

Full Length Research Paper

Semi-artificial method of induced breeding of the African catfish (*Clarias gariepinus*, Burchell, 1822) under varying broodstock ratios using Ovaprim®

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Modern methods of producing African catfish (*Clarias gariepinus*) fingerlings require that the male brooder is sacrificed to obtain milt for artificial fertilization of the eggs stripped from the female under hormonal induction. This study assessed the semi-artificial technique of producing catfish larvae with different broodstock ratios using Ovaprim®, a synthetic spawning inducing hormone. The treatments with 3 replicate each were: T1 (artificial spawning with 1:1 female: male ratio), T2 (semi-artificial spawning with 1:1 female: male ratio), and T3 (semi-artificial spawning with 2:1 female: male ratio). The relative fecundity of brooders in T1, T2, and T3 was 68 ± 6.31 , 78 ± 12.29 , and 65 ± 8.18 , respectively with no significant difference ($P \geq 0.05$). Percent fertilization for T1 ($81 \pm 1.52\%$), T2 ($75 \pm 2.51\%$) and T3 ($62 \pm 2.50\%$) was significantly different ($P \leq 0.05$). The observed percent hatchability (85 ± 2.51 , 83 ± 3.21 , and $82 \pm 2.50\%$) in respect of T1, T2, and T3 was not statistically different ($P \geq 0.05$). Differences in total egg weight (96 ± 3.30 , 72 ± 10.53 , and 59 ± 0.50 g; $p=0.099$), and total larval production ($57,700 \pm 3672$; $42,423 \pm 6972$ and $34,078 \pm 762$; $p=0.002$) for T1, T2 and T3, respectively, were statistically significant between artificial spawning and semi-artificial spawning. Larval survival was significant ($P \leq 0.05$) between T1 (84 ± 2.31) and T3 (92 ± 2.50) but both did not differ significantly ($P \geq 0.05$) from T2 (87 ± 2.51). In conclusion, the semi-artificial spawning of *C. gariepinus* with Ovaprim® could be beneficial to fish farmers if done at a broodstock sex pairing ratio of 1:1.

Key words: *Clarias gariepinus*, induced breeding, Ovaprim®, semi-artificial, aquaculture.

INTRODUCTION

In the past, fish farmers have collected their fish fingerlings from the wild, but due to challenges such as erratic supply and poor quality, it is not reliable to source fingerlings from natural waters to sustain commercial

aquaculture (Olumuji and Mustapha, 2012; Ali et al., 2016, 2020). Increase in demand for fingerlings, necessitated by the remarkable growth in the fish culture industry, has exacerbated the requirement for artificial

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propagation of catfish fingerlings (Nwokoye et al., 2007). The major challenge to captive breeding of *Clarias gariepinus*, which is their inability to breed under captive conditions (Adebayo and Fagbenro, 2004) due to stress induced ovarian atresia (Lubzens et al., 2010), must however, be resolved to help meet this demand.

Thus, to instigate the release of eggs, hormones are used to overcome the limitations of producing the eggs in captivity. This is necessary for the catfish to proceed through spawning without the limitation of ovarian atresia. Hormonal induction is carried out either by injecting natural hormone extract from the pituitary gland of same fish species or other fish species (tilapia and carp) (Fagbenro et al., 1993) or the injection with synthetic hormones (Salami et al., 2006). Nwokoye et al. (2007) and Ngueku (2015) revealed that hormones such as Human Chorionic Gonadotrophin (HCG), Decorticosterone Acetate (DOCA), Pituitary extracts, Ovaprim®, Ovatide and Ovaryprim have been used to induce reproduction in the African catfish with success. However, in all the techniques developed to induce artificial reproduction in the African catfish, the female is stripped and the male is killed in order to obtain their eggs and milt, respectively. Some attempts at sparing the male catfish is by abdominal incision to extract milt from the gonads (Yisa et al., 2013), hand stripping of milt from live male (Viveiros et al., 2002) and removal of one testis and suturing (Egwui and Nwanko, 2015), but these techniques even though successful to some extent have limitations. For instance, abdominal incision requires special care to ensure survival and post-surgery recuperation, whereas hand stripping can fail because of anatomical blockage in African catfish males, and sutured males, used for breeding only twice. This work seeks to demonstrate that the African catfish can be bred commercially without sacrificing the male catfish, hence, reducing the overall cost of fingerling production. The technique of inducing the male African catfish in captivity to spontaneously release milt for fertilization during spawning, will not only save it for subsequent breeding but also provide a simpler method for breeding the African catfish under hatchery conditions.

MATERIALS AND METHODS

This study was carried out at Flosell Farms Limited, a reputable farm that produces tilapia and catfish (table fish and fingerlings), located at Sogakope in the Volta Region of Ghana. Mature brood stocks (27 females and 18 males) were obtained and kept in separate rectangular concretetanks (one for males and another for females) for one month during which period they were fed with 35% crude protein Skretting Fish Feed (Skretting, Egypt) at 5% body weight. Twelve (12) females and nine (9) males were selected for the breeding experiment based on their ripeness. Ripeness of females was determined morphologically by the swelled abdomen and readiness to spawn (eggs ooze out easily when the abdomen is gently pressed). The reddening of the genital papilla was used as an indicator of ripeness in the males (Natea et al., 2017; Ngueku, 2015; Olumuji and Mustapha, 2012).

The total length (cm) and corresponding weight (g) of the brooders were measured prior to hormone administration. The condition of the brooders was determined using the Fulton's condition Factor (K) from the relationship (Ricker, 1975; Barnham et al., 1998):

$$K = (W/L^3) \times 100.$$

where W is the mean weight and L^3 is the total length of the fish in cm cubed.

Experimental design

The male and female brooders were grouped into three (3) treatments with three (3) replicates each. The treatment groups with female to male ratios were as follows:

- Treatment 1 (T1, Control): Artificial spawning with 1:1 ratio
- Treatment 2 (T2): Semi-artificial spawning with 1:1 ratio
- Treatment 3 (T3): Semi-artificial spawning with 2:1 ratio

T1 (artificial spawning) was made up of one matured female and one matured male brooder, where both male and female brooders were injected with Ovaprim®, the synthetic spawning hormone (Syndel, USA) and kept in separate plastic tanks for a latency period of 11 h. After the latency period, the female was stripped and the male was sacrificed, and the gonads were removed to obtain the milt. This treatment was designated as the Control group because it is the most common method used by hatcheries in the production of catfish fingerlings (Abdulraheem et al., 2012). T2 and T3 were the semi-artificial spawning, where both female and male brooders were injected with Ovaprim® and left for 11 h to allow for natural spawning, fertilization and incubation. T2 had a female: male ratio of 1:1 and T3 consisted of a female: male ratio of 2:1.

Hormone injection

Ovaprim® was used as the spawning inducing hormone. All the brooders were disinfected in a salt bath (5 g sodium chloride per litre of water), after starving for a period of 24 h and weighed before Ovaprim® was administered. The brooders were injected intramuscularly with the hormone using a syringe with a needle inserted 2.00 to 2.50 cm at an angle of 45° (Abdulraheem et al., 2012). A dosage of 0.50 ml/kg brood stock weight (BW) and 0.25 ml/kg BW of the hormone was administered to the females and males, respectively.

Stripping and fertilization for artificial spawning

Injected female brooders were placed in circular plastic tanks (1.5 m³) fill with water to 30 cm depth with continuous aeration and flow-through. The female brooders were stripped into dry bowls and weighed after the latency period. The total number of eggs was determined by taking three representative samples (1 g each) and counting the eggs with the aid of an egg counter. Relative fecundity was estimated as follows:

$$\text{Relative Fecundity} = (\text{Total no. of eggs} / \text{body weight of female}).$$

The males were sacrificed by dissecting to remove the testes. In order to collect sperms, the testes were cut into pieces with a sterile surgical blade and the milt was extracted and used to fertilize the eggs. The eggs were fertilized by mixing the milt with little quantity (25 ml) of saline solution in a Petri dish and milt mixture was poured over the eggs and mixed with a clean feather after adding clean

Table 1. Body weight, length and condition factor (K) for female *Clarias gariepinus* broodstocks.

Parameter	T1	T2	T3
Female body weight (g)	1000 ± 132.28 ^a	600 ± 50.00 ^b	616 ± 60.55 ^b
Female body length (cm)	51 ± 2.51 ^a	43 ± 2.46 ^b	43 ± 2.54 ^b
K – Factor	0.75 ± 0.05 ^a	0.76 ± 0.07 ^a	0.75 ± 0.09 ^a

Values are means ± standard deviations. Means in the same row with different superscripts differ significantly ($P \leq 0.05$).
Source: Authors

water to activate the sperms and prevent the coagulation of the eggs. The fertilized eggs were spread evenly on an incubation tray, which was a 50 cm × 60 cm rectangular frame of Polyvinyl Chloride (PVC) pipes, overlaid with a 2 mm nylon mesh. The tray was placed in a circular plastic tank with water at 40 cm deep with continuous aeration in a flow-through system. Hatching occurred approximately 22 h after incubation and the hatching trays were removed from the tanks when the hatched larvae had penetrated the nylon mesh and gathered at the edges and at the bottom of the tank.

Semi-artificial spawning

For this treatment, injected female and males were placed in hapas (Cloth hatchery), made with nylon netting material (2 mm mesh size) within the circular plastic tanks and allowed to spawn. Brooders were carefully removed from the hapas with a scoop net after the 11 h latency period and weighed to determine the weight of eggs spawned. The mass of eggs spawned was determined by calculating the difference in weight of each female catfish before and after spawning. The overall number of eggs reproduced was estimated by multiplying the weight of spawned eggs by quantity of eggs in 1 g (Tiamiyu et al., 2015). Eggs spawned were incubated in the same tank for a period of 24 h. The hapas and unhatched eggs were removed to prevent fungal infection from egg shells. The relative fecundity was then estimated.

Estimation of percent fertilization, hatchability and survival

Percent fertilization was determined using Ella method (Ella, 1987) as described by Ataguba et al. (2012). A glass tube (30 cm in length and 2.5 mm in diameter) was used to siphon eggs from the egg mass. Samples were taken from areas of the hapa or hatching tray with the fertilized egg mass. Good and bad eggs were counted by viewing the glass tube against a source of light to determine total numbers of good and bad eggs. The fertilized eggs were seen as bright and shiny while unfertilized eggs appeared opaque and white. Fertilization success was estimated as:

$$\% \text{Fertilization} = [(N-b)/N] \times 100$$

where N = the total number of eggs spawned, b = number of bad eggs, b was estimated by the ratio:

$$b = [(y/x) \times N],$$

where y = number of bad eggs counted and x = total number of eggs in the samples.

Hatchability and survival rates were calculated at 30 h and 5 days after hatching (Adebayo and Popoola, 2008). These rates were calculated as:

$$\% \text{Hatchability} = [(\text{No. of hatched eggs in a sample} / \text{Total no. of eggs in a sample}) \times 100]$$

$$\% \text{Survival} = [(\text{No. of larvae alive} / \text{Total no. of hatchlings}) \times 100]$$

Statistical analysis

Data collected were subjected to one-way Analysis of Variance (ANOVA) test. The test of significance (at α level of 0.05) for egg weight, egg number, relative fecundity, hatchability rate and survival rate were compared for statistical differences using Tukey's HSD with PRIMER 6.0 software.

RESULTS

Broodstock characteristics

The weight of female broodstock used for the experiment was in the range of 600 to 1000 g (Table 1). Artificial spawning with 1:1 female to male ratio (T1) had the highest mean female weight (1000 ± 132.28 g) followed by semi-artificial spawning with 2:1 ratio (T3). Semi-artificial spawning with 1:1 ratio (T2) showed the lowest mean female weight (600 ± 50.00 g). The statistical differences in mean female brood stock weight among T1, T2 and T3 was significant ($P \leq 0.05$), but mean weight variation in T2 and T3 was not significant ($P \geq 0.05$).

Induced spawning of *C. gariepinus* broodstock

Spawning was observed in all treatment groups as indicated in Table 2. Mean number of eggs counted was highest (67,573 ± 2,315.65) in artificial spawning (T1) and lowest (41,300 ± 350.00) in semi-artificial spawning with 2:1 ratio (T3) ($P \leq 0.05$), but the difference in means of semi-artificial spawning (T2) (50,633 ± 7,377.72) and T3 (41,300 ± 350.00) were not significant ($P \geq 0.05$). The mean total egg weight estimated was 96 ± 3.30, 72 ± 10.53 and 59 ± 0.50 g for T1, T2, and T3, respectively. The highest mean total egg weight (96 ± 3.30 g) was recorded in T1 and the lowest (59 ± 0.50 g) was in T3. Differences in means were significant ($P \leq 0.05$) between T1 and T3 but not between T2 and T3 ($P \geq 0.05$).

Mean relative fecundity was 68 ± 6.31, 78 ± 12.29 and 65 ± 8.18 for T1, T2 and T3, respectively (Table 2). T2 showed the highest value (78 ± 12.29) and T3 recorded the lowest value (65 ± 8.18) with no significant difference

Table 2. Spawning characteristics of *Clarias gariepinus* in the different treatments.

Parameter	T1	T2	T3
Egg number	67,573 ±2,315.65 ^a	50,633 ±7,377.72 ^b	41,300 ±350.00 ^b
Total egg weight (g)	96 ±3.30 ^a	72 ±10.53 ^b	59 ±0.50 ^b
Relative fecundity	68 ±6.31 ^a	78 ±12.29 ^a	65 ±8.18 ^a

Values are means ± standard deviations. There is a significant difference between means in the same row with different superscripts ($P \leq 0.05$).

Source: Authors

Table 3. Mean percentage fertilization, hatchability, larval survival and mean larval production.

Parameter	T1 (Control)	T2	T3
Fertilization (%)	81 ±1.52 ^a	75 ±2.51 ^b	62 ±2.50 ^c
Hatchability (%)	85 ±2.51 ^a	83 ±3.21 ^a	82 ±2.50 ^a
Larval production	57,700 ±3,672 ^a	42,423 ±6,973 ^b	34,078 ±1,321 ^b
Larval survival (%)	84 ±3.21 ^a	87 ±2.51 ^a	92 ±2.50 ^b

Values are means ± standard deviations. There is a significant difference between means in the same row with different superscripts ($P \leq 0.05$).

Source: Authors

($P \geq 0.05$).

Fertilization, hatchability, larval production and larval survival

Table 3 shows the mean percent fertilization obtained during the experiment as 81 ± 1.52, 75 ± 2.51 and 62 ± 2.50% for artificial spawning (T1), semi-artificial spawning with 1:1 ratio (T2) and semi-artificial spawning with 2:1 ratio (T3), respectively. T1 recorded the highest value (81 ± 1.52%) with T3 having the lowest value (62 ± 2.50%) and differences among all treatment means were significant ($P \leq 0.05$). Mean percent hatchability were 85 ± 2.51% (T1), 83 ± 3.21% (T2) and 82 ± 2.50% (T3), with no significant difference ($P \geq 0.05$) as indicated in Table 3. Mean larval production was highest in T1 (57,700 ± 3,672) followed by T2 (42,423 ± 6,973) and lowest in T3 (34,078 ± 762). The difference in means were significantly different ($P \leq 0.05$) between artificial and semi-artificial but not significant between T2 and T3 ($P \geq 0.05$).

Mean percent larval survival recorded during the experiment was 84 ± 2.31, 87 ± 2.51, and 92 ± 2.50% with T3 recording the highest (92 ± 2.50%) followed by T2 (87 ± 2.51%) and T1 (84 ± 2.31%) (Table 3). The difference in means of the treatments was significant ($P \leq 0.05$) but there was no significant difference between T2 and T1 ($P \geq 0.05$).

DISCUSSION

The individual weights of the brood stocks, as well as the

sex pairing weights are relevant in the determination of the fecundity, fertilization and hatchability, and the overall production of larvae by the African catfish. Brood stock weight between 300 and 800 g was ideal for spawning, since larger fish were difficult to handle and often resulted in significant loss of eggs (Graaf and Janssen, 1996). Other studies have reported the successful spawning of the African catfish weighing between 500 and 3000 g for both female and male brooders (Adebayo and Popoola, 2008; El-Hawarry et al., 2016; Okomoda et al., 2016). In a study conducted (Ataguba et al., 2012), brood stock combination of 824 g (female) and 619 g (male) resulted in the best fecundity, fertilization and hatchability. The variations observed in the weights of the brood stock used in this study (600 - 1000 g) could have accounted for the differences observed in total egg weight, egg number, percent fertilization and the eventual larval production. According to Atuguba et al. (2012), increase in brood stock size leads to significant increase in fertilization and hatchability of *C. gariepinus*.

The Condition Factor (K) recorded for both male and female brood stock were <1, indicating poorer state of well-being (Keyombe et al., 2015). The body condition of fish under natural conditions changes as a result of development of gonads, food abundance and other environmental factors (Pope and Willis, 1996), hence fecundity could have been affected by the condition of brooders used in this study. Well-fed female brood fish will give excellent result with respect to fecundity, latency period, and egg mass, size and yolk (Ngueku, 2015). Condition factor greater than 1 gives an indication of well-being of fish (Datta et al., 2013).

Results from this study indicated that spawning was

successful for all treatment groups (T1, T2 and T3). Relative fecundity across treatments was not significantly different and this gives a good indication of good response to Ovaprim®. Fecundity is used as an index to determine the reproductive capability of fish and the extent of the efficiency of the inducing agent (Okere et al., 2015). Ovaprim® significantly increases ovulation and spawning in matured female African catfish (Watson et al., 2009; Sharaf, 2012). The relative fecundity values (68 ± 6.31 , 78 ± 12.29 and 65 ± 8.18) obtained in this research were lower than the 129.06 value obtained by Ngueku (2015) who concluded that well-fed female will give excellent result with respect to fecundity, latency period, and egg mass, size and yolk.

The mean weight of egg observed in this study was significantly higher ($p < 0.05$) in artificial spawning treatment group than the semi artificial spawning treatment groups. This is accounted for by the significant variation in the mean weight of female brooders used in this experiment. Females used for artificial spawning were significantly heavier than those used for the semi artificial spawning, hence the difference in mean egg weight. A ripe female African catfish can release a mass of eggs that is about 15 to 20% of its total body weight with stripping, but usually spawns only 5 to 15% of the total body weight under semi artificial spawning techniques (Graaf and Janssen, 1996). The weight of eggs spawned as observed for the semi artificial spawning group in this experiment was found to be within the range of 9 to 12% of the body weight of brood stock used, which is similar to that reported (De Graaf et al., 1995).

Fertilization rate was best at $81 \pm 1.52\%$ in artificial spawning treatment group, and this is comparable with 83.7% (Ataguba et al., 2012), but lower than the 88.3% (Ngueku, 2015) and 92.7% reported by Kasi et al. (2015), due to difference in species and cultural systems. The difference observed in fertilization rates for the artificial and semi artificial breeding techniques from this study were statistically significant ($P \leq 0.05$).

Percent fertilization was lower for semi artificial treatment groups (T2 and T3) than the artificial treatment group (T1). Fish sperm motility after activation is usually short-lived, hence losing their capacity for fertilization of eggs (Cejko et al., 2016). The African catfish sperm has a relatively short (about 90 s) period of motility after contact with water, even though the eggs can remain active for a relatively longer period, fertilization is affected if the sperms do not reach the eggs (Biegniewska et al., 2010; Kucharczyk et al., 2019). Sperm activation tests was not carried out in this study but could have caused the difference in fertilization rates observed between the artificial and semi artificial methods of propagation, since fertilization occurred under artificial (T1) and natural (T2 and T3) conditions.

Percent hatchability was high for all treatment groups (85 ± 2.51 , 83 ± 3.21 and $82 \pm 2.50\%$) and the differences observed were not significant ($P \geq 0.05$). These results are similar to what was reported by Ataguba et al. (2012)

and Kasi et al., (2015). Other authors (Delince et al., 1987; De Graaf et al., 1995) have however, reported lower values (4 - 59%) with different substrates and at different seasons. Adebayo and Popoola (2008) used different synthetic hormones and reported hatchability within the range of 51 to 73%. The use of Ovaprim® in this study resulted in a better hatchability, due to its influence on egg size compared with natural hormones. Eggs size positively correlates with hatchability and hatching rate is also influenced by breeding history, type and age of fish, as well as water quality. Particularly, water quality parameters recorded in this study were within the recommended range for hatching catfish eggs and could also account for the good hatchability.

Larval survival rate was observed to be highest ($92 \pm 2.5\%$) in semi artificial spawning (2:1 female to male ratio) and lowest ($84 \pm 2.31\%$) in artificial spawning (1:1 female to male ratio). These rates are higher than the 40 to 42% (Olumuji and Mustapha, 2012), 66 to 73% (Abdulraheem et al., 2012) in other studies. Survival rate of larvae is influenced by the size of the receptacle and aeration (Adebayo and Popoola, 2008), in addition to effect of high dissolved oxygen concentration (Nwaduwe and Ayinla, 1993; Sahoo et al., 2008). The higher survival rates observed in this study could be attributed to the effect of Ovaprim® on ovulated eggs. Ovaprim® induced the release of larger eggs compared with natural hormones and eggs size correlate positively with larval length and survival (Rideout et al., 2005). Larger eggs provide more energy for larvae development; the larger the yolk sac, the more energy is available for survival (Olaniyi and Akinbola, 2013; Kucharczyk et al., 2019).

In this study, significantly ($P \leq 0.05$) high percent of larvae survived in the treatment T3 than in T1 and T2, due to larval density that was traced to the low larval production in T3, thus, giving larvae more dissolved oxygen and larger space to move than in T1 and T2 experiments, that had relatively higher numbers of larvae produced.

In conclusion, semi artificial spawning of *C. gariepinus* with Ovaprim® could be beneficial to fish farmers if done at a broodstock sex pairing ratio of 1:1.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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