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Effect of artificial food on the survival and growth tadpoles of *Hoplobatrachus occipitalis* (Günther 1858) in pond-raised

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ABSTRACT

The current study aimed at evaluating the effect of artificial food on the survival and growth tadpoles of *Hoplobatrachus occipitalis* in rectangular enclosures (2 m x 1 m x 0.5 m) placed in a pond until the onset of metamorphosis. Three different mediums: medium 1 (E1) = tadpoles fed with artificial food containing 27% protein, medium 2 (E2) = tadpoles fed with spirulina and medium 3 (E3) = no-fed tadpoles were tested in triplicate on tadpoles of initial weight 0.04 g. The tadpoles were fed daily at 10% of their biomass at a frequency of 3 times/day. After 28 days of rearing, the highest final weight, final length, specific growth rate and survival rate, respectively 2.16 ± 0.21 g, 62.59 ± 1.81 mm, 1328 ± 0.32% and 80.33% were recorded with tadpoles of medium 1 fed with the artificial food containing 27% protein. Growth and feed utilization parameters recorded in medium E1 tadpoles were significantly higher ($p<0.05$) compared to other culture mediums. The results obtained show the possibility of rearing the tadpoles of *Hoplobatrachus occipitalis* with an artificial feed in a pond.

Keywords: *Hoplobatrachus occipitalis*, tadpoles, artificial food, medium, rearing.

INTRODUCTION

The study of the living beings which surround us raises in all the spirits a great attention especially when these have an interest in the human consumption. Frogs are found in all regions of the world offering a temperate climate, although these animals are particularly fond of the tropics and subtropics (Morin, 2008). They are poikilotherms whose growth is directly linked to the temperature of the medium. Amphibians have a dual respiratory system depending on the medium in which they find themselves (Neveu, 2004; Morin, 2008). These animals are of capital importance for humans both in terms of food and economics (Neveu, 2004; Mareike et al., 2011; Godomé et al., 2018). Frogs are eaten by various populations in sub-Saharan Africa, where they constitute a very important food resource (Onadeko et al., 2011). This consumption mainly concerns *Hoplobatrachus occipitalis* captured in the wild (Godomé et al., 2018; Coulibaly and Zigui, 2021.). The trade of this frog is very flourishing in many west African countries (Benin, Burkina Faso, Ghana, Guinea, Côte

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d'Ivoire, Nigeria), where specimens are sold fresh, dried or fried (Mareike et al., 2011). This trade generated heavy captures of the amphibian population which led to a reduction in natural stocks (Collins et al., 2003). This has led some researchers to look into the possibility of its breeding. This involves mastering its reproduction, its diet and its behavior in captivity (Tohé, 2009; Godomé et al., 2018). In Côte d'Ivoire, the amphibian fauna is relatively little studied. The available studies focus on biological diversity, eating, habits and reproduction (Kouamé, 2009; Assemian et al., 2015; Konan et al., 2016; Tohé et al., 2016). Rearing trials carried out in a controlled medium (Godomé, 2018, 2019) and in ponds (Hardouin, 2000; Munyuli, 2002) on *H. occipitalis* encourage the determination of zootechnical bases of this species. However, the basic techniques of rearing from breeding to assessing the food needs of this species are poorly understood. Acquiring this knowledge is essential for the successful breeding of this frog. Indeed, the breeding of a species would be a disadvantageous and risky operation if one does not control its needs to ensure good growth of the tadpoles. This work therefore aimed at assessing the effect of an artificial food on the survival and growth tadpoles of *Hoplobatrachus occipitalis* in ponds.

MATERIALS AND METHODS

Study site

The tadpoles of *Hoplobatrachus occipitalis* used in the present experiment were obtained by natural reproduction in ponds from broodstock collected on the site of the Agro-piscicole de la Mé company (Figure 1). 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.

Experimental process

After hatching, the tadpoles were transferred to plastic bins for a period of three (03) days where they were fed with spirulina. After this period, they were transferred to different enclosures in the evening to avoid thermal shock. Four hundred and fifty (450) tadpoles with 0.04 g of weight and 17.45 mm of lengh were used for this experiment. Tadpoles were distributed in 9 enclosures of dimension (2m x 1m x 1m) arranged in a pond of 180 m² (Figure 2). Each enclosure was loaded with 50 tadpoles. The height of the water in the pond was kept at 50 cm. Three mediums were tested in triplicate in enclosures for 28 days:

Medium 1 (E1): tadpoles + plankton + industrial food (Contains 27% protein);

Medium 2 (E2): tadpoles + plankton + spirulina;

Medium 3 (E3): tadpoles + plankton.

The intakes of industrial feed (E1) and spirulina (E2) are made 3 times a day (8 a.m., 11 a.m. and 3 p.m.). Tadpoles are fed 10% of their biomass. Each enclosure is independent of the others and the water in the pond was fully exposed to the sun to stimulate natural temperature conditions. The tadpoles were followed to metamorphosis to determine the best growth medium.

The water quality was monitored by measuring of temperature, dissolved oxygen and pH. To measure these parameters, a portable multimeter (OxyGuard, Handy Polaris) and a pH meter (VOLTCRAFT, PH-100ATC) were used. The measurements were taken every day at 7:30 a.m. and 3 p.m. A fishery was taken every five days to determine the growth parameters of the tadpoles. The food ration was adjusted accordingly. The different measurements taken related to the total length and weight of the various individuals.

Statistical analysis

The data collected was used to calculate the zootechnical parameters. For each parameter, the mean and range were calculated. The statistical software R (version 3.4.2) was used for the analyses with 5%

probability significance threshold. A one-way analysis of variance (ANOVA 1) was carried out in order to compare the zootechnical parameters in the different treatments. The Student-Newman-Keuls test (SNK test) was used to make pairwise comparisons of the different treatments when significant differences were observed between the treatments, growth and survival rate of *Hoplobatrachus occipitalis* tadpoles.

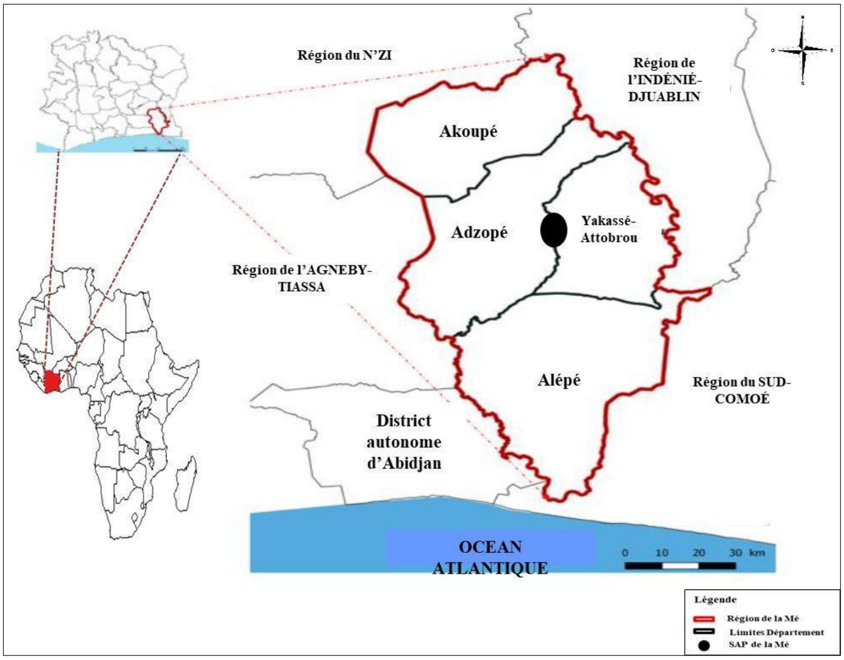

Figure 1 : Location of Agro-piscicole de la Mé company (Côte d'Ivoire).

Figure 2: Enclosures used for rearing *Hoplobatrachus occipitalis* tadpoles.

RESULTS

During the experimentation, there was no significant difference (p>0.05) between temperature, pH and dissolved oxygen (Table 1). The mean temperature and pH values varied from 26.47 ± 0.93 to 33.13 ± 0.72°C and 6.43 ± 0.31 to 7.61 ± 0.38, respectively. The highest values were observed in the afternoon. Oxygen concentrations varied between 2.8 ± 0.56 and 3.5 ± 0.23 mg/l with a slight drop at the end of aging. The lowest dissolved oxygen values were also observed in the afternoon.

The growth performance and survival rate of *H. occipitalis* tadpoles relative to food are shown in Table 2. The recorded survival rates vary between 38.67 ± 3.61% and 81.33 ± 3.31%. The highest survival rate was observed in enclosures fed with artificial food (E1), while the weakest survival rate (38.67 ± 3.61%) was observed in enclosures with non-fed tadpoles (E3). The differences between the survival rates are significant at the threshold α = 0.05 (SNK, p<0.05) affected by the different types of medium. The survival rate in enclosures fed with spirulina was 45.67 ± 2.03%. Final weight, daily weight gain, specific growth rate, and feed conversion rate were significantly influenced by each treatment. The average weight of tadpoles fed with artificial food significantly increased from until the end of the testing. The rapid increase of tadpole weight is observed from the 17th day of rearing (Figure 3). The maximum value of the final weight was observed in enclosures fed with artificial food (E1), ie 2.16 ± 0.21 g. The lowest mean value was recorded with no-fed tadpoles, ie 1.69 ± 0.26 g. Tadpoles fed with spirulina obtained 1.76 ± 0.3 g. The highest final length (p <0.05) was observed in tadpoles fed with artificial food, ie 62.59 ± 1.81 mm. The final lengths obtained in media 2 and 3 were similar and are respectively 60.29 ± 3.28 and 58.74 ± 3.23 mm. Figure 4 shows the differences between tadpoles at the start and at the end of the experiment. The specific growth rate and the feed conversion index were significantly affected (p<0.05) by the different treatments. Tadpoles raised in medium 2 and 3 showed low specific growth rate which are respectively 12.56 ± 0.57 and 12.43 ± 0.53%/d compared to medium 1 which is 13.28 ± 0.32%/d.

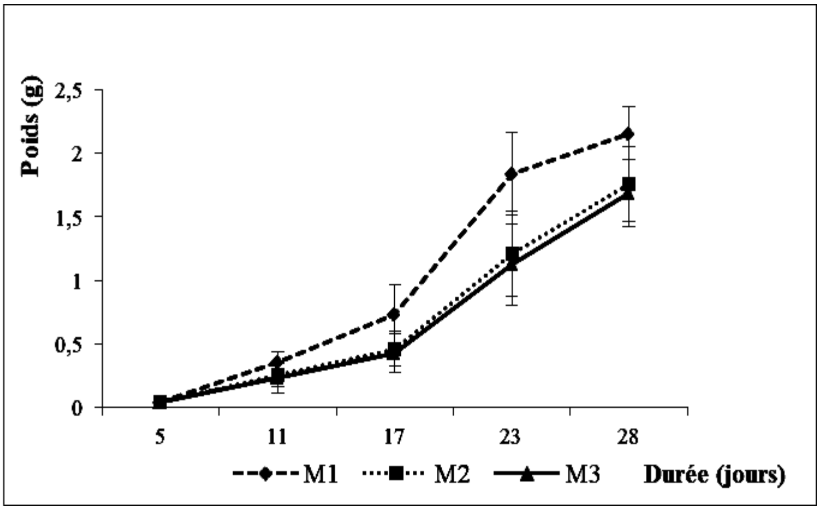

Figure 3: Effect of different fed on the body weight of *Hoplobatrachus occipitalis* tadpoles during 28 days.

Figure 4 : Morphological appearance of *Hoplobatrachus occipitalis* tadpoles.

- Tadpole at the start of the experiment: A: dorsal view, B: ventral view (6 days).
- Tadpole at the end of the experiment: C: dorsal view, D: ventral view (28 days).

Table 1: Evolution of temperature (T), pH, and dissolved oxygen (DO) throughout the breeding period.

		week 1	week 2	week 3	week 4
pH	Morning	6,49 ± 0,25[a]	6,81 ± 0,26[a]	6,72 ± 0,33[a]	6,43 ± 0,31[a]
	Evening	7,09 ± 0,37[a]	7,04 ± 0,24[a]	7,61 ± 0,38[a]	7,02 ± 0,15[a]
T (°C)	Morning	26,47 ± 0,95[a]	26,52 ± 0,74[a]	26,7 ± 0,42[a]	26,97 ± 0,22[a]
	Evening	32,4 ± 1,93[a]	31,17 ± 1,83[a]	33,13 ± 0,72[a]	32,8 ± 2,29[a]
DO (mg/L)	Morning	3,37 ± 0,12[a]	3,35 ± 0,57[a]	3,33 ± 0,35[a]	3 ± 0,56[a]
	Evening	3,1 ± 0,2[a]	3,5 ± 0,23[a]	3,07 ± 0,33[a]	2,8 ± 0,56[a]

[ab] values on same line and not affected by same letter are significantly different (p <0.05).

Table 2: Zootechnical parameters of *Hoplobatrachus occipitalis* tadpoles fed with different types of food for 28 days.

Parameters	Medium 1	Medium 2	Medium 3
SR (%)	80,33 ± 3,31[b]	45,67 ± 2,03[a]	38 ± 3,61[a]
Li (mm)	17,45 ± 0,0[a]	17,45 ± 0,0[a]	17,45 ± 0,0[a]
Lf (mm)	62,59 ± 1,81[b]	60,29 ± 3,28[a]	58,74 ± 3,23[a]
Pi (g)	0,04 ± 0,0[a]	0,04 ± 0,0[a]	0,04 ± 0,0[a]
Pf (g)	2,16 ± 0,21[b]	1,76 ± 0,3[a]	1,69 ± 0,26[a]
DWG (g/j)	0,07 ± 0,01[b]	0,06 ± 0,01[a]	0,05 ± 0,01[a]
SGR (%/j)	13,28 ± 0,32[b]	12,56 ± 0,57[a]	12,43 ± 0,53[a]

[ab] values on same line and not affected by same letter are significantly different (P< 0.05).
Survival Rate (SR), Initial Length (Li), Final Length (Lf), Initial Weight (Pi), Final Weight (Pf), Weight Gain (GP), Daily Weight Gain (GPJ), Specific Growth Rate (TCS).

DISCUSSION

Temperature is the most important factor interfering with the growth of frogs because it directly affects the metabolism. The mean temperature values of all tests performed are identical and fall within the favorable range for rearing frogs (15 to 35°C) recommended by Petersen and Gleeson (2011). According to FAO (2006), an interval of 28 to 42°C is necessary for good growth of tadpoles. In addition, the lowest oxygen concentrations were observed during the last weeks of breeding. These low concentrations would be due to the no-renewal of water in our medium during the entire period of breeding thus causing a strong dissolution of organic matter from the products of metabolism discharged into the medium (aquatic plants and animals present in the pond). In addition, pH values observed during the four weeks were similar and are within the range (6.5 - 9) recommended by the Ministry of Sustainable Development, Medium and the Fight against Climate Change (2014). According to Godomé et al. (2018), a pH range 6.2 and 7 and temperature equal or upper than 28°C are suitable for the growth of *Hoplobatrachus occipitalis*. In general, water quality in the rearing structures is optimal and remains within the required standards of Summerfelt (2000). In this experiment, the survival rates of *H. occipitalis* tadpoles ranged from 38.67 ± 3.61% to 81.33 ± 3.31%. The highest survival rate is observed in enclosures fed with artificial feed, while the weakest survival rate (38.67 ± 3.61%) is observed in enclosures with no-fed tadpoles. The high survival rate in enclosure fed with artificial feed could be attributed to the quality of the feed but and its availability throughout the testing period. Similar results were recorded with the tadpoles of *Rana catesbeiana* (Munguia-Fragozo et al., 2015), *Pelophylax saharicus* (Meher et al., 2014), *Litoria aurea* (Browne et al., 2003) and *Hoplobatrachus occipitalis* (Godomé et al., 2019). The low survival rates observed in media 2 and 3 could be related to the lack of sufficient food but and the high level of cannibalism of the tadpoles of *H. occipitalis* (Ogoanah and Uchedike, 2011; Buston and Sperry, 2017). In fact, *H. occipitalis* is carnivorous in the larval state and the cannibalism of the earliest tadpoles is very important. In this study, the medium 3 is used to observe evolution of tadpoles in the natural medium. The low survival rate observed is in agreement with the work of Neveu (2004) who estimated the survival rate in the wild to be very low (about 6%) whereas in controlled breeding survival rates of 92% can be obtained. The final weight of *H. occipitalis* tadpoles reared in the different media was significantly different over the entire rearing period. The highest value (2.15 ± 0.21 g) of final weight is noted in enclosures fed with artificial feed. These results clearly indicate that the feed containing 27% protein has a significant effect on the growth of tadpoles. The use of artificial feed for tadpole rearing has been achieved with satisfactory results in *Rana angolensis* (Munyuli, 2002), *Parachanna obscura* (Kpogue et al., 2013), *Rana catesbeiana* (Munguia-Fragozo et al., 2015) and *Hoplobatrachus occipitalis* (Godomé et al., 2018, 2019). Furthermore, the final weight with the artificial feed is approximately twice that of those obtained by Godomé et al. (2018, 2019). These authors obtained average weights ranging from 0.48 to 0.95 g on tests carried out in circular tanks. This could be explained by the fact that the tadpoles have a greater space of movement in enclosures. They also have natural foods present in the pond such as zooplankton, phytoplankton (Peres and Oliva-Teles, 2005). In this study the final length of the tadpoles varied from 58.74 ± 3.23 to 62.59 ± 1.81 mm. Tadpoles fed the artificial food obtained the longest length (62.59 ± 1.81 mm). The development in size of tadpoles is subject to the consumption of food allowing remarkable changes in morphology, physiology and behavior during the metamorphosis process. This life stage differentiates Anurans from other aquatic vertebrates and affects nutritional experiences due to weight loss in the animal to achieve metamorphosis. The final length values obtained are greater than those (24.5 - 46.71 mm) recorded by Munguia-Fragozo et al. (2015) on *Rana catesbeiana* tadpoles reared in plastic containers. This difference could be explained by temperature variation, differences in experimental facilities, feed rates and water quality management. The statistically higher specific growth rate (13.28 ± 0.32%) with the artificial food is thought to be related to the quantity and quality of the food consumed. Indeed, according to Peres and Oliva-Teles (2005), the increase in weight is synonymous with the availability of aquatic animals and synonymous with the availability of food.

Conclusion

At the end of this study, it can be learned that the use of artificial feed for rearing positively affects the growth and survival rate of *Hoplobatrachus occipitalis* tadpoles in ponds. Development of a complete feed based on the nutritional requirements of tadpoles of this species is recommended for optimum growth performance.

COMPETING INTERESTS

The authors declare that there is no competing interests.

AUTHORS' CONTRIBUTIONS

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

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