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Suitable habitat for rearing the edible frog *Hoplobatrachus occipitalis* in ponds and concrete basins in a West African pre-forest ecosystem (Daloa – Côte d'Ivoire)

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Abstract
This study was undertaken to determine the suitable habitat for the rearing of one of the most prized edible frogs in Africa, particularly in Côte d'Ivoire. For this purpose, three environments were selected in ponds and concrete basins. The design of these environments took into account the semi-aquatic and semi-terrestrial lifestyle of the frogs. We then placed wild male and female specimens of the species studied in these habitats in order to test and improve them. Thus, regular measurements of the morphological parameters of the frogs and the physico-chemical parameters of the environment were taken. In the 1.8 m^3 concrete basins, the average survival rate was 13.41% after 30 days, whereas it was 75.00% and 83.33% after 60 days in the 3 m^3 concrete basins and 13.5 m^3 pond enclosures respectively. The average pH of the aquatic environments was 9.99 in the 1.8 m^3 basins, 8.87 in the 3 m^3 basins and 6.76 in the enclosures. The 3m^3 concrete basins and mesh enclosures maintained milder temperatures (29.83 °C to 30.52 °C), with high air temperatures. This study has shown that the improved environments of the 3m^3 basins and mesh enclosures are suitable for rearing adult *Hoplobatrachus occipitalis* frogs.

Keywords: Ranaculture, *Hoplobatrachus occipitalis*, basins, mesh enclosures

Introduction
The demand for edible frogs for human consumption is clearly increasing in some parts of Africa. In Côte d'Ivoire, there has been a sharp increase in the consumption of the frog Hoplobatrachus occipitalis in many parts of the country, especially in the forest because of its so-called pleasant taste (Tohé *et al.*, 2016) [22]. Also a recent study showed a good adaptation of *Hoplobatrachus occipitalis* reproduction in highly anthropized environments (Aliko *et al.*, 2018) [1]. Thus, to ensure the availability of this animal protein on the market and also to preserve wild populations of this species in the wild, of this species in the wild, it is therefore necessary to establish its breeding. However, modern frog farming for commercialization (ranaculture) does not currently exist in our country, unlike in some developed countries (France, Canada, USA...) and Asia. This activity is not practiced in an organized and rational way (Richard, 2008) [18] although it has been the subject of a few attempts in the past. In order to address these shortcomings and to respond to the numerous requests for precise information on frog farming, it would be important to create suitable farming infrastructures for ranaculture. The objective of this study is therefore the establishment of ideal environments for the rearing of the edible frog Hoplobatrachus occipitalis in ponds and concrete basins on the fish farm. In order to have basic data for successful ranaculture in Côte d'Ivoire.

Materials and Methods
Study site
The project was set up at the APDRACI (Association for fish farming and rural development in humid tropical Africa of Côte d'Ivoire) fish farm in Daloa (Figure 1), whose geographical coordinates are 6°51 north latitude and 6°27 west longitude. Daloa is the capital of the Haut Sassandra region in the centre-west of Côte d'Ivoire. This farm is located at the exit of the city on the Daloa-Issia axis, about 500 m from the old corridor. It has nineteen fish ponds, a large dam, three large concrete basins, six covered hatcheries, a water tower and other facilities necessary for fish farming.

Methodology for creating the different habitats

Based on preliminary studies of the concrete tanks and also the work of Hardouin (1997) [11]. This made it possible to set up 2 types of concrete basins. 4 basins 1 of 1.8 m^3 (1.5 m x 1.2 m x 1 m) and 4 basins 2 of 3 m^3 (2.5 m x 1.2 m x 1 m). A 75 pipe drainage system and a 25 pipe pressure supply system were installed and operated continuously to maintain good water quality. To accommodate the nature of the frogs, the basins were divided into two environments (terrestrial and aquatic). In the aquatic environment there is 20 cm of water and in the terrestrial environment 2 groups of plants. On the one hand, seedlings of certain food crops producing large flowers such as *Abelmoschus esculentus*, *Cucumis sativus*, *Phaseolus vulgaris* were planted before the start of the trial. On the other hand, plants from the frogs' natural environment were transplanted, such as *Ludwigia abyssinica*, *Cyperaceae*, *Asteraceae*, *panicum sp*. The four net pens were constructed using rafters 3m apart, each forming a 9 m2 square, with one part of the rafters on the dam and the other part in the pond. This ensures that each enclosure is part land and part water. Then two rows of mosquito netting (2 mm mesh diameter) were attached to the rafters using a 4-point spike. Thirty centimeters of the first row of netting was placed underground and the whole area was rammed and reinforced with solid bricks. Then the intersection of the two nets was wrapped and sewn together. A fishing net (15 mm mesh diameter) was used to close the top of the pens (Figure 2).

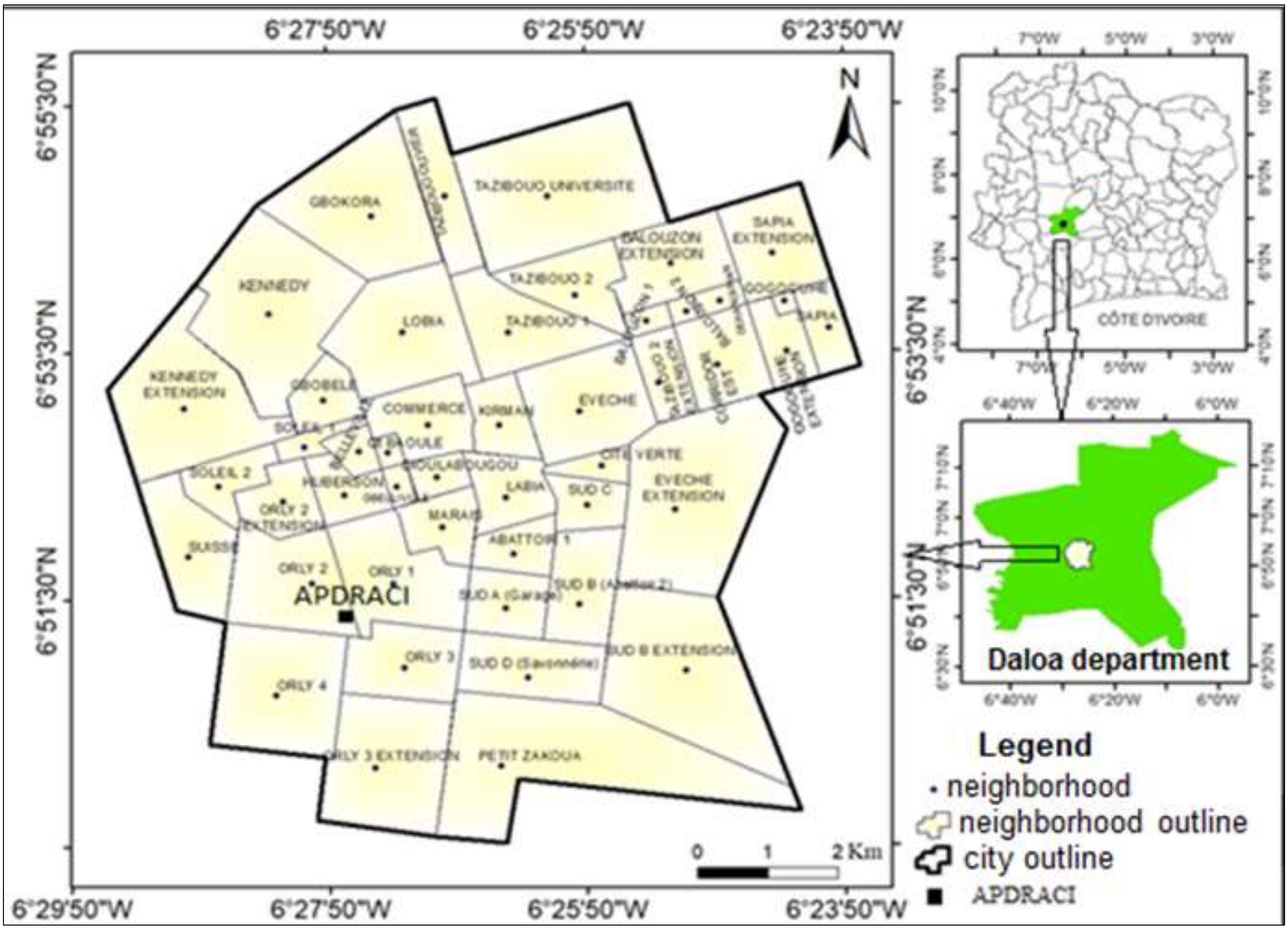


Fig 1: Geographical location of the city of Daloa (INS 2014) [13]

Techniques to prevent frog escape

This component was of paramount importance given the agility and exceptional jumping abilities of the frog Hoplobatrachus occipitalis. In order to prevent escapes from the concrete basins, three protective barriers were installed. The smooth interior of the basins to avoid providing jumping anchor points, then mesh fences following the contours of the basins and finally a net fence of the site. The design of the net enclosures in the ponds took into account the prevention of frog escape.

Fig. 2: Different habitats in the trial

A & B: 1.8m^3 concrete basin or basin 1; C & D: 3m^3 concrete basin or basin 2; E & F: 13.5m^3 mesh enclosure.

Experimental units

Frogs from healthy male and female wild strains of Hoplobatrachus occipitalis were collected from the same natural environment using the standard techniques of Heyer *et al.* (1994) [12] & Rödel and Ernst (2004) [19]. The frogs were divided by habitat according to their sex in the different devices as follows:

- 15 male frogs in the 1.8 m^3 basin (a), the 3 m^3 basin (a) and the mesh enclosure (a)
- 15 male frogs in the 1.8 m^3 basin (a'), the 3 m^3 basin (a') and the mesh enclosure (a')
- 15 female frogs in the 1.8 m^3 basin (b), 3 m^3 basin (b) and mesh enclosure (b)
- 15 female frogs in the 1.8 m^3 basin (b'), the 3 m^3 basin (b') and mesh enclosure (b').

Feeding

For the feeding of the wild frogs, several techniques have been implemented. Light traps were made and adapted to the concrete basins and pond enclosures to attract insects at night. Fry larvae were progressively released into the water of all the concrete basins and pond enclosures. The latter were less mobile and therefore easier prey for the frogs. There was also a gradual release of insect larvae or maggots that had been produced from fish remains and cow dung. All these feeding techniques were carried out concurrently to provide a varied diet for the frogs in captivity.

Identification and monitoring

The evaluation of the physico-chemical parameters of these environments was carried out on the one hand by taking daily measurements of the pH, water temperature and conductivity of the aquatic environment in all habitats. On the other hand, the relative air humidity, air temperature and luminosity were measured daily. These measurements were carried out at three times of the day, namely at 8 am, 12 noon and 6 pm. The assessment of the adaptability of the wild frogs was carried out by taking the weight and size of each individual every two weeks and counting the deaths. The choice of a large gap between two measurement sessions avoids stress (Barnett *et al*., 2001) [3] and avoids impairing the antibacterial properties of the amphibian skin (Mattute *et al.*, 2000; Nasciemento *et al.*, 2003) [15, 16]. To recognise each individual a sewing thread was attached to the hind leg before their webbing. For the identification and tracking of each individual in the different environments, sewing threads of different colours and combinations were attached to the abdomen of each individual. Each thread was tied loosely to avoid disturbing the frog and the negligible weight of the thread did not constitute an obstacle to its movement (figure 3).

Statistical tests

The Kruskal-Wallis, Anova, Mann-Whitney and Student's t tests were performed with the Statistica version 7.1 program (Statsoft, 2005). While the means and standard deviations were performed with the program Past version 3.10.

Fig 3: Male specimen of Hoplobatrachus occipitalis

Results

Physico-chemical parameters

Relative air humidity and air temperature

At 8:00 am, the air humidity averaged 63.33 ± 1.97%, 61.75 ± 1.29% and 60.34 ± 1.68% in basins 1, basins 2 and the pens respectively (Figure 4). The average air temperatures are in the same order of 28.11 ± 0.29, 27.93 ± 0.28 °C and 28.38 ± 0.21 °C. On the other hand, at midday (12:00) the ratios are reversed, so that on average, for air humidity's of 21.26 ± 1.08%, 18.78 ± 0.49% and 22.15 ± 0.69%, the air temperatures are respectively 42.72 ± 1.87°C at the level of basins 1, 39.22 ± 1.41 °C at the level of basins 2 and 34.96 ± 0.53°C at the level of the enclosures At 6 p.m. the ratios evolve similarly to those of 8 a.m. but with an increase in air humidity and a decrease in air temperature. Thus, on average, for air humidity's of 77.18 ± 1.66%, 73.71 ± 1.64% and 72.73 ± 1.14%, the air temperatures are respectively 25.25 ± 1.16 °C in basins 1, 26.54 ± 0.26 °C in basins 2 and 25.58 ± 0.22 °C in the mesh enclosures.

Hydrogen potential (pH)

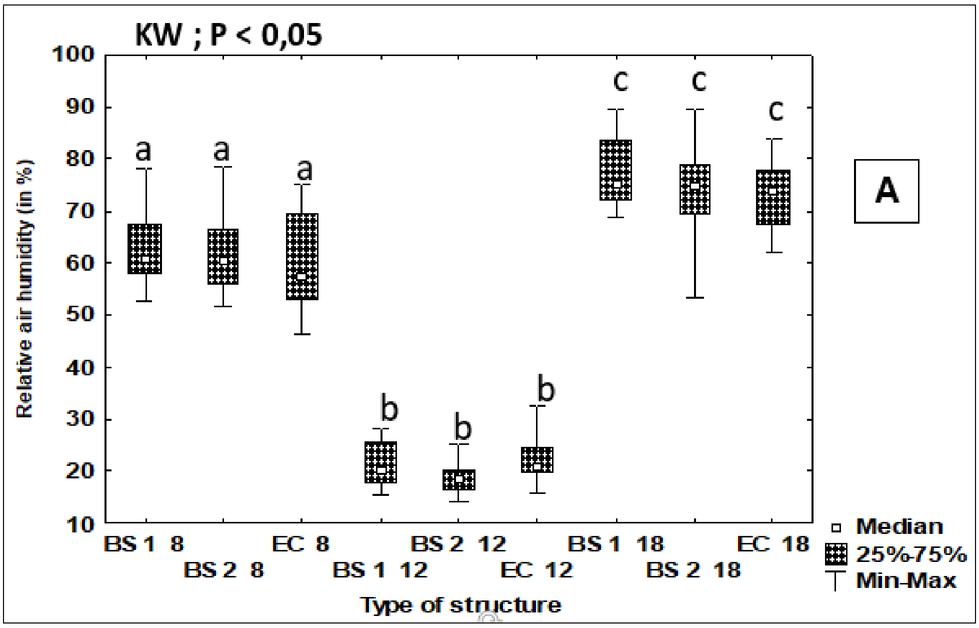
A variation in pH is observed between the three sites at 8h, 12h and 18h (figure 5A). At the beginning of the day (8 am), the pH is on average 9.99 ± 0.08 and 8.55 ± 0.08 respectively in basins 1 and 2, whereas it is 6.63 ± 0.03 in the pens. At noon (12:00) the pH was on average 09.87 ± 0.08, 8.92 ± 0.07 and 6.79 ± 0.03 respectively in basins 1, basins 2 and the mesh enclosure. As for the pH at sunset (18:00), it averaged 10.11 ± 0.08, 9.17 ± 0.07 and 6.89 ± 0.02 for basins 1, basins 2 and mesh enclosure respectively. Statistical analyses showed a significant difference between the pH averages for the three environments at 8 am, 12 pm and 6 pm (P< 0.05).

Water temperature

Water temperatures in general were slightly lower at 8am and 6pm but higher at midday at all three sites (Figure 5B). They are also higher in basins 1 and lower in the mesh enclosure from sunrise to sunset. At 8 am, the average water temperature was 30.32 ± 0.13 °C, 29.55 ± 0.11 °C and 29.73 ± 0.09 °C in concrete basins 1 and 2 and in the respectively. At 12:00, the average temperature was 31.77 ± 0.17 °C, 30.52 ± 0.09 °C and 29.83 ± 0.09 °C in concrete basins 1 and 2 and in the mesh enclosure, respectively. At 6 pm the water temperatures were 30.44 ± 0.13 °C in concrete tank 1, 30.04 ± 0.08 °C in concrete basins 2 and 29.15 ± 0.10 °C in the pens. The analysis of homogeneity of variance followed by the Kruskal-wallis test at the 5% threshold showed that the water temperatures differed significantly between the three environments at 8 am, 12 pm and 6 pm (P<0.001). But taken in pairs there was no significant difference at 8 am between basins 2 and mesh enclosure (Mann-whitney, P=0.35) and between basins 1 and basins 2 at 6 pm (Mann-whitney, P=0.12).

Conductivity

The conductivity of the water is generally higher in the concrete basins than in the basins and does not vary throughout the day (figure 5C). At 8 am it averaged 285.13 ± 10.85 US/cm in pond 1, 286.67 ± 8.68 US/cm in basins 2 and 131.60 ± 4.87 US/cm in the mesh enclosure. The average conductivity at noon was 296.25 ± 10.29 US/cm, 309.34 ± 7.41 US/cm and 129.04 ± 7.02 US/cm respectively in basins 1, basins 2 and mesh enclosure. At 18:00 hrs the conductivity values are approximately the same on average, i.e. 280.23 ± 9.26 US/cm, 301.34 ± 7.88 US/cm and 126.16 ± 4.51 US/cm respectively in basins 1, basins 2 and the mesh enclosure. Statistical tests show that there is a significant difference between basins and mesh enclosure at 8h, 12h and 18h (P< 0.001). The statistical tests show that there is no significant difference between the concrete basins 1 and 2 at 8 am (P= 0.72), 12 pm (P= 0.58) and 6 pm (P= 0.08).



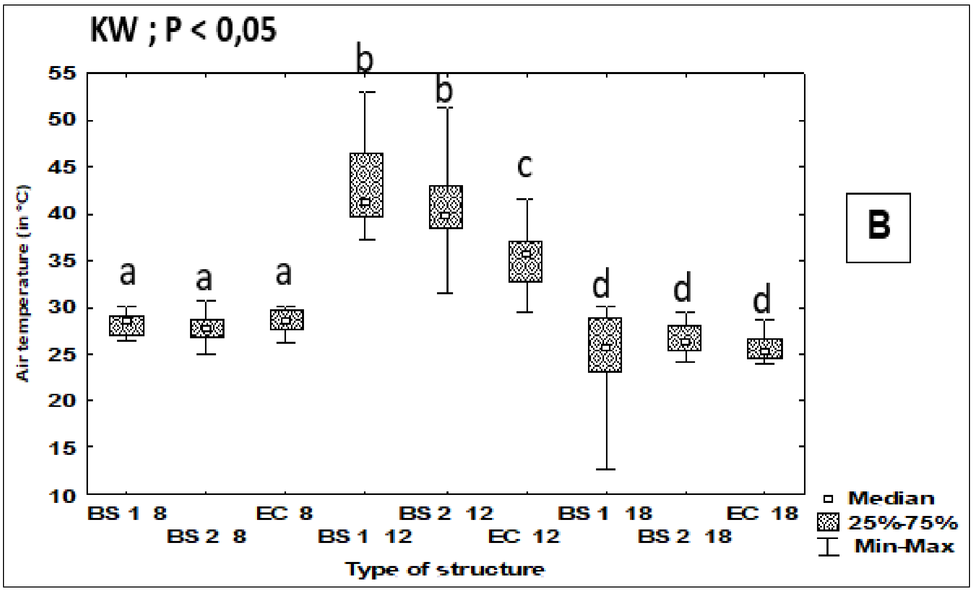
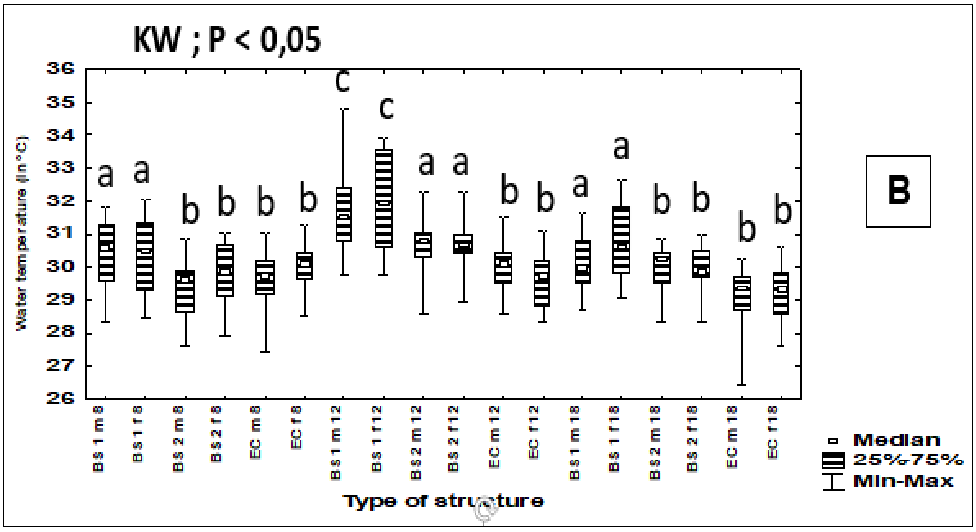


Fig 4: Air-related physico-chemical parameters of the different habitats at 8 am, 12 pm and 6 pm

A: relative air humidity, B: air temperature; BS: concrete basin, EC: mesh enclosure. P = results of the Kruskal-Wallis and Mann-Whitney tests at the 0.05 significance level. [abc] mean values on the same line that are not assigned the same letter are significantly different (p<0.05).



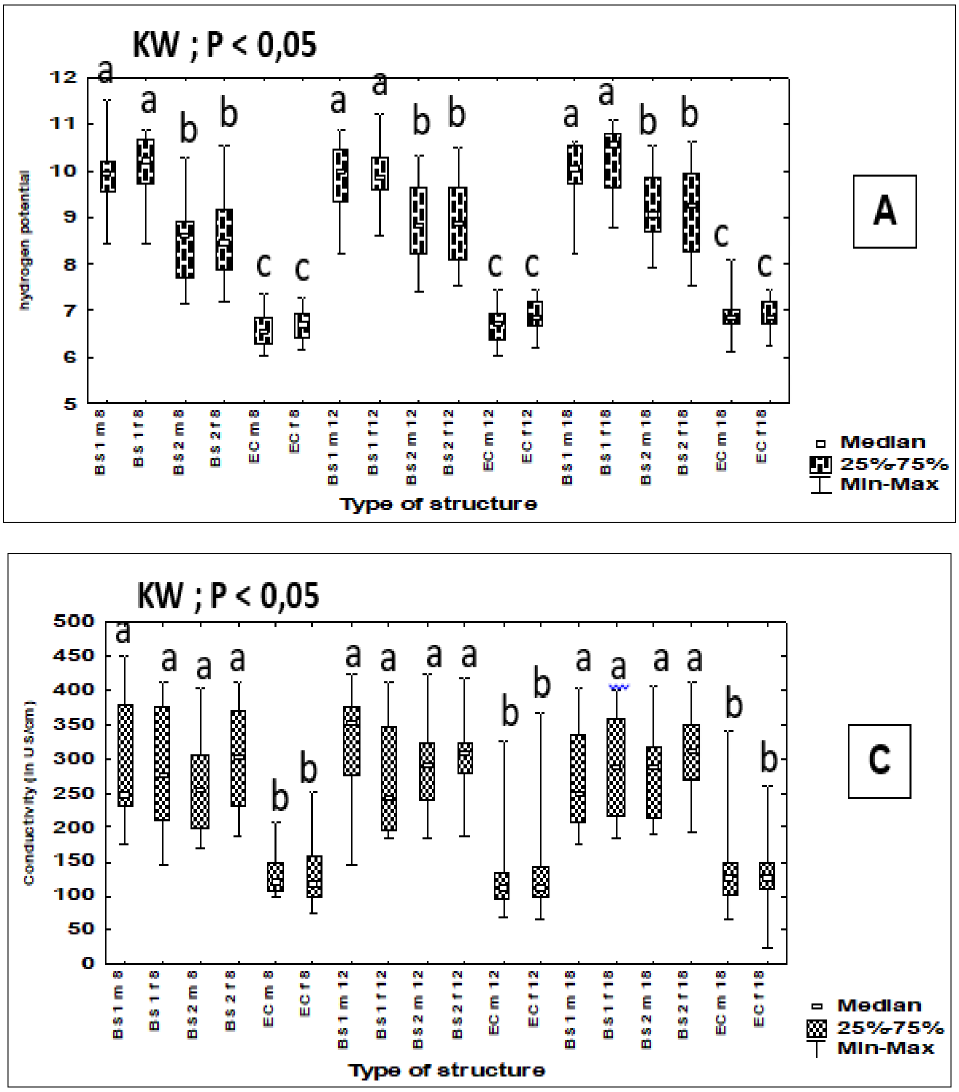


Fig 5: Physico-chemical parameters related to the water of the different habitats at 8 am,12 pm and 6 pm

A: hydrogen potential, B: water temperature, C: conductivity; BS: concrete basin, EC: mesh enclosure, m: male, f: female. P = results of the Kruskal-Wallis and Mann-Whitney tests at the 0.05 significance level. [abc] mean values on the same line that are not assigned the same letter are significantly different ($p<0.05$).

Morphological parameters

Males in different environments

In concrete basins 1, there was a significant drop in the number of surviving male frogs from day 1 to day 30 with a total loss of remaining frogs beyond that. Whereas in concrete basins 2 and the mesh enclosure there is a significantly high number of surviving male frogs from day 1 to day 60 (figure 6). For the body weights of surviving male frogs in basins 2 and the mesh enclosures, there is a general sawtooth pattern, so that after a loss there is a gain and vice versa and this to varying degrees. The survival rates of male frogs are very low in concrete basins 1 and high in the other two environments, with a slight preponderance of frogs surviving in mesh enclosure compared to tanks 2. The average survival rate on day 30 was 47.78 ± 17.16% in basins 1, but 83.33 ± 4.12% in basins 2 and 87.33 ± 2.71% in the mesh enclosure. Statistical tests of pairwise comparisons show that there is no significant difference between the survival rates of male frogs in basins 2 and in the mesh enclosures (Mann-whitney; P = 0.23), whereas there is a significant difference between basins 1 and basins 2 (Mann-whitney; P = 0.0008) and between basins 1 and the mesh enclosures (Mann-whitney; P = 0.0007).

Females in the different environments

For the females, we observed a similar evolution to that of the males, in terms of survival rates and frog weights, but to a greater extent (figure 7). The average survival rate of frogs on day 30 was 57.83 ± 15.23% in basins 1, while the average survival rate of frogs in basins 2 and the average survival rate of frogs in mesh enclosure was 89.33 ± 3.61% and 91.33 ± 1.73% respectively. Survival rates of female frogs in basins 2 and mesh enclosure were not significantly different (Mann-whitney; P = 0.78), while there was no significant difference between basins 1 and basins 2 (Mann-whitney; P = 0.0008) and between basins 1 and mesh enclosures (Mann-whitney; P = 0.0006). A comparison of the survival rates of male and female frogs in the three environments shows that there is no significant difference between them (Test *t*; P=0.52).

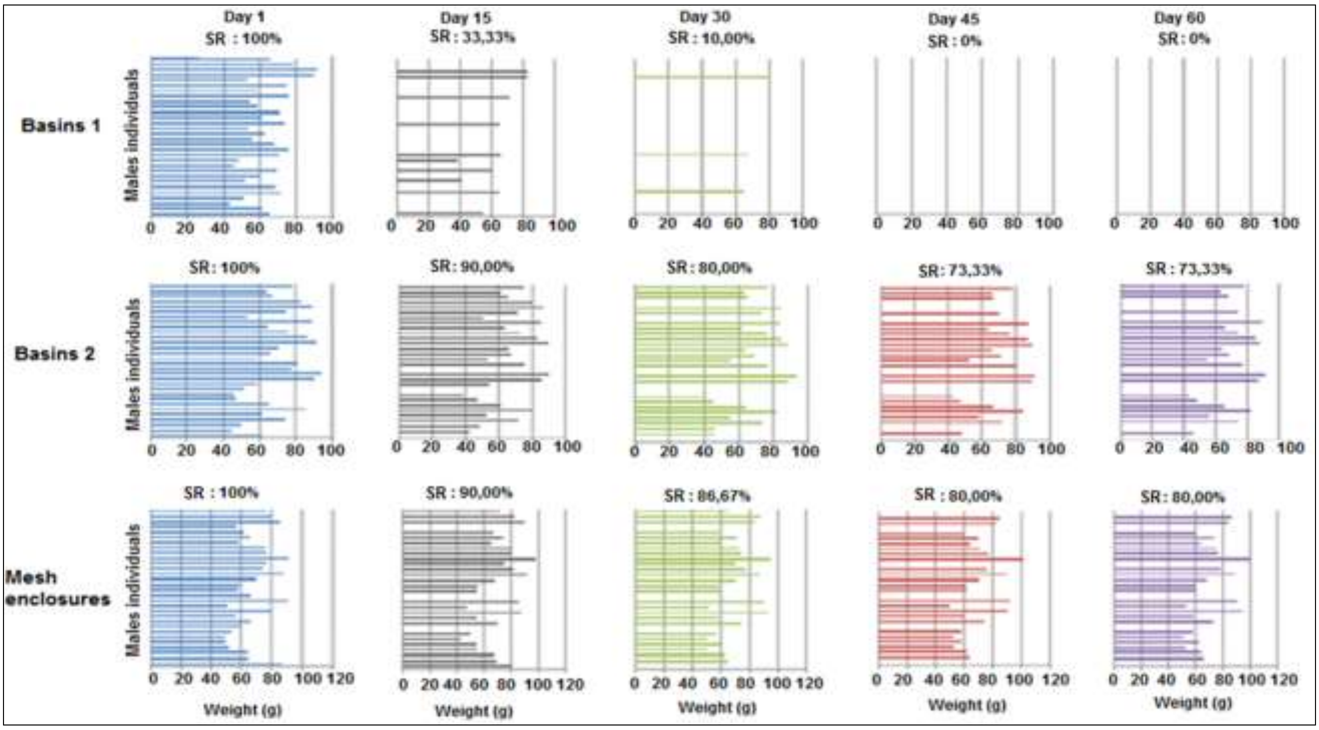


Fig 6: Weights of male Hoplobatrachus occipitalis frogs in concrete basins 1 and 2 and mesh enclosure in from day 1 to day 60 SR: survival rate

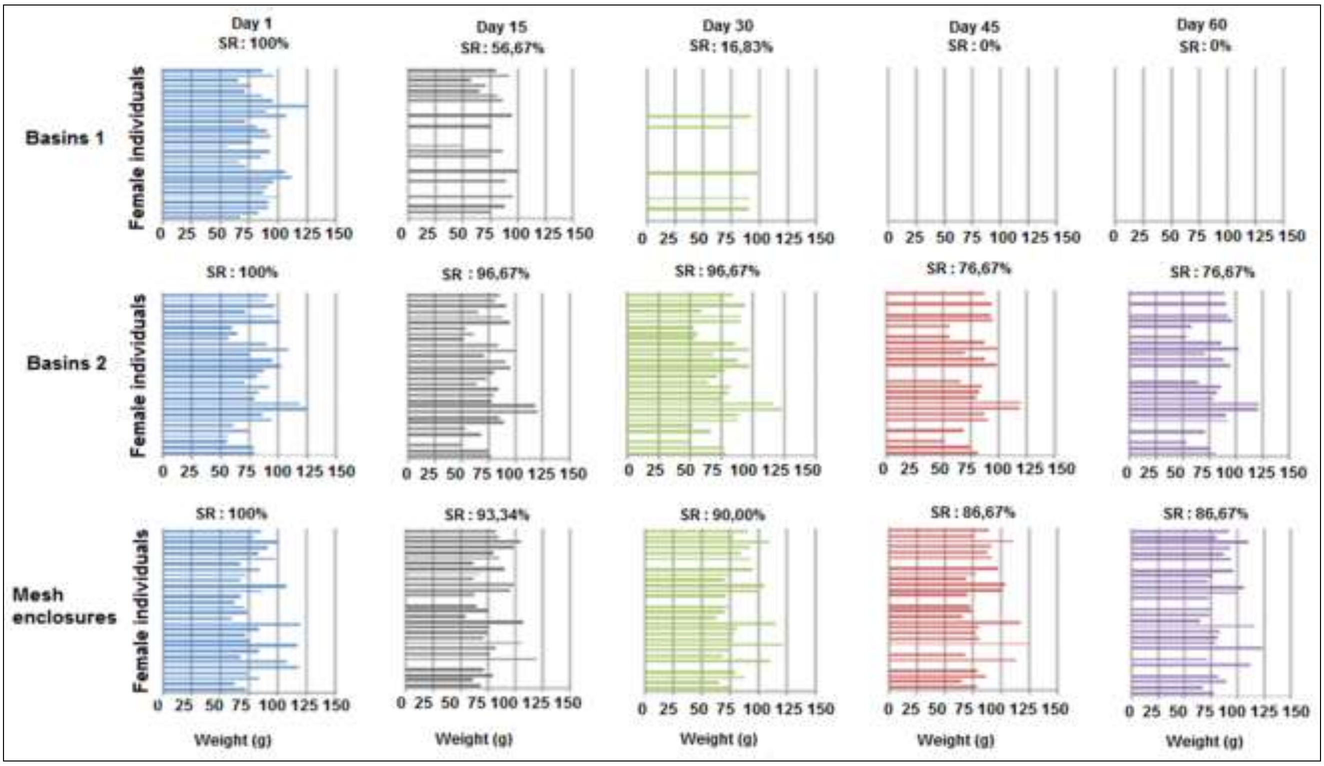


Fig 7: Weight of female Hoplobatrachus occipitalis frogs in concrete basins 1 and 2 and mesh enclosure from day 1 to day 60 SR: survival rate

Discussion

The regular measurement of pH in the three environments showed a major difference between the pH of the concrete basins (1 and 2) and the mesh enclosure. Indeed, the average pH of the concrete basins ranged from 10.11 to 8.55, whereas the average pH of the mesh enclosure ranged from 6.33 to 6.89. This result could be explained by the high pH of the Portland cement used to design the concrete basins. This is supported by the Scientific and Technical Centre for Construction (CSTC 2004), according to which concrete is a basic material and its pH is above 12.5. The acidic pH of the water in the mesh enclosures could be explained by the acidification of the aquatic environment of the basins as a result of the decomposition or humification of dead plants before the installation of the device. From 8 am to 6 pm, the average pH increased from 9.99 to 10.11; from 8.55 to 9.17 and from 6.63 to 6.89 respectively in basins 1, basins 2 and the mesh enclosure. This increase could be explained by two phenomena, photosynthesis and respiration by aquatic plants. Indeed, at daybreak, the pH is at its lowest due to the accumulation of CO_2 in the water through respiration during the night. As the day progresses, photosynthesis increases as the light intensity increases. More and more CO_2 is released from the water and absorbed by the plants, causing the pH to rise. This statement is corroborated by (Guy *et al.*, 1993) [9] according to which in a high pH environment photosynthesis is intense. The average pH in the basins and in basins 2 are closest to the ideal pH, which is in the range (6.5 - 9) recommended by MDDELCC (2014a) [2]. This statement is also supported by Laurentides (2013) [14]. According to which the pH range that allows for the protection of aquatic life is between 6.5 and 9.0 according to the criteria established by the MDDETP. Thus the pH of basins 1 is far from the ideal pH, which is confirmed by these authors Eric *et al*. (2006) [8] according to which a high pH is unfavourable to the development of amphibians. There is a significant difference between the pH of the three environments, which shows that these habitats have different aquatic environments from each other.

The water temperature of the three habitats (29.15 °C to 30.44 °C) is higher than the air temperature (25.25 °C to 28.38 °C) at sunrise (8 am) and sunset (6 pm) in the three habitats with high air humidity (60.34% to 77.18%). This could be explained by the fact that the period of the experiment corresponds to the harmattan period. Thus at these times the sunshine was low (233.50 lux to 2576 lux), The air has become colder while the different environments have retained heat. This is important for frogs as they need the water temperature to be above 26 °C (Carmona *et al.*, 1997) [4]. Basins 1 retains more heat due to its smaller environment.

At midday (12:00) the water and air temperatures and humidity are reversed, which is believed to be due to the increased sunlight (27326 lux to 34363.17 lux). Basins 2 and mesh enclosures manage to keep milder temperatures (29.83 °C to 30.52 °C) compared to basins 1 (31.77 °C), despite higher air temperatures (34.96 °C to 39.22 °C) and lower air humidity (18.78% to 22.15%). This could be explained by the fact that these environments are larger with larger amounts of water from basins 2 and pens in addition to the infiltration of water into the basins for the mesh enclosures and the presence of mud at the bottom of the water for basins 2. The average air temperature in the basins was even lower at midday (34.96 °C) than in the concrete basins (39.22 °C to 42.72 °C) due to the presence of several water bodies in the fish basins that soften the air. On the one hand there is no significant difference between basins 2 and mesh enclosures at 8 am and between basins 1 and 2 at 6 pm, and on the other hand there is a significant difference between the three environments at 12 pm. This could be explained by the fact that when it is hot, each environment reacts differently and in this case it is the basins 2 and the mesh enclosures that manage to maintain milder temperatures for the frogs.

The conductivity of the water was higher in the concrete basins (309 US/cm to 280.23 US/cm) than in the mesh enclosures (126.16 US/cm to 131.60 US/cm). This could be explained by the fact that the fresh water in the basins has a lower conductivity compared to mineral water. The water in the basins was close to mineral water due to the presence of concrete and the water in the basins came from a well on the farm that was dug into the bedrock. This would explain why its conductivity is higher. This statement is corroborated by Hade (2002) [10] and Wasc (2003), according to whom fresh water has a conductivity of less than 200 US/cm and mineral water has a conductivity between 200 US/cm and 1000 US/cm, which is in line with our results. Statistical tests confirm these results, so there is no significant difference in the conductivity of the aquatic environments of the concrete basins 1 and 2, which are mineral waters, whereas they are significantly different from the freshwater basins. Analysis of the survival rates of male and female frogs showed that they were higher in basins 2 after 60 days (73.33% to 80.00%) and mesh enclosures (76.67% to 86.67%) than in basins 1 after 30 days (10.00% to 16.67%). Statistical tests confirm these results as the first two do not differ significantly from each other whereas the opposite is true for the survival rates of the frogs in basins 1. These results could be broadly explained by the fact that basins 2 and the mesh enclosures have environments or habitats that have been more conducive to the life and wellbeing of the *Hoplobatrachus occipitalis* frogs compared to the basins 1 environment. Firstly, it is important to note that basins 1 and 2 share common advantages for frog life, including the two environments (terrestrial with vegetation and aquatic), the presence of a palm roof to reduce sunlight (Hardouin, 1997) [11] and the same food supply (light trap, fry, maggots) and finally a continuous water supply and drainage system.

In addition to all these elements, basins 2 has a larger surface area of 3 m^3 which is almost double that of basins 1 with 1.8 m^3. This advantage allows the frogs in basins 2 to reduce competition for food, for aquatic and terrestrial resting space and to offer more food. Also the presence of mud at the bottom of the water avoids contact between the frogs and the concrete slab and therefore creates an environment close to the natural environment. In terms of the physical and chemical parameters of basins 2, the pH (8.55 to 9.17) is close to the ideal pH (6.5 to 8.5), so this basins keeps the water temperature milder even when the air temperature is high, thus providing a refuge for the frogs. This assumption was also made by Stewart (1984) [20] who reported that in captivity amphibians need to have a body of water large enough for them to submerge in. All of these features help to reduce captive stress and thus create the conditions for increased survival as observed.

The mesh enclosures had greater advantages than the concrete basins 2, as they were natural living environments for the frogs with a terrestrial part made up of plants from their living environment and an aquatic part with reasonable spatial restrictions. Indeed, the living space was 13.5 m^3 larger than in basins 2 and the concrete was replaced by less aggressive mosquito netting. In terms of physico-chemical parameters, this environment has an ideal pH of 6.63 to 6.89 and, like basins 2, the enclosures have allowed for milder water temperatures (29.83 °C) with higher air temperatures. In addition to this at midday to the high sun in the basins the air temperature was more moderate which was of interest to the frogs as they were able to remain in the terrestrial environment in search of prey. This is supported by Stewart (1984) [20] that in captivity, temperature must be controlled and kept within limits that allow the amphibians to function. All of this may explain why the mesh enclosures had the highest survival rates.

Basins 1 had the lowest survival rates and therefore a very high mortality rate, this would be due to a major handicap of this environment which was the restricted living space of the frogs (1.8 m^3). Indeed, this parameter led to overpopulation and competition for food and space. Also this resulted in an increase in pH (10.11) due to the proximity to the concrete and also in high water (31.77 °C) and air (42.72 °C) temperatures. The result of all these consequences was an increase in the feeling of captivity and therefore a higher stress level that would have inhibited the feeding reflex. This was confirmed by the finding of empty abdomens of a large number of dead frogs in these basins. Our results are supported by the Canadian Council on Animal Care, which states that amphibians and reptiles are sensitive to heat, cold, dehydration and stress (CCAC, 2004) [5]. This dependence of amphibians on their living environment is also confirmed by EAZA, (2008) [7] which states that the permeability of their skin makes amphibians extremely vulnerable to small temperature changes. We found a slight increase in the survival rates of female Hoplobatrachus occipitalis frogs compared to males in all three environments (57.83% and 47.78% in basins 1 ; 89.33% and 83.33% in basins 2; 91.33% and 87.33% in mesh enclosure), although there was no significant difference between these survival rates. These results could be explained by the fact that the females of this species are often twice the size and mass of the males. So the females had more nutrient reserves with their large egg stock, which then allowed them to endure long periods of starvation whereas the males being smaller in size have few nutrient reserves and therefore need to feed more frequently. This result could also be due to the more active temperament of the males, which tend to be more on the move, resulting in more energy expenditure, whereas the females are calmer, conserving their resources and therefore better able to live in a confined environment.

Finally, the evolution of frogs' masses could be explained by the fact that the stress caused by captivity prevented them from feeding at first, resulting in a loss of mass, then those who were able to overcome this stress and feed were able to recover more or less the lost mass and survive. Also due to the limited resources the frogs did not have access to food at the same time, which explains the different weight evolution of each individual. This statement is confirmed by their behaviour during our nocturnal observations around the light traps. Indeed, when an insect was caught in the light trap, each frog tried its luck to swallow it. Thus according to Deborah *et al.* (2008) [6], captive amphibians must be provided with suitable prey. Also according to Hardouin (2000) [11], many insects will be attracted if lighting is installed (electric bulbs, paraffin lamps.).

Conclusion

The first thing we learned from this study is that the ideal environment for ranaculture is very difficult to find, given the nature and the very complex way of life of frogs and particularly of the species studied. However, despite these difficulties, we believe that we have found two environments that come close to this ideal environment, namely the concrete basins 2 and the mesh enclosure, in view of the high survival rates and the physico-chemical parameters that are close to the frogs' natural environment. We hope that further studies will perfect these environments for the well-being of the frogs in captivity and the preservation of the natural stock of *Hoplobatrachus occipitalis* frogs.

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