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## Research Article

# Effects of inbreeding depression on the success of artificial reproduction in the African catfish *Clarias Gariepinus* (BURCHELL, 1822)

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## Abstract

The objective of this study was to establish an effective method of artificial reproduction and larval rearing to improve the fry production of the African catfish *Clarias gariepinus*. Thus, a hormonal treatment using ovaprim was used to induce maturity in males and females. Two breeding trials were first conducted on captive populations by crossing a male and female Senegalese strain (♂ss/♀ss), and a male and female Beninese strain (♂bs/♀bs). A third reproduction test was carried out by crossing ♀ss/♂bs and ♂ss/♀bs but for this test, the ♀ss and ♂ss are wild breeders that were collected from the natural environment. For the first two breeding tests (♂ss/♀ss and ♂bs/♀bs, respectively), fertilized eggs either did not hatch or some hatched but the fry did not survive. Although the number of broodstock used in these first two breeding tests is small, this lack of hatching and poor larval survival may reflect inbreeding depression. The third breeding test was successful as females and males, respectively, produced large quantities of eggs (13g of eggs for ♀ss and 32g of eggs for ♀bs) and sufficient quantities of sperm to fertilize the eggs (approximately 12g for the Benin strain and 5g for the Senegal strain). Hatching rates of 90% and 60% were obtained for the ♀ss/♂bs and ♀bs/♂ss breeding's, respectively, after incubation of fertilized eggs on water hyacinth (natural substrate) and pompon (artificial substrate). Comparisons of growth rates of larvae fed two different diets (combination of natural and artificial food, and artificial food alone) showed that artificial food alone was more effective for growth, especially after one month of rearing. The high larval mortalities recorded especially at the end of the experiment were mainly due to poor water quality. Thus, this study provided a better understanding of the conditions in the hatchery and larval culture systems that are critical to the success of artificial reproduction and optimal growth of *C. gariepinus* fry.

## Introduction

Large-scale aquaculture can be a sustainable alternative to meet the ever-increasing demand for animal protein in the world and thus limit the exploitation of natural fish stocks [1-3]. Indeed, with the increase of the world population and the sophistication of fishing techniques, capture fisheries are no longer able to meet the demand of populations, hence the need to develop fish farming [2,4]. The expansion of aquaculture will make it possible to provide fish products to the population, but this will depend on the productivity of the farming systems [5,6]. This will relieve pressure on wild fish stocks threatened by overfishing.

Aquaculture, the currently rapidly and steadily growing food production sector, provides nearly 50% of the fish consumed in the world [7-9]. It is therefore considered the largest potential sector for fish production [9]. Indeed, the global aquaculture production has increased exponentially from 60 million tons in 2010 to 97.2 million tons in 2013 with a production of 99.1% of freshwater fish [2,10]. In Senegal, aquaculture production increased from 334 tons in 2011 to 1095 tons in 2014 [11]. This production is mainly dominated by Nile tilapia *Oreochromis niloticus*, African catfish *Clarias gariepinus*, crocodiles, mollusks (mussels and oysters), and micro and macro algae. Most of this comes from fish farming, which has been responsible for an impressive growth in fish supply, with a rate of 7% in 1974, 26% in 1994, 39% in 2004, and 42% in 2014 [12].

It is widely recognized that aquaculture will mitigate and assist overfishing while preserving the oceans from the damage caused by intensive and destructive fishing. It is also widely accepted that the promotion of fish farming activities and the reduction of capture fisheries will help boost this food production sector. Aquaculture has aroused the interest of decision-makers in many African countries, including Senegal, where it is undergoing rapid development given the importance of its potential. In Senegal, there is a national will to promote aquaculture and the breeding of indigenous species such as *C. gariepinus* which has very good nutritional values due to its high protein content and poverty. The African catfish is a popular species for aquaculture due to its good feed conversion, disease resistance, low water quality requirements, the possibility of rearing it at high density (intensive) as well as the quality of its flesh [13-16]. *Clarias gariepinus* spawners produce large quantities of eggs and sperm throughout the year, ensuring the possibility of artificial reproduction [15,17-19]. The species also can accept a wide variety of inexpensive artificial food [20,21]. *Clarias gariepinus* has a great capacity to adapt to rearing conditions, even to very poor environmental conditions [22].

Despite these qualities mentioned above and these assets, the main problems in Senegal are the lack of control of breeding and rearing conditions for *C. gariepinus* larvae, which leads to significant mortalities. *Clarias gariepinus* does not reproduce in ponds because it is not subject to the stimuli related to the rise in water level and the flooding of lateral areas. In addition, its reproductive rate is very low in the wild [23]. Therefore, it is necessary to use the artificial reproduction method developed by many authors [24-32] to meet the needs of fish farmers. Although artificial reproduction can help to optimize fish production in farming systems, it can cause inbreeding that leads to reduced growth performance, fertility, and survival of the offspring. It is, therefore, necessary to properly plan and control outcrossing in breeding programs to avoid inbreeding depression that can affect the hatching rate and larval survival. It has been shown that inbreeding can negatively affect reproductive success and fry survival, especially in small facilities that cannot maintain a large number of parent spawners [33]. Indeed, inbreeding in these fish facilities can reduce genetic variation for the fitness and adaptability of larvae.

Therefore, the objective of this study is to develop an efficient method for breeding and rearing *C. gariepinus* larvae in a controlled environment. Thus, different series of crosses between parents of two different strains (Senegalese and Beninese) of *C. gariepinus* were performed to optimize the hatching rate of fertilized eggs and the survival of the larvae and to obtain new generations more adapted to the rearing conditions of the larvae. Induced reproduction was performed by hormonal induction using ovaprim. The study of larval growth under artificial conditions consisted in evaluating and comparing the effects of artificial food and natural food (live prey) on the growth performance of the fry.

## Materials and methods

### Study area and crossbreeding

The experiments were carried out in the aquaculture greenhouse of the University of Gaston Berger for two months and 15 days. The farm has a pumping station for water supply, with a starting pressure of 3.5 bars. This pump is immersed in the Djeuss, a branch of the Senegal River located about 1 km from the farm. Inside the farm, motor pumps and reception channels are installed or developed for agricultural, livestock, and aquaculture activities.

The climate of the study area is sub-Saharan to Sahelian, characterized by two alternating seasons: a dry season from October to July and a rainy season from August to September. The terrain is flat and the maximum temperatures recorded in April-May are generally between 35°C and 37°C. The minimum temperatures recorded in January are around 16°C. The soil of the farm is sandy to sandy clay with a pH close to neutral (6.7 to 7.7). The farm has aquaculture infrastructures composed of 03 rooms including a hatchery and a larval rearing room. The aquaculture production of the farm concerns mainly the Nile tilapia *O. niloticus* and the African catfish *C. gariepinus*.

For this study, a total of 12 broodstock (6 males and 6 females) were used for the three artificial reproduction tests that were successively performed. The first and second breeding tests were performed twice using a total of 8 broodstock (4 breeders for each breeding test). The first test was performed by crossing a male and a female of Senegalese strain ( $\delta ss/\eta ss$ ) while the second was performed by crossing a male and a female of Beninese strain ( $\delta bs/\eta bs$ ) (Figure 1). Given they did not give the expected results; these two breeding tests were repeated with the same number of breeders. The Senegalese strain for these two breeding trials is a captive strain maintained under laboratory conditions for several generations. These two artificial reproduction trials lasted approximately one month. The third breeding trial was conducted with 4 breeders by crossing  $\eta ss/\delta bs$  and  $\delta ss/\eta bs$  but for this trial, the  $\eta ss$  and  $\delta ss$  are wild breeders that were collected from the wild (Figure 1). They were caught in the Djeuss River by a local fisherman. The Beninese broodstock used in the three rearing trials belongs to an exotic strain maintained at the SIA (Station d'Innovation Aquacole) in Saint-Louis for generations. This broodstock was not fed the first day after their transfer to the holding tanks to avoid inducing stress that could affect reproductive success. They were fed with an artificial feed manufactured locally by the ANA (Agence Nationale de aquaculture). Feeding began on the second day after transfer to the holding tanks. During their stay in the holding tanks, the broodstock was manually fed twice a day with a feeding ratio of 10% of body weight.

### Selection, sexing and storage of broodstock

The selection broodstock for artificial propagation is of particular importance, especially for females whose stage of maturity must be carefully verified to ensure successful artificial propagation [34]. Thus, the breeding trials in this study began on August 21, 2020, at 7:00 am with the fishing

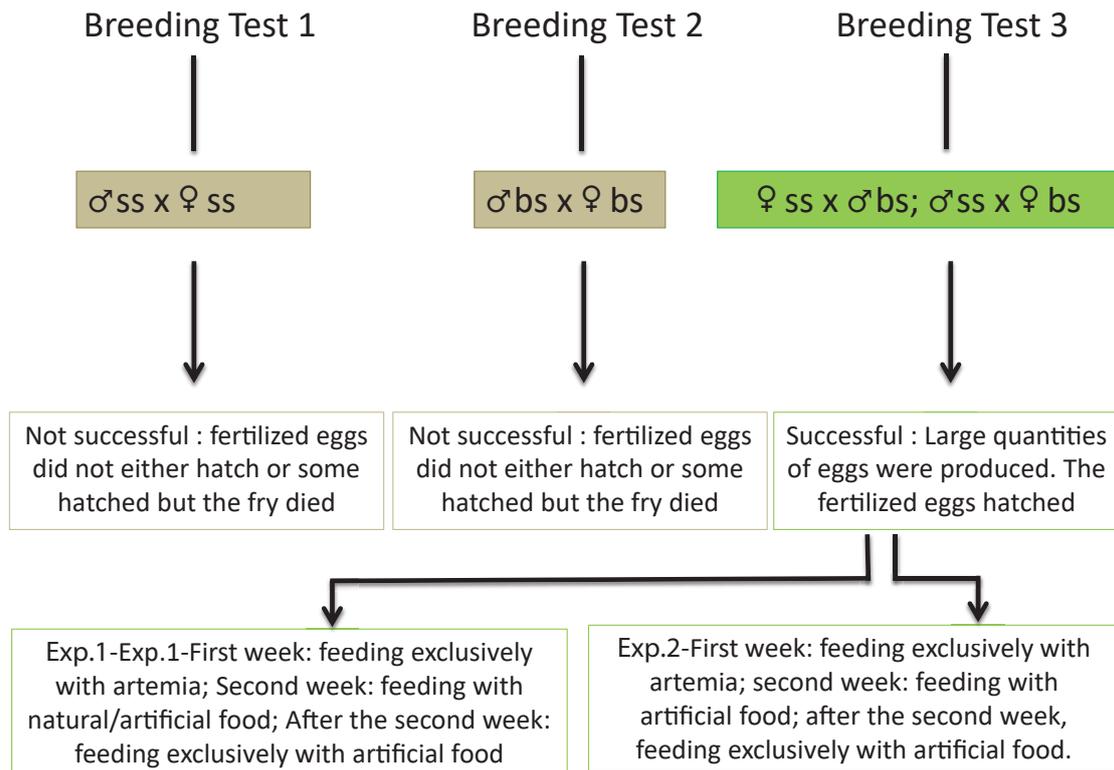


Figure 1: Setup of the feeding and larval growth experiment.

and selection of broodstock in the storage tank. Males of *C. gariepinus* have specific criteria that were used to distinguish them from females. They have a urogenital papilla and females have a longitudinal slit in the middle of their round urogenital papilla. They have a swollen and hard stomach when they are almost mature and ready for reproduction (natural, artificial, or induced reproduction).

### Hormone preparation and injection of broodstock

The hormonal injection of the broodstock was performed the same day, a few hours after their selection. They were first weighed with an electronic scale. The precise dose of ovaprim to be injected into each individual was then calculated according to its size (body weight) (0.5 ml/kg body weight for females and 0.25 ml/kg for males). The doses used for males and females are shown in Table 1. To inject the hormone, females were laid face down with the cloth over their eyes and their heads well supported. The dose of ovaprim was withdrawn with a syringe and injected gradually into the dorsal muscle. Upon removal of the needle, the injection site was massaged to prevent the solution from coming out. The injections of the males were performed in the same manner. After injection, the male and female parents were stored in separate tanks and were not disturbed until milt and eggs were collected. Thus, the latency time for an average temperature of 28°C was determined.

### Milt harvest and manual egg extraction (stripping)

Sperm in *C. gariepinus* cannot be collected by abdominal massage. It is therefore necessary to sacrifice the male. Thus, the male was first weighed before being sacrificed by

Table 1: Ovaprim doses injected to broodstock selected for the artificial reproduction.

Sex	Wight (kg)	Dose per kg	Dose per ml
Female 1 of the Senegalese strain	0.40	0.50	0.200
Female 2 of the Senegalese Beninese strain	0.75	0.50	0.375
Male 1 of the Senegalese strain	0.50	0.25	0.125
Male 2 of the Beninese strain	0.75	0.25	0.187

decapitation with a knife. It was then dissected with a pair of scissors from the anus to the pectoral fins. The testicles were extracted, dried, and cleaned with absorbent paper to eliminate the traces of blood present on the surface. They were then crushed to collect the sperm freely. To collect the eggs, the female was carefully held on both sides with a wet towel. Light pressure was applied to the belly to collect the oocytes in a clean, dry bowl. This was done until a drop of blood appeared, which showed that there was no egg left in the ovaries.

### Egg fertilization

Fertilization of the eggs was done artificially using the dry method, which involves mixing the eggs and sperm and adding water to activate the sperm around the eggs and allow for fertilization [35]. Thus, the sperm was first mixed with the eggs and a volume of water was added to the mixture to activate the sperm. This was then mixed with a spoon and an equal volume of physiological liquid was added to the eggs while stirring gently for two minutes. This same operation was repeated a second time to accelerate fertilization. After one minute, the fertilized eggs immediately began to swell and became sticky.

## Incubation and hatching of eggs

The incubation was done in two breeding tanks of 300L on natural and artificial supports. The natural supports are water hyacinths, aquatic plants floating in water with submerged roots while the artificial supports are pompons. Fertilized eggs attached to these supports were then incubated. The oxygen supply system was then activated during the incubation period to optimize the hatching rate. The eggs began to hatch on August 22, 2020, at approximately 2:00 pm with green eggs that have developed flagella and are continuously moving. However, the larva has not yet been released. There were also white-colored eggs that were inert and a foamy layer began to develop on them. By 5:00 p.m., which is 24 hours of incubation, almost all the fertile eggs had hatched. Since there were few unhatched fertile eggs left in the tanks, the racks were left until the next day (August 23). They were then gently removed to avoid dumping the detritus into the rearing water, which would be a source of pollution. The incubation tanks were siphoned off to remove any detritus remaining at the bottom.

The hatching rate (HR) was calculated using the following formula:

$$HR = \frac{NL}{NIE} \times 100$$

With NL= number of larvae and NIE = number of incubated eggs. The number of incubated eggs was obtained by estimation.

## Larval rearing and feeding

Larval rearing, which began on day 8 post-hatch (September 1, 2020), was conducted in triplicate in six different 30L aquaria, with a density of 60 individuals per aquarium. Larvae, divided into two batches of three aquaria, were fed exclusively artemia for the first week (Figure 1). Then for the second week, one of the batches (experiment 1: Exp.1; triplicate: A1, A2, and A3) was fed with natural food alone (phytoplankton), and the other one (experiment 2: Exp.2; Triplicate: A4, A5, and A6) with natural food (phytoplankton) mixed with a little artificial food (Figure 1). After the second week, the fry of both experiments was exclusively fed with artificial food until the end of the experiment (Figure 1).

These experiments were conducted in triplicates using three aquaria for each experiment. The natural food, composed mainly of phytoplankton and zooplankton, was collected in the grow-out tank of the aquaculture farm using a plankton net. Each batch was fed a feed rate of 14.8% of body weight divided into six parts that were distributed throughout the day. This avoids having uneaten food remains in the tanks, which can impact water quality and cause stress to the fry. Physicochemical parameters (temperature, pH, and dissolved oxygen) were measured daily. Temperature and oxygen content was measured using a multi-function oximeter that simultaneously measures temperature and dissolved oxygen. The pH of the water was measured with a pH meter.

## Mortality and Growth

The mortality rate was monitored during the experiment

by counting the number of dead individuals in each aquarium during each estimate. The mortality rate (MR) was then calculated using the following formula:

$$MR = (Nd - Ni) * 100$$

Where Nd = Number of dead and Ni = Initial number of larvae.

The growth rate (GR) was calculated using the following formula:

$$GR = (Wf - Wi) / Wf * 100$$

where Wi = initial weight and Wf = final weight.

The normality of the distribution was tested with the Shapiro-Wilk test and the homogeneity of variances with the Levene test. The distribution was normal but the variances were not homogeneous. Therefore, Welch's t-test was used to compare the means of physicochemical, mortality, and growth parameters between fry-fed live prey and those fed artificial feed. All analyses were performed using the 'ADE4' library of 'R' software. The significance level was set at 5%.

## Results

### Spawner response and hatch rate

Hormone induction accelerated the maturation of the broodstock. Each of them weighed more than 200 g, which is the lower limit of weight at the age of sexual maturity of *C. gariepinus*. Indeed, according to Viveen, et al. [23], *C. gariepinus* specimens are sexually mature after seven to ten months with an average body weight that varies between 200 and 500 g. The collection of a large quantity of eggs eight hours after ovaprim injection indicates a favorable response of females to hormonal induction. The eggs, after fertilization, started to increase in size. Fertilized eggs were greenish with a red spot appearing in each mature egg while unfertilized eggs were whitish.

The incubation time in this study was 24 hours at a water temperature of 28–29°C. For the first two rearing trials (♂ss/♀ss and ♂bs/♀bs) that were conducted twice, either fertilized eggs did not hatch after incubation or some eggs had hatched but the larvae did not survive. Indeed, 12 hours after incubation, the eggs were already starting to turn whitish and 12 hours later, they started to give off an unusual odor and develop a coating all around. The eggs were therefore rotten, which corresponds to a hatching rate of 0%. For the third experiment, the hatching rate of the breeding test ♀ss/♂bs was 90% while that of ♀bs/♂ss was 60%. At hatching, after about 24 hours of incubation, the larvae bear yolk sac vesicles that constitute their nutritional reserve. At this stage, they appear very small, needle-shaped, very active, and always seeking to hide in dark corners.

### Physicochemical parameters

The temperature was not significantly different between experiments (Welch's test;  $p > 0.05$ ). It varied between 26.87 and 30.23°C for Exp.1 where fry was fed artificial food alone and

between 26.93 and 30.20°C for Exp.2 (fry fed natural food), with an average of  $28.65 \pm 0.74^\circ\text{C}$  and  $28.68 \pm 0.77^\circ\text{C}$ , respectively. At the beginning of the experiment, the temperature tends to decrease gradually (between September 2 and 6) (Figure 2). Then, it gradually increases after this stage to reach a maximum of 30°C on September 26. Thereafter, it varies slightly from around 30°C until October 1<sup>st</sup>. It then gradually decreases until the end of the experiment with a minimum of 26.9°C.

Curve comparisons show that the mean temperature is not significantly different between replicates but also between treatments (Welch's test;  $p > 0.05$ ) and its evolution over time is quite similar for all aquaria (Figure 2). Dissolved oxygen levels varied between 1,300 and 6,970mg/l for Exp.1 and between 2,770 and 6,370 mg/l for Exp.2, with a mean of  $3,969 \pm 0.096$  and  $3,955 \pm 0.004\text{mg/l}$ , respectively. Mean dissolved oxygen was not significantly different between Exp.1 and Exp.2 (Welch's test;  $p > 0.05$ ). Overall, the pattern of variation in oxygen levels in the six aquaria is similar throughout the larval rearing cycle (Figure 3). For Exp. 1 (A1, A2, A3) oxygen levels decrease from September 2 to 26 and increase between days 26 and 28. It then decreases to a minimum between September 30 and October 2 and begins to increase again. Although the variations are sometimes more pronounced, the oxygen content of Exp. 2 (A4, A5, and A6) shows a similar overall pattern (Figure 3) except from September 30 to October 02 where an opposite pattern (increase followed by a decrease for A1, A2 and A3 and a decrease for A4, A5, and A6) was observed. The lowest oxygen concentrations were recorded for Exp. 2 (A4, A5, and A6) from September 30 to October 02 (Welch's test;  $p < 0.05$ ). The average pH measured during the larval rearing period was  $7.174 \pm 0.010$  and  $7.239 \pm 0.015$  for Exp.1 and Exp.2, respectively. The pH in the fry rearing tanks did not vary significantly with time during the experimental period (Figure 4) (Welch's test;  $p > 0.05$ ). There was also no significant difference in pH levels between Exp.1 and Exp. 2 (Figure 4) (Welch's test;  $p > 0.05$ ).

### Mortality and survival of larvae

During the first two weeks of rearing (September 1 to 13), the mortality rate was high for Exp.1 (A1, A2, and A3) while it was very low for Exp.2 (A4, A5, and A6) (Figure 5; Table 2) (Welch's test;  $p < 0.05$ ). Then in the latter, the mortality rate gradually increased to reach its maximum on September

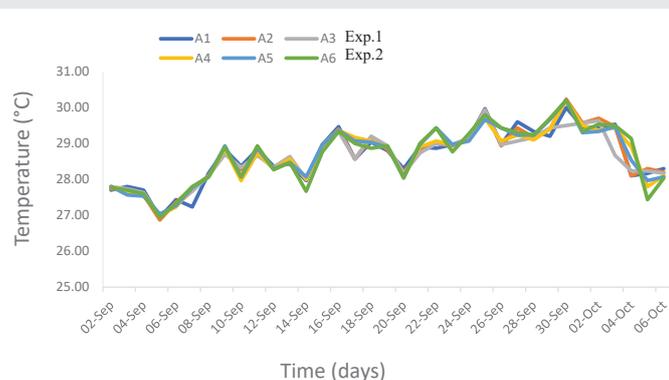


Figure 2: Evolution of temperature in the larval rearing tanks during the experimentation.

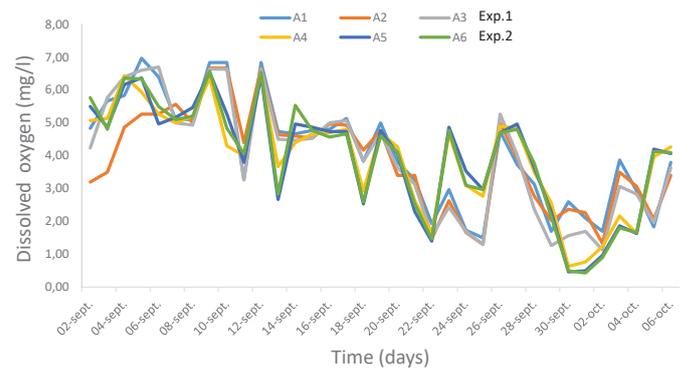


Figure 3: Evolution over time of dissolved oxygen in the larval rearing tanks.

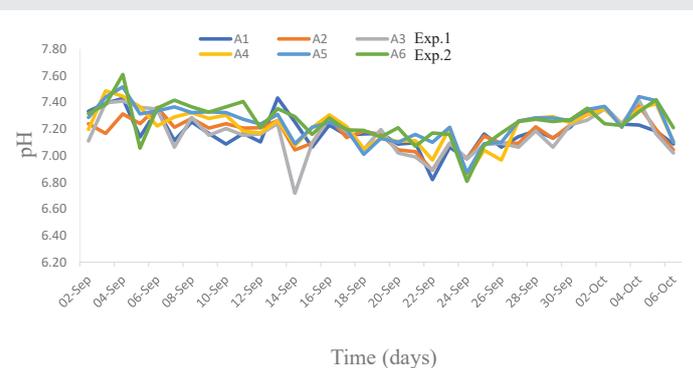


Figure 4: Evolution of water pH in the larval rearing tanks throughout the experimental period.

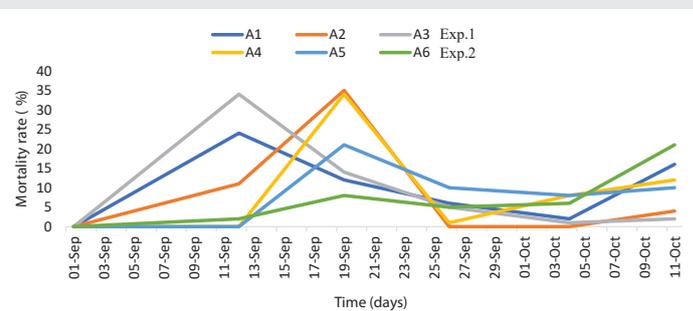


Figure 5: Mortality rates in the rearing tanks of the Exp.1 and Exp.2.

19, then decreased and reached a maximum on September 27 (Figure 5; Table 2). During this time, the average mortality in Exp.2 decreases to reach its minimum on October 5. The highest mean mortality (Welch's test;  $p < 0.05$ ) was recorded on day 13 for Exp.1 and day 19 for Exp.2. On day 45, corresponding to the end of the experiment, the average mortality rate was 91.96% for Exp.1 (i.e., a survival rate of 8.04%) and 76.45% for Exp.2 (i.e., a survival rate of 23.55%) (Figure 5; Table 2).

### Larvae growth

The average body weight increased from September 12 to 26 for both Exp.1 and Exp.2 (Figure 6) but it was not significantly different between them (Welch's test;  $p > 0.05$ ). Then, it increased again for Exp.2 while it decreased for Exp.1 to reach a minimum on October 4 and then increased until the end

of the experiment (Figure 6). The average body weight was significantly higher for Exp.2 than for Exp.1 (Welch's test;  $p < 0.05$ ) from September 30 to October 10 (end of the experiment) (Figure 6). Overall, the average growth rate of fry-fed artificial food (Exp.1) during the last two weeks of the experiment was higher than that of those fed the natural and artificial feed (Exp.2) (Welch's test;  $p < 0.05$ ).

The growth rate increased with time for both experiments, but the variations were more pronounced for Exp.1 than for Exp.2. It was higher at the beginning of the experiment and lower at the end (Figure 7) (Welch test;  $p < 0.05$ ). Overall, the growth rate of Exp.1 and Exp.2 showed a similar pattern between September 12 and 19, during which time it gradually decreased. It reached its minimum on September 19 (43.73% for Exp.1 and 42.37% for Exp.2) (Figure 7). It then increases until September 26 and starts to decrease for Exp.1 and remains relatively constant for Exp.2 until the end. Overall, fry-fed the artificial feed (Exp.1) had a higher growth rate (Welch's test;  $p < 0.05$ ) during the last two weeks of the experiment than those fed the natural and artificial feed (Exp.2). In the end, the growth rate was significantly higher for Exp.1 (50.35%) compared to Exp.2 (39.84%) (Welch test;  $p < 0.05$ ).

## Discussion

### Spawner response to hormone induction and hatch rate

The broodstock responded favorably to the hormonal

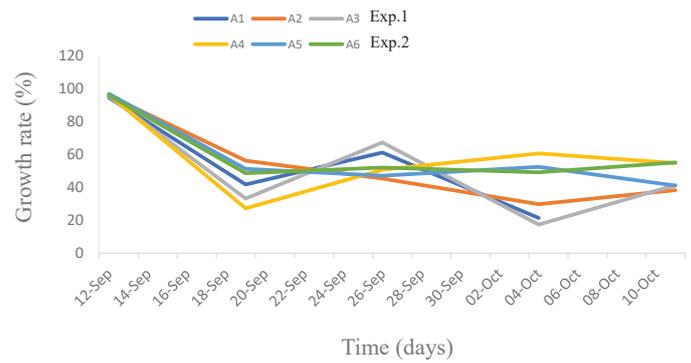


Figure 7: Growth rate of larvae during the experiment.

injection, which may have accelerated their maturity. The response rate of females was good, as shown by the high quality of the number of eggs released especially for ♂ss/♀bs (32g of eggs). For the males, the amounts of semen collected were sufficient to fertilize the eggs of the females (5g for ♂ss and 10g for ♂bs). There were no hatched eggs for the first breeding trials (♂ss/♀ss) after incubation. All eggs had turned white even before the end of incubation. The second breeding (♂bs/♀bs) yielded a few larvae after hatching but they all died immediately thereafter. The lack of larvae after incubation for the first reproduction and the mass mortality after the second brood may be explained by contamination of the natural supports with traces of the chlorine used to clean them. These natural supports could have accelerated the degradation of the unfertilized eggs, thus provoking the pollution of the water as evidenced by the odor emitted. However, the experiments were not repeated with a clear control for contaminants to assess whether this was the only reason for the mortality problems observed in these 2 experiments. Further experiments are needed to determine if traces of chlorine remaining after cleaning natural incubation media can cause mass larval mortality.

Although the number of broodstock used in these first two reproductions is small, this lack of hatching and poor larval survival may also reflect inbreeding depression. Indeed, the parents used in these first two breeding attempts are descendants of the same female and would have the same ancestors. Consistent with this conclusion, several studies have reported the deleterious effects of inbreeding on fish reproductive fitness [36–38]. However, additional studies exploring the effects of inbreeding on reproduction with sufficient numbers of broodstock are needed to confirm this conclusion.

In the third breeding (♀ss/♂bs and ♀bs/♂ss), the white eggs remained stuck to the hyacinth roots and pompons while the larvae after hatching precipitated to the bottom of the pools. The incubation time of the eggs in this study, which was 24–36 hours at 20–30°C hours, appeared to be one of the best average incubation times reported for *C. gariepinus*. Indeed, an average incubation time of 48 hours with some temperature variation has been observed in controlled laboratory experiments [39,40]. According to Fiogbe [41], the temperature of the water in the larval rearing tanks plays a determining role

Table 2: Mortality and survival rates in the rearing tanks during the experiment.

Experiments	Exp.1			Exp.2		
	A1	A2	A3	A4	A5	A6
Aquariums	A1	A2	A3	A4	A5	A6
Initial numbers	60	60	60	63	67	60
01-sept	0	0	0	0	0	0
12-sept	24	11	34	0	0	2
19-sept	12	35	14	34	21	8
26-sept	6	0	5	1	10	5
04-oct	2	0	1	8	8	6
11-oct	16	4	2	12	10	21
Final numbers	60	50	56	55	49	42
Mortality rate (%)	100.00	83.33	93.33	87.30	73.13	70.00
Average mortality rate (%)	91.96			76.45		
Average survival rate (%)	8.04			23.55		

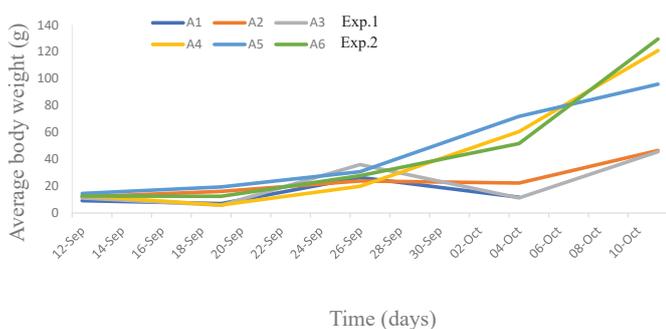


Figure 6: Average body weight of larvae in the rearing tanks during the experimental period.

in the development of the embryo and therefore affects the total incubation time. It has been shown in *C. gariepinus* that, depending on the temperature, it takes 20 to 57 hours for the eggs to fully hatch [42]. The results of this study indicate a good hatching rate estimated at 90% and 60% for the ♀ss/♂bs and ♀bs/♂ss crosses, respectively. This difference in hatching rate could be explained by the smaller amount of milt of the ♂ss compared to the ♂bs.

## Larvae growth

Two days after hatching, the larvae still carrying yolk sacs stood in the corners of the incubation tanks under the pumps. The larvae became fry after three days and their feeding with external food became essential. As reported in many studies [34,41,42] it is important to feed *C. gariepinus* fry with natural foods after yolk resorption. According to Imouro Toko [43], at this stage, *C. gariepinus* larvae prefer live food (artemia, failing that, zooplankton). Therefore, in this study, the larvae were fed artemia for one week, after which their average weight was 5 mg. A time-dependent decrease in growth rate was observed for Exp.1 and Exp.2 from September 12 to 19, during which time the fry was fed exclusively with natural live food (zooplankton and phytoplankton). This phase is marked by massive mortalities which were first more important for exp.1 (from September 1 to 12) and then for exp.2 (from September 13 to 19). Weaning the fry from the natural diet on September 19 and replacing it with the artificial diet associated with plankton caused a slight increase in growth rate and a decrease in mortality rate in Exp.2. For Exp.1, on the other hand, replacing the natural diet on September 19 with the artificial diet alone did not cause significant changes in the growth rate and mortality of larvae. However, changing the diet from a combination of artificial and natural feeding to artificial feeding alone on October 4 led to an increase in mortality rate and a slight decrease in the growth rate of the fry in Exp.1. These results reflect the importance of natural feeding (Phyto- and zooplankton) in the growth and survival of larvae during the first three weeks of rearing of *C. gariepinus* larvae. Fry fed with this live food showed certain behavioral characteristics. Indeed, they were more active and moved around the aquaria. These results also indicate that although the artificial food/plankton combination appears to be more beneficial than artificial food alone, it must be applied over a sufficiently long period to be more effective and for the effects on fry growth to be more apparent. The fluctuations in growth rate observed during the first two weeks of larval rearing appear to be due to the high light levels in the rearing room that were illuminated by sunlight. According to Poll and Goss [44], low light intensity, at least, can influence the growth of *C. gariepinus* larvae because they require a dark environment for maximum development. Interruption of the water recirculation system due to power outages could also have impacted fry growth.

The temperature in the aquaria of Exp.1 and Exp.2 was between 26 and 30°C and is within the favorable range for the growth of *C. gariepinus*. Therefore, the differences in growth between experiments observed in this study are probably not due to variations in the water temperature of the rearing aquaria.

Although low oxygen levels can have negative impacts on *C. gariepinus* growth and cause mortality, growth performance in both experiments did not coincide with periods of low oxygen levels. This suggests that variations in dissolved oxygen are not the causes of growth differences between experiments. Regarding pH, the curves showed similar trends between Exp.1 and Exp.2, except for aquarium A3 where pH dropped between September 12 and 14, which is likely due to CO<sub>2</sub> accumulation in the fry rearing aquarium.

*Clarias gariepinus* is a species with good growth performance that reaches market size in a very short time (4-5 months) when the diet used contains the appropriate amount of protein, fat, and vitamins. In this study, the fish in Exp.2 fed with the mixture of natural and artificial food were slightly larger than those fed with natural feed alone after the second week of larval rearing. However, at the end of larval rearing on day 45, the size of the fry was smaller for Exp.2 (1.10-1.21g) than for Exp.1 (2.20-2.36g). Overall, these results show that the gradual replacement of the natural feed by the artificial feed is more beneficial for the growth of the larvae than the abrupt weaning, i.e. the sudden replacement of the natural feed by the artificial one. Consistent with our results, studies have shown that the average weight of *C. gariepinus* fry after 45 days of rearing is 1 to 10g, sometimes even less than 1g depending on the feed used and the rearing conditions. In both experiments, growth is heterogeneous although the fish with the largest sizes were observed in Exp.2 (A4, A5, and A6). Indeed, in this experiment, the largest individual in A4 weighs 6.61g and measures 6.4cm while in A5 the largest is 6.2cm long and weighs 6.31g. For A6 the head of the batch measures 8cm with a weight of 12.89g. This difference in individual size could be due to cannibalistic behavior within the aquariums. Indeed, the biggest fish can kill the smallest.

The high mortality rates (91.96% for Exp.1 and 76.45% for Exp.2) recorded throughout the experiment may be due to a malfunction in the water circulation system that may have resulted in decreased oxygen levels. Although the survival rates (8.04 and 23.55%) observed in this study under fully controlled conditions are low, they are higher than those reported by [45] in semi-artificial environments (4.5% for his first experiment and 10.7% for the second) but lower than those of Viveen, et al. [39], which were 50%.

## Conclusion

This study strengthened our understanding of the conditions required for successful artificial reproduction of African catfish, achieved for the first time between Senegalese and Beninese strains. Hormonal induction by ovaprim injection accelerated broodstock maturity and induced spawning. Females responded to hormonal induction 8 hours after ovaprim injection. However, the results indicate that the broodstock must be appropriately selected for successful reproduction. This is because inbreeding can lead to a loss of variability in broodstock, fertilization and egg hatching, and larval survival. The egg incubation time in this study was 24 hours at 28-29°C with a hatching rate of 90% and 60% for Exp.1 and Exp. 2, respectively. The results also show that the natural live

food (zooplankton) is the preferred food of the larvae during the first phase of the rearing cycle. Indeed, in addition to its nutritional value and high digestibility, this live food is easily detected and captured by the larvae, due to its small size and swimming movements in the water column. However, in the advanced stages, artificial dry food seems to be more suitable for larval growth. Indeed, the replacement of the natural diet with the artificial diet resulted in better growth performance for the Exp.2 larvae compared to the Exp.1 larvae fed with the natural diet. These results show that artificial reproduction of *C. gariepinus* is feasible and promising in local hatcheries but that broodstock must be appropriately selected to avoid inbreeding depression that could compromise its success. The results also show that changing the diet at the appropriate time of larval growth is crucial for successful growth. The Physico-chemical conditions and the maintenance of good water quality by a recirculation system must be well controlled to avoid high mortalities during the rearing cycle.

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