A COMPARATIVE STUDY ON THE BREEDING PERFORMANCE OF *BIDORSALIS HETEROBRANCHUS* AND *HETEROBRANCHUS LONGIFILIS*, USING THREE DOSES OF OVAPRIM

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ABSTRACT

Comparative analysis was carried out on induced breeding of *Heterobranchus bidorsalis* and *Heterobranchus longifilis*, using ovaprim at three doses, viz 4ml/Kg, 5ml/Kg and 6ml/Kg of female broodfish weight. Latency period and time of hatching were similar for both fish species. The dosage of 4mg/Kg gave 2.66% fertilization among *H.bidorsalis* eggs and no fertilization among *H. longifilis* eggs. No hatching occurred at this hormone dosage with 5mg/Kg of hormone of dosage, mean fertilization and hatching values were 49.17±5.73 and 38.75 ±5.10 respectively for *H. bidorsalis* and 37.42±7.00 and 26.08 ± 3.82 respectively for *H. longifilis*. However, the dosage of 6mg/kg gave the best results of 65.58±4.54 and 59.75±4.41 for fertilization and hatching rates respectively in *H. bidorsalis*. In *H. longifilis*, the same dosage gave fertilization and hatching rates of 51.75±4.47 and 47.16±4.73 respectively. Mean water temperature in the broodstock ponds was 28.42^{o}C ± 1.55, dissolved oxygen was 1.64mg/L±0.56 during the study period. Students t-test should no significant difference (P>0.03) in survival of fry of fish species after 10 days of rearing in concrete tanks.

INTRODUCTION

The expanding catfish production activities is presently the most important aspect of the aquaculture industry in Nigeria. The most cultured species include *Clarias gariepinus*, and *Clarias anguillaris*, but many consumers in southern Nigeria still demand for *Heterobranchus* species.

There are three known species of *Heterobranchus* in Africa, namely, *Heterobranchus bidorsalis*, *Heterobranchus longifilis* and *Heterobranchus isopterus* (Teugels *et al.*, 1990). The first two species are very common in southern Nigeria where they are known to attain large sizes in ponds, especially in polyculture with tilapias.

Several studies have been carried out on both species with different results (Nunez-Rodriguez *et al.,* 1995; Nwadukwe, 1993.

As a result of increased fish culture activities in Nigeria, various individuals have locally engaged in seed propagation of *Heterobranchus* spp. and their hybrid with *C.gariepinus* (Nwadukwe, 1995a,b). Without a good scientific knowledge of species identification, this would probably lead to the production of unknown sub-species with varying genetic make-up. The easiest morphological tool that can be used in differentiating both *Heterobranchus* species is the structure of the adipose fin (Teugels *et al.,* 1990).

The present study is aimed at investigating the effects of various doses of ovaprim on oocyte maturation and ovulation in *H. bidorsalis* and *H. longifilis* under similar conditions. This will guide fish breeders in applying a more cost-effective hormone age when handling any of the two species.

MATERIALS AND METHODS

Artificial propagation of *H. bidorsalis* and *H. longifilis*, using ovaprim as an inducing agent was carried out at the fish hatchery and farm of the African Regional Aquaculture Centre (ARAC) at Aluu, Port Harcourt, Nigeria.

Physico-chemical parameter of pond water

Some physico-chemical parameters of *Heterobranchus* broodstock pond were monitored during the study period. They included water temperature which was determined for 5 days in a week, using a mercury-in-glass thermometer; pH, which was determined with a pH meter (Aquatic ecosystem, Florida; and dissolved oxygen content (DO), which was monitored with a DO meter (YSI, model 51B, Ohio). Ammonia-nitrogen and nitrite-nitrogen

were monitored for 3 days in a week, using the Koi reagent system.

Broodfish

Mixed-sex fingerlings of *H.bidorsalis* and *H. longifilis* were procured from a private fish farm in Rivers State and reared in ponds for a period of two years when they became sexually-mature adults. The species were identified, using the method that was described by Teugels *et al* (1990).

Spawning Procedure

A total of four spawning trials were carried out for each fish species. During each trial, broodfish of both species were seined out of the pond. Females were selected, based on the degree of distention of their abdomen as earlier described by Nwadukwe *et al* (1993). Males were selected based on the dark tip of the genital papillae as well as on their aggressive behaviour during handling. Each selected broodfish was weighed to the nearest gram, using a "Salter" balance the fish were also measured individually in measuring board.

Hormone injection and Latency period.

The effects of three dosage of ovaprim were investigated during each spawning trial and the doses include. 4ml/kg; 5ml/kg and 6ml/kg of female broodfish weight. These doses were selected, based on the manufacturer's recommended dosage of 5ml/kg of fish weight. Higher values were not used for this study since these would not be economically feasible.

During each spawning trial, nine (9) gravid females of each species were injected intramuscularly with the hormone: three females (in triplicate) of each species received each hormone dosage. Latency period was determined by checking for ovulated eggs at 20-30min intervals from 6h post-injection.

Fertilization, Egg Incubation and Hatching

Males of *H. bidorsalis* and *H. longifilis* were sacrificed and their milt was separately collected and stored in 0.9% saline solution as described by Nwadukwe *et al* (1993). For each hormone dosage, eggs were stripped from each of the three injected females and the eggs were separately mixed with milt from the of the same species. Egg fertilization was effected after sperm dilution with clean water. The eggs were allowed to incubate and hatch in rectangular hatching troughs and in large concrete tanks.

Data Collection.

Hundred (100) eggs were collected at random and incubated each of three 1-litre plastic bowls containing water. This was done for each hormone dosage and for each fish species. Time of sperm dilution was regarded as time of egg fertilization and the eggs were allowed to incubate in the bowls.

Percent fertilization and percent hatching were calculated as follows:

% fertilization = x 100

% hatching = x 100

as described by Nwadukwe *et al* (1993)

Fry Rearing

After hatching five-day-old fry of each species were separately reared in concrete tanks (each species in triplicate) for a period of 10 days. Each tank measured 1mx1mx1m and was filled up to the 50cm level with clean bore hole water from the hatchery. Three tanks contained 100 fry of *H. bidorsalis*, while each of the other 3 contained 100 *H.longifilis* fry. All the fish were fed *ad libitum* with *Artemia* cysts for the first 5-days and thereafter, with powdered (ground) Coppens feed for the remaining 5 days. Seventy percent (70%) of the water in each of the tanks was renewed with clean fresh water at 5-day interval

At the end of the 10-day period, each of the tanks was drained and the fingerlings were counted and measured.

Data Analysis

The number of eggs that were fertilized under each of the three hormone doses (treatments) were subjected to one-way ANOVA at 5% level of significance (Wahua, 1999).

The same was done for the number of fry that hatched out under the three treatments. These were carried out separately for each fish species.

Student's t-test (p=0.05) was also used to test for any significant differences in the

results of fertilization, and also hatching, as well as fry survival in the tanks between both fish species.

RESULTS

Physico-chemical parameters

The mean values of the physico-chemical parameters of *Heterobranchus* broodstock pond are presented in Table I. Mean surface water temperature was 28.42^{0}C ±1.55, while dissolved oxygen concentration was 6.28mg/l ± 1.65. Mean nitrite-nitrogen content was 0.06mg/l ± 0.56 during the study period.

Broodfish

Female *H. bidorsalis* ranged from 550g to 800g, while the males weighed between 600g and 800g. Among *H. longifilis* broodfish, the females were between 525g and 850g and the males ranged from 700g to 725g.

Latency period, fertilization and Hatching

After hormone injection, latency period was observed to fluctuate between 8h at 28^{0}C and 10h at 26^{0}C among both *Heterobranchus* species. At the end of 8h after fertilization, unfertilized eggs become dead and opaque and this continued till 10h after which no more eggs were observed to turn opaque (die). Hatching commenced at 18h and continued till 30h after which no further hatching was observed. The remaining unhatched eggs were later observed to gradually become whitish (opaque).

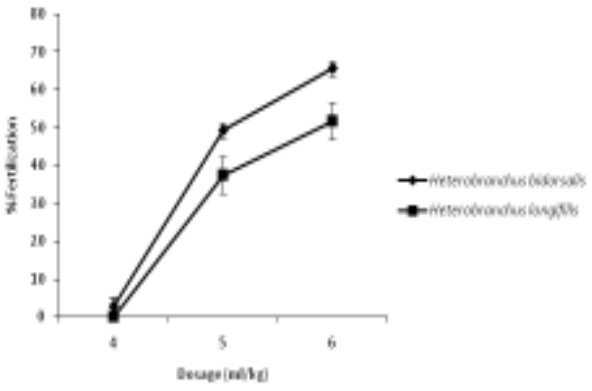

Fig.1: Shows the results of fertilization and hatching rates among both Heterobranchus species.

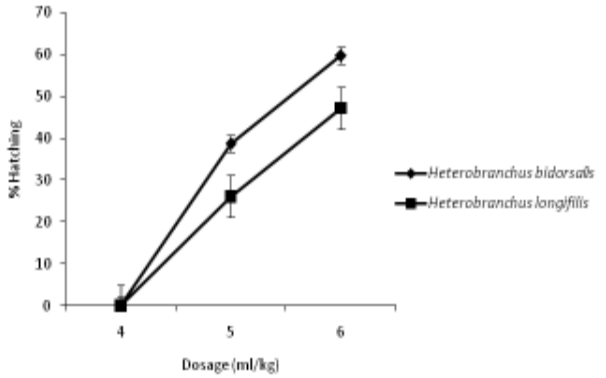

Fig. 2. Hatching rates among fertilized eggs of H. bidorsalis and H. longifilis that were injected with ovaprim at different doses.

Hormone dosage 4mg/kg of female broodfish weights

Among *H. bidorsalis* species, only 32 out of the 1200 incubated eggs remained yellowish in colour up to 20h after fertilization. This phenomenon was observed during each of the four spawning trials. However, no hatching was observed in any of the incubation bowls at the end of 35h after fertilization.

Among *H. longifilis* species, all the incubated eggs became opaque between 8 and 9h after fertilization. Hatching did not occur in any incubation bowl among *H. longifilis* eggs under the hormone dosage of 4mg/kg.

5mg/kg of Female Broodfish Weight

Fertilization rate ranged from 38% to 58% among incubated eggs of *H. bidorsalis* females that were injected with ovaprim at a dosage of 5m/kg of female broodfish weight. Mean hatching rate was 49.17% ± 5.73 (SD) during the study period.

In the incubation bowls containing *H. longifilis* eggs, mean fertilization rate was 37.42% ± 7.00 (SD), while hatching rate ranged between 20.00% and 31.00% (Fig I).

6mg/kg Female Broodfish Weight

Mean fertilization rate was 65.58% ± 4.54 (SD) among the *H. bidorsalis* eggs when the female broodfish was injected at a dosage of 6mg/kg. the highest fertilization value of 71.00% was obtained during the two spawning trials in 2008. Hatching rates ranged from 52% to 66%. For *H. longifilis* eggs, mean fertilization and hatching rates were 51.75% ± 4.47 (SD) and 47.17% ± 4.73(SD) respectively (fig 1)

Comparing the 3 Hormone Treatments/ Fish Species

When comparing the effect of the three hormone dosage on the eggs of *H. bidorsalis* ANOVA showed a significant difference in the number of fertilized eggs ($p<0.5$). A significant difference ($p<0.5$) was also found among the number of hatched eggs. The same effect was also found when considering the effect of the three hormone dosage on the eggs of *H. longifilis*.

When comparing the two *Heterobranchus* fish species, student's t-test showed a significant difference in egg fertilization values when each (individual) hormone dosage was considered separately ($p<0.5$). The same effect was obtained when the hatching values of eggs of both species were considered under each separate hormone dosage ($p<0.5$).

Fry Rearing

At stocking, the fry of *H. bidorsalis* ranged between 5.8 and 6.5 with a mean value of 6.2mm ± 0.03. *H. longifilis* fry were found to be between 6.0 and 6.5mm with a mean value of 6.3mm ± 0.03.

After five days of rearing fry weight ranged from 26 to 44mg with a mean value of 38.60mg ± 5.00 among *H. bidorsalis*. During the same period weight range was between 26 and 42mg (mean value ≡ 34.60mg ± 5.20) for the fry of *H. longifilis*. (Table 2)

At the end of the 10-day rearing period, a total of 209 fingerlings of *H. bidorsalis* was harvested from the three tanks giving a survival rate of 69.67%. The fingerlings weighing between 83 and 152mg with a mean value of 115.00mg ± 0.02 (SD). They also measured between 19 and 22mm in length. A total of 218 fingerlings of *H. longifilis* was also harvested from the other three tanks, showing a survival rate of 72.67%. the fingerlings ranged from 19-21mm in length and weighed between 84 and 138mg, with a mean weight of 102.00mg ± 0.02 (SD) (Table 2)

When comparing both species, student's t-test showed no significant difference in the total number harvested fingerlings ($p>0.05$).

Table I. Some physico-chemical parameters of *Heterobranchus* broodstock ponds.

Water Parameters	Mean Values (±SD)
Water Temperature (^{0}C)	28.42±1.55
PH	7.50±0.34
Dissolved oxygen content(mg/ℓ)	6.28±1.65
Ammonia-nitrogen(mg/ℓ)	1.64±0.56
Nitrite-nitrogen(mg/ℓ)	0.06±0.01

Table 2, early growth and survival of *H.bidorsalis* and *H. longifilis* fry in concrete tanks.

Rearing period (days)	Fish species					
	H.bidorsalis			*H.longifilis*		
	range,	mean,	survival(%),	range,	mean,	survival(%)
Stocking	5.8-6.5,	6.2cm, ±0.03(SD).	—	6.0—6.5,	6.3cm, ±0.03(SD).	—
DAY 5	26-44,	38.60mg, ±5.00(SD)	—	26-42,	34.60mg, ±5.20(SD)	—
DAY 10	83-152,	115.00mg, ±0.02(SD).	69.67%,	84-138,	102.00mg 0.02(SD)	72.67%

DISCUSSION

The study revealed that sexually mature female *H. bidorsalis* and *H. longifilis* broodfish were induced to undergo artificial breeding with varying success when ovaprim was used. In order to facilitate breeding, it is essential that the brooders should be in good condition so as to ensure smooth transition from one gonadal developmental stage to the other. This involves the well-being of the fish and this largely depends on favorable extrinsic factors such as good water quality, steady supply of food and a habitat with low amount of diseases and infections. The results of this study showed that the values of the physico-chemical parameters of the broodstock ponds were favorable for good physiological condition of the broodfish as documented by Boyd (1982).

The use of ovaprim as an inducing agent for artificial catfish reproduction has been established for *C. gariepinus* (Okoro *et al.*, Halics 2006). *Clarias* and *Heterobranchus* belong to the same family, Clariidae and exhibit relatively similar habits. However, the present result indicated that *Heterobranchus*

spp required slightly higher doses of ovaprim (5-6ml/kg) than *C. gariepinus* (4-5ml/kg as in Okoro *et al.,* 2006) in order to effect egg maturation and ovulation. This trend seem to be similar to earlier studies on these catfishes using carp pituitary gland (Nwadukwe *et al.,* 1993).

It was also interesting to note that from this study, *H. bidorsalis* required relatively low doses of ovaprim than *H. longifilis* to achieve comparable egg fertilization and hatching results. During stripping, it was observed that *H. bidorsalis* had smaller eggs than *H. longifilis* it could not be confirmed if egg size was related to the effect of hormone dosage on egg maturation and ovulation. This aspect requires further genetic investigation. Ovaprim is synthesized from Salmon pituitary glands and the manufacturer's recommended dosage of 5mg/kg of fish weight produced poor results in *H. longifilis*. The present study revealed some similarities in size and appearance among *H. bidorsalis* and *C. gariepinus* eggs. Both *Heterobranchus* species that were used for this study were of similar age group and were reared in the same pond, under similar environmental conditions. Therefore, it was assumed that the process of oogenesis up to vitellogenesis, should have progressed in a similar pattern. In addition, the process of egg maturation and ovulation after hormone administration should also have progressed similarly if not for variations in the genetic characters within individual species. Thus, the present result have demonstrated that fish breeders should differentiate between *H. bidorsalis* and *H. longifilis* when embarking on *Heterobranchus* breeding exercises. Also, it should be noted that *H. bidorsalis* will give good breeding results when ovaprim is applied at the manufacturer's dosage, but that *H. longifilis* requires relatively higher hormone dosage for effective egg maturation and ovulation.

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