STUDIES ON THE REPRODUCTIVE POTENTIAL OF HOMOPLASTIC AND HETEROPLASTIC PITUITARY HORMONES IN *Heterobranchus bidorsalis* (GEOFFROY SAINT HILAIRE, 1809)

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ABSTRACT

Artificial induce breeding of gravid Heterobranchus bidorsalis was carried out using two hormonal materials – homoplastic and heteroplastic hormones. The study which involved 10 trials was carried out with 60 gravid female and 20 mature male. The broodfish used for the study were 18 months hatchery produced H. bidorsalis. The hormonal treatments led to the following results in terms of percentage weight loss (3.16 and 3.06%); fertilization rate (9522.77 ± 348.13 and 8,857.93 ± 255.57); and hatchability (9,180.13 ± 343.37 and 8,476.83 ± 345.95) for homoplastic and heteroplastic hormones respectively. The mean numbers of dead eggs were 396.10 ± 19.15 for homoplastic hormone injected catfish and 425.53 ± 17.09 for those injected heteroplastic hormone. Recorded deformed of larva were low (35.80 ± 1.11 and 34.27 ± 1.43) respectively for catfish injected homoplastic and heteroplastic hormones. Survival of hatchlings was high (99.61 and 99.59 %) for gravid catfish injected homoplastic and heteroplastic hormones respectively. There was no significant difference (P < 0.05) in weight of pre and post female spawners. Although the two tested hormones investigated were effective inducers, homoplastic hormone is recommended as it recorded better results.

Keywords: Homoplastic and Heteroplastic hormones, Induced spawning, Hatchability, *Heterobranchus bidorsalis*

INTRODUCTION

Nigeria is a coastal state with lots of fisheries resources both marine and inland waters. About 12 million hectares and 500,000 hectares (Gaffar, 1996), was estimated to be suitable for aquacultural development in freshwater and marine environments. The growing aquaculture industry has led to the high demand for fish fingerlings. Capture and culture fisheries play major roles in fish production contributing an average of 84.2 % of the total domestic output between 1990 and 1994 (CBN, 1994). Gaffar (1996) reported that out of 650,000 metric tons of total annual fish output in Nigeria, 350,000 metric tons were produced locally 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 (-0.6 %) averaging 298.8 thousand metric tons per annum against annual demand of 1.5 million metric tons.

Otubusin (1996) reported that Food and Agricultural Organization (FAO) indicated that to maintain the present per caput fish consumption levels of 13.0kg per year, 91 million metric tons of food fish would be required. Such an increase can only be achieved through aquaculture. Farming culturable fish species under controlled environment has proved be a successful method of increasing fish supply. In a national diagnostic survey of water resources of Nigeria carried out in 1983, it was revealed that about 200 ha of ponds are under cultivation with another 836 ha under construction and 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 Freshwater Fisheries Research (NIFFR), New Bussa. The survey showed that there had been immense awareness of profitability of fish farming in the country within the last one decade. 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 revealed that out of 80 % of fish hatcheries identified in different parts of the country, 48% were government owned. FAO (1990) reported that the major constrain of fish farming in Nigeria is the inadequate availability of quality and fast growing fish seed. Based on a 1992 United Nations Development Project (UNDP) assisted base line study (Fish Network, 1994) the total fingerlings requirement of Nigeria was 250,000 million while the domestic production stood at 7.2 million.

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

Despite the break through reported for artificial propagation of catfish (Richter and Van der

Hurk, 1982; Madu *et al.,* 1987; Madu *et al.,* 1989) the demand for the fish seed still exceeds supply. Various types of fish have been induced to spawn using various hormonal materials (Nwadukwe, 1993; Eyo, 1996, 1997, 2000; Nwuba and Aguigwo, 2002). Some of the hormonal materials include HCG (Eyo, 2002), clomiphene citrate (Aguigwo, 1991), Pituitary extract (Janssen, 1985; Haniffa *et al.,* 2000) and ovaprim (Abol-Munafi *et al.,* 2006).

The present study compared the effects of Homoplastic and Heteroplastic hormones on artificial breeding of *Heterobranchus bidorsalis* with the following objectives. Comparison of the level of ovulation inducement of the two hormonal materials, establishment of spawn efficacy of *H. bidorsalis* injected with the hormonal materials, determination of percentage hatchability and survival of the eggs and larva respectively and establishment of cost benefit of the hormones

MATERIALS AND METHODS

The study Area: This study which lasted 70 days (May – July, 2002) was carried out using fish hatchery facilities at the Aquafish farm, Ihudim, Ihiala, Anambra State. The farm which covers an area of 5 hectares has the following developed structures and facilities: indoor fish hatchery complex with 10 incubation concrete tanks (1 x 1.5 x 1 m) each. 20 earthen nursery ponds (10 x 15 x 1.2 m). Three brood stock ponds (20 x 10 x 1.2 m) each. Seven production ponds (30 x 80 x 1.5 m) each. A borehole and 5000 gallon concrete reservoir. A perennial river – Ulasi is located about 300 m from the farm.

Brood Fish Procurement and Management: Broodfish used for the study were 18 months old domesticated *H. bidorsalis* fingerlings produced at Aquafish hatchery. All broodfish were selected by external morphological characteristics using the methods described by Ayinla and Nwadukwe (1988). Female brood fish were selected on the basis of ovarian biopsy of the oocytes as described by Legendre (1986). The 60 selected gravid females and 20 mature males had weight range of 310 to 550 grams. The female broodfish were kept separate from the males in earthen ponds (10 x 15 x 1.2 m). They were fed Aquafish pelleted fish feed (35 % Crude Protein) twice daily (7 and 5 pm) on 5 % of total fish biomass, 7 days of the week. The brood stock were acclimated in their new environment (10 x 15 x 1.2 m earthen fish pond) for 15 days at mean temperature of 28 ± 2^{O}C and normal photoperiodic regimes (12 hour light and 12 hour darkness).

Experimental Design and Induced Spawning: Three treatments replicated trice with three fish per replicate was used. Two hormonal materials (homoplastic and heteroplastic hormones) were used. Control fish were administered 1 ml of O.6 % saline solution.

Ten induced spawning trials were carried out using 60 gravid females and 20 mature males. Prior to each trial, pituitary gland was extracted from mature fishes for homoplastic and heteroplastic hormones respectively (Viveen *et al.,* 1985). Each gland was then transferred into a glass tube containing acetone. The acetone was decanted after eight hours and refilled. This was kept in a cool place for 24 hours after which it was finally decanted, dried and stored pending use.

Hormone Injection: Before hormone injection, vitellogenic females were randomly seined out from the ponds and kept singly in aerated 50 litres aquarium with 25 litres of aerated water for 12 hours. This was to allow them remove their gastro-intestinal content (Viveen *et al.,* 1985).

The injection of hormonal materials was done between six and seven pm during each experiment. During each trial the acetone dried pituitary was macerated in a porcelain mortar with a known volume (1 ml /kg body weight of fish) of 0.6 % saline solution. It was allowed to settle after which the supernatant was drawn with 5 ml hypodermic syringe with 0.6 mm gauge needle. The weighed gravid fish were 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 backflow of the injected fluid. The control fish were injected 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 ± 2^{O}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 towards the tail onto a 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 by spoon. One mature male was used for 3 females. The whole process took 3 minutes to accomplish.

Incubation of the fertilized eggs was carried out in 1 x 1.5 x 1 m concrete tank that was partitioned into three equal compartments. It was equipped with water flow through facilities.

Nylon mesh net (1 mm) was suspended above the floor and the fertilized eggs were spread in single layers on it for incubation. Water parameters were monitored. Temperature was measured by centigrade thermometer, pH was monitored using Hanna Hep pH meter and optimum oxygen level was maintained with RESUN LP-100 low noise air-pump.

Sample of 200 eggs was taken from each of the treatments at random and incubated in aerated aquaria (36 x 24 x 18 cm). Dead eggs were removed after 10 hours (Nwadukwe, 1993).

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

Hormone	Mean wt. before spawning (g)	Mean wt. after spawning (g)	Mean wt. loss	% wt. loss	T-value	P-value
Homoplastic hormone	423.83 ± 14.19	410.42 ±13.92	13.09	3.16	0.67	0.25
Heteroplastic hormone	424.43 ± 12.39	411.43 ± 11.97	12.34	3.06	0.76	0.23

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

Hormone	Mean number of fertilized eggs	% fertilized	F-value	P-value
Homoplastic hormone	9,522.77 ± 348.13	96.01	13.18	0.001
Heteroplastic hormone	8,851.93 ± 255.57	95.42	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	0.00
Heteroplastic hormone	425.53 ± 17.09	4.55	235.32	0.00

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	9,180.13 ± 343.37	96.40	15.86	0.001
Heteroplastic hormone	8,857.93 ± 255.57	8476.83 ± 345.95	95.70	15.86	0.001

Percentage hatchability and larval deformity were calculated (Haniffa 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 were fed to satiation with incubated *Artemia naupli.* Percentage survival was calculated at the end of 5 days.

Data Analysis: The data collected for the trials were pooled together and analysed for central tendencies using descriptive statistics. Analysis of variance with F-LSD post ad hoc test was used to separate differences in treatment means. Multiple regression and correlation statistics was used to establish linear relationships between variables. All analysis were carried out using Microsoft Excel 2006. The output is presented in tables.

RESULTS

Effect of Hormonal Treatment on Body Weight of Pre and Post Spawners of *H. bidorsalis:* The effects of the two hormonal materials of gravid female *Heterobranchus bidorsalis* are presented in Table 1. The mean pre-spawning weight was 423 ± 12.39 g for gravid *H. bidorsalis* injected with heteroplastic hormone. There was non significant difference ($P < 0.05$) in weight of female fishes before hormonal treatments. Similarly, all gravid *H. bidorsalis* recorded no significant weight loss after spawning. The non significant weight difference was 410.42 ± 13.92 g for homoplastic hormone and 411.4 ± 11.97 g for females injected with heteroplastic hormone. The T- value was not significantly different for the hormonal treatments.

The percentage weight loss emanating from spawning as induced by hormonal treatments were 3.16 % for fishes injected with homoplastic hormone and 3.06 % for those administered ovaprim. Although homoplastic hormone recorded higher percentage weight loss, the result was not significantly different ($P. > 0.05$) from that of heteroplastic hormone.

Effect of Hormonal Treatment on Number of Fertilized Eggs: Gravid female *H. bidorsalis* injected with homoplastic hormone recorded higher number of eggs (9522.77 ± 348.13) while females injected with heteroplastic hormone recorded (8857.93 ± 255.57).

Also, the percentage fertilization was higher for homoplastic injected *H. bidorsalis* (96.01 %) while those injected heteroplastic hormone recorded (95.42 %) (Table 2). The analysis of variance test of the number of fertilized eggs indicated significant difference ($P < 0.001$) for the hormonal treatments.

Effects of Hormonal Treatment on Number of Dead Eggs after Fertilization in *H. bidorsalis:* Gravid female *H. bidorsalis* injected with homoplastic hormone recorded lower mean number of dead eggs (396.10 ± 19.15). Those injected with heteroplastic hormone recorded (425.53 ± 17.09).

Furthermore, the percentage dead eggs were lower (3.99 %) in homoplastic injected female *H. bidorsalis* than that of gravid *H. bidorsalis* injected heteroplastic hormone (4.55 %). The analysis of variance test of number of dead eggs showed significant difference ($P < 0.001$) for the hormonal treatments (Table 3).

Effects of Hormonal Treatment on Hatchability of Eggs at Same Environmental Variables for *H. bidorsalis:* The effects of different hormonal treatments on the hatchability of gravid *H. bidorsalis* are presented in Table 4.

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

Hormone	Mean number	Mean number of deformed larvae	% deformity	F-value	P-value
Homoplastic hormone	9180.13 ± 343.37	35.80 ± 1.11	0.39	86.63	0.001
Heteroplastic hormone	8,476.83 ± 245.95	34.27 ± 1.43	0.40	86.63	0.001

Table 6: Effect of hormonal treatment on percentage survival of *H. bidorsalis* larvae under unvaried environment

Hormone	Mean number of larvae	Mean number of hatchlings	% survival
Homoplastic hormone	9,180.13 ± 343.37	9,144.33	99.61
Heteroplastic hormone	8,476.83 ± 245.95	8,442.56	99.59

Table 7: Costs Benefit of hormonal treatment on *H. bidorsalis*

Hormone	Total wt. of fish (g)	Cost of hormone ₦
Homoplastic hormone	12,715	6,350.00
Heteroplastic hormone	12,733	6366.00

Gravid *H. bidorsalis* injected with homoplastic hormone recorded higher number of hatched eggs (9180.13 ± 343.37). Female *H. bidorsalis* administered heteroplastic hormone recorded (8476.83 ± 345.95). Similarly, percentage hatchability was 96.40 % for female *H. bidorsalis* injected homoplastic hormone and 95.70 % for those injected heteroplastic hormone. The analysis of variance test of eggs hatchability showed significant difference ($P < 0.001$) for the hormonal treatments.

Effects of Hormonal Treatment on Larval Deformities of *H. bidorsalis:* Record of deformed larva arising from hatchlings of *H. bidorsalis* injected with hormonal materials are shown in table 5. Gravid female of *H. bidorsalis* injected with homoplastic hormone recorded higher mean number of deformed larva (35.80 ± 1.11). Females of *H. bidorsalis* injected with heteroplastic hormone recorded lower number of deformed larva (34.27 ± 1.43).

However, percentage deformity was (0.39 % and 0.40 %) respectively for female *H. bidorsalis* injected with homoplastic and heteroplastic hormones respectively. Analysis of variance test for deformed eggs, showed significant difference ($P < 0.001$) for the hormonal treatments.

Effect of Hormonal Treatment on Percentage Survival of *H. bidorsalis* Larvae Under Unvaried Environment: Gravid female *H. bidorsalis* injected with homoplastic hormone recorded percentage survival of 99.61% while those injected heteroplastic hormone recorded 99.59 % (Table 6).

Cost Benefit of Hormonal Treatment in *H. bidorsalis:* Comparative costs of hormonal materials used on *H. bidorsalis* are shown in Table 7. Gravid female of *H. bidorsalis* which weighed a total of 12.72 kg were injected homoplastic hormone worth N6350 while female *H. bidorsalis* that weighed a total of 12.73 kg were administered heteroplastic hormone worth ₦ 6366:00K.

DISCUSSION

Effects of Hormonal Treatment on Body Weight of Pre and Post Spawners: Results presented in 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 411.43 ± 1.97 g for female injected with homoplastic and heteroplastic hormones respectively. The non significant differences may be as a result of 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 induced breeding technique.

Delince *et al.* (1987) reported that a 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. gariepinus* and noted that the quantity of ovulated eggs was between 15-20 % of its own body weight. In another study, Eyo and Mgbenka (1992) 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 from successful spawning trials.

Effects of Hormonal Treatment on Number of Fertilized Eggs: The result in Table 2 indicated that spawners injected with homoplastic hormones recorded significantly higher number of fertilized eggs (9522.77 ± 348.13 eggs) fertilized eggs arising from injection of heteroplastic hormone was (8857.93 ± 255.57 eggs). In another study, using HCG to induce breed *Channa punctatus,* Haniffa and Sridhar (2002), had fertilized egg output ranging from 1253 ± 126 eggs for *C. punctatus* weighing 65 – 85 g injected 3000 IU HCG. The difference in egg output of Haniffa and Sridhar when compared to this study may be due

to difference in species, weight of spawners and the hormonal material used. In another study Oladosu *et al.* (1993) induce breeding *H. bidorsalis* (mean weight 707.72 ± 4.28.22) with carp pituitary recorded mean egg out put of 33460 ± 20.571 eggs. This study reported lower fertilized eggs output than Oladosu *et al.* may be due to weight difference in spawners used.

Effect of Hormonal Treatment on the Number of Dead Eggs after Fertilization in *H. bidorsalis:* 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 homoplastic hormone recorded 396.10 ± 19.15 while those administered heteroplastic hormone had 425.53 ± 17.09. The percentage of dead eggs was 3.99 % for females injected homoplastic hormone and 4.55 % for those injected heteroplastic hormone. In another study, Nwadukwe (1993) induce breeding *Heterobranchus longifilis* with frog (*D. occipitalis*) pituitary hormone recorded higher dead eggs percentage (29 %). The lower percentage achieved in the present study may be attributed to the efficacy of the hormones.

Effect of Hormonal Treatment on Hatchability of *H. bidorsalis:* Results on Table 5, showed higher hatchability (9,180.13 ± 343.37 larva or 96.40 %) of eggs for female *H. bidorsalis* injected with homoplastic hormone while those administered heteroplastic hormone recorded slightly lower number of hatched larva (8,476.83 ± 345.95 larva or 95.70 %). In a similar study Fagbenro and Adebayo (2004), using homoplastic hormone suspension on *H. bidorsalis* reported hatchability percentage of 86 %.

Effect of Hormonal Treatment on Larval Deformities of *H. bidorsalis:* In this study spawners injected homoplastic hormone recorded slightly higher number of deformed larvae (35.80 ± 1.1) when compared with those injected heteroplastic hormone (34.27 ± 1.43). Percentage deformity followed the same pattern (0.12 % and 0.39 % respectively. In a similar study, Nwadukwe (1993) using frog pituitary to induce breed *H. longifilis* reported higher percentage deformity of 7.17 ± 1.72. The higher percentage deformity recorded by Nwadukwe may be Nwadukwe may be attributed to the low potency of frog pituitary.

Effect of Hormonal Treatment on Percentage Survival of *H. bidorsalis* under Unvaried Environment: Percentage survival of hatchlings was 99.61 and 99.59 % respectively for gravid *H. bidorsalis* injected homoplastic and heteroplastic hormone suspension. The result recorded in this study is similar to those reported by earlier studies. Salami *et al.* (1994) induce breeding *C. gariepinus* with non-piscine pituitary extract (HCG) reported a better survival rate due to HCG administration than carp pituitary extracts.

Similarly, in another study Nwadukwe (1993) using frog pituitary extract to induce breed *H. longifilis* reported a survival rate of 66 to 90% for hatchlings after one week. The slight difference in these results when compared with that of this study may be attributed to species of fish and hormonal material used.

Cost Benefit of Hormonal Treatment in *H. bidorsalis:* Table 7 showed the comparative costs of the hormonal materials used homoplastic hormone which recorded better result in the parameters investigated cost same (₦ 6350:00K for 12.7 kg of fish injected). Although the two hormones tested were effective inducers, homoplastic hormone is recommended as it recorded better results.

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