the mouth cages of the tadpoles at these different stages were significantly different (Anova; $p < 0.05$).

Table 4. Mouth cage measurements of *Hoplobatrachus occipitalis* tadpoles at different growth stages.

Settings	Tadpoles ages			
	05 days	10 days	15 days	20 days
Average length of oral cage (mm)	1.04 ± 0.09[a]	1.72 ± 0.08[b]	2.38 ± 0.19[c]	4.02 ± 0.15[d]
Average width of oral cage (mm)	0.34 ± 0.05[a]	0.76 ± 0.05[b]	1.58 ± 0.07[c]	2.02 ± 0.08[d]
Average size tadpole (mm)	13.8 ± 0.41[a]	28.38 ± 0.77[b]	40.19 ± 0.97[c]	48.30 ± 2.03[d]

[abcd] mean values in the same column that are not assigned the same letter are significantly different ($p<0.05$).

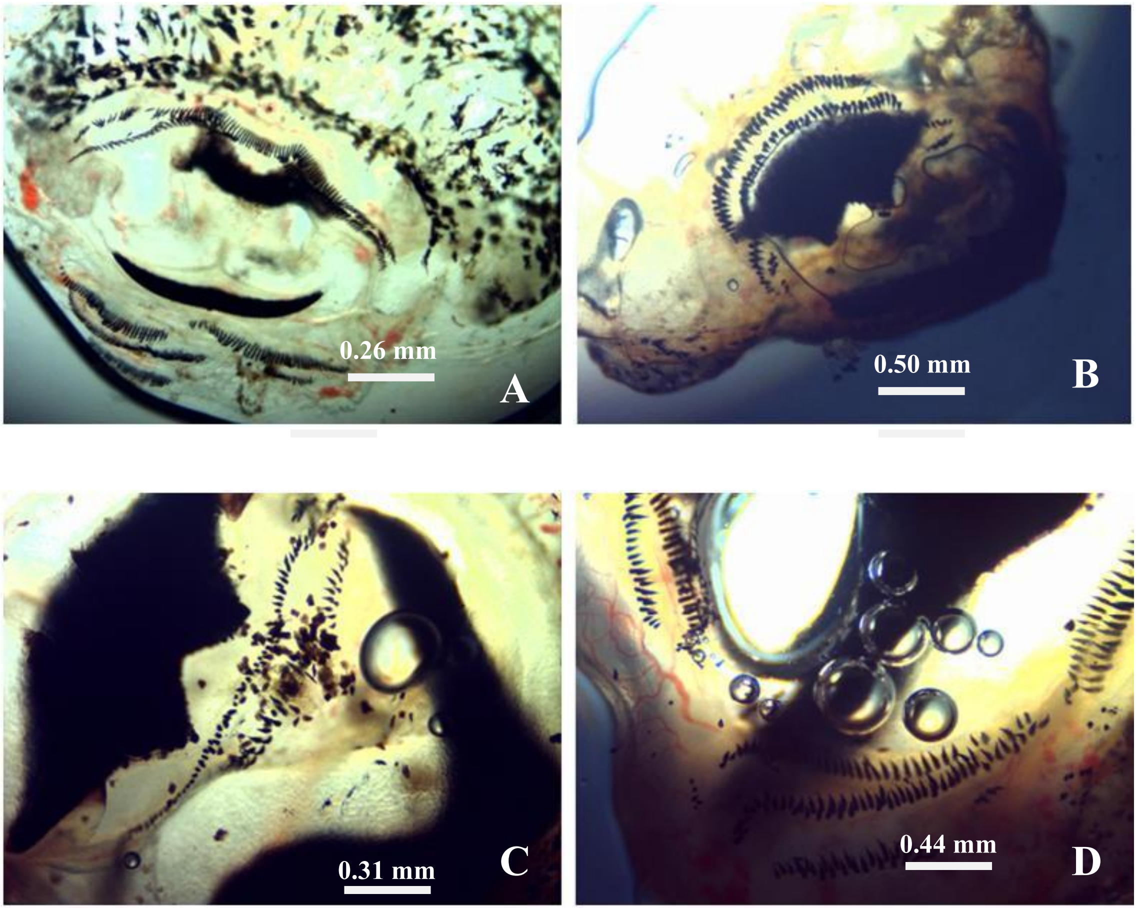


Figure 12. Photograph of the oral cage of *Hoplobatrachus occipitalis* tadpoles at different stages of their growth. A: 5 days; B: 10 days; C: 15 days; D: 20 days.

This study was part of the introduction of raniculture in Côte d'Ivoire. With regard to the physico-chemical parameters that prevailed during this trial, the relative humidity of the air and the air temperature evolved in opposite directions during the 2 waves, because when the temperature of a system increases, the relative humidity decreases. As for pH, the average values of 6.70 in screened enclosures and 8.80 in concrete basins are within the range (6.5-9) recommended by MDDELCC (2014a), and therefore favourable to aquatic life. Over the two waves, the pH remained low in the ponds (6.7-7.4) compared with the concrete basins (8.2-8.8). This is thought to be due to the greater humification of the vegetation in the ponds, which lowers the pH (Guy *et al.*, 1993). The higher pH in concreted ponds is also due to the proximity of the concrete (CSTC, 2007). The average water temperature was higher in the concrete ponds (29.5-29.9°C) than in the screened enclosures (27.2-28.1°C). The warmer water in the concrete basins could be explained by the restricted environment and the smaller quantity of water, compared with the mosquito net enclosures installed in ponds (CSTC, 2007). Survival rates (SR) for tadpoles were relatively low, to the point of zero (52.05% to 0%). This would appear to be due to the weakness of the various habitats to the action of predators. On the one hand, the various devices were vulnerable because they were being improved. We also found that tadpoles are highly prized by virtually all predators in the natural urban environment (snakes, lizards, birds, etc.). This was confirmed by Gunzburger & Travis (2005) who noted a very high mortality rate of Hyla cinerea tadpoles due to the combined effect of three predators. Nevertheless, a significant improvement in survival rates between the first wave (34.8-0%) and the second wave (52.05-7.52%) is thought to be due to improvements made to the two devices to control predators. This was achieved in particular by hermetically sealing the enclosures (sewing the edges of the enclosures and installing effective devices to close the basins) with fishing nets and mosquito netting. The final environment of the concrete basins resulted in much higher survival rates (52.05-18.2%) than the final environment of the mosquito net enclosures (35.15-7.52%). These results could be explained by the fact that the improved environment of the mosquito net enclosures is very exposed to predators and climatic hazards, given the vulnerability of the tadpoles. On the other hand, the improved environment of concrete basins offers several advantages, including stability, permanent oxygenation (El-Sherif & El-Feky, 2009), a more effective predator control system and a higher temperature. The latter parameter has been confirmed by

Neveu (2004). According to this author, insufficient temperature slows the growth of tadpoles and mortality can occur. TS are lower at higher densities, which is probably due to cannibalism Smith (1983) and competition for food. This is corroborated by Ouattara (2004), who maintains that the decrease in TS with increasing density could be linked to increasing aggressiveness. The doubling in tadpole weight observed from day 10 to day 20 showed that the 40.52% protein feed was well assimilated by *Hoplobatrachus occipitalis* tadpoles. The weights of the tadpoles were not significantly higher in ponds than in ponds, which may be due to a greater variety of feed given the nature of this environment. Taken together, these results show that the improved environment of the 3 m3 concrete basins presents the best characteristics for optimal growth of *Hoplobatrachus occipitalis* tadpoles.

The size of the mouth cage of *Hoplobatrachus occipitalis* tadpoles increased in proportion to their morphology and age. The light dentition of the tadpoles at the start and then their stronger, sharper dentition could be due to the feeding method. Initially, tadpoles are microphagous, grating plants and algae before feeding on small invertebrates (Hardouin, 2000; Neveu, 2004). The tadpoles therefore become more or less carnivorous, hence the presence of sharp teeth.

CONCLUSION

This study shows that *Hoplobatrachus occipitalis* tadpoles have the best chance of reaching metamorphosis in large numbers in concrete basins. The physico-chemical parameters are close to those found in the frogs' natural environment. As for the evolution of the mouth cage, this study clearly showed the diet of the tadpoles, thus facilitating their feeding in the rearing environment. In short, these studies make it possible to optimise the rearing of the *H. occipitalis* frog and to preserve the natural stocks of this species.

ACKNOWLEDGMENT

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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