Research Article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Effects of Spirulina on the Survival and Growth of Larvae of *Hoplobatarchus occipitalis* (Günther, 1858)

Louis César AGOU[1*], Blayda TOHE[1] and Beh Romaric KONATE[1]

[1]Nangui Abrogoua University, Laboratory of Environment and Aquatic Biology, UFR-Sciences and Environmental Management, 02 BP. 801 Abidjan 02, Ivory Coast.
E-mail: agoualc29@gmail.com; Tel: (+225) 07 48 46 43 68

ABSTRACT

The effect of three different foods on the survival of *Hoplobatrachus occipitalis* tadpoles during the first days of life was studied in three different environments. The T1 medium contains tadpoles fed with an industrial food containing 40 % protein, the T2 medium has tadpoles fed with fertilized pond water and the T3 medium contains tadpoles fed with spirulina. All mediums were tested in triplicate on tadpoles with an average initial weight of 0.08 g in plastic trays. The tadpoles were fed daily at 6 % of their biomass distributed every two hours. After seven days of breeding, the highest average final weight and survival rate which are respectively 0.2 ± 0.02 and 75.33 ± 3.78 % were recorded in tadpoles fed with spirulina. These different values recorded with the tadpoles of medium 3 were significantly higher ($p<0.05$) compared to the other two treatments.

KEY WORDS: *Hoplobatrachus occipitalis*, tadpoles, spirulina, survival rate.

***Corresponding Author:-**

Louis César AGOU

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

E-mail: agoualc29@gmail.com; Tel: (+225) 07 48 46 43 68

INTRODUCTION

The survival rate is an important parameter in breeding. Like most aquatic species, many frogs carry out the first part of their life cycle in water with a very varied diet. They are generally predators that only attack living and mobile prey. Frogs are generally herbivorous in the larval state and carnivorous in the adult state [1]. From the start of its diet, at the tadpole stage, the animal preferably consumes green algae and other aquatic organisms. In the adult stage, it becomes extremely selective and consumes only mobile preys [2]. However, some species such as *Hoplobatrachus occipitalis* which is the subject of the present study are carnivorous in the larval state as well as in the adult state. Its preys are usually invertebrates: molluscs, annelids, arthropods [3.4]. However, the carnivorous nature of *Hoplobatrachus occipitalis* tadpoles complicates the first few days of captive breeding. Indeed, the level of cannibalism is high as large tadpoles target other tadpoles as prey [5]. In a recent study [6], live zooplankton (*Artemia salina*) was used to feed the tadpoles of *Hoplobatrachus occipitalis* during the first days of rearing. However, the high cost of living zooplankton limits its use for tadpole production. It is therefore important to research new methods of feeding tadpoles in order to limit cannibalism. It is in this context that takes place the present study which uses spirulina as an accessible and less expensive food for tadpoles during the first days of breeding.

MATERIEL ET METHODES

The spawners of *Hoplobatrachus occipitalis* used in the present experiment were collected on the site of the Agro-Piscicole de la Mé Society. 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).

Three days after hatching, the tadpoles collected with a dip net are transferred to rectangular plastic experiment tanks. The experiment is conducted for seven days in nine plastic tanks containing 15 liters of water each. Tadpoles with an initial average weight of 0.08 g are subjected to three treatments: T1 (tadpoles fed with industrial food), T2 (tadpoles fed with fertilized pond water) and T3 (tadpoles fed with spirulina (*Arthrospira platensis*)). The test is carried out in triplicate and the tadpoles are fed manually every day, every two hours from 8:00 a.m. to 6:00 p.m. at 6% of their body weight [7]. The water in each tank was renewed morning and evening (7:30 a.m. and 5:30 p.m.). As for the water in the pond, it is taken every morning from a fish pond fertilized with chicken droppings and pig manure and stored in a 60-litre tank.

Table No. 1 : Bromatological and mineralogical composition of industrial food grading at 40% protein.

	Proportion (%) per 100g
Soybean meal	45
Fishmeal	41
Rice bran	8,5
Bicarbonate	0,5
Vitamin mineral complex	0,25
Shellfish	1,5
Salt	1,25
Palm oil	2
Dry matter (%)	90,14
Ash (%)	16,34
Crude protein (%)	40,25
Crude fat (%)	7,73
Gross energy (Kcal/Kg)	4348,45

Definition of different environments:

- **Treatment 1:** The tadpoles in this medium are fed with an industrial food titrating at 40% protein. Its composition is given in table 1.

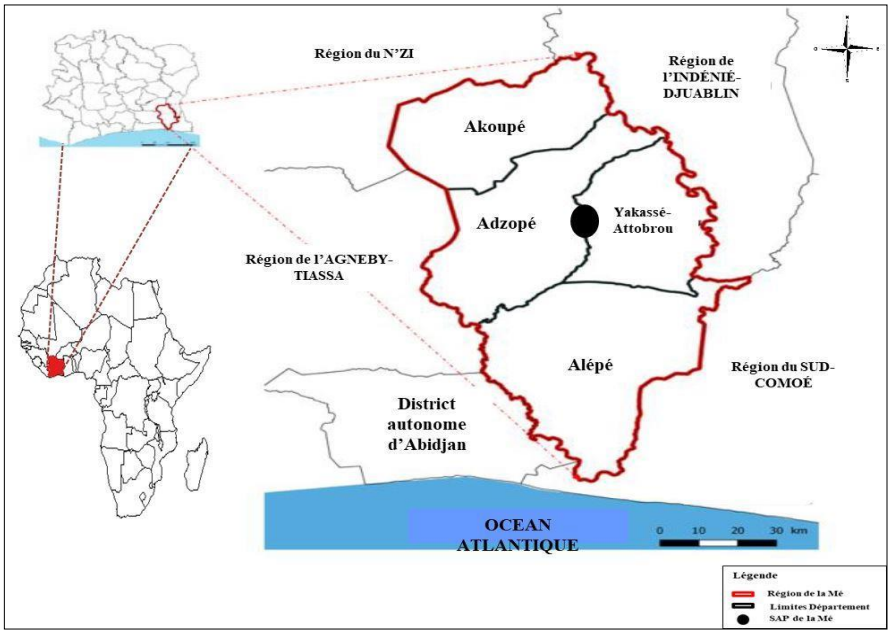

Fig. 1 : "Location of Agro-Piscicole de La Mé Society (Côte d'Ivoire)"

- **Treatment 2:** The water used for the study of this environment was collected in a fish pond fertilized with organic fertilizers (50% chicken droppings + 50% pig manure) at a rate of 0.2 kg of fertilizer / m^2 two days before impoundment in the pond. Thereafter, every week, the fertilization of the pond continues with a supply of 2 g / m^2 of fertilizers. This fertilization rate is the one used by the Agro-piscicole de La Mé Society (SAP de la Mé) for tilapia *Oreochromis niloticus* breeding.

- **Treatment 3**: The tadpoles in this environment are fed with spirulina produced in situ by SAP de la Mé. One gram of spirulina is distributed each day in the tanks during the 7 days of breeding. The nutrient composition of spirulina is shown in Table 2 below.

Table No. 2 : "Bromatological and mineralogical composition of spirulina (Arthrospira platensis) (SAP de la Mé)"

Proportion (%) per 100 g	
Proteins (%)	65
Carbohydrates (%)	15
Lipids (%)	6
Minerals (%)	8
Humidity (%)	5
Energy (KJ)	1672
	Values in mg
Beta - carotene (mg)	150
Gamma Linolenic Acid	1000
Phycocyanin	8500
Chlorophyll - a	700
Vitamin B 12	0,2
Vitamin E	10
Iron	110
Magnesium	250
Calcium	120
Phosphorus	800

Gamma linolenic acid (OMEGA-6): cell regeneration and resistance to infections;

Beta - carotene (Pro-VIT-A): vision, growth and resistance to infections;

Vitamin E (antioxidant): resistance to infections and cell regeneration;

Vitamin B 12: production of red blood cells;

Phycocyanin (anti-oxidant): cell cleansing;

Iron: production of red blood cells;

Magnesium: cell growth and regeneration;

Calcium: bone regeneration; Phosphorus: bone regeneration.

Statistical analyzes

The data collected was used to calculate zootechnical parameters. For each parameter, the mean and the standard deviation were calculated. The R statistical software (version 3.4.2) was used for the analyzes with a probability significance level of 5%. A one-way analysis of variance (ANOVA 1) was carried out in order to compare the survival rate and the final weight of the tadpoles in the different treatments. The Student-Newman-Keuls test (SNK test) was used to make comparisons of the different treatments when significant differences were observed between the treatments, the growth and the survival rate of the tadpoles of *Hoplobatrachus occipitalis*.

RESULTS AND DISCUSSION

Physico-chemical characteristics of the rearing environment

The physico-chemical parameters of the water in the different tanks are recorded in Table 3. The average pH values oscillate between 6.78 ± 0.15 and 8.33 ± 0.13. The highest pH value (8.33 ± 0.13) is noted in the tanks fed with spirulina and the lowest (6.78 ± 0.13) is noted in the medium with fertilized water. These pH values are influenced by the different treatments (SNK, $p<0.05$). As for the temperature values, they are between 27.3 ± 0.18 °C (T2) and 27.43 ± 0.14 °C (T1). They are similar in all tanks (ANOVA, $p>0.05$). The dissolved oxygen contents recorded vary between 3.34 ± 0.20 mg / l (T3) to 4.45 ± 0.22 mg / l (T1) passing through 4.07 ± 0.37 mg / l (T2). These mean values are statistically identical (SNK, $p>0.05$) between T1 and T2 and they are different from those of T3 (SNK, $p<0.05$).

Table No. 3 : "Mean values of temperature (°C), pH and dissolved oxygen (DO) of water in *Hoplobatrachus occipitalis* tadpole rearing tanks"

	T1	**T2**	**T3**
	Artificial food	**Fertilized water**	**Spirulina**
pH	6.8 ± 0.1^{a}	6.78 ± 0.15^{a}	8.33 ± 0.13^{b}
T (°C)	27.43 ± 0.14^{a}	27.3 ± 0.18^{a}	27.34 ± 0.15^{a}
DO (mg/L)	4.45 ± 0.22^{a}	4.07 ± 0.37^{a}	3.34 ± 0.2^{b}

Table No. 4 : "Evolution of survival rate and final weight of tadpoles of *Hoplobatrachus occipitalis* subjected to three different foods for 7 days in plastic tanks"

	T1	**T2**	**T3**
	Artificial food	**Fertilized water**	**Spirulina**
Ni	100	100	100
Pmi (g)	0,08 ± 0,01	0,08 ± 0,01	0,08 ± 0,01
Nf	12,67 ± 2,44^{a}	7 ± 1,33^{b}	75,33 ± 3,78^{c}
Pmf (g)	0,15 ± 0,02^{a}	0,14 ± 0,02^{a}	0,2 ± 0,02^{b}
TS (%)	12,67 ± 2,44^{a}	7 ± 1,33^{b}	75,33 ± 3,78^{c}

^{abc} mean values on same line, following by different letters are significantly different (P < 0.05).

Ni: Initial number; Nf: Final number; Pmi: Initial average weight; Pmf: Final average weight; SR: Survival rate.

Survival rate and final weight

Throughout the rearing period, the tadpole survival rate varies from 7 ± 1.33 to 75.33 ± 3.78% (Table 4). The highest survival rate is recorded in tanks fed with spirulina (75.33 ± 3.78%) while the lowest is observed in tanks fed with fertilized pond water (7 ± 1.33%). Tadpoles fed the artificial diet recorded a survival rate of 12.67 ± 2.44%. The values of the final mean weight were statistically influenced (SNK, $p<0.05$) by the different treatments and are between 0.14 ± 0.02 and 0.2 ± 0.02 g. The highest Pmf (0.2 ± 0.02 g) is observed in tadpoles fed with spirulina. No significant difference was observed between the T1 and T2 treatments.

The evolution of the average final weight (Pmf) of the tadpoles according to the three treatments is illustrated in figure 2. After the first day, there is a shift between the T1, T2 and T3 treatments, which is clearer on the 7th day.

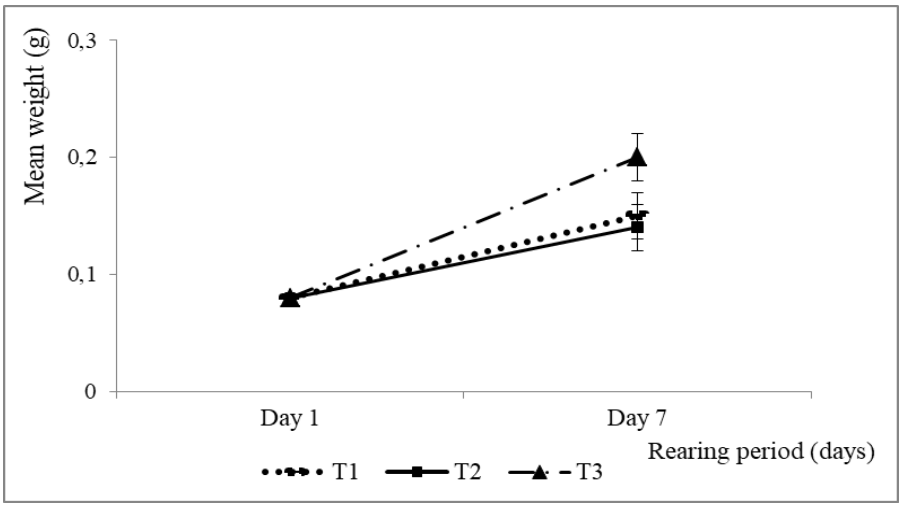

Fig. No. 2 : **"Evolution of *Hoplobatrachus occipitalis* tadpoles submitted to three different foods for 7 days in plastic tanks. T1: Tadpoles fed with artificial food; T2: Tadpoles fed fertilized pond water; T3: Tadpoles fed with spirulina**

DISCUSSION

The average values of the physico-chemical parameters of the water recorded in the tanks during the period of the experiment are between 27.3 ± 0.18 and 27.43 ± 0.14 °C for the temperature, 6.78 ± 0.15 and 8.33 ± 0.13 for pH and 3.34 ± 0.2 and 4.45 ± 0.22 mg/L for dissolved oxygen. No difference is observed in the mean temperature values. However, tanks fed with spirulina recorded higher pH and lower dissolved oxygen content, respectively 8.33 ± 0.13 and 3.34 ± 0.2 mg/L. These results could be explained by the dissolution of the spirulina powder which is accompanied by a strong activity of the tadpoles leading to the release of the waste products of the metabolism thus making the medium an alkaline character [8, 9]. Survival rates (SR) recorded in this trial were affected by different foods. These are between 7 ± 1.33 and 75.33 ± 3.78%. The lowest TS values are observed in the tanks fed with pond water and artificial food respectively of 7 ± 1.33 and 12.67 ± 2.44%. These results suggest that the water of the fertilized pond contains plankton (phytoplankton and zooplankton) in insufficient quantity to meet the nutritional intake of the tadpoles, which would lead to a high rate of cannibalism in the tanks concerned. Indeed, the *H. occipitalis* frog is carnivorous in the larval state and the cannibalism of the earliest tadpoles is very important [3, 10]. Similarly, the low survival rates recorded in the tanks fed with artificial food could be justified by the fact that the tadpoles were unable to assimilate the food during the first days of rearing, which favored high mortality observed. The slow dissolution of the powder prevented tadpoles (Pmi = 0.08 ± 0.01 g) with a smaller oral cavity from assimilating the food. Tadpoles

fed Spirulina recorded the highest survival rate (75.33 ± 3.78%) at the end of the test. According to [11], spirulina is an algae used for its nutritional and therapeutic effects. It is both a complete food and a medicine. These authors mentioned the effect of spirulina on cardiovascular diseases by reducing the values of lipid parameters as well as its antidiabetic effects by reducing hemoglobin and blood sugar in diabetic patients. Similarly, immuno-modulatory, anti-inflammatory, anti-cancer, anti-anaemic effects of spirulina have been proven. It would strengthen the immune system, tone and vitality. It also acts on the maintenance of weight and blood sugar, while strengthening the capacities of resistance and endurance. Thus, the high survival rate in tadpoles fed with spirulina could be explained not only by the nutritional virtues of this food as a food supplement but also by the therapeutic effectiveness of this food. The composition of spirulina in different nutrients as well as its high digestibility makes it an excellent food supplement [9]. The average final weights recorded during this test are between 0.14 ± 0.02 and 0.2 ± 0.02 g. The highest final weight obtained in tadpoles fed with spirulina could be explained by the high protein content (65%). Indeed, recent studies have shown that the growth of frog larvae is correlated with the increase in protein content in the food [6, 12, 13]. In addition, Spirulina protein is very digestible (75 to 88%) because it does not contain cellulose. Its proteins are qualified as complete proteins because spirulina contains all the essential amino acids, i.e. 47% of the total weight of proteins [14,15]. Furthermore, the total lipid composition is characterized by a good balance between saturated fatty acids and polyunsaturated fatty acids. The composition of the main fatty acids reveals the presence of a high concentration of essential fatty acids, including omega-3 and omega-6 which would prevent the accumulation of cholesterol in the body [16]. Spirulina is a known food source of γ-linolenic acid, after human milk and some uncommon vegetable oils but also a vitamin-rich algae [17]. It contains a relatively high concentration of provitamin A, vitamin B1, vitamin B 12 and β-carotene [17, 18, 19] It is used as a partial supplement or a complete protein replacement in fish feed [20]. It has had positive effects on the growth performance of Nile tilapia (*Oreochromis niloticus*) [21]. Indeed, fish feed containing 30% spirulina improved feed efficiency and overall health of Nile tilapia. In addition, a 25% substitution level improved the immune response of fish by increasing the number of granular hemocytes and reducing apoptosis [22]. Spirulina-containing feeds also improved the survival rate of abalone (*Haliotis midae*) [23]. Similarly, fish meal and soybean meal can be partially replaced by spirulina in the diet of poultry, cattle, frogs and other domestic animals [23, 24, 25].

CONCLUSION

At the end of this study, it appears that spirulina is an adequate food for the feeding of tadpoles because it would increase to more than 75% the survival of tadpoles of *Hoplobatrachus occipitalis*.

ACKNOWLEGMENTS

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

REFERENCES

1. Combes R, Knoepffler LP. "Les amphibiens et le milieu." Vie et Milieu, Observatoire Océanologique. 1970; 159-173.
2. Morin R. "Élevage de la grenouille." Document d'information DADD-10. Ministère de l'Agriculture, des Pêcheries et de l'Alimentation. 2008; 9.
3. Rödel MO. "Herpetofauna of West Africa." Edition chimaira, amphibians of the West African savanna, frankfurt/m. 2000; 335.
4. Konan JCBYN, Kouamé NG, Kouamé AM et al. "Feeding Habits of Two Sympatric Rocket Frogs (Genus Ptychadena) in a Forest Remnant of Southem-Central Ivory Coast West Africa." Entomol Omithol Herpetol. 2016; 5: 176.
5. Hardouin J**.** "Élevage commercial de grenouilles en Malaisie." Tropicultura. 1997; 15: 209-213.
6. Godomé T, Adjahouinou DC, Vodounnou DSJV et al. 2019. "Effect of protein rate on the growth, feed efficiency and composition of *Hoplobatrachus occipitalis* (Günther, 1858) tadpoles' carcass in controlled medium." South Asian Journal of Life Sciences. 2019; 7 (1): 1-7.
7. Godomé T, Sintondji SW, Azon MTC et al. "Artificial reproduction and embryogeny of the tiger frog *Hoplobatrachus occipitalis* (Günther 1858)". Zoological Society. 2020; 9 p.
8. Jordan**.** "Sugar as a source of carbon for spirulina (*Arthrospira platensis*) culture." International Biotechnology. 1999; 96-97.
9. Magermans P, Dengis C, Detienne X et al. "Formation du monde entrepreneurial et associatif à la culture de spiruline en Haïti." International Journal of Tropical Ecology and Geography. 2019; 3: 453-460.
10. Hardouin J**.** "Un élevage de grenouilles-taureaux aux Philippines." Tropicultura. 1991; 9 (1): 34-36.
11. Barry M, Ouédraogo MS, Sibiry G. "Intérêt thérapeutique de la spiruline chez l'homme: revue général." International Journal of Biological and Chemical Sciences. 2015; 8: 27-40.

12. Carmona-Osalde C, Olvera-Novoa MA, Rodríguez-Serna M, Flores-Nava A. "Estimation of the protein requirement for bullfrog (*Rana cutesbeiana*) tadpoles, and its effect on metamorphosis ratio." Aquaculture. 1996; 141: 223-231.
13. Olvera-Novoa MA, Ontiveros-Escutia MV, Flores-Nava A. "Optimum protein level for growth in juvenile bullfrog (*Rana catesbeiana* Shaw, 1802)." Aquaculture. 2007; 266: 191-199.
14. Costa JAV, Colla LM, Duarte P et al. "Modelling of *Spirulina platensis* growth in fresh water using response surface methodology." World Journal of Microbiology and Biotechnology. 2002; 18: 603-607.
15. Bujard E, Braco U, Mauron J et al. "Composition and nutritive value of blue green algae (*Spirulina*) and their possible use in food formulations." 3rd. International Congress of Food Science and Technology, Washington, 1970.
16. Hug C, Von der wied D. "La spiruline dans la lutte contre la malnutrition, bilan et perspectives." Antenna Technologies. 2011; 30.
17. Cruchot H. "La Spiruline, bilan et perspective." Thèse docteur en pharmacie. Université de France-Comite, 2008.
18. Belay A. "Mass culture of *Spirulina platensis* - The earthrise farms experience in *Spirulina platensis* (*Arthrospira*)" Ed. Avigad Vonshak, Taylor & Francis. 1997; 131-158.
19. Sall MG, Dankoko B, Badiane M. et al. "La spiruline: une source alimentaire à promouvoir." Médecine d'Afrique Noire. 1999; 46 (3): 140-141.
20. Nandeesha MC, Gangadhara B, Manissery JK, Venkataraman LV. "Growth performance of two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels of *Spirulina platensis*." Bioressource Technology. 2001; 80 (2): 117-120, 2001.
21. Velasquez SF, Chan MA, Abisado RG. "Dietary Spirulina (*Arthrospira platensis*) replacement enhances performance of juvenile Nile tilapia (*Oreochromis niloticus*)." Journal of Applied Phycology. 2016; 28 (2): 1023-1030.
22. Macias-Sancho J, Poersch LH, Bauer W. "Fishmeal substitution with Arthrospira (*Spirulina platensis*) in a practical diet for *Litopenaeus vannamei* : effects on growth and immunological parameters." Aquaculture. 2014; 426: 120-125.
23. Britz PJ. "The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae*." Aquaculture. 1996; 140 (1): 63-73, 1996.

24. El-Sayed AFM. "Evaluation of soybean meal, spirulina meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings." Aquaculture. 1994; 127 (2): 169-176.
25. Toledo PH, Suazo R, Viana MT. "Formulated diets for giant Chilean frog *Calyptocephalella gayi* tadpoles." Ciencia e Investigación Agraria. 2014; 41 (1): 13-20.