



GLOBAL JOURNAL OF SCIENCE FRONTIER RESEARCH
AGRICULTURE AND VETERINARY SCIENCES
Volume 12 Issue 8 Version 1.0 Year 2012
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

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Abstract - A reputable fish farm in Abeokuta, Ogun State, Nigeria were used to carry out studies on the induced breeding of *Clarias gariepinus* under varying brood stock ratios. Six gravid females weighing 1.00 kg each and 6 reproductively matured males weighing 1.00 kg were used for the three induced breeding trials in ratios of 2:1, 1:2 and 2:3, female/male respectively. Females were injected at a dosage of 0.50 ml ovaprim/kg body weight and 0.25 ml/kg body weight for males. Hatching starts after 24 hours of incubation and lasted for 6 hours. Dissolved oxygen, pH, ammonium ion, nitrate ion levels and temperature were monitored. Mean weight of eggs produced is 285.00 g \pm 65.00 with a relative percentage weight of eggs to body weight of 26 to 33%. Also, fecundity was the same in all the treatments with a value of 66,000+100 eggs. The lowest pseudo-gonadosomatic index of 41.50+6.50 was recorded in treatment 1, while the highest value of 51.00+3.00 was recorded in treatment 2. The effect of the varying brood stock (female:male) ratios in all the trials were not significantly different at ($P < 0.05$) as indicated by the number of fertilized eggs, number of hatched eggs, % larval production and survival.

Keywords : *Brood stock, Clarias, fertilization, induced breeding.*

GJSFR-D Classification : *FOR Code: 070201*



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Induced Breeding of African Catfish (*Clarias gariepinus*) Under Varying Brood Stock Ratios

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Abstract - A reputable fish farm in Abeokuta, Ogun State, Nigeria were used to carry out studies on the induced breeding of *Clarias gariepinus* under varying brood stock ratios. Six gravid females weighing 1.00 kg each and 6 reproductively matured males weighing 1.00 kg were used for the three induced breeding trials in ratios of 2:1, 1:2 and 2:3, female/male respectively. Females were injected at a dosage of 0.50 ml ovaprim/kg body weight and 0.25 ml/kg body weight for males. Hatching starts after 24 hours of incubation and lasted for 6 hours. Dissolved oxygen, pH, ammonium ion, nitrate ion levels and temperature were monitored. Mean weight of eggs produced is 285.00 g ± 65.00 with a relative percentage weight of eggs to body weight of 26 to 33%. Also, fecundity was the same in all the treatments with a value of 66,000±100 eggs. The lowest pseudo-gonadosomatic index of 41.50±6.50 was recorded in treatment 1, while the highest value of 51.00±3.00 was recorded in treatment 2. The effect of the varying brood stock (female:male) ratios in all the trials were not significantly different at (P<0.05) as indicated by the number of fertilized eggs, number of hatched eggs, % larval production and survival.

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I. INTRODUCTION

Over the past decades, aquaculture has grown in leaps and bounds in response to an increasing demand for fish as a source of protein globally (Akinrotimi *et al.*, 2007a). The single most important drawback of large-scale commercial culture of several fish species is the deficiency of quality seed of uniform size and free of diseases, parasites, and pests at the time of stocking in culture ponds (Marimuthu *et al.*, 2009). Odedeyi (2007) noted that the largest mature *C. lazera* (*gariepinus*) would usually give the best spawn weight in induced breeding, but there is no literature available as to whether the fish with the best spawn would equally give the best fry survival and best growth performance. A major pre-requisite for successful fish farming enterprise is a reliable and consistent source of fish seeds (fingerlings) of the commercially important species (Nwuba and Aguigwo, 2002). The surest and

most reliable source of supply is to produce the fingerlings under a controlled system, usually in a hatchery as earlier emphasized by Ezechi and Nwuba (2007). The objective of this study was to determine the most appropriate male-female ratio of catfish (*Clarias gariepinus*) brood stock for induced breeding.

II. MATERIALS AND METHODS

a) Experimental site

The hatchery facilities of a reputable private fish farm known as 'Aqua Consult Ltd' situated in Abeokuta, the Ogun State capital in Nigeria was used for the study. The hatchery has an indoor flow through system with ten holding concrete tanks (six 2 m × 6 m and four 2 m × 3 m) equipped with 101.60 mm diameter inlet and outer polyvinyl chloride (PVC) pipes. The concrete incubation tank measured (2 m × 3 m × 1 m) also with inlet and outlet PVC pipes, respectively.

b) Catfish brood stock for the trial and hormone injection

Gravid female and matured male were obtained from the brood stock pond of the farm mentioned above. A total of 6 females and 6 males were used for the three hatching trials in each tank in the ratio of female: males 2:1, 2:2 and 2:3 (weight in kg). Oxygen in each tank was maintained above 6.00 mg/l and temperature ranged from 25.70 to 27.00°C. All the fish were weighed and starved for 24 hours before ovaprim was administered using 2.00 ml capacity syringe with 1^{1/4}" needle for injecting the fish. The needle was inserted 2.00 to 2.50 cm intramuscularly at an angle between anterior part of the dorsal fin towards the direction of the tail. The females were injected at a dosage of 0.50 ml/kg body weight and 0.25 ml/kg body weight for males.

For the first trial, the eggs from the two females were stripped after a latency period of 11 hours into a bowl and the total egg weight was weighed using a weighing balance. The male was sacrificed and the testes collected and pierced with a needle and the milt squeezed on the eggs contained in the bowl and thoroughly mixed dry for a minute with a plastic spoon. Clean sterilized water was added and the eggs mixed for another two minutes. The fertilized eggs contained in bowl were incubated by spreading them on submerged

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Hapa netting in a prepared incubation tank. This procedure was repeated for the second and third trials respectively.

Fecundity was estimated according to Hogendon (1977) and Haylor and Oyegunwa (1993) by the equation:

$$Total\ No\ of\ Eggs = 66.60 [Female\ body\ weight\ (g)] \text{-----} Eq.1$$

The pseudo-gonadosomatic index (PSI) also taken as sensitivity to ovaprim was calculated with the relation:

$$PGSI = \frac{100(Weight\ of\ eggs\ collected\ by\ stripping)}{Body\ weight\ before\ injection - Weight\ of\ eggs\ stripped} \text{-----} Eq.2$$

After 24 hours of incubation, hatching started and lasted for 6.00 hours. During this time, water parameters such as, temperature, pH and dissolved oxygen concentration in the incubation tank were assessed. Percentage larval production, larval survival and mortality were calculated as follows:

$$\% Larval\ production = \frac{100(Number\ of\ eggs)}{Number\ of\ fertilized\ eggs} \text{-----} Eq.3$$

$$\% Larval\ survival = \frac{100(Number\ of\ normal\ larval)}{Number\ of\ egg\ hatched} \text{-----} Eq.4$$

$$\% Mortality = \frac{100(Number\ of\ deformed\ larvae)}{Number\ of\ egg\ hatched} \text{-----} Eq.5$$

After the third day of hatching, the larvae have absorbed their yolk sacs and they were distributed into the prepared twelve compartments in the outdoor nursery tanks of 2.80 m × 1.70 m each and were fed with *copens* fish feed.

c) Statistical analysis

Data collected were subjected to statistical analysis using one-way analysis of variance (ANOVA) test and the differences were tested for significance (P<0.05) using Duncan’s Multiple Range Test (Duncan, 1955).

III. RESULTS

a) Latency period, fecundity and pseudo-gonadosomatic index

The dosages of ovaprim administered were 0.50 ml for females of 1000 g each and 0.25 ml for males weighing 1000 g each. Latency period for the females was from 11 to 11 hrs, 20 mins. (Average 11.10 h) (Table 1). Weight of eggs was highest in treatment II and lowest in treatment I with a relative percentage weight of egg to body weight of 33 and 26% respectively.

The pseudo-gonadosomatic index (PSGI) ranged from 35% to 49%. This also showed a direct relationship with mean weight of eggs of fish. Treatment II had the highest PGSI of 49% followed by treatment III (46%) while treatment I had the least PGSI of 35%.

Table 1: Latency period and pseudo-gonadosomatic index of *C. gariepinus* under varying female: male brood stock ratios

| Parameter | Treatment I | Treatment II | Treatment III |
|-----------------------------------|-------------|--------------|---------------|
| Brood stock ratio (F:M) | 2:1 | 2:2 | 2:3 |
| Mean body weight (g) | 1000.00 | 1000.00 | 1000.00 |
| Mean dosage of ovaprim (ml/kg BW) | 0.50 | 0.50 | 0.50 |

| | | | |
|-----------------------------|------------|------------|------------|
| Mean latency period (h:min) | 11:00±0:06 | 11:10±0:07 | 11:15±0:03 |
| Mean weight of eggs (g) | 260.00 | 330.00 | 317.00 |
| % WE/BW | 26.00 | 33.00 | 32.00 |
| Mean PGSI (%) | 41.50±6.50 | 51.00±3.00 | 46.50±3.50 |

b) Hatchability rate and larval survival

The milt from the male fish was milky and sticky in nature. The fertilized eggs were transparently greenish brown in colour, while the unfertilized ones were whitish in colour. Incubation was for 24 hours after which hatching started and lasted for six hours. Treatment I with ratio of female to male of 2:1 had a total fecundity of 117,882 eggs, mean fertilized eggs, 41,259±1631.50, mean hatchability, 58% and with mean percentage larval survival of 73%.

Treatment II with ratio of female to male of 2:2 had a total fecundity of 117,882 eggs; mean fertilized eggs of 45,455±1165.50 eggs; mean hatchability of 63% and mean percentage larval survival of 67% (Tables 1 and 2).

Treatment III with female: male of 2:3 had a total fecundity of 117,882 eggs, mean fertilized eggs of 46,600±0.00 mean hatchability of 56% and with percentage larval survival of 6.7% (Tables 1 and 2).

Table 2 : Mean values of percentage larval production and survival in ovaprim induced *C. gariepinus* under varying brood stock (female: male) ratios

| Parameter | Treatment (female: male) | | |
|------------------------------|-----------------------------|-----------------------------|----------------------------|
| | I (2:1) | II (2:2) | III (2:3) |
| No of stripped eggs | 58,941±2330.99 ^a | 64,935±1664.99 ^a | 66,600±0.00 ^a |
| No of fertilized eggs | 41,259±1631.50 ^a | 45,455±1165.50 ^a | 46,600±0.00 ^a |
| No of hatched eggs | 23,964±5497.50 ^a | 28,734±1272.50 ^a | 25,931±410.00 ^a |
| Percentage larval production | 58.0±10.49 ^a | 63.2±1.19 ^a | 55.6±0.89 ^a |
| No of deformed larvae | 66,94±2730.49 ^a | 96,46±565.49 ^b | 86,91±121.49 ^b |
| No of Normal larvae | 14,270±2666.99 ^a | 19,088±706.99 ^b | 17,239±288.49 ^b |
| Percentage larval survival | 73±5.500 ^a | 66±0.000 ^a | 66±0.500 ^a |

abc mean values in each row having the same superscripts are not significantly different at P>0.05.

c) Physico-chemical parameters

The mean temperature recorded in the tank during the trial was 28°C. The pH value was 7.20;

dissolved oxygen concentration 6.40 mg; NH₄ 0.50 mg/l and 0.05 mg/l for NO₂ (Table3).

Table 3 : Mean values quality parameters monitored during the experiment

| Parameter | T1 (2:1) | T2 (2:2) | T3 (2:3) |
|--------------------------------|----------|----------|----------|
| Temperature (°C) | 28.00 | 28.00 | 28.00 |
| pH value | 7.20 | 7.20 | 7.20 |
| DO ₂ (mg/l) | 6.40 | 6.40 | 6.40 |
| NH ₄ (mg/l) | 0.50 | 0.50 | 0.50 |
| NO ₂ (mg/l) | 0.05 | 0.05 | 0.05 |
| Turbidity (NYU) | 5.00 | 5.00 | 5.00 |
| Total solids (mg/l) | 257.00 | 257.00 | 260.00 |
| Acidity (mg/l) | 0.10 | 0.10 | 0.10 |
| Total hardness (ppm) | 60.00 | 60.00 | 60.00 |
| Ca ²⁺ (ppm) | 42.00 | 43.00 | 50.00 |
| Mg ²⁺ (ppm) | 18.00 | 17.00 | 12.00 |
| Chloride (ppm) | 33.00 | 33.00 | 33.00 |
| Co ₂ residual (ppm) | Nil | Nil | Nil |
| Bacterial counts/100 ml | TNC | TNC | TNC |
| Coliform counts (mpn) | Nil | Nil | Nil |

IV. DISCUSSION

The induction of ovulation and spawning in the African catfish *C. gariepinus* using ovaprim injection was effective on a single intramuscular injection of 0.50 ml for female brood fish 1000 g each and 0.25 ml for males weighing 1000 g each. The maximum latency period of 11 hrs, 20 mins recorded in this study could be ovaprim dosage-dependent as was observed for mammalian gonadotropin, methyltestosterone and partially purified Salmon gonadotropin in grey mullet *Mugil cephalus* (Shehadeh *et al.*, 1973) and for HCG and LH. RH-A in Mudskipper, *Boleosphthalamus pectinirostris* (Zhang *et al.*, 1989) or it could be due to the physiological make up of the fish (Haylor, 1993).

The pseudo-gonadosomatic index also used as index of sensitivity to ovaprim reached up to 49%, indicative of the fact that a high number of eggs could be collected when fish is induced with ovaprim. According to Richter *et al.* (1985), this is also an indication that ovaries of the fishes used in the experiment have reached the postvitellogenic stage. Also it has been observed that activity of dosage administered actually defined on the readiness of the females, their age, size, sensitivity amongst other factors (Woyhavorish and Horvath, 1980).

The temperature range of 25.70 to 27.00°C (mean 26.40°C) recorded throughout the experiment was higher than 22°C which Viveen *et al.* (1986) observed for *C. gariepinus* that exhibited latency period in excess of 15 hours. Zonneld *et al.* (1988) obtained their best results at 25°C. The pH of 7.00 to 8.00 was within normal range for culture fishes (Viveen *et al.*, 1986). Woynovorich and Horvath (1980) stated that a number of environmental factors such as temperature, pH, dissolved oxygen and calmness to play decisive role in ovulation and that temperature is of vital importance.

The incubation time of 24 hrs in the present study was higher than that recorded by Viveen *et al.* (1986) using carp pituitary suspension (CPS). Pillay (1993) reported that at 22°C hatching of fertilized eggs extends incubation time. In the present study the yolk sac was absorbed within three days as reported in Viveen *et al.* (1986) but differed from the six days reported by Pillay (1993). The temperature of the incubation tanks could be responsible for the differences. The high larval production (58%, 63.2% and 55.6%) in the three treatments out of which 73%, 66% and 66% survived respectively, indicates an overall good egg quality and effectiveness of ovaprim, including ovulation and spawning in the African catfish. There was no significant difference ($P < 0.05$) among number of fertilized eggs, number of hatched eggs, % larval production and survival in all three treatments.

V. CONCLUSION

This study was conducted at the peak of breeding period of the species, hence the possibility of the experimental fish having attained the resting phase were able to positively respond to the single knockout dose of ovaprim administered. Ovaprim dosage varied with sex of the fish. However, the response in the male could not be ascertained because the sperm could only be collected by sacrificing the males. In the female, it was easier to follow the response to ovaprim since eggs were examined after lapse of the minimum prescribed latency period of 10 hrs. The maximum latency period of 11 hrs, 20 mins recorded in this study could be ovaprim-dosage dependent or due to the physiological make up of the fish.

From this study, it was observed that the fecundity value (66,600 eggs per unit body weight) of the female brood stocks were constant because they have the same weight. But weight of stripped eggs per female fish per treatment varied despite the same body weight.

High number of eggs could be collected when fish is induced with ovaprim, this was indicated by the 54% obtained as the pseudo-gonadosomatic index value, which was used as an index of sensitivity to ovaprim. It was an indication that the ovaries of the fishes used in the experiment had reached the postvitellogenic stage. Also, the height sensitivity was recorded in the biggest fish with the highest percentage body weight of eggs.

It was confirmed from this study that the standard ratio of 2:1 of male to female in fish breeding was not significant, hence a lower number of male brooder can be used to get the same result as this enhances the prudent use of male brood stock as indicated by treatment I (2:1) with the least number of male and highest percentage larval survival of 73%.

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