IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
July 2022 Vol.:22, Issue:1

Comparative Study of Natural and Artificial Reproduction and Evolution of Larva of *Hoplobatrachus occipitalis* (Günther 1858)

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Louis César AGOU*, Blayda TOHE, Beh Romaric KONATE

Laboratory of Environment and Aquatic Biology, UFR-Sciences and Environmental Management, Nangui Abrogoua University, 02 BP. 801 Abidjan 02, Ivory Coast.

Submitted: 20 June 2022
Accepted: 25 June 2022
Published: 30 July 2022

Keywords: Eggs, *Hoplobatrachus occipitalis*, Ovaprim, Natural and artificial reproductions

ABSTRACT

The comparative study of the natural and artificial reproduction of *Hoplobatrachus occipitalis* eggs at different stages until metamorphosis was carried out in a pond from April to May 2020 at the Agro-piscicole de la Mé Company. Thirty (30) spawners including 10 males and 20 females were released into a pond to induce natural mating. Simultaneously, 6 males and 6 females received an intramuscular injection of the hormone (Ovaprim®) at respective doses of 6 µL/g and 8 µL/g of body weight. After the eggs hatched, 50 tadpoles were reared in a pen (2 m x 1 m x 0.5 m) and placed in a pond. The tadpoles fed with a food containing 27% protein were monitored until metamorphosis. Egg laying after injection of the hormone occurs after 4h 30 min. while the first pond eggs were harvested after 13 days. The fecundity and hatching rates obtained at the pond level were 80.06 ± 2.78% and 81.86 ± 3.81% for natural reproduction against 90.04 ± 2.06% and 83 ± 4.24% for artificial reproduction. The number of eggs laid estimated under the effect of the hormone is 1428 ± 382.8. The average relative fecundity evaluated at the level of artificial reproduction is 9.77 ± 1.73 eggs/ g. Egg hatching is observed from 26 hours at 26.35 ± 0.21°C. The hind and forelegs appear respectively on the 8th and 28th day for the earliest tadpoles, while the resorption of the tail is complete 8 days after the eruption of the forelegs.

INTRODUCTION

In West Africa, *Hoplobatrachus occipitalis* which is the largest of all edible frogs is widely exploited for human consumption in many countries [1]. Most of its consumed specimens are taken from natural stocks [2]. In addition, the destruction of favorable habitats for the benefit of agriculture and urbanization accentuates the disappearance of the species [3]. The renewal of its population becomes an essential condition for its preservation and sustainable consumption. However, controlling its reproduction is one of the key factors for successful breeding [4]. According to [5], artificial propagation has several advantages, including year-round reproduction, genetic improvement of stocks and hybrids, and compensation for past attacks. The use of pituitary hormones or their analogs to induce ovulation in amphibians is well established in some species of laboratory anurans, including the northern leopard frog (*Rana pipiens*) and the wood frog (*Rana sylvatica*) [6-7]. Several reproductive hormones have been shown to induce ovulation and spermiation in anuran species by acting either at the pituitary or directly on the gonads [8]. Furthermore, studies carried out on *Rana angolensis*, [9], *R. catesbeina*, *R. esculenta*, and *R. tigerina*, [2-10] and on *R. temporaria* [11] have shown satisfactory results with natural reproduction of broodstock. Very few works relating to the reproduction of *H. occipitalis* in the natural environment [12] and a controlled environment with the use of synthetic hormones [13] are in our possession. However, according to [14], captive breeding is important for maintaining the viability of endangered amphibian species across the world and would allow viable oocyte production through hormone induction. Thus, the present work which aims to make a comparative study between natural reproduction and artificial reproduction by hormone induction on *Hoplobatrachus occipitalis* broodstock would be of undeniable importance for the sustainable production of this species of edible frog.

MATERIAL AND METHODS

The broodstock of *H. occipitalis* used in this experiment was collected at the site of the Agro-piscicole de la Mé Society (SAP de la Mé). This site is located in the south-east of Côte d'Ivoire between latitudes 05°11'15" and 06°41'15" North and longitudes 3°15'00" and 4°11'15' ' West (Figure 1). Thirty (30) broodstock including 10 males and 20 females (ratio 1:2) were released the same evening in a constructed pond (227 m²), and the water depth of the pond is maintained

at 10 cm. Early in the morning, the second group of 6 males and 6 females received an intramuscular injection of Ovaprim® (Syndel, Canada) at respective doses of 6 µL/ g and 8 µL/g of body weight. The broodstock used had a mass and length of 120 and 171 g and 9.8 and 12.2 cm respectively for females, 82 and 109 g, and 9 and 10.9 cm for males [12]. Selected females have well-developed abdomen to be sure they are carrying eggs. The injection was performed at 7 a.m. in the thigh muscles of each frog as recommended by [15]. Then, the pairs of frogs were distributed in 6 rectangular plastic bins (45 cm x 35 cm x 30 cm). To avoid the penetration of the sun's rays and to accelerate the maturation of the gametes, the plastic bins are covered by racks. Treated broodstock was monitored every 30 minutes to determine the exact egg laying time. During these operations, the water temperature was 26.5 ± 0.1°C, the dissolved oxygen 6.32 ± 0.17 mg/L, and the pH 7.34 ± 0.25 in the 6 bins. In addition, all the edges of the pond are inspected every morning for a month to collect the maximum eggs. Once the harvest is complete, the eggs collected are kept in plastic bins and exposed under a shed at room temperature until hatching. The egg storage water is changed 3 times/day to prevent the proliferation of bacteria.

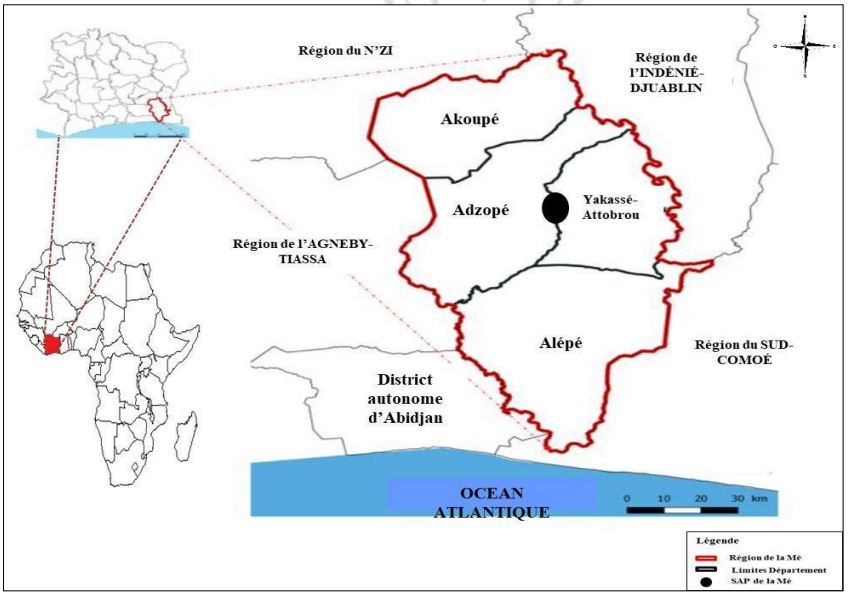

***Figure 1** : Location of Agro-piscicole de la Mé Society (Côte d'Ivoire).*

After egg hatching, 50 randomly selected tadpoles with an average weight of 0.04 g were reared in a pen (2 m x 1 m x 0.5 m) placed in a pond (figure 2). Tadpoles are fed with powdered food

containing 20% protein until metamorphosis. The ingredients used in the production of the food are obtained in the local market (Table 1). The food ration was 10% of body weight, distributed at 8 a.m., 11 a.m., and 3 p.m. A control fishing is carried out every 3 days to observe the different morphological changes that appear on these tadpoles. These changes relate to:

- The general appearance of the tadpoles upon hatching;

- The time of appearance of the hind and forelimbs;

- The resorption of the tail;

- The modification of the oral cavity.

***Figure 2**: Enclosure used for tadpoles rearing*

Table 1: Mineralogical composition of the industrial food used to feed tadpoles

Components	Percentage (%)	Proteins (%)	Lipids (%)	Carbohydrates (%)	Starch (%)
Copra meal	11	2,46	0,98	2,4	0,04
Soybean meal	25	10,88	5	8,6	0,45
Cottonseed meal	18	7,2	3,6	6,19	0,32
Rice bran	18,5	2,41	0,31	3,76	1,11
Bran	24	3,6	3,37	8,96	4,36
Salt	1,25	-	-	-	-
Sodium bicarbonate	0,5	-	-	-	-
Minerals	0,25	-	-	-	-
Shellfish	1,5	-	-	-	-
Total	100	27	13	30	6

Reproduction parameters such as absolute fecundity, relative fecundity, fecundity rate, and hatching rate allowed a comparison between the two studied reproductive systems.

Absolute Fertility: Absolute Fertility (AF) is the total number of eggs produced by the female. It designates the total number of eggs, or ova before fertilization, present in a female studied.

$$AF = \text{eggs extracted by pressure} + \text{eggs released into the water}$$

Relative Fecundity: Relative Fecundity (RF) indicates the number of eggs (or ovules) per unit of live weight.

$$RF = \frac{\text{Absolute Fertility}}{\text{Female weight (g)}}$$

Fertilization Rate: Fertility Rate (FR) is the number of eggs fertilized about the number of eggs laid.

$$FR = 100 * \frac{\text{number of fertilized eggs}}{\text{number of eggs laid}}$$

Hatching Rate: The Hatching Rate (HR) is the ratio of the number of hatched tadpoles to the number of fertilized eggs.

$$HR\ (\%) = 100 * \frac{\text{Number of tadpoles hatched}}{\text{Number of fertilized eggs}}$$

RESULTS

Study of natural and artificial reproduction systems

Spawning in natural reproduction was observed 13 days after placing the broodstock in the pond and after heavy rain, during the night. The recorded spawning temperature was 26.4 ± 0.2°C. On the other hand, the laying after the induction of the hormone occurs after 4 hours 30 minutes (Table 2). Eggs are observed over 8 days in the pond with a daily variation in the number of eggs collected.

Table 2: Spawning time after administration of the hormone (Ovaprim®) to* Hoplobatrachus occipitalis *females.

Females	body weight (g)	Size snout-vent (mm)	Spawning times
1	126	10.6	7 h
2	140	11.5	5 h 30 mn
3	123	10.4	6 h
4	171	11.8	4 h 30 mn
5	156	11.4	6 h 30 mn
6	145	11.6	10 h 15 mn

The fertilization and hatching rates obtained are respectively 80.06 ± 2.78 and 81.86 ± 3.81% (Table 3) for natural reproduction against 90.04 ± 2.06 and 83 ± 4.24 % (Table 4) for artificial reproduction. In this study, the total number of eggs laid by *H. occipitalis* females after injection of the hormone is estimated at 1428 ± 382.8. The average relative fecundity is 9.77 ± 1.73 eggs/ g for artificial reproduction (Table 4).

Table 3: Mean values of reproductive parameters of* Hoplobatrachus occipitalis *in the natural reproductive system.

Days	**Number of eggs collected**	**Number of fertilized eggs**	**Number of hatched eggs**	**Fertilization rate (%)**	**Hatching rate (%)**
D1	380	309	235	81,32	76,05
D2	635	517	459	81,42	88,78
D3	713	553	471	77,56	85,17
D4	503	411	357	81,71	86,86
D5	350	250	200	71,43	80
D6	450	374	301	83,11	80,48
D7	835	691	532	82,75	76,99
D8	1020	828	667	81,18	80,56
Mean ± standard deviation	611 ± 19	492 ± 16	403 ± 13	80,06 ± 2,78	81,86 ± 3,81

Table 4: Mean values of the reproduction parameters of* Hoplobatrachus occipitalis *after injection of Ovaprim (Artificial reproduction).

Parameters	**Mean ± standard deviation (n = 6 females)**
Number of eggs laid	1419 ± 387
Number of fertilized eggs	1269 ± 316,5
Number of hatched eggs	1058 ± 282
Absolute Fertility	1428 ± 382,8
Relative fecundity (eggs/g)	9,77 ± 1,73
Fertilization rate (%)	90,04 ± 2,06
Hatching rate (%)	83,36 ± 4,24

General characteristics of *Hoplobatrachus occipitalis* eggs

Each spawning egg is enveloped by a gelatin membrane (Figure 3A). This only disappears at the time of hatching. The egg is large and has two poles: the animal hemisphere of black color and the vegetative hemisphere of white color (Figure 3B). Under a magnifying glass, no morphological difference is observed between unfertilized eggs and fertilized eggs. This difference appears in newly laid unfertilized eggs as the egg shows degeneration of the animal hemisphere in favor of the vegetative hemisphere. The average egg diameter is 2.7 ± 0.01 mm.

Evolution of eggs and hatching

At the level of the fertilized egg, the nuclear mass undergoes a slight regression in favor of the peri-yolk space. The gelatin becomes clearer: we are at the embryo stage which occurs around 18 hours after spawning (Figure 3C). Complete hatching of the eggs was observed from 26 h at 26.35 ± 0.21°C. The positioning of the head, tail, and yolk sac is clear. Upon hatching, the eyes appear as well as external gills on each lateral side of the tadpoles (Figure 3D). The total length of the tadpoles at this stage is 9 ± 0.1 mm. The embryo feeds off the vitelline for the first 3 days.

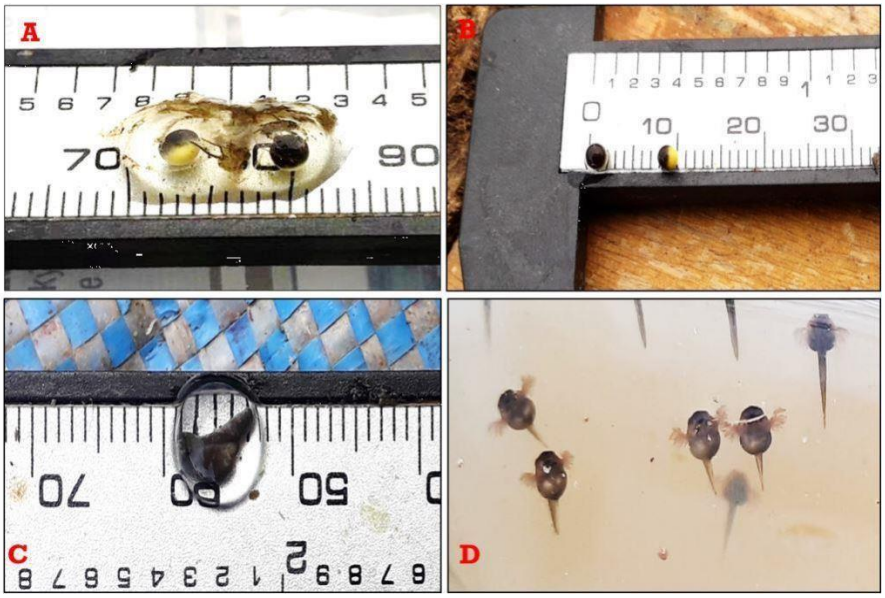

***Figure 3**: General appearance of Hoplobatrachus occipitalis eggs. A: cluster eggs maintained by gelatin; B: differentiation of the animal (black) and vegetative (white) hemispheres; C: head and tail differentiation and gelatin clearing; D: gill differentiation.*

Tadpole development towards metamorphosis

The morphological changes in the tadpoles begin from the first week of rearing. The outline of the hind legs (Figure 4Ad) occurs from the 8th day. They become more visible from the 13th day. From this point on, small black horny teeth are observable in the tadpole's mouths (Figure 4Bf). The nostrils are taking shape and the eyes are getting larger. The abdomen is clear and its contents are visible to the naked eye. The elongation of the tail is pronounced as it reaches a maximum length of 40 ± 2 mm with rapid development from the second week. At this stage, the tadpoles are strictly aquatic.

From the 28th day, we first note the appearance of the front legs below the skin of the tadpole: the right front leg is always the first to erupt (Figure 5A). The appearance and development of the front legs are very fast and occur in less than 48 hours (Figure 6). After the appearance of the front legs, we witness the resorption of the tail. The disappearance of the tail is complete 7 to 8 days after the eruption of the front legs (Figure 7). The eyes become bulging with the development of the eyelids and the formation of the eardrum on each lateral side of the head. The mouth widens and the teeth disappear to give way to a small tongue. The final coloring of the frog appears and it is amphibious at this stage.

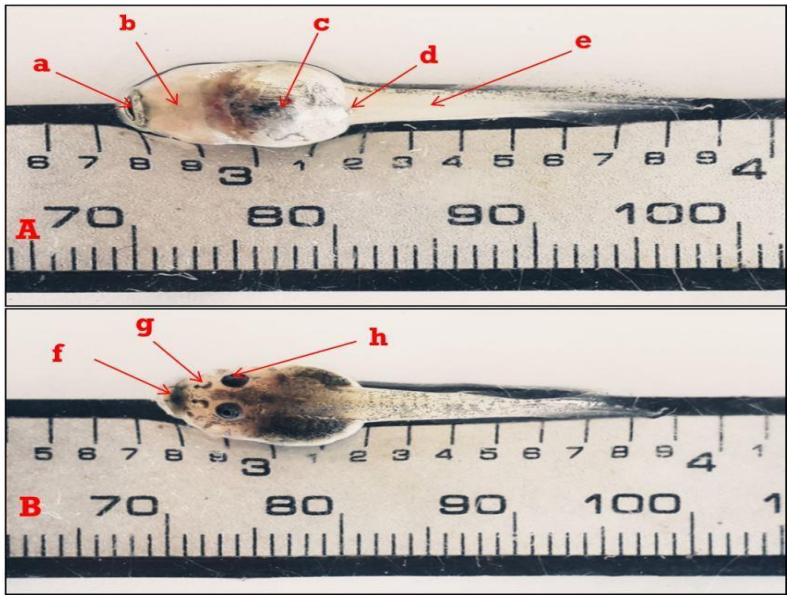

Figure 4*: Pre-metamorphosis modifications occurring on the tadpole. A: ventral view, B: dorsal view. a: mouth, b: trunk, c: abdomen; d: hindleg bud, e: tail; f: tooth; g: nostril; h: eye.*

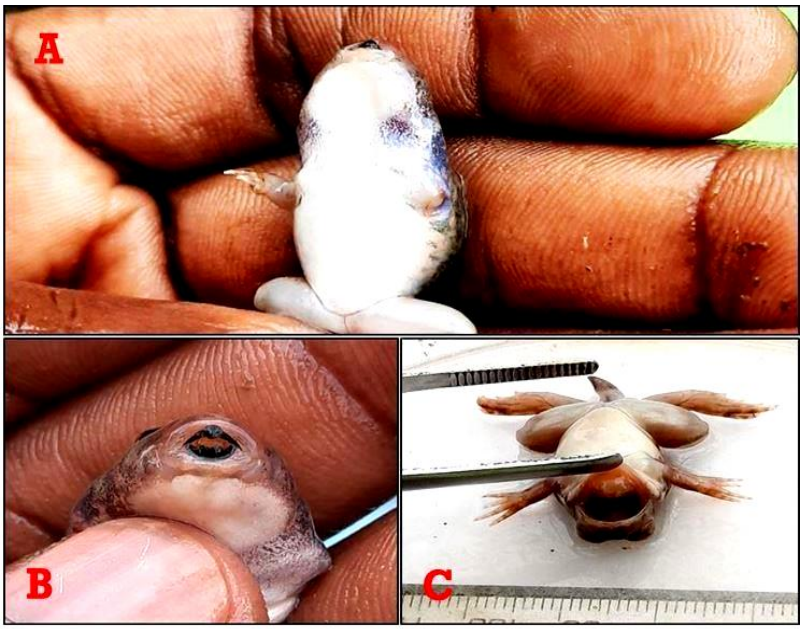

Figure 5*: Changes occurring during metamorphosis. A: Appearance of the forelimbs; B: Appearance of the mouth with the presence of teeth (pre-metamorphosis); C: the appearance of the mouth with the absence of teeth (post-metamorphosis).*

Figure 6*: Tadpole of Hoplobatrachus occipitalis on the first day of appearance of the forelimbs (A: dorsal view; B: ventral view).*

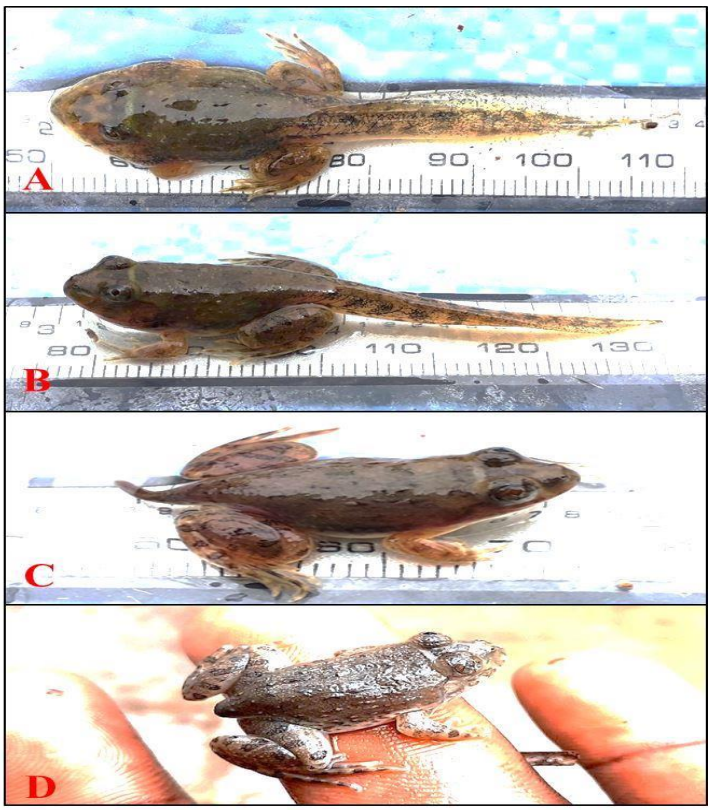

Figure 7*: Resorption of the tail of tadpoles after the appearance of the front legs.*

DISCUSSION

The general appearance of the eggs of *Hoplobatrachus occipitalis* shows similarities with the eggs of other amphibians with some differences. When laid, the eggs of *H. occipitalis* are opaque, ovoid in shape, black-white in color, and covered with a translucent membrane. These observations are similar to those mentioned by [15]. The average egg size of *H. occipitalis* at spawning is 2.7 ± 0.01 mm. This value is similar to those (2.74 mm) reported by [12] in the Banco National Park and 2.73 mm by [15] in the wetlands of the University of Abomey-Calavi (Benin). It is slightly lower than those recorded by [16] (2.9 to 3.7 mm) and [17] in the Comoé National Park (Côte d'Ivoire) which is about 3 mm. However, our values are higher than those indicated by [18] which reports that the eggs of *H. occipitalis* in the Outamba-Kilimi, region in Sierra Leone have sizes between 1.6 and 1.8 mm. The absolute fecundity observed in the present study (1428 ± 382.8) is very close to that indicated by [15] (1467 ± 217.504) but it is lower than that mentioned by [12] on the same species. The latter reported an absolute fecundity of

approximately 3225 oocytes. This difference could be explained by the size of the females. Indeed, during our experiment, the large broodstock laid more eggs than the smaller ones. Elsewhere, fecundity values in *H. occipitalis* are in the range 469 to 3752 [16] and in the range 800 to 1500 [19-17]. The fertilization rate recorded during this work (90.04 ± 2.06%) is very close to that mentioned by [15] who obtained a fertilization rate of 93.07 ± 3.23%. Ovaprim® is a hormone that is relatively efficient for inducing spermiation and spawning in *H. occipitalis* [20-15]. Also, the dose of Ovaprim® (0.008 ml/g) used is optimal for final maturation and egg release [15]. The hatching rate obtained in this study is 83.36 ± 4.24%. This value is lower than that (88.36 ± 3.15) recorded by [15] for the same species in Benin. This difference could be explained by the hatchery system used. Indeed, these authors used bins connected to a water renewal system with a regular supply of oxygen, whereas the water in our bins is renewed only 3 times per day, resulting in a reduced quantity of dissolved oxygen. Furthermore, the temperature recorded at hatching during the test, which is 26.35 ± 0.21°C at 26 h after laying, is similar to that measured by [15]. These authors observed a temperature of 26.4 ± 0.3°C during the hatching of *H. occipitalis* eggs 27 h after laying. On the other hand, the hatching time differs according to the species of frogs. According to [21], the hatching of the *Hyla Arborea* frog takes place after 52.30 hours at 20°C. Hatching of eggs of *Colostethus machalilla* takes place after 197 hours at temperatures between 18°C and 23°C [22], whereas that of the *genus Engystomops* takes place after 29 hours in the same temperature range [23].

CONCLUSION

This study shows that the breeding of *Hoplobatrachus occipitalis* could be carried out both naturally and in an environment controlled by hormonal induction. Hormonal induction has several advantages such as the rapid production of fertilized eggs and the availability of tadpoles at any time of the year.

ACKNOWLEDGMENTS

The authors thank Agro-piscicole de la Mé Company for the site access permit for carrying out this study and for the financial support.

COMPETING INTERESTS

The authors declare that there are no competing interests.

AUTHORS' CONTRIBUTIONS

All authors have made an adequate effort on all parts of the work necessary for the development of this manuscript according to their expertise. All authors read and approved the final manuscript.

REFERENCES

1. Mohneke M, Onadeko AB, Rödel MO. Medicinal and dietary uses of amphibians in Burkina Faso. *Afri. j. of the herp.*, 2011; 60:78-83.
2. Tohé B, Son P, Konate BR. Frog trade: A reality in Abidjan district, Ivory Coast. *World J. of Adv. Res. and Rev*. 2022; 13(02):371-376.
3. Mareike H. & Rödel M.O., 2011. The diet of the African Tiger Frog, *Hoplobatrachus occipitalis*, in northern Benin. *Salamandra*. 2011, 47(3):125-132.
4. Webber H, Riordan PE. Criteria for candidate species for aquaculture. *Aquaculture*. 1976; 7:107-123.
5. Woynarovich E, Horvàth L. La reproduction artificielle des poissons en eau chaude : manuel de vulgarisation. FAO Doc. *Tech. Pêches*. 1981, 191p.
6. Light P, Pickoff H. Species specificity in the response of an *in vitro* amphibian (*Xenopus laevis*) ovulation assay to mammalian lutenizing hormones. *Gen. and Comp. Endo*. 1976; 29:552-555.
7. Light P, Porter D, Millar RP. Specificity of amphibian and reptile pituitaries for various forms of gonadotropin-releasing hormones in vitro. *Gen. and Comp. Endo*. 1987; 66:248-255.
8. Reinier MM, Ross VH, Catherine BC. Hormonal induction of spermiation, courting behavior, and spawning in the southern bell frog, *Litoria raniformis*. *Zoo Biology*. 2010; 29:774-782.
9. Munyuli BMT. Elevage contrôlé des grenouilles au Kivu (République Démocratique du Congo), Cahiers Agricultures. 2002; 11 (4), 6p.
10. Hardouin J. Élevage commercial de grenouilles en Malaisie. *Tropicultura*, 1997; 15:209-213.
11. Neveu A. La raniculture est-elle une alternative à la récolte? Etat actuel en France. Institut National de la Recherche Agronomique (INRA). *Prod. Ani*. 2004; 17:167-175.
12. Tohé B, Assemian NE, Kouamé NG. Reproduction of African Tigrine Frog *Hoplobatrachus occipitalis* in Banco National Park (Ivory Coast). *Int. J. of Sci and Res*. 2016; 5:577-581.
13. Hardouin J., 1991. Un élevage de grenouilles-taureaux aux Philippines. *Tropicultura*. 9(1):34-36.
14. Clulow J, Clulow S. Cryopreservation and other assisted reproductive technologies for the conservation of threatened amphibians and reptiles: bringing the ARTs up to speed. *Repro., Fert. and Dev*. 2016; 28: 1116-1132.
15. Godome T, Sintondji SW, Azon MTC, Tossavi CE, Fiogbe ED, Ouattara NI. Artificial reproduction and embryogeny of the tiger frog *Hoplobatrachus occipitalis* (Günther 1858). *Zool. Soc*. 2020, 9p.
16. Channing A. Amphibian of Central and Southern Africa. Cornell University Press, Ithaca, New York. 2001, 496p.
17. Rödel MO. Herpetofauna of West Africa, vol. i : amphibians of the West African savanna, Edition chimaira, frankfurt/m. 2000, 335p.
18. Zug GR. Amphibians and reptiles of the Outamba-Kilimi region, Sierra-Leone. *J. of the Herp. Ass. of Africa*. 1987; 33:1-4.

19. Barbault R. Stratégie de reproduction et démographie de quelques amphibiens anoures tropicaux. *Oikos*. 1984; 43:77-87.
20. Kouba AJ, Vance CK, Willis EL. Artificial fertilization for amphibian conservation: current knowledge and future considerations. *Theriogenology*. 2009; 71:214-227.
21. Sayim F, Kaya U. Embryonic development of the tree frog, *Hyla arborea*. *Biologia*. 2008; 63:588-593.
22. Del Pino EM, Ávila ME, Pérez OD, Benítez MS, Alarcón I, NOBOA V, Moya IM. Development of the dendrobatid frog Colostethus machalilla. *Inter. J. of Dev. Biol*. 2004; 48:663-670.
23. Romero-Carvajal A, Sàenz-Ponce N, Venegas-Ferrín M, Almeida-Reinoso D, Lee C, Bond J, Ryan MJ, Wallingford JB, Del Pino EEM. Embryogenesis and Laboratory Maintenance of the Foam-Nesting Tùngara Frogs, Genus *Engystomops* (= Physalaemus). *Dev. Dyn*. 2009; 238:1444-1454.