

Full Length Research Paper

Induced propagation of African clariid catfish, *Heterobranchus bidorsalis* (Geoffrey Saint Hillarie, 1809) using synthetic and homoplastic hormones

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Induced spawning of African giant catfish (*Heterobranchus bidorsalis*) was successfully carried out using synthetic hormone (Ovaprim) and natural hormone (homoplastic hormone-pituitary extract from *H. bidorsalis*). The study which was carried out at Aquafish Farm, Ihiala, Anambra State, Nigeria, lasted 70 days (May to July). Sixty gravid females and twenty mature males of *H. bidorsalis* (weight range of 310 to 550 g) were used for the study. In all, 10 trials were carried out with a control. The results showed that ovaprim performed significantly better ($P < 0.01$) in almost all the parameters investigated. The two hormonal materials gave slightly different results in terms of pre and post hormonal induced spawning mean somatic weight loss of 423.83 ± 14.19 g and 446.00 ± 13.37 g, mean number of dead eggs of 396.10 ± 19.15 and 194.90 ± 11.00 , hatchability of $9,180.13 \pm 343.37$ and $11,162.27 \pm 362.00$ hatched larvae, 35.80 ± 1.11 and 12.37 ± 1.54 deformed larvae, and 99.61 and 99.88% survival were recorded respectively for homoplastic hormone and ovaprim, respectively. Comparative cost benefit analysis showed that ovaprim which recorded better results, was also relatively cheaper. Ovaprim worth ₦3467.00 was used for induced breeding of *H. bidorsalis* with combined body weight of 13.38 kg while pituitary hormone was extracted from ₦6350.00 worth of *H. bidorsalis* and used for induced breeding of gravid *H. bidorsalis* with combined body weight of 12.72 kg. Because of its relatively cheap cost, ease of handling and better survival of hatchlings from *H. bidorsalis*, ovaprim is highly recommended for hatchery users.

Key words: Homoplastic hormone, Ovaprim induced spawning, hatchability, *Heterobranchus bidorsalis*.

INTRODUCTION

The high demand for fish fingerlings in the phenomenal growing aquaculture industry has stimulated the need for artificial propagation of cultural warm water fishes. Statistics of global fish production shows that fish farming represents about 15% of the global fish yields and was expected to exceed 20% by the year 2000. FAO (1995) noted that inland capture fisheries yields had continuously increased from 6.5 million tons recorded in 1984 up to 1989. Since 1990, catches appeared to have stabilized or even declined slightly. Considering both inland and marine capture fisheries, in 1989 world fish production reached 100.3 million tons. Production increased slightly

from 1992 to 101.3 million tons in 1993 (FAO, 1995).

The increase in total production between 1992 and 1993 came entirely from aquaculture. In Nigeria, capture fisheries and aquaculture play leading roles in fish production, contributing an average of 84.2% of the total domestic output between 1990 and 1994 (CBN, 1994). According to Gaffar (1996), out of the approximately 650,000 metric tons annual fish output in Nigeria, 350,000 metric tons was locally produced with inland water and aquaculture accounting for 110,000 metric tons and 18,000 metric tons, respectively. Ogbe and Odiba (1996) reported that between 1990 and 1994, Nigeria's fish output experienced a negative growth (-0.06%) averaging 298.8 thousand metric tons per annum against annual demand of 1.5 million metric tons.

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fish consumption levels of 13.0 kg per year, 91 million tons of food fish would be required (Olubusin, 1996). Such increase in the production of food fish was considered feasible if aquaculture production could be doubled in the next 16 years. Olubusin (1996) noted that the only means of meeting up with the projected fish demand in the country was through a pragmatic option of intensive fish farming. Rearing culturable fish species under controlled environment has proved to be a successful method of enhancing fish supply. Tobor (1996) estimated that Nigeria has the potential to produce up to 1.5 million metric tons of fish through aquaculture.

A natural diagnostic survey of water resources of Nigeria carried out in 1983, revealed the existence of about 200 ha of ponds under construction at the time of the survey and a total of about 2700 ha proposed for execution (Ita et al., 1985). In 1994, a national survey of aquaculture development in Nigeria was conducted by the Nigeria Institute of Fresh water Fisheries Research (NIFFR), New Bussa. The survey revealed that there has been general awareness of profitability in fish farming in the last decade compared with the situation in the previous three decades. It was noted that 80% of all the existing fish farms in every state of Nigeria were developed within the last decade (Ita, 1996). The survey also showed that out of 80% of fish hatcheries identified in different parts of the country, 48% was government owned. FAO (1990) reported the existence of 2000 earthen ponds, 3000 concrete ponds and 36 ha of commercial fish farms in Nigeria.

It has been noted that fish farming is hardly imaginable without availability of fish seed (Chondar, 1980). It is an established fact that inadequate supply of quality and fast growing fish seed was a major constrain of fish farming in Nigeria. Based on a 1992 United Nations Development Project (UNDP) assisted base line study (Fish Network, 1994) the total annual fingerlings requirement for Nigeria was 250,000 million while the domestic production stood at 7.2 million.

Among the culturable food fish in Nigeria, catfish is the most sought after fish species, very popular with fish farmers and consumers and commands a very good commercial value in Nigerian markets (Ezenwaji, 1985; Oladosu et al., 1993; Ayinla et al., 1994;). The catfish is very important to the sustainability of the aquaculture industry in the country.

However, inspite of the break through reported for its artificial propagation (Richter and Van der Hurk, 1982; Madu et al., 1987; Madu et al., 1989), the demand for fish seed still outstrips the supply. Richter and Van der Hurk (1982) reported that the problem of inadequate supply of fish seed can only be solved through induced breeding by the application of various inducement materials. Various types of fishes have been induced to spawn, using various hormonal materials (Nwadukwe, 1993; Eyo, 1997, 1998, 2000; Nwuba and Aguigwo, 2002). Some of these hormonal materials (natural and synthetic) include cHCG

(Eyo, 1997; 1998), HCG (Eyo, 2002); clomipogene citrate (Aguigwo, 1991), pituitary extract (Janssen, 1985; Haniffa et al., 2000) and Ovaprim (Manosroi et al., 2004; Abol-Munafi et al., 2006).

Our present study which compared the effect of synthetic (ovaprim) and natural (homoplastic) hormones in induced breeding of African giant catfish, *H. bidorsalis* had the following specific objectives thus: to compare the level of ovulation inducement of the two hormonal materials, establishing of their spawning efficacy, determining the percentage hatchability of the fertilized eggs, and establishing their cost benefits.

MATERIALS AND METHODS

The study area

This study was carried out between May and July 2002 using fish hatchery facilities at the Aquafish Farm, Ihudim, Ihalala, Anambra State, Nigeria. The farm which covers 5 hectares of land has the following facilities; indoor hatchery complex with 10 incubation tanks (1 x 1.5 x 1 m³ each), 20 earthen nursery ponds (10 x 15 x 1.2 m³ each), three brood stock ponds (20 x 10 x 1.2 m³ each), seven production ponds (30 x 80 x 1.5 m³ each), and a bore hole and 5000 gallon concrete reservoir. Furthermore, a perennial river, Ulasi, is located about 0.3 km from the farm.

Broodfish management

Hatchery raised 18 months gravid broodstocks were selected. All broodfish were selected by external morphological characteristics, using the method of Ayinla et al. (1994). Female fish were selected on the basis of ovarian biopsy of Legendre (1986). Sixty females and 20 males catfish (weight range of 310 - 550 g) were selected. The broodfish were kept separate from males in ear-then pond (10 x 15 x 1.2 m³). They were fed aquafish pelleted fish feed (35% crude protein) twice daily (7 am and 5 pm) on 5% of total fish biomass, 7 days of the week. The broodstocks were acclimated in their new environment (10 x 15 x 1.2 m³ earthen pond) for 15 days at mean temperature of 28 ± 2°C and normal photoperiodic regimes (12 h light and 12 h darkness). The water pH and alkalinity were between 7.0 – 7.1 and 111.11 – 113.44 mg/l, respectively.

Experimental design and artificial spawning

Three treatments in triplicate with 3 fish per replicate were used. Two hormonal materials (ovaprim and homoplastic hormones) were used. Control fish were administered 1 ml of 0.6% saline solution. Ten artificial spawning trials were carried out using 60 gravid females and 20 mature males of *H. bidorsalis*. The study lasted 70 days. Prior to each trial, pituitary gland was extracted from mature *H. bidorsalis* using the methods of Viveen et al. (1985). Each gland was then transferred into a sealed test tube containing acetone. The acetone was decanted after 8h and then refilled with acetone. This was kept in a cool place for 24 h after which it was finally decanted dried and stored pending use.

Hormone Injection

Prior to hormone injection, vitellogenic females were randomly

Table 1. Effect of hormonal treatment on the weight before and after spawning of *H. bidorsalis*.

| Hormone | Mean wt. before spawning | Mean wt. after spawning | Mean wt. loss (g) | % weight loss | T-value | P-value |
|---------------------|--------------------------|-------------------------|-------------------|---------------|---------|---------|
| Homoplastic hormone | 423.83 ± 14.19 | 410.42 ± 13.92 | 13.09 | 3.16 | 0.67 | 0.25 |
| Ovaprim | 446.00 ± 13.37 | 430.80 ± 12.91 | 15.23 | 3.41 | 0.82 | 0.21 |

seined out from the ponds and kept singly in aerated 50 litres aquarium with 25 litres of aerated water for 12 h. The injection of hormonal materials was done between 6 and 7 pm during each trial. During each experiment for homoplastic hormone, the weighed and stored acetone dried pituitary gland (donor fish has equal weight with recipient fish) was macerated in a porcelain mortar with a known volume (1 ml 1 kg body wt. of fish) of 0.6% saline solution. The pituitary suspension was drawn with 5 ml hypodermic syringe with 0.6 mm gauge needle. The weighed fish was then covered with towel and injected intramuscularly above the lateral line towards the dorsal section and pointed towards the ventral side. After withdrawal of the needle, the fish was finger-rubbed to avoid back flow of the injected fluid. Ovaprim which is in liquid form was administered at 0.5 ml/kg body weight of female fish (Legendre, 1986; Haniiffa and Sridhar, 2002). The control fish was injected 5 ml of 0.6% saline solution. The injected fish were returned separately into their respective 50 litre aquaria.

Stripping, fertilization and Incubation

Stripping took place 10 h after injection at a mean temperature of $28 \pm 2^\circ\text{C}$. This was carried out by holding the fish at the head and tail by an assistant. The ovulated eggs oozed out on slight pressure by thumb onto the plastic bowl. Incisions were then made on the sperm sac which was collected minutes prior to stripping by sacrificing the mature male. Milt was squeezed over the eggs. The two sex products were then mixed with plastic spoon. To this 0.6% Saline solution was added and further agitated. Spermatozoa from one mature male were used to fertilize eggs stripped from three females. The process from tripping to fertilization took three minutes to accomplish.

Incubation of the fertilized eggs was carried out in $1 \times 1.5 \times 1 \text{ m}^3$ concrete tank that was partitioned into three equal compartments. It was equipped with water flow-through facilities. Nylon mesh (1 mm) was suspended above the floor for spreading of the fertilized eggs. The eggs were spread in single layers on the suspended nylon meshed net for incubation. Water parameters were monitored. Temperature was measured with centigrade thermometer, pH was monitored using Hanna Hep pH meter and optimum oxygen level was maintained with RESUN LP- 100 low noise air - pump.

A sample of 200 eggs was taken from each of the treatments at random and incubated in aerated aquaria ($36 \times 24 \times 18 \text{ cm}^3$). Dead eggs were removed after 10 h (Nwudukwe; 1993), while percentage hatchability and larvae deformity were calculated (Haniiffa and Sridhar, 2002). The number of eggs released was calculated following the gravimetric method (Lagler, 1982; Legendre, 1986).

The nylon meshed net was removed with the egg shells while the hatched larvae clustered at dark corners of the incubation tank. Three days after hatching, post-yolk fry was fed to satiation with incubated *Artemia nauplii*. Percentage survival was calculated at the end of 5 days.

Data analysis

The data collected for the 10 trials were pooled together and analyzed for their central tendencies using descriptive statistics. Analysis of variance with F-LSD post hoc test was used to separate differ-

ences in treatment means. Multiple regression and co-relation statistics was used to establish linear relationships between variables. All analysis was carried out using Microsoft Excel 2006 and the output is presented in tables.

RESULTS

Effects of hormonal treatment on pre and post spawning body weight of *H. bidorsalis*:

The effects of the two hormonal materials on the weight of female *H. bidorsalis* gravid spawners are presented in Table 1. The pre-spawning weights were 423.83 ± 14.19 g for spawners injected with homoplastic hormone and 446.00 ± 13.37 g for gravid *H. bidorsalis* injected with ovaprim. There was non-significant difference ($P < 0.05$) in weight of female fishes before hormonal treatments. Similarly, all gravid *H. bidorsalis* recorded no significant loss in weight after spawning. The non-significant weight differences were 410.42 ± 13.92 g for homoplastic hormone and 430.80 ± 12.91 g for females injected with ovaprim. The t-values were not significantly different for all the hormonal treatments.

Also, the percentage weight loss arising from spawning as induced by hormonal treatments was 3.16% for fishes injected with homoplastic hormone and 3.41% for fishes injected with ovaprim. Ovaprim injected spawners had a higher percentage weight loss, although the result was not significantly different ($P > 0.05$) from those injected homoplastic hormone.

Effects of hormonal treatment on number of fertilized eggs

Gravid female *H. bidorsalis* injected with ovaprim had the highest mean number of eggs ($11,349.23 \pm 364.59$ eggs) while female fishes injected with homoplastic hormone recorded (9522.77 ± 348.13 eggs) (Table 2). Similarly the percentage fertilization was highest for ovaprim (98.31%), while female fishes injected with homoplastic hormone recorded (96.01%). The analysis of variance test of the number of fertilized eggs indicated significant difference ($p < 0.001$) for all the treatments.

Effects of hormonal treatment on number of dead eggs after fertilization

Female *H. bidorsalis* injected with ovaprim recorded the

Table 2. Effect of hormonal treatment on the number of fertilized eggs of *H. bidorsalis*.

| Hormone | Number of eggs spawned | Mean number of eggs fertilized | % fertilization | F-value | P-value |
|---------------------|------------------------|--------------------------------|-----------------|---------|---------|
| Homoplastic hormone | 9918.83 | 9,522.77 ± 348.13 | 96.01 | 13.18 | 0.001 |
| Ovaprim | 11,544.13 | 11,349.23 ± 364.59 | 98.31 | 13.18 | 0.001 |

Table 3. Effect of hormonal treatment on the number of dead eggs after fertilization in *H. bidorsalis*.

| Hormone | Mean number of dead eggs | % of dead eggs | F-value | P-value |
|---------------------|--------------------------|----------------|---------|---------|
| Homoplastic hormone | 396.10 ± 19.15 | 3.99 | 235.32 | 0.001 |
| Ovaprim | 194.90 ± 11.74 | 1.69 | 235.32 | 0.001 |

Table 4. Effect of hormonal treatment on hatchability of eggs at same environmental variables for *H. bidorsalis*.

| Hormone | Mean number of eggs fertilized | Mean number of eggs hatched | % Hatchability | F-value | P-value |
|---------------------|--------------------------------|-----------------------------|----------------|---------|---------|
| Homoplastic hormone | 9522.77±348.13 | 9180.13±343.37 | 96.40 | 15.86 | 0.001 |
| Ovaprim | 11,349.23±364.59 | 11,162.27±360.00 | 98.35 | 15.86 | 0.001 |

least mean number of dead eggs (194.90 ± 11.74 dead eggs) and those injected with homoplastic hormones recorded 9396.10 ± 19.15 dead eggs (Table 3). Furthermore, the percentage of dead eggs was lowest in ovaprim (1.69%) and 3.77% of dead eggs was recorded for gravid fishes injected with homoplastic hormone. The analysis of variance test of number of dead eggs indicated significant difference ($P < 0.001$) for the treatments.

Effects of hormonal treatment on hatchability of eggs

The effects of different hormonal treatments on the hatchability of gravid *H. bidorsalis* are presented in Table 4. The highest number of hatched eggs ($11,162.27 \pm 362.00$ larvae) was recorded for gravid *H. bidorsalis* injected with ovaprim. Female *H. bidorsalis* administered homoplastic hormone had 9180.13 ± 342.37 larvae. Similarly, percentage fertilization was 98.35% for ovaprim injected *H. bidorsalis* and 96.40% for those injected with homoplastic hormone. The analysis of variance test of eggs hatchability showed significant difference ($P < 0.001$) for all the treatments.

Effect of hormonal treatment on larval deformities

Records of deformed larvae arising from different hormonal materials injections are shown in Table 5. Gravid females of *H. bidorsalis* injected with ovaprim had the lowest mean number of deformed larva (12.37 ± 1.54 larvae), while females of *H. bidorsalis* injected with homoplastic hormone had 35.80 ± 1.11 deformed larvae. Furthermore, ovaprim injected *H. bidorsalis*, recorded very low percentage larval deformity (0.12%) while homoplastic injected *H. bidorsalis* had 0.39% larval deformity. Ana-

lysis of variance test for deformed larvae, showed significant difference ($P < 0.05$) for all the treatments.

Effect of hormonal treatment on percentage survival

Gravid female *H. bidorsalis* injected with ovaprim recorded the best percentage survival (99.88%), while those injected with homoplastic hormone recorded 99.61% survival (Table 6).

Costs benefit of hormonal treatment

Table 7 shows the comparative cost of the hormonal materials used. Gravid female of *H. bidorsalis* which weighed a total of 13.38 kg was injected with ovaprim worth N3,467 while female *H. bidorsalis* fish that weighed 12.72 kg was injected with homoplastic hormone extracted from fish worth N6,350.00.

DISCUSSION

Our results on Table 1 indicated non-significant difference ($P > 0.05$) in weight of female spawners before hormonal treatment and after spawning. The non-significant weights were 410.42 ± 13.92 g and 430.80 ± 12.91 g for females injected with homoplastic hormone and ovaprim respectively. The non-significant differences may be attributed to the fact that the ovarian weight is usually a negligible fraction of the somatic (body) weight. de Graaf et al. (1995) reported similar finding for *Clarias gariepinus* breed, using artificial propagation techniques. Delince et al. (1987) reported that spent ovary of *C. gariepinus* represented about 10–20% of its initial weight. Viveen et al. (1985) reported about 700 eggs per gram in *C. gariepi-*

Table 5. Effect of hormonal treatment on larval deformities of *H. bidorsalis* under similar environment.

| Hormone | Mean number of eggs hatched | Mean number of deformed larvae | % deformity | F-value | P-value |
|---------------------|-----------------------------|--------------------------------|-------------|---------|---------|
| Homoplastic hormone | 9,180.13±343.37 | 35.80±1.11 | 0.39 | 86.63 | 0.001 |
| Ovaprim | 11,162.27±362.00 | 12.87±0.72 | 0.12 | 86.63 | 0.001 |

Table 6. Effect of hormonal treatment on percentage survival of *H. bidorsali*.

| Hormone | Mean number of larvae | Mean number of hatchlings | % survival |
|---------------------|-----------------------|---------------------------|------------|
| Homoplastic hormone | 9,180.13±343.37 | 9,144.33 | 99.61 |
| Ovaprim | 11,162.27±362.00 | 11,149.40 | 99.88 |

Table 7. Comparative costs of hormonal treatment in *H. bidorsalis*.

| Hormone | Total wt of fish (g) | Cost of hormone (₦) |
|---------------------|----------------------|---------------------|
| Homoplastic hormone | 12,715. | 6,350.00 |
| Ovaprim | 13,380 | 3467.00. |

nus and noted that the quantity of ovulated eggs was between 15 - 20% of its own body weight. Eyo and Mgbenka (1992) had earlier established linear relationship between fecundity, ovarian weight, length, GSI and somatic weight of *C. gariepinus*. This relationship is important in estimating fecundity from ovarian weight, length, GSI and somatic weight, hence facilities required for successful spawning trials.

In this study, spawners injected with ovaprim had the significantly higher number of fertilized eggs (11,349.23 ± 364.59 eggs). Fertilized eggs resulting from homoplastic hormone injection to female *H. bidorsalis* were (9, 522.77 ± 348.13 eggs) (Table 2). In a similar study using ovaprim to induce breed the catfish *H. fossilis*, Haniffa and Sridhar (2002) had fertilized egg output ranging from 6,336 ± 800 eggs for *H. fossilis* weighing 80 -105 g given 1000 IU ovaprim. The difference in egg output of Haniffa and Sridhar when compared to this study even when the same quantity of ovaprim was used may be due to differences in species and weight of spawners *H. bidorsalis* being more fecund than *H. fossilis*. In another study, Oladosu et al. (1993) induced breeding *H. bidorsalis* with carp pituitary recorded 743.66 ± 14.84 eggs. This present study reported more fertilized eggs output than Oladosu et al. (1993). It may be due to weight differences in spawners used.

This study showed that there was significant difference ($P < 0.05$) in the number of dead eggs recorded for female fish injected with the hormonal materials. The female fish injected ovaprim recorded low number of dead eggs (194.90 ± 11.74 dead eggs) while those injected homoplastic hormone recorded a higher number (396.10 ± 19.15) (Table 3). The percentage of dead eggs was (1.69%) for females injected with ovaprim and 3.99% for

females injected with homoplastic hormone. In another study, Nwudukwe (1993) induced breeding *H. longifilis* with frog hormone recorded higher dead percentage (29%). The lower percentage recorded in the present study may be attributed to the efficacy of the hormones.

Results on Table 4 indicated higher hatchability, (11,162.27 ± 362.00 or 98.35% larvae) of eggs for female *H. bidorsalis* injected with ovaprim while those injected with homoplastic hormone recorded slightly lower number of hatched larvae. (9180.13 ± 37 larvae or 96.40%). In a similar study, Haniffa and Sridhar (2002) using ovaprim (at a dose of 0.5 m/kg body weight) induced breeding of spotted murrel (*Channa punctatus*) reported fertilization percentage of 70%. In another study, Haniffa et al. (1998) reported a fertilization rate of 95 - 98% for *C. striatus* with ovaprim as the hormonal material. This result is similar to that reported in the present study.

In our study, spawners injected with ovaprim recorded low deformities (12.87 ± 0.72 larvae) when compared with those injected with homoplastic hormone (35.80 ± 1.11 larvae) (Table 5). Percentage deformity followed the same pattern - (0.12%) and (0.39%) respectively. In a similar study, Nwudukwe (1993) using frog pituitary extract to induce breed *H. longifilis* reported a survival rate of 66 - 90% for hatchlings after one week. The slight difference in these results when compared with those in the present study may be attributed to species of fish and hormonal material used.

Gravid female *H. bidorsalis* injected with ovaprim recorded the best percentage survival (99.88 %). In a similar study using ovaprim to induce breed, the catfish *H. fossilis*, Haniffa and Sridhar (2002) had better survival rate of juveniles compared to when other hormonal materials were used. Furthermore, Nwudukwe (1993) using frog pi-

tuitary extract to induce breed *H. longifilis* had lower survival rate of juveniles than what was recorded in our study. Generally, hormonal products have correlating effect on animal growth, as most hormones are either growth promoters or inhibitors, depending on dosage.

Table 7 showed the comparative costs of the hormonal materials used. Ovaprim which recorded better performance in all the parameters evaluated, cost less (N3467.00) for induced breeding of *H. bidorsalis* of total body weight 13.38 kg as against N6350.00 for induced breeding of *H. bidorsalis* total body weight 12.72 kg using homoplastic hormone. The overall better performance of ovaprim induced *H. bidorsalis* confirms earlier reports that GnRH analogues are more potent than natural hormones. Zohar (1995) reported that GnRH analogues are advantageous because they resist enzymatic degradation when injected into gravid fish resulting in a more prolonged stimulation of hormone released when compared to the native GnRH peptide.

Conclusion

From all the reproductive parameters investigated, ovaprim injected gravid *H. bidorsalis* had higher number of spawned and fertilized eggs, hatchlings, low number of deformed larvae, high survival rate and comparatively lower cost of administration, thus much beneficial over homoplastic hormones for induced breeding of the clariid catfish. Furthermore, the rigorous procedures involving sacrifice of the donor catfish are eliminated, although males are usually sacrificed for milt, a necessity for fertilization

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