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Evaluating the Efficacy of Pituitary Gland Extracts and Ovaprim in Induced Breeding and Fry Quality of *Clarias gariepinus*, Burchell (Pisces: Claridae)

Efe Okere, Ebere Samuel Erondu, Nenibarini Zabbey*

Department of Fisheries, Faculty of Agriculture, University of Port Harcourt, PMB 5323 Choba, Port Harcourt, Rivers State, Nigeria

Email address:
nenibarini.zabbey@uniport.edu.ng (N. Zabbey), zabbeyn@yahoo.com (N. Zabbey)

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Abstract: This study compared the effectiveness of Ovaprim and pituitary gland extract (PGE) in induced spawning of the African mud catfish, *Clarias gariepinus*, using reproductive output and fry quality indices. At a mean temperature of 26.0 ±0.70^{0}C, latency period for Ovaprim and PGE were 613 and 745 minutes, respectively. Workers fecundity was significantly higher ($p<0.05$) for brooders treated with Ovaprim (36086.00 ±7215.50eggs) than PGE induced spawners (20978.00 ±6782.15 eggs). Hatching rates also followed the same trend, in which significantly higher hatching success was recorded for Ovaprim ovulated eggs (83.5%) than PGE induced eggs (63.7%). Fry survival rate was 81.90 ±1.10% for Ovaprim treated fish, while PGE induced fish fry had 77.73±1.33%; percentage deformed fry was significantly minimal for Ovaprim treated. However, all Ovaprim-treated spent fish died few hours post stripping, contrary to PGE spent brooders that were fully recovered. Production cost analyses revealed that the use of Ovaprim resulted in about 25% cost reduction. It is thus concluded that Ovaprim is superior to PGE in induction of breeding in *Clarias gariepinus*. This notwithstanding, the mortality suffered by all the spent fish treated with Ovaprim raises food safety concerns. This however, needs to be validated.

Keywords: Hatching Success, Induced Breeding, Fecundity, Spawners, Latency

1. Introduction

The African mud catfish, *Clarias gariepinus* (Burchell, 1822) is an important food fish in Nigeria. It is well relished and has high market value (Oladosu *et al*., 1993; Ayinla *et al*., 1994). *C. gariepinus* is the most important aquaculture fish species in Nigeria because of its hardiness, ability to survive hypoxic condition, ability to accept pelleted feed, fast growth in captivity and high market value (Ekunwe and Emokaro, 2009; Adewolu and Adeoti, 2010).

Until the late 1980s, most of the fish seeds, including *C. gariepinus*, used for aquaculture in Nigeria were collected from the wild (Osuigwe and Erondu, 1997). But aquaculture that is dependent on wild-bred fish seeds is fraught with several disadvantages such as inadequate supply of fish seed required to meet the production target of the farmer. Meanwhile, adequate supply of quality seeds is an essential prerequisite to successful aquaculture production (Rottmann *et al*., 1991). Furthermore, stunted individuals are often procured from the wild as it is difficult to differentiate between siblings and cohorts at early life history stages. To guarantee adequate supply of fingerlings with known age and genetic background, several studies have been carried out, which recent findings have led to improved techniques in induced breeding of *C. gariepinus* (Viveen *et al*., 1986; Nwokoye *et al*., 2007; Akinwande *et al*., 2009; Ataguba *et al*., 2009).

In order to bridge fingerling demand-supply gap, hatchery techniques have been developed for seed production of some culturable fish species. These are either through natural breeding in captivity, or by induced breeding using exogenous hormone (Ndimele and Owodiende, 2012). Induced breeding involves the use of some exogenous ovulating agents, which trigger the ripening of mature eggs (Ajah, 2007). In Africa, various hormonal substances (Ovaprim, carp pituitary, human chorionic gonadotropin, frog pituitary extracts, etc,) have been used to induce breeding in fish with varying magnitudes of success (Nwadukwe 1993; Okoro *et al*., 2007). There is, therefore, the need to carry out comparative studies on the effectiveness of these induction agents in order to define the

viable options. This will provide valuable information that will promote sustainable hatchery propagation of fish in Nigeria.

This study compares the efficacy of Ovaprim and catfish pituitary gland in induction of breeding in *C. gariepinus,* using appropriate reproductive and cost-benefit indices.

2. Materials and Method

2.1. Collection and Selection of Broodfish

Ten gravid female *C. gariepinus* fish and nine mature male fish with weight ranging from 550g – 1000g were purchased from a fish farm in Port Harcourt, Rivers State. All broodfish were selected by external morphological characteristics, using the method of Ayinla *et al.* (1994). Females were selected on the basis of their bulging abdomen as well as egg colour. The selected fish were kept in an outdoor concrete tank (2.5m x 2.0m x 1.5m) at the University of Port Harcourt Demonstration Farm for seven days prior to the breeding date. They were fed 5% of total biomass with Coppens pelleted fish feed (48% crude protein) twice daily. Feeding was suspended a day prior to the hormonal treatments.

2.2. Water Holding Facilities

Eighteen litres of bore-hole water was introduced into ten plastic containers (20-litre capacity) arranged in rows of fives on an elevated platform in the hatchery section of the Demonstration Farm. The dimension of the plastic containers was 40cm x 30cm x 26cm. The fish holding containers were equipped with a simple flow through system with an overhead reservoir containing water properly conditioned before use. Each of the plastic containers was filled with calcium carbonate treated water (50g of $CaCO_3$to 1000 liters of water) and kept standing for twelve hours prior to the time of injecting the broodfish.

2.3. Pituitary Extraction and Preparation

Pituitary glands were extracted from five of the male broodfish. They were weighed so as to get a corresponding weight to that of the recipient fish. The head of the male donor was cutoff after stunning the fish, and subsequently the lower jaw was also cut off. The ventral side of the brain was opened to expose the pituitary gland. Glands were collected with a pair of tweezers and placed in a beaker containing 2ml of 0.9% normal saline solution. Each of the glands was crushed in a mortar using a pestle. Two millimeter of 0.9% normal saline solution was added and the suspension decanted and collected into a 2ml syringe. The freshly collected pituitaries were immediately injected into the female spawners. This was done in the evening hours at about 9pm.

2.4. Administration of Hormone

The ten gravid female were divided into two groups of five fish with one set of fish representing the replicates for each treatment. Ovaprim and pituitary extract were administered on each set of gravid female fish. The mean weight of female brooders used in pituitary and Ovaprim treated fish was 815.5 g. Pituitary suspension was drawn into a 2ml syringe, and then injected intramuscularly above the lateral line of the fish toward the dorsal section and pointed to the ventral side. After withdrawal of the needle, the fish were gently rubbed at the site of injection to avoid back flow of the injected fluid. Each female from the second group was injected with a dose of 0.5ml Ovaprim/kg of body weight (Haniffa and Sridhar, 2002). The injected fish were kept separately in well-labeled containers measuring 40cm x 30cm x 26cm containing water. The containers with the injected fish were covered with heavy boards so as to prevent the fish from leaping out.

2.5. Preparation of Milt

Four male fish were killed, dissected carefully and their milt sac obtained. The weight of each male was obtained and recorded alongside the weight of each of the gonad. A small incision was then made on the lobes with a sharp razor blade and the milt squeezed into a dry Petri dish. Milt was washed into the Petri dish with 0.9% normal saline solution (Ayinla, 1991; Nwadukwe *et al.,* 1993).

2.6. Stripping, Fertilization and Incubation of Eggs

Latency period was recorded for each of the fish in each group and stripping took place within 10 and 12 hrs after injection at a mean temperature of 24.05^{o}C. With slight pressure at the ventral part of the abdomen, ovulated eggs oozed out freely and were collected into a dry Petri dish of known weight. This was done for each treated female fish, and collected eggs were weighed and recorded. Workers fecundity was then determined from the data. A sample of 1g was collected from the stripped eggs from each female and fertilization was done by pouring the prepared milt onto the eggs. The mixture of 1g was incubated separately on the spawning substrate (*kakaban*) placed in water in each of the plastic containers (Szabo *et al.,* 2002).

Basic water quality parameters were determined. Temperature was measured with mercury in-glass thermometer. Dissolved Oxygen and pH were measured using pH meter (WTW pH 330) and DO (Model MW600) meter, respectively. The flow through system was used so as to enhance proper aeration. Post hatching, dead eggs were removed by siphoning. The *kakabans* were removed and percentage hatchability determined by recording the number of dead eggs in each container.

Larvae were reared with constant water supply and by the third day their yolk sacs were fully absorbed and the fry were seen swimming in the containers. Fry Survival rate was determined by records of number of dead fry in each treatment medium. Live larvae were fed with decapsulated Artemia, five times daily.

2.7. Determination of Reproductive Success Parameters

Latency period (time taken from injection of female brood fish to time of stripping) was recorded.

Workers fecundity was estimated by counting the number of eggs stripped from each female broodfish as follows:

F = Total weight of eggs x no. of egg per gram

Relative workers fecundity was estimated by dividing the number of eggs stripped (fecundity) by the length per fish.

Percentage hatchability was determined by estimating the number of unhatched egg as follows:

$$\%\ hatchability = \frac{Total\ no\ of\ egg\ incubated - no\ of\ unhatched\ egg}{Total\ no\ of\ egg\ incubated} \times 100\%$$

The Survival rate per rearing tank was determined at the end of the experimental period with the formular:

$$\%\ survival = \frac{number\ of\ survived\ fry}{Total\ no\ of\ fry\ stocked} \times 100\%$$

The percentage of deformed fry per rearing tank was determined as follows:

$$\%\ deformity = \frac{number\ of\ deformed\ fry}{Total\ number\ of\ fry} \times 100\%$$

2.8. Statistical Analysis

The data obtained were statistically analyzed using student's t-test and for all the analyses, probability values < 0.05 were considered significant.

3. Results

3.1. Physico–Chemical Parameters

Mean pH in the incubation tanks was 6.92±0.08, while that of the rearing tank was 6.80±0.18. Mean temperature and DO in the incubation tank were 26.0 ±0.70 and 6.9± 0.32 mg/l, respectively, while in the rearing tank the values were 26.8±0.83^{0}C and 6.26 ±0.45mg/l, respectively, as shown in Table 1. There was no significant difference in pH and temperature in both tanks despite the higher values recorded in the incubation tank, but there was significant difference in the DO values recorded (P<0.05).

3.2. Latency Period

The latency period ranged from 10:13 to 12:25 hours (Table 2). Ovaprim recorded a mean latency period of 10:25 hours while pituitary recorded 11:49 hours. There was a significant difference between the latency periods of the treatments (P< 0.05).

3.3. Workers Fecundity /Relative Workers Fecundity

Average number of eggs stripped from pituitary treated fish was 20978.4 ±0.50 while 36086±72 eggs were recorded from Ovaprim treated fish as shown in Table 2. Mean relative workers fecundity was also computed and the values recorded were 453.3±2 124.95 and 781.67± 11 eggs for pituitary and Ovaprim treatments, respectively. There was significant difference in both workers fecundity and relative workers fecundity for both treatments (P< 0.05).

3.4. Egg Hatchability

The period of hatching ranged from 10:20 to 24:06 hours for pituitary treated fish, which recorded a longer hatching period at a temperature of 26^{0}C. A mean percentage hatchability rate of 63.77.±29% was recorded for pituitary treated fish while 83.53 ±2.77% was recorded for Ovaprim treated fish. There was significant difference between the percentage hatchability of the two groups (p <0.05) as shown in Table 2.

3.5. Percentage Mean Fry Survival

Females injected with pituitary recorded 77.7±1.33% while Ovaprim treated fish recorded a mean value of 81.9% fry survival. There was significant difference between the treatments (P< 0.05)

3.6. Percentage Deformity of Fry

The percentage deformity of fry is presented in Table 3. Ovaprim treated fry had the lowest mean percentage of deformed fry (0.1± 0.28 %), while fry from pituitary gland injected females had 0.9±0.50% deformity. Student's t–test showed significant difference (P <0.05) for both treatments.

3.7. Percentage Mean Fry Weight

Ovaprim treated females had higher mean fry weight (0.12±0.023g), whereas fry from female treated with pituitary gland had lower mean fry weight (0.09±0.018g). Student's t–test showed significant difference in this parameter (p<0.05).

3.8. Survival of Spent Brood Stock

Spent fish from both treatments were kept in two separate recovery tanks. Ovaprim treated spent fish were relatively less active and died one after the other after six hours. However, pituitary treated spent fish showed signs of recovery and no death was recorded in this group six hours post stripping.

3.9. Cost Benefit Analysis of Hormonal Treatment

Table 4a shows the comparative mean cost per quantity of Ovaprim and pituitary gland required to induce spawning in *C. gariepinus*. Two hundred and fourteen naira eight kobo (N214.8±171.84 ~ $1.08) was used to procure 1.74ml of Ovaprim, as against N204.48±163.58 used to procure 19mg of pituitary. Table 4b shows the comparative cost benefit analysis required for a gram of egg when Ovaprim and pituitary are

used as inducing agents. Females injected with pituitary gland were stripped of a total of 257g of eggs and the cost of pituitary per gram of egg was ₦19.95. For females injected with Ovaprim a total of 334g of eggs were stripped, implying ₦15.9 per gram of egg.

Table 1. *Mean values of Physico-Chemical parameters of water.*

Ovaprim/pituitary	Incubation tank	Rearing tank
Temperature(^{0}C)	26.0 ±0.71	26.8±0.84
pH	6.9 ±0.08	6.8± 0.19
Dissolved oxygen (mg/L)	6.9 ± 0.34	6.26 ± 0.45

Table 2. *Mean values of reproductive indices of C. gariepnus brood fish induced to breed with Ovaprim and pituitary gland.*

	Pituitary	Ovaprim
Workers fecundity	20978.4±6782.15^b	36086.0± 7215^a
Relative workers fecundity	453.3±124.95^b	781.7± 11^a
Latency period (minutes)	709.0±34.00^b	625.0±08.00^a
Percentage hatchability (%)	83.5±2.77^a	63.8±4.29^b

$^{a-b}$Values with the same superscript are not significantly different ($p<0.05$)

Table 3. *Survival and rate of deformity of progeny of C. gariepinus brood fish induced to breed with Ovaprim and pituitary gland extract.*

	Pituitary gland	Ovaprim
% of deformed fry	0.9 ±0.50^a	0.1 ±0.28^b
(%)Mean fry survival	77.7 ±1.33^b	81.9 ±1.10^a
Mean fry weight (g)	0.09 ± 0.023^b	0.12 ±0.023^a

$^{a-b}$Values with the same superscript are not significantly different ($p<0.05$)

Table 4a. *Comparative cost of Ovaprim and PGE required for inducing spawning in C. gariepinus.*

Parameter	Total Quantity	Mean cost (in naira)
Ovaprim	1.74ml	214.8±171.84
Pituitary	19mg	204.48±163.58

Table 4b. *Comparative cost benefit analysis of Ovaprim and PGE for inducing breeding in C. gariepinus.*

	Mean egg wt (g)	Total cost (naira) per gram
Pituitary gland extract	257.0±41.12	19.95±3.19
Ovaprim	334.0±53.44	15.9±2.54

4. Discussion

Prolonged latency period has indirect implication on the quantity and quality of eggs produced as well as the quality of the fry. Thus, the shorter the latency period, the better the reproductive output. The latency period recorded in this experiment is similar to De Leeuw *et al.* (1985), who reported a latency period of 12:3hrs when *C. gariepinus* was injected with Gly^{10}(D-Ala^6) LHRH – ethylamide and pimozide. Richter *et al.* (1987) recorded 16hrs latency period, using the same treatment as De Leeuw *et al.* (1985) and Kouril *et al.* (1992). Tan-Fermin and Emata (1993) recorded a latency period of 12 to 16 hrs when *C. gariepinus* and *C. macrocephalus* were induced to breed using pituitary and Ovaprim. Mohammed *et al.* (2000) recorded a latency period of 26hrs for fish injected with 3000 IU HCG. Francis (1992) also recorded 26hrs latency period for *Heterobranchus fossilis* and *C. batrachus.* The difference in the latency period is generally attributable to variable potency of the different hormonal materials used as well as the responses of the different fish species and temperature. Prolonged time of spawning is a drawback of using certain substance in induced breeding (De Leeuw *et al.*, 1985; Kouril *et al.*, 1992; Bruzuska, 2000). The shorter latency period recorded in Ovaprim treated fish than those treated with pituitary gland extract is a reflection of the superiority of the former in induction of breeding of *C. gariepinus*.

Workers fecundity is an important index in determining the reproductive capacity of fish that are undergoing artificial spawning and is, therefore, a measure of the efficiency of the inducing agent. From the values recorded for this index, it is obvious that Ovaprim is more effective in induction of ovulation in *C. gariepinus.* The hatching time recorded in this study was similar to that of Haniffa *et al*. (2000) who recorded hatching duration of 39 – 43 hours for *C. gariepinus* treated with pituitary gland, 23 hours for Ovaprim induced eggs and 36 – 38 hours for eggs induced with HCG at a mean temperature of 25± 0.35^{0}C. The hatching duration recorded in this study was a function of combined effect of temperature and the potency of the hormonal agent. The percentage hatchability is analogous to the data reported by Olubiyi *et al*. (2005) on *H. longifilis*, induced with Ovaprim. Nwadukwe (1993) recorded 63% mean hatchability in *H. longifilis* induced with pituitary gland. Similarly, Ude *et al*. (2005) reported 67% hatching success for *C. gariepinus* induced with LHRHa. Hatching success is also hormonal dose dependent. Haniffa and Sridhar (2002) recorded different hatching rates at variable hormonal doses. The hatching rate was 50.5% for eggs from brooders injected with 0.3ml/kg Ovaprim, while hatching rate was increased to 60% when 0.5ml/kg was administered.

Fry survival rate depends on several factors such as feed availability, pH, temperature, dissolved oxygen, ammonia, nitrite, nitrate, etc (Ajah, 2007). In addition, type of hormonal agent also determines fry survival rate as recorded in this study. Nwokoye (1985) reported 75 – 80% fry survival rate following pituitary gland induced breeding in *C. gariepinus*. In this study, Ovaprim showed superior fry survival rate over PGE.

Spawners induced with Ovaprim were characterized by aggressive swimming behaviour. The persistent intense swimming activity is energy sapping, which aggravated post stripping weakness in the Ovaprim treated individuals, and may have contributed to their mortality. However, Ovaprim proved to be more cost effective than pituitary gland. Nwokoye *et al*. (2007) reported similar production cost effectiveness in *H. bidorsalis* when induced with Ovaprim. It is inferred that both Ovaprim and pituitary can be used effectively for artificial breeding of *C. gariepinus*. However, Ovaprim has more comparative advantages in terms of reproductive outputs and fry quality. Notwithstanding the above merits, absolute mortality suffered by Ovaprim treated spent fish is indicative of substance toxicity, which may have food safety implications. Also loss of broodfish in Ovaprim

treated lot has cost implication, which to some extent could offset the comparative advantage the synthetic hormone has over PGE. Future studies should be directed at validating the toxicity of Ovaprim.

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