# Comparative study of larvae production by the Nile tilapia (*Oreochromis niloticus*, Linné, 1758) Bouaké strain between earthen ponds and hapas

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#### Abstract :

The aim of this study was to contribute to the development of tilapia aquaculture in Côte d'Ivoire by increasing the productivity of Oreochromis niloticus fry in fish hatcheries. Thus, the production of Oreochromis niloticus larvae was compared between two tilapia breeding systems, earthen ponds and hapas in ponds. Over 98 days, 900 broodstock fish were allowed to reproduce in two ponds of 500 m2 and 900 others in 20 hapas of 50 m2, each installed in two ponds of 1200 m2. The sex ratio was one male for two females at a density of 0.9 breeders/m2 at a density of 450 breeders per pond and 45 breeders per hapa. After breeding broodstock in both systems, larvae were harvested and counted every 14 days. The survival rate and growth of the broodstock, as well as the reproductive performance of the females, were evaluated. There was no significant difference between the survival rates of the broodstock in the two breeding systems (98.1 ± 0.4% in ponds and 97.4 ± 0.5% in hapas). Broodstock, however, showed significantly higher growth in ponds than in hapas. The quantity of larvae produced by females in ponds was significantly higher than that of females bred in hapas (p < 0.05). Hence, the best reproductive performance was recorded in broodstock stocked in ponds (absolute productivity: 93.1 larvae per female, relative productivity: 0.3 larvae per g of female, system productivity: 3.9 ± 0.2 larvae·m-2·day-1).

Keywords : hapa, larvae production, Oreochromis niloticus, pond, reproductive behaviour

### **1 INTRODUCTION**

Among tilapia species, *Oreochromis niloticus* is the most widely cultivated because of its rapid growth, adaptation to certain breeding conditions, and ease of breeding in captivity (de Graaf et al., 1999; Gómez-Márquez et al., 2003). In Côte d'Ivoire, two strains of the subspecies Oreochromis niloticus, which originally existed in the tributaries of the Volta River (Burkina Faso) and the Nile basin (Uganda), were introduced in 1957 and 1968 (Lazard, 1990). Interbreeding between these two strains at the Bouaké fish research station, current National Agronomic Research Centre, resulted in the Bouaké strain (Lazard, 1990), mainly grown in Côte d'Ivoire. In an effort to promote aquaculture in Côte d'Ivoire, the Ivorian State created structures from which fish farmers could obtain broodstock and fry of O. niloticus from wellknown strains, including the Bouaké strain (Lazard, 1990). However, due to the economic difficulties faced by the country, exchanges of tilapia strains were carried out directly between fish farms. This resulted in a mixture of wild and Bouaké strains and therefore there has been little control over the origin of the tilapia O. niloticus strains currently present on the farms. At the zootechnical level, poor growth performance was observed after several production cycles on various fish farms. In an effort to remedy this situation, fry from specimens of the O. niloticus Bouaké strain transferred to Brazil in 1971 (Freato et al., 2012) were reintroduced to Côte d'Ivoire in 2014 with the goal to revive continental aquaculture.

The captive production of most aquaculture species requires mastery of the breeding steps, which makes it possible to obtain larvae of high quality and in sufficient quantity. Among tilapia species, in particular *O. niloticus*, major factors that can influence the performance of broodstock breeding have been widely studied. They include the sex ratio (Khalfalla et al., 2008), photoperiod (Onumah et al., 2010), salinity (El-Sayed et al., 2005), and feed quality (Ng and Wang, 2011). Ponds are the oldest breeding systems and are used in Asia and Africa to this day for the production of *O. niloticus* larvae and fry (Lazard et al., 1990; Bwanika et al., 2004; Qiuming and Yi, 2004; Trong et al., 2013). This system is similar to the natural environment of

this species, in which males build and defend nests where spawning and egg fertilization occur during breeding (Rana, 1988; Lacroix, 2004). With the increase in fry demand associated with the development of fish farming by producers who have not yet mastered O. niloticus breeding methods, other breeding systems are increasingly being used in hatcheries to maximize fry production. These include concrete tanks, raceways, and hapas suspended in earthen ponds (Siddiqui et al., 1997; Dhraïef et al., 2010; Barman and Little, 2011). These systems allow better control of broodstock and also facilitate larvae and fry harvesting (Bocek, 2004; Adel, 2012). Hapas are the most widely used systems in Asia for the production of O. niloticus fry (Little et al., 1997), but in Côte d'Ivoire, only a few hatcheries combine hapas in earthen ponds or other systems for larvae production. Indeed, the choice of breeding system depends on the financial capacity of the production company. As a result, most fish farms choose direct breeding in ponds. The productivity between the breeding systems for the O. niloticus Bouaké strain has not yet been compared due to its recent introduction. We aimed to compare the production of O. niloticus larvae in earthen ponds and in hapas suspended in ponds using a sex ratio of one male to two females, as recommended by Salama (1996). The objective was to improve the productivity of fish fry hatcheries for the further development of fish farming in Côte d'Ivoire.

### 2 MATERIALS AND METHODS

#### **2.1 Ethical statement**

The National Polytechnic Institute Félix HOUPHOUËT-BOIGNY has approved the procedures and methodologies followed in the present study to minimize suffering.

## **2.2 Broodstock**

In total, 1,800 individuals of three-year-old broodstock, including 600 males and 1,200 females of the *O. niloticus* Bouaké strain, were used. This broodstock was the result of a selection of the third generation of *O. niloticus* specimens from Brazil introduced to Côte d'Ivoire in 2014.

Males of the broodstock weighed  $400.7 \pm 41.2$  g and were  $27.2 \pm 1.6$  cm in length and females  $290.7 \pm 31.6$  g and  $24.9 \pm 1.6$  cm.

#### 2.3 Breeding systems

The breeding systems consisted of four ponds, two of  $500 \text{ m}^2 (25 \text{ m} \times 20 \text{ m})$  for bottom breeding and two of 1,200 m<sup>2</sup> (40 m × 30 m) for breeding in hapas. Ten hapas of 50 m<sup>2</sup> each (10 m x 5 m for a water height of 0.9 m) were installed so as to obtain a total area of 500 m<sup>2</sup> within the hapas in each 1,200 m<sup>2</sup> pond. Breeding hapas were replaced by clean ones every 14 days at the time of larval harvest to ensure good water exchange. Two additional closed ponds of 500 m<sup>2</sup> were used as relays to receive the broodstock during the larval harvest in earthen ponds. All the ponds were supplied with fresh water by gravity at a flow rate of 7 L.min<sup>-1</sup> from a dam water reservoir. The water drain in each pond consisted of a monk with planks and a 3 mm mesh grid to adjust the water level. The average water depth in the ponds was 1.20 m.

## 2.4 Constitution of the experimental batches in ponds and hapas

Two batches of broodstock of 150 males and 300 females each (sex ratio: one male for two females) were distributed in two ponds of 500 m<sup>2</sup> at a density of 0.9 individuals.m<sup>-2</sup>. In two other 1,200 m<sup>2</sup> ponds, ten hapas were installed and each hapa received 15 males and 30 females at the same density of 0.9 individuals.m<sup>-2</sup>, for a total of 150 males and 300 females in each pond with hapas. The dissolved oxygen concentrations and the water temperature in each pond were recorded once daily using an oximeter (Model Extech DO700). The level of nitrates (NO<sub>3</sub><sup>-</sup>), nitrites (NO<sub>2</sub><sup>-</sup>), and ammonium (NH<sub>4</sub><sup>+</sup>) and the pH were measured twice a week by colorimetry using specific kits (JBL PROAQUATEST COMBISET POND) for each chemical component. The broodstock were fed an industrial feed (Tilapia Prime Growth Feed, 4.5 mm, RAANAN Fish Feed West Africa Limited) containing 30% crude protein twice a day at a ratio of 3% of their biomass per day. Feed consumption was visually confirmed since floating pellets were

distributed and immediately eaten. Also, broodstock were not fed on the days of the larval harvesting and the day before.

## 2.5 Larva harvesting

Larval harvests were carried out every 14 days after loading the broodstock into the breeding systems until the end of the trial, which lasted 98 days. A few hours before harvesting the larvae in the earthen ponds, the water level was lowered by removing a few boards from the monk. Using a large fine mesh net (1 mm) held by two people on either side of the bank of the pond, the net was slowly drawn across the surface of the water to collect the larvae that appeared. The water level was again lowered as before and the harvesting continued until the ponds were completely emptied. Then, the broodstock was caught using a 14 mm mesh net. The mouth of each female was checked to recover any mouthbrooding larvae. At each larval harvest, a sample of broodstock (50 males and 100 females) was taken from each breeding system to measure the weight with a SARTORIUS scale (range 3,100 g and accuracy 0.01 g) and the length of each specimen using an ichthyometer before their transfer to a relay pond.

To harvest the larvae in hapas, a PVC pipe was slid horizontally along the hapa so as to sweep its length to gradually bring together the broodstock and larvae in a corner. The larvae were then collected using a 1 mm mesh net. The females were sampled using a 6 mm mesh landing net and their mouths checked. Then, a sample of 50 males and 100 females (five males and ten females per hapa) was taken from all the hapas in each pond to record the weight and length of each individual before they were returned to their home hapa.

The larvae collected in each breeding system were counted individually using plastic spoons and then transferred to a tank for larval rearing.

### 2.6 Zootechnical parameters

The survival rate (SR) of broodstock for each sex in each breeding system was calculated using the formula  $SR = 100 \times$  (final number of broodstock / initial number of broodstock) (Ntumba,

2018). The weight of the broodstock recorded at each larval harvest in each system allowed us to calculate the average daily gain (ADG) of males and females according to the formula ADG  $(g.day^{-1}) =$  (final average weight - initial average weight) / duration (days). The absolute productivity (AP) and relative productivity (RP) were calculated according to Dhraïef et al. (2010) and Suloma et al. (2017), respectively, using the formulas AP = number of larvae harvested / number of females and RP = number of larvae harvested / (average weight of females × number of females). The productivity of each breeding system was determined using the formula Ps = number of larvae harvested / (breeding duration × system area exploited) according to Abou-Zied and Ali (2012).

## 2.7 Statistical analysis

The physicochemical parameters of the water in the ponds and the broodstock size data in the two breeding systems were analysed using Tukey's test. A KHI-2 test was used to compare the survival rate of the broodstock at the end of the experiment. The Kruskall-Wallis non-parametric test was used to compare the number of larvae harvested, the absolute and relative productivity of the females, and the productivity of the two breeding systems. The analyses were carried out using Statistica 10.0 software and the significance level was 0.05 for all tests.

#### **3 RESULTS**

## 3.1 Physicochemical parameters of water in the ponds and hapas

The physicochemical parameters recorded in the ponds were  $28.5 \pm 3.6^{\circ}$ C for the temperature and  $5.7 \pm 1.5 \text{ mg.L}^{-1}$  for the dissolved oxygen. The respective values were  $29.1 \pm 2.4^{\circ}$  C and  $3.2 \pm 0.9 \text{ mg.L}^{-1}$  for the temperature and dissolved oxygen in the hapas. There was no significant difference (P > 0.05) between the temperatures in the two systems, unlike for dissolved oxygen, for which the values were significantly higher in the earthen ponds (P < 0.05) than those recorded in the hapas. The nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), and ammonium (NH<sub>4</sub><sup>+</sup>) levels were < 15 mg.L<sup>-1</sup>, 0.05 mg.L<sup>-1</sup>, and 0.1 mg.L<sup>-1</sup>, respectively, in the ponds and hapas. The pH was between 7.5 and 8.4 in the two breeding systems, without any significant difference (P > 0.05).

## **3.2 Zootechnical parameters**

The survival rate was 98.3  $\pm$  0.5% for male and 97.8  $\pm$  0.2% for female spawners in the ponds. In the hapas, the survival rate of broodstock was 96.7  $\pm$  0.9% for males and 97.5  $\pm$  0.7% for females. There was no significant difference in the broodstock survival rate between the two breeding systems ( $\chi 2 = 1.07$ , d.f. = 1, P > 0.05 for males and  $\chi 2 = 0.15$ , d.f. = 1, P > 0.05 for females). Male growth was similar for both breeding systems up to the second larval harvest (Figure 1, Tukey HSD Test, P > 0.05). However, the weight of males in the earthen ponds was significantly higher than that of males in the hapas from the third larval harvest to the seventh (i.e., after 4 months, Tukey HSD Test, P < 0.05). For females, the weight was significantly higher (Tukey HSD Test, P < 0.05) for those reared in ponds compared to those reared in hapas from the second larval harvest to the last. The ADG for males was  $0.62 \pm 0.05$  g.day<sup>-1</sup> in ponds and  $0.28 \pm 0.04$  g.day<sup>-1</sup> in hapas.

The number of larvae harvested and the absolute productivity (Figure 2) in the ponds were significantly higher than those observed in the hapas (Kruskal-Wallis test:  $H_{(3, N = 28)} = 20.38$ ; P < 0.001 for the number of larvae and  $H_{(3, N = 28)} = 20.31$ ; P < 0.001 for absolute productivity). The same significant difference was obtained for relative productivity (Kruskal-Wallis test:  $H_{(3, N = 28)} = 19.86$ ; P < 0.001).

In the ponds, the relative productivity increased, with an increase in the weight of the females between the first and third harvests, followed by a decrease between the third and seventh harvests. In the hapas, the relative productivity of the females decreased between the first and seventh harvests, whereas their weight increased (Figure 3). The pond breeding system showed average productivities  $(3.7 \pm 0.8 \text{ and } 4.1 \pm 0.6 \text{ larvae.m}^{-2}.\text{day}^{-1}$  one value per pond

without hapas) that were significantly higher than those of the hapa breeding system ( $2.3 \pm 0.5$  and  $2.7 \pm 0.4$  larvae.m<sup>-2</sup>.day<sup>-1</sup> one value per pond with hapas) over the 98 days of the experiment (Kruskal-Wallis test:  $H_{(3, N = 28)} = 20.4$ ; P = 0.0001).

### **4 DISCUSSION**

We aimed to compare the number of larvae produced by females of *O. niloticus* at a sex ratio of one male for two females in earthen ponds and hapas installed in ponds. The results show that the earthen pond breeding system is more productive than the hapa breeding system installed in a pond.

The physicochemical parameters recorded in the two breeding systems were well within the ranges recommended for the breeding of tilapia *O. niloticus*. Indeed, breeding of *O. niloticus* is possible when the temperature is between 28 and 32°C (Lazard, 2009). Dissolved oxygen concentrations in the ponds were significantly higher than those of water in the breeding hapas (5.7 mg.L<sup>-1</sup> vs. 3.2 mg.L<sup>-1</sup>). This could be due to the lower renewal rate of water in the hapas due to the progressive obstruction of the net mesh by suspended matter. However, these values are still above the tolerable dissolved oxygen limit (3 mg.L<sup>-1</sup>) for the breeding of *O. niloticus* (Meyer and Meyer, 2007; Malcolm et al., 2000).

Broodstock survival rates in both breeding systems were over 96%. These values are consistent with those obtained by Ntumba (2018). The high survival levels observed in the Bouaké strain show the hardiness and ease of adaptation of *O. niloticus* in a rearing environment (Balarin and Halton, 1979; Malcolm et al., 2000). The growth of male and female broodstock in ponds was higher than that obtained in hapas. Similar results have been reported by Ouattara et al. (2005), who showed a difference in growth in tilapia depending on the farming system. Indeed, the growth of tilapia is higher in ponds than in floating cages and concrete ponds. Such differences in the growth of broodstock between the two breeding systems could be linked to the high level of confinement stress experienced by broodstock in the hapas. The low growth

in hapas could also be linked to low oxygen levels as observed in several studies (Kolding et al., 2008; Abdel-Tawwab et al., 2015; Makori et al., 2017) and low water renewal (Mires, 1982). Indeed, hapas show lower water renewal relative to ponds, in which the water is completely renewed, and the sediment evacuated after each harvest. The greater water renewal in ponds also eliminates harmful substances, possibly provides additional food items and improves breeding (Little et al., 1994; Bhujelr, 2000).

The reproductive parameters (absolute and relative productivity) of females in ponds were significantly higher than those of females in hapas. The absolute productivity values obtained with females at each harvest (93.1  $\pm$  18.7 larvae per female in ponds and 38.3  $\pm$  9.4 in hapas) are lower than those of Adel (2012), who used the same sex ratio of one male for two females (210.0  $\pm$  45.2 larvae per female). The absolute and relative productivities obtained were also lower than those of Abdel and Kamel (2011) using different strains of *O. niloticus*. This could be related to the smaller size (165.5  $\pm$  11.5 g and 151.43  $\pm$  7.98 g) of the females used by the aforementioned authors than those that we used in the two breeding systems. The decline in fertility observed in the two breeding systems between the start and end of the experiment could be linked to the increase in the weight of the females according to the results of Tsadik (2008). Indeed, the frequency of spawning and fertilization, as well as hatching rates of *O. niloticus* females decrease over 30 months.

The difference between the reproductive parameters observed in the two systems could be due to a confinement stress effect. Indeed, ponds are similar to the natural living environment of *O. niloticus*, in which males build egg-laying nests to attract females during breeding (Rana, 1988; Lacroix, 2004; Mendonça et al., 2010). Male and female broodstock in ponds can thus fully express courtship displays, whereas in hapas, in which the fish are more confined, males do not have access to the bottom and hence the possibility to build laying nests to attract and court females. This could have the disadvantage of decreasing reproductive success, although it has been shown by Mendonça and Gonçalves-de-Freitas (2008) that females of O. niloticus spawn in the absence of substrate. In addition, a stable hierarchy within a tilapia population may inhibit breeding (Turner, 1986; Fishelson, 1983). Indeed, in hapas, where the spawners are more confined, the social hierarchy is established quickly and remains stable due to low water renewal (dos Santos Gauy et al., 2018; Gonçalves-De-Freitas et al., 2019). The small number of larvae obtained in the hapas corroborates the results of Behrends et al. (1993), in which the confinement of the broodstock in small breeding units, such as hapas, led to aggression between the males and thus affected larva production. Indeed, the reduced living space for the broodstock in hapas leads to competition for space, which increases social interactions, causing stress, and thus affecting breeding efficiency. On the contrary, for bottom breeding in ponds, the broodstock is transferred to a new relay pond after each harvest, thus breaking the previously established hierarchy and the dominance of territorial males with renewal of the water (Little et al., 1993; Gonçalves-De-Freitas et al., 2008). In addition, it has been shown that female O. niloticus in ponds can mate with territorial males from neighbouring nests during the same reproductive phase due to successive polyandry (Ruwet, 1963). Given this possibility, it is very likely that females in ponds mate with more fertile males to produce more larvae (Avise et al., 2002) than females in hapas because those in hapas only mated with the 15 males present in the hapa. In addition, the inability to build nests in the hapas for spawning constrains the females to deposit their eggs directly in the net. Females might be disturbed by their congeners when the eggs are taken back into the mouth for mouthbrooding (Lévêque et al., 2006). According to Rifai (1980), egg fertilization and larvae production are limited in hapas, either due to the inhibition of reproductive behaviour or eggs falling through the mesh.

#### **5 CONCLUSION**

This study shows that breeding of the *O. niloticus* Bouaké strain in ponds allows to obtain more larvae than breeding of this strain in hapas. The results can be generalized to commercial hatcheries to improve their performance to provide sufficient fry to other fish farmers.

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#### AUTHOR CONTRIBUTION

Dieudonné Djétouan Akian, Crespin Luc Quenum, Joël Alain Kouakou Houra, and Kouakou Yao designed the research. Dieudonné Djétouan Akian, Crespin Luc Quenum, and Joël Alain Kouakou Houra performed the research. Dieudonné Djétouan Akian, Crespin Luc Quenum, Joël Alain Kouakou Houra, and Daniel N'Zi Koua analysed the data. Dieudonné Djétouan Akian, Crespin Luc Quenum, Daniel N'Zi Koua, Joël Alain Kouakou Houra, Frédéric Clota, and Marie-Laure Bégout wrote the manuscript. Kouakou Yao supervised the study. All authors have agreed to submit this article for publication.

#### CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## **DATA AVAILABILITY STATEMENT**

Sufficient data has been provided in the form of figures in this article. The raw digital data will be made available in the SEANOE database which is free and gives a DOI with the dataset.

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Figures

**FIGURE 1** 



## PowerPoint

Growth of Oreochromis niloticus males (a) and females (b) in ponds and hapas. Values represent the mean ± SE



## FIGURE 2

Open in figure viewer PowerPoint

Number of larvae harvested (a) and absolute fecundity (number of larvae per female) of Oreochromis niloticus females (b) in ponds and hapas. Letters a and b indicate significant differences (p < 0.001)



Relative fecundity of *Oreochromis niloticus* females in ponds and hapas at each larval harvest. Values represent the mean  $\pm$  SE and an asterix indicates a significant difference (Kruskal–Wallis test, p < 0.001)