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Research Article

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Artificial propagation and larval breeding tests of the african sharptooth catfish, *Clarias gariepinus* conducted in captive conditions

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Abstract

The African catfish Clarias gariepinus is the second most cultured species in many African countries, including Senegal. Despite its economic importance in local aquaculture, artificial propagation of the species that would allow obtaining quality seed throughout the year has not yet been mastered adequately. This study aimed at producing C. gariepinus quality and mass supply seed to enhance local production. Three different types of incubation (Macdonald's bottle, water lettuce and wire mesh frame) were tested to identify the most efficient and accessible method for the producers at low cost and with a high hatching rate. The hatching rates obtained for the three different incubation methods were 26.9%, 10.0% and 6.3% for the Macdonald bottle (MB), wire frame (WF) and water lettuce (WL), respectively, while the corresponding survival rates were 91.6%, 70.4% and 77.8%.77.8%. The high hatching rate obtained with the MB could be due to the influence of permanent water circulation, which is absent in the other two hatching systems. The survival rates decreased 15 days after hatching and were 3.24%, 2.04% and 7.16%, respectively. The survival rates obtained three days after hatching were satisfactory, but the significant decrease at 15 days after hatching especially during the post- weaning phase seems to be due to the food quality, and unrelated to the hatching system. The daily individual growth, weight gain and body size of the larvae was significantly higher for larvae fed with natural food compared those that received artificial food. These results indicate that larvae preferred and fed the natural food which was permanently available in the rearing tanks. The artificial food deposited on the bottom of the tank changes appearance over time and may not be appreciated and efficiently consumed by the larvae, which may negatively impact their growth performance.

Keywords: *Clarias gariepinus*, Ovaprim, African sharptooth catfish, Fry, Natural Food, Reproduction

Introduction

Fisheries and aquaculture are importance sources of food for the world population, especially in the poor countries with nutrition limited income [1,2]. The contribution of the aquaculture sector to supply fish for human consumption for the first time overtook that of fisheries captures in 2015 [2]. Since then, aquaculture remains a fast-growing sector and its contribution towards food security and nutrition continues to increase. Aquaculture is experiencing a considerable and indisputable boom, while the state of fishery resources shows that 33.1% of worldwide stocks exploited by marine fisheries were classified as overfished in 2015 [3]. Overfishing not only has negative ecological consequences for the entire ecosystem, but it also leads to a drop in fish production (90 million tonnes in recent decades) [3], which in turn can have negative socio-economic impacts. Thus, aquaculture is an alternative to fill the gap linked to the decrease in fishing as a food production sector, given the constraints linked to food security. However, progress remains comparatively less visible on the African continent, despite its considerable potential. Among the number of problems recognized as slowing down the development of aquaculture operations on the African continent, is the lack of hatchery which mass produce larvae throughout the year [4].

The African sharptooth catfish *Clarias gariepinus* Burchell, 1822,

is a species of high economic value which is widely encountered in calm continental waters, lakes, swamps and rivers, but also in fast flowing rivers [5-8]. The species inhabits shallow and turbid waters as well as clear and deep waters [9,10]. Its commercial importance, wide environmental tolerance range and relative ease of breeding and rearing offspring make the species the second most common aquaculture species on the African countries after tilapia [11,12]. Like many African freshwater fish species, reproductive success in *C. gariepinus* is optimal during the long rainy seasons [13]. Observations made on males and females of different stocks in experimental environments have shown that they are mature and capable to reproduce from an age of 7 to 10 months [14]. Likewise, increases in temperature and photoperiodicity in the natural environment promote gonadal development and sexual maturity, which are followed by spawning during rising waters [7]. The quantity of eggs obtained from a female by manual extraction represent up to 25% (relative fertility) of her body weight [15]. On average, there are 600 to 800 eggs per gram of ovary, and a spawner of around 500g body mass can lay 75,000 to 100,000 eggs (absolute fertility). The spawning areas of *C. gariepinus* are freshly flooded water bodies, particularly where the water is calm and shallow. Male sperm, which are immobile in the seminal glands, become mobile after contact with water. This mobility is enabled by a flagellum, which becomes active within 30 to 60 seconds [16]. The courtship display can last several hours after which the female lays her eggs in small groups. Then, the male drops his sperm above the eggs and the female disperses the fertilized eggs with her tail. There is no parental care, which has a strong impact on the survival rate [15].

Clarias gariepinus is a species of high economic value that has great potential for aquaculture in many countries. Its introduction into several areas has been shown to be beneficial due to its adaptability to different environments [14]. However, the species reproduces in captivity only if all required conditions for the reproduction in natural environment are fulfilled, which often is difficult to realize. The species can only reproduce in an artificial environment with human intervention, which generally consists of imitating environmental conditions of the wild or performing a hormonal induction. The first attempt at artificial reproduction of *C. gariepinus* dates back to the 1970s-1980s [14,17-18]. Then, the implementation of nursery in the farming facilities and the manufacturing of artificial food began to be mastered, which allowed the development of semi-intensive and, subsequently, intensive farming of this species [19]. Since then considerable research on artificial reproduction has been conducted, and has resulted in significant progress in yield, which is essentially linked to the rate of fertilization, hatching and survival rate of the larvae [15,19-26] The success of this artificial method is also linked to the incubation conditions including the quantity of eggs, water temperature, pH, dissolved oxygen and the state of the broodstock (stress, maturation stage) [21]. Most of these studies used synthetic hormones such as ovaprim, human chorionic gonadotropin (HCG) and deoxycorticosterone acetate (Doca) or pituitary gland extract of *C. gariepinus* and Nile tilapia *Oreochromis niloticus* to induce the artificial reproduction. This artificial reproduction method allowed obtaining larvae throughout the year.

The main objective of this study was to offer local producers an efficient artificial propagation method and a good larval breeding technique that will permit the mass production of *C. gariepinus* in the local farms. The implementation of such an approach can potentially improve the fertilization and hatching rates, optimize the egg protection against enemies and unfavorable environmental conditions, and create optimal conditions for larval survival and growth. The decision to focus on this species is motivated by its economic importance but also its high tolerance to poor water quality, the ability to accept a variety of food, and good resistance to disease. The species has a good growth rate and its flesh is appreciated.

Material and Methods

Experimental facility and acquisition of spawners

The experiments of this study were conducted in the facility of ISRA/IRD located in the IRD campus of Bel-Air in Dakar, Senegal. This facility has four 3000L broodstock tanks, six aquaria that serve as a sex reversal table for producing tilapia monosex male populations (male tilapia are preferred for culture because their faster growth) from fry that have already absorbed the yolk sac, and 12 aquaria for the magnification of the fry having left the inversion table. It has also five 1000L tanks used for the mass enlargement of the fry, and for male broodstock housing and the reproduction, an incubator where eggs are placed for hatching, eight Macdonalds bottles and a 1000L tank which is not affiliated with any circuit and which allows infected individuals to be quarantined for treatment. These various installations are supplied with oxygen by an air compressor connected to airstones which aerates the water to provide constant supply of oxygen for the fish.

The spawners used in the experiments originated from the Technopole wetlands of the Dakar region, more precisely the Gadaye wetland of Guédiawaye commune. A total of 10 spawners (six males and four females) were caught on August 31, 2018, by a local fisherman. This broodstock was transported to the experimental facility using a cooler box, where they were weighed and acclimated for one month in two tanks, separating the male and female broodstock.

The fish were weighed, their length was measured (Table 1), and they were then transferred to two 1000L tanks that are hereafter referred to as B1 (for males) and B2 (for females). During acclimation in controlled conditions, the brood fish were treated twice in 40L of water containing 35% salinity for 2 minutes for disinfection and wound healing. Fish were fed with both live preys (from fertilized tanks) and artificial food.

Table 1: Spawner body weight before and after acclimatization

Individuals (♀or ♂)	Body weight (g) before acclimatization	Body weight (g) after acclimatization	Body weight loss
Male	1396.0	960.0	436.0
Male	630.7	521.8	108.9
Male	1414.6	1174.4	240.2
Male	888.8	842.5	46.3
Male	676.5	551.8	124.7
Male	295.9	238.5	57.4
Female	964.2	768.2	196.0
Female	525.6	432.9	92.7
Female	494.2	438.8	55.4
Female	454.7	358.2	96.5

Measurement of Physicochemical Parameters

Water temperature and dissolved oxygen were monitored throughout the period of the experiment in acclimation tanks, incubators and larval rearing tanks. The strict control of these physicochemical parameters can contribute towards improving the yield of the artificial reproduction [23]. Therefore, they were measured twice a day, in the morning at 9 a.m. and in the evening at 3 p.m. Particularly temperature has a strong influence on the latency and incubation times, because the females only mature when the temperature is 22°C or higher [27]. The best growth rates of *C. gariepinus* were obtained at water temperature ranging from 20 to 30°C with an optimum of 27°C for juveniles and 25°C for adults [4, 28-30]. *Clarias gariepinus* adults can withstand low oxygen levels but larvae are more sensitive and their oxygen requirements have to be fulfilled for optimal growth performance [31].

Pituitary Gland Extraction, Milt Collection and Female Induction

Females with a protruding genital papilla, and a rounded and swollen belly were selected. For males, the largest individual was selected to have sufficient amount of pituitary gland extracts for the injection of single female. After the acclimation, one male and one female of 521.8 g, 438.8 g weight and 44 cm and 41 cm length, respectively, were selected. The male was anesthetized, sacrificed, and the pituitary gland was extracted. The pituitary gland was ground and mixed with 2 ml of physiological fluid (9 g of sea salt in one liter of water), and this solution was injected immediately into the female to induce spawning. The male's testes were then extracted and kept in the refrigerator at a temperature of 4°C. At the time of fertilization, the testicles were incised and pressed with a syringe, and the milt was collected.

Table 2: Hatching rates and survival rates after the york sac resorption and 15 days after hatching. Macdonald's bottle (MB), wire frame (WF) and water lettuce (WL)

	MB	WF	WL
Number of incubated eggs	8715	8715	8715
Hatching rate	26.89	10.04	6.25
Survival rate 15 days after the york sac resorption	91.60	70.37	77.77
Survival rate 15 days after hatching	3.24	2.04	7.16

Egg Collection: Stripping Fertilization of Eggs

The eggs were collected on the subsequent day after at 7:00 a.m., 11 hours after the female was injected. The female was held by two operators, one holding the tail and the other the head, and light pressure was applied to the belly of the female. A quantity of 60 g of eggs was collected, which indicates that the females responded favorably to the induction. Three egg samples of 15g were fertilized for three different incubation methods. Each sample received three drops of semen, followed by mixing and addition of water with twice the weight of the eggs. The mixture was stirred for one minute and rinsed three times before being incubated. The latency time was calculated as the difference between the time of injection of pituitary extract and oocyte collection (stripping).

Egg Incubation and Hatching

After fertilizing, the eggs were incubated using three different methods (Figure 1) to determine which was most efficient based on the hatching and the survival rates after resorption of the yolk sac: 1) wire frame (WF), which involved placing a wooden wire frame into a 20 L incubation tray; 2) water lettuce (WL), which involved adding eggs to several specimens of the free-floating aquatic plant, water lettuce (*Pistia stratiotes*) from Technopolis wetlands that were placed in a 20L tank to incubate 15 g of eggs; and 3) Macdonald bottles (MB) that were placed on a permanent circuit receiving15g of eggs (Figure 1). The incubation time was calculated as the time interval between the start of incubation and the end of the hatching. The hatching rate was calculated using the following formula:

$$\text{Hatching rate} = \frac{\text{Number of larvae obtained}}{\text{Total number of eggs}} * 100 \quad (1)$$

Comparison of The Effects of Artificial and Natural Foods On Larval Growth

The second stage of the experiment involved evaluating the impact of live food on the larvae. This was done by distributing 400 larvae in four tanks (D1, D2, D3, and D4) containing100 larvae each. D1 and D2 are duplicates and D3 and 4 are also duplicates. The experiment began on the third day after hatching, corresponding to the resorption, i.e. the day on which the larvae lose their yolk sacs. Larvae in two of the tanks were fed with Daphnia (ration: 3litters/day) whereas the larvae in the other two tanks were fed with formulated artificial food (ration: 70% of the biomass/day). The Daphnia were reared in 5 L bottle and fed with microalgae grown a 10L culture medium. The tanks D1 and D2 received artificial food up to 59.26 mg per meal three times a day whereas the tanks D3 and D4 received natural food (daphnia) 1L per meal, three times a day. From the fourth day, tanks D1 and D2 received 118.52 mg (2 × 59.26 mg) per meal, three times a day. The experiment ended on day 9, which corresponds to the first day of weaning. The survival rate was obtained by the following formula:

$$\text{SRays} = \frac{\text{NLa}}{\text{NHL}} * 100 \quad (2)$$

with SRays = survival rate after resorption of the yolk sac; NLa = number of larvae after resorption and NHL = number of hatched larvae.

The size of the larvae was evaluated by measuring the fork length. Average weight gain was calculated from the following formula:

$$\text{Average weight gain (g)} = \text{Final weight (g)} - \text{initial weight (g)} \quad (3)$$

Daily individual growth (DIG), also called daily weight gain (DWG), allows assessing the daily weight gain of farmed fish. It was determined from the following relationship:

$$\text{DIG} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Aging time (days)}} \quad (4)$$

Analyses of variation (ANOVA) and Student-t test were used to compare means between conditions or groups for physicochemical variables and for the hatching, survival and growth parameters. The validity of these tests was first confirmed using Shapiro-Wilk and Bartlett tests to verify the normality and homogeneity of data, respectively. These analyses were completed using the 'ADE4' library in 'R' software. The significance level was set 5% for statistically different means between factors.

Results

Physicochemical Parameters

There was no significant difference in water temperature and dissolved oxygen content between morning and evening during the acclimation and incubation phases (Student test; $p > 0.05$). Likewise, the water temperature and dissolved oxygen content did not vary significantly between morning and evening and between replicas during the larval rearing phase (ANOVA; $p > 0.05$). The average water temperature in the acclimation tanks in the tank B1 varied between 27.7 and 28°C whereas that of B2 and varied between 27.33 and 28.59°C (Figure 2A). The water temperature was not significantly different between B1 and B2 (Student test; $p > 0.05$). The average water temperature in the different incubation tanks kept in the same enclosure varied was 27.25-28.35°C, 26.4-28.35°C and 26.3-27.15°C for the incubators MB, WF, and WL, respectively (Figure 3A). This was no significant difference in water temperature between the incubation tanks MB, WF and WL (Student test; $p > 0.05$). The average water temperature in the larval rearing tanks was 25.21°C and 25.85°C for the tanks D1/D2 and D3/D4, respectively (Figure 4A). There was no significant difference in water temperature between replicas D1/D2 and D3/D4 during the larvae rearing (ANOVA; $p > 0.05$).

The average dissolved oxygen levels during the acclimation varied between 4.5 and 9.5 and from 5.09 to11.65 in tanks B1 and B2, respectively (Figure 2B). The average dissolved oxygen content did not vary significantly between B1 and B2 during the acclimation period (Student test; $p > 0.05$). The dissolved oxygen levels during the incubation phases ranged from 14.27- 14.80, 14.80-15.50 and 15.30-16.25 in the tanks MB, WF and WL, respectively (Figure 3B). This was no significant difference in dissolved oxygen content between incubation tanks (Student test; $p > 0.05$) (Figure 3B). The average dissolved oxygen was from 13.51, 14.31, 14.20 and 14.42 in the larval rearing tanks D1, D2, D3 and D4, respectively (Figure 4B). The average dissolved oxygen level was 13.77 for D1/D2 and 14.31 for D3/D4. These were not significantly different between in dissolved oxygen levels between D1/D2 and D3/D4 (ANOVA; $p > 0.05$).

Figure 1: The different types of incubators used for egg incubation: wire frame (WF), water lettuce (WL) and Macdonald's bottle (MB).

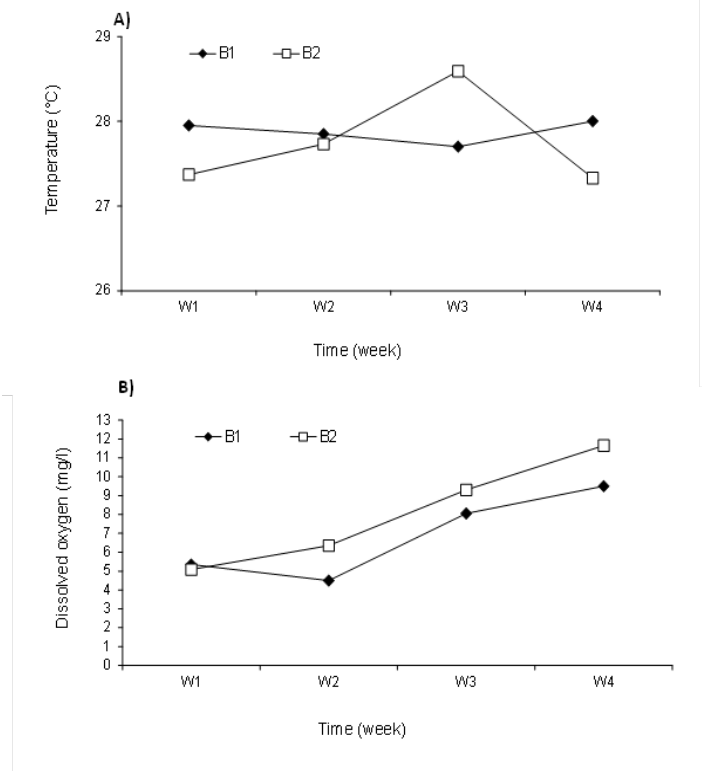

Figure 2: Temporal variation of water temperatures (A) and dissolved oxygen concentrations (B) in the acclimatization tanks (B1 and B2)

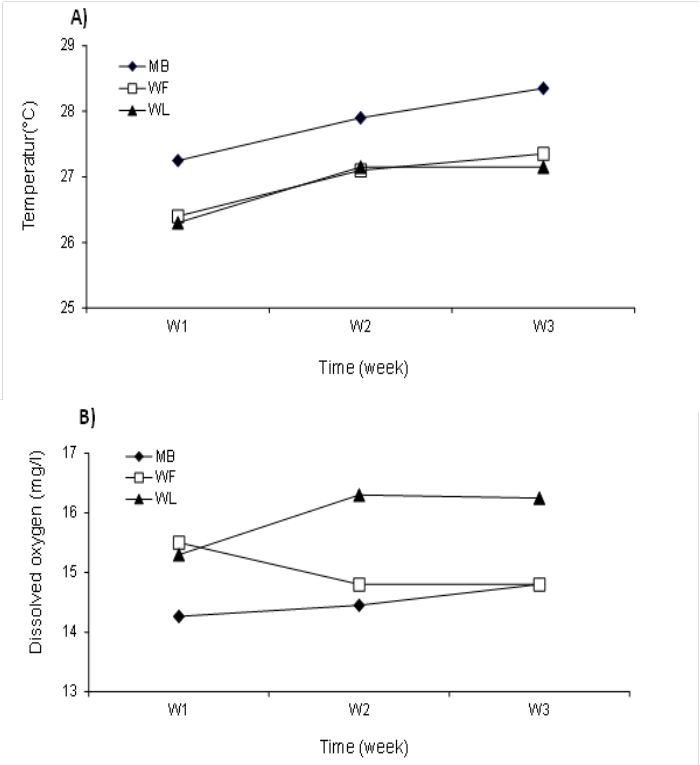

Figure 3: Temporal variation of water temperature (A) and dissolved oxygen concentrations (B) in the three different types of incubators: Macdonald's bottle (MB), wire frame (WF) and water lettuce (WL)

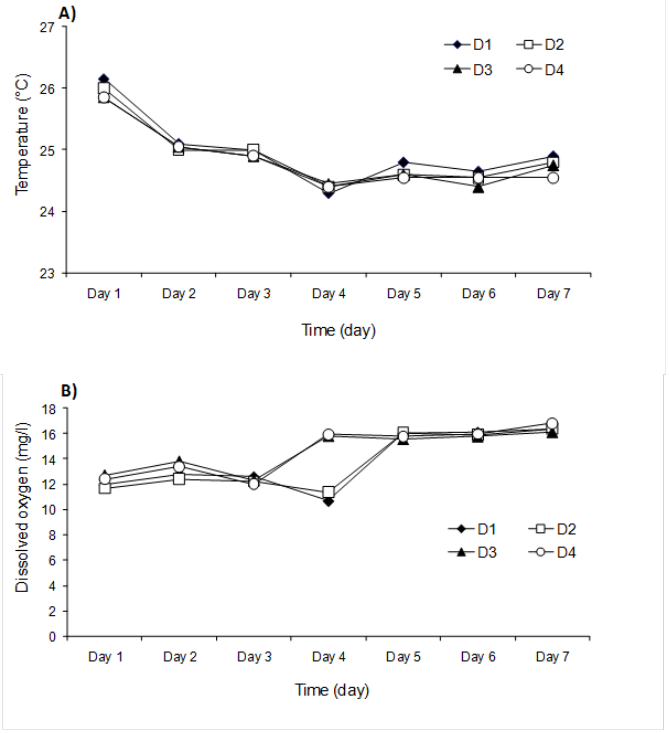

Figure 4: Temporal variation of water temperature (A) and dissolved oxygen concentrations (B) in the larval rearing tanks (D1, D2, D3, and D4). D1 and D2 are duplicates and D3 and D4 are also duplicates

Spawner Acclimation

There was no mortality of the broodstock during the acclimation period. All individuals were visibly healthy since any injuries was found. There was a significant drop in the weight of individual broodstock during the acclimatization phase. The difference in body weight between the beginning and the end of the acclimation was from 436.0 to 46.3 g (Table 1). The weight loss between males ranged from 436.0 to 46.3 g and that of females ranged from 196 to 55.4 g. The average weight loss did not significantly differ between males and females (ANOVA; $P > 0.05$).

Latency and Incubation Times

The ova obtained after stripping had a diameter of 1.2 mm. One gram of ova contained 581 ova, which gives a total of 8715 ova for the 15 g (Table 2). The latency was 11 hours within a temperature range of 27 to 28°C. Incubation of the eggs commenced 30 minutes after the egg collection, i.e. 24 hours after all the eggs were hatched.

Hatching Rate and Survival Rate

The hatching rates obtained for the three different supports are 26.89%, 10.04% and 6.25% for the MB, WF and WL, respectively (Table 2). The highest hatching rate was obtained with the MB, followed by the WF (Student test; $p < 0.05$). The lowest rate was obtained with the WL.

The survival rates were 91.6%, 70.37% and 77.77% for MB, WL and WF, respectively (Table 2). The highest survival rate was obtained with MB, followed WL and WF (Student test; $p < 0.05$). Survival rates decreased 15 days after hatching and were 3.24%, 2.04% and 7.16%.

Table 3: Growth parameters of larvae fed with natural and artificial food. D1 and D2 are duplicates and D3 and D4 are also duplicates. Ni: Number initial of larvae; Wi: initial weight; Wf: final weight; Wg: weight gain; DIG: daily individual growth

Growth parameters	D1	D2	D3	D4
Ni	100	100	100	100
Wi (mg)	2.54	2.54	2.54	2.54
Wf (mg)	3.11	2.82	6.68	7.68
Average W (mg)	2.96		7.18	
Size (mm)	5	5	10	10
Wg	0.57	0.28	4.14	5.14
AverageWg	0.42		4.94	
DIG	0.81	0.04	0.59	0.73
Average DIG	0.42		0.66	

Larval Performance According to The Type of Food

Tanks D1, D2, D3 and D4 had the same density and equal number of larvae (100 larvae) (Table 3). The initial average weight of larvae in each tank was 2.54 mg. The survival rate during the larval rearing phase was higher in thanks D4 (95%) and D3 (82%) than in tanks D1 (29%) and D2 (25%). There was no significant difference in the final average weight between D1 and D2. Likewise, the final average weight was not significantly different between D3 and D4. The final average weight was also higher in tanks D3/D4 and (7.18 mg) and lower in the tanks D1/D2 (2.95 mg) (Student test; $p < 0.05$) (Table 3).

No significant difference in the average weight gain was observed between D1 and D2, but also between D3 and D4 (Student test; $p > 0.05$) (Table 3). The average weight gain was higher in tanks D3/D4 (4.64) than in tanks D1/D2 (0.42) (Student test; $p < 0.05$) (Table 3). The average size was not different between D1 (5 cm) and D2 (5 cm) and between D3 (10 cm) and D4 (10 cm). The average size and daily individual growth (DIG) were all significantly higher in D3/D4 and compared to D1/D2 (Student test; $p < 0.05$) (Table 3).

Discussion

The water temperature and dissolved oxygen levels observed in this study were within the range of standards and optimum values reported as having no significant influences on the acclimation and maintenance of *C. gariepinus* spawner stock in captivity. Although the optimum temperature for juveniles and adults is 27°C and 25°C, respectively, the species can tolerate a wide range of water temperature ranging from 20 to 30°C [4, 28-30]. The optimal temperature for maximum growth rate varies between 25 and 33°C with the highest growth performance being observed at 30°C and a decline in the rate from 25°C [28]. During the whole experimental period of this study, the water temperature varied between 24.2 and 28.9°C and was thus in the range of the optimal temperatures for the species [4, 28-29]. The dissolved oxygen content, which ranged from 4.4 and 16.9 mg/l, was also within the range of dissolved oxygen concentrations for efficient food intake and for the success of the artificial reproduction. Although dissolved oxygen is not often a limiting factor for *C. gariepinus* because of its capacity to breathe atmospheric air and to leave the water using its pectoral fins, the oxygen concentration required for good growth of fingerlings is around 3mg/l [27]. The African sharptooth catfish is a bottom dweller, and the photoperiod would be a very important factor for its survival and growth under both natural and controlled conditions. Indeed, a tendency for larvae and juveniles to grow faster under short light periods has been observed [31-35]. Overall, these results indicate that variations in water temperature and dissolved oxygen levels in this study did not negatively influence the success of the artificial reproduction of *C. gariepinus*.

The brooders received daily artificial food, in addition to the live food (Daphnia) from the fertilization of the tanks during the acclimation phase. The exponential development of the Daphnia population in the storage tanks and the leftover foods strongly suggest that the fish did not consume all food. Thus, the drop in the weight of the brooders may be explained by their refusal to feed due to the stress caused by the transfer under controlled conditions. However, this drop in weight did not appreciably impact the success of the artificial reproduction because the females responded favorably to the injection.

The latency time in this study was 11 hours with an average temperature of 27.4°C and is equal to that reported by Ahotondji [36] for *C. gariepinus* at a water temperature of 25°C. It has been reported that the latency time decreases as the water temperature increase [16]. The incubation time in this study was 24h at a temperature that varied between 27 and 29°C, in accordance with the results of Ahotondji [36], who reported an incubation time of 23h at a temperature of 27°C [36]. The hatching rate obtained with the Macdonald's bottle (MB), the wire frame (WF) and the water lettuce (WL) with the pituitary gland extracts of the male were 26.89%, 10.04% and 6.25%, respectively. The incubated eggs were laid by the same female, which suggests that the pituitary inducer did not influence the results obtained. On the other hand, the results obtained with the Cadre, Jacinthe and Bouteille Zoug (44.00±13.6, 39.10±8.4 and 66.77±2.18, respectively) are more satisfactory [19]. The highest hatching rate obtained in this study with the Macdonald's bottle can be explained by the influence of the permanent circulation of water, which was absent in the water lettuce and the wire mesh hatching systems.

The survival rate obtained three days after hatching is satisfactory, but it decreases significantly 15 days after hatching especially during the post-weaning phase, which seems to be due to the food provided to the larvae. Indeed, after the resorption the larvae must be feed with natural food to ensure their survival and optimize their growth performance, as it is evident from the results of the comparative feeding experiment.

The daily individual growth, the weight gains and the size of the larvae showed significant differences between larvae fed with nat-

ural food (Daphnia) and those fed with artificial food. The weight and size of the larvae that received the natural food were at least twice that of the larvae fed with the artificial food. These differences cannot be due to variations in water temperature and dissolved oxygen levels because these parameters were not significantly different between rearing tanks. The natural food was available all the time in the rearing tanks, which indicates that the larvae could feed any time. This can explain their higher growth rate compared to larvae fed with the artificial food. The artificial food became deposited at the bottom of the tanks and changed appearance over time, and may thus not have been appreciated and consumed by the larvae, which may have negatively impacted their growth performance. These results are in accordance with those of Rukera-Tabaro et al. [38] where larvae reared on fertilized pond had higher growth rates compared to those cultured in basins that received the artificial food directly.

Conclusions

The main objective of this study was to produce fry of *C. gariepinus* from a local strain captured in the wild and acclimated under controlled conditions. Three different types of incubation (MB, WL and WF) were tested to identify the most efficient and accessible method to producers at low cost and with a high hatching rate. Such an efficient reproduction method could allow solving the main problem with which aquaculture production is confronted, which is the supply of sufficient quantities of *C. gariepinus* larvae. The results obtained in this study are encouraging, although preliminary. Comparisons of the hatching rate, survival rate and especially the feeding of the larvae after absorption of the yolk sac indicated that the incubation on the Macdonald's bottle provided the best results. The results also indicated that the survival rate of the larvae after resorption of the yolk sac depends largely on the nature of the food received during this phase, with the live food (Daphnia) being more efficient than the artificial food. Altogether, these results indicate how aquaculture production and therefore increase the quality and quantity of food supply can be boosted. However, many challenges such as conducting the same study in a pond where natural food production could contribute to improving results remain to be faced. Indeed, *C. gariepinus* can take advantage of various food sources in the natural environment, which could allow solving the problems related to the mass productions of fry in Africa, and especially Senegal.

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