SPAWNING PERFORMANCE OF AFRICAN GIANT CATFISH (*HETEROBRANCHUS BIDORSALIS*, (GEOFFRROY SAINT-HILAIRE, 1809) INDUCED WITH OVATIDE AND OVARY-PRIM IN SEMI-ARID REGION (SOKOTO), NIGERIA

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ABSTRACT
The efficiency of two synthetic hormones analogue ovary-prim and ovatide on breeding performance of Heterobranchus bidorsalis in Sokoto, North-Western Nigeria were investigated. Three brood fish were injected intramuscularly with ovary-prim and ovatide at manufacturers recommended dose 0.5 mlkg-^{1} and 0.2mlkg-^{1} respectively. The males received half dose of the hormones according to treatment, with respect to their body weights, treatments were set up in a completely randomized design (CRD). The results showed that a latency period of 11 hours was recorded at temperature range of 28^{o}-30^{o}C. The egg output was estimated at about 109,000 with ovary-prim and 104,640 with ovatide as recorded during the breeding exercises. Fertilization rate was greater (78%) with ovary-prim as against 76% recorded for ovatide but were not significantly (P > 0.05) differed. The hatching success was however significantly (P < 0.05) greater (86%) with ovatide than 72% with ovary-prim. Percentage survival also followed the same trend. It was hence concluded that while both synthetic hormones are good for inducing ovulation, ovatide is a more effective synthetic hormone analogue for induced spawning and seed production of Heterobranchus bidorsalis in the prevailing climatic condition of semi-arid environment of Sokoto, North-Western Nigeria.

KEYWORDS: *Heterobranchus bidorsalis,* Spawning performance, ovary-prim, ovatide, hormones

INTRODUCTION
Reproduction in fish is controlled by several factors which include sex steroids in the regulation of reproductive processes (Kime, 1993). These reproductive processes are controlled through the brain-pituitary gonadal axis, the brain is stimulated by environmental cues like water rise, temperature, feeding, rainfall,

and photoperiod to release gonadotropin releasing hormones (Zohar *et al*., 2001). Hence ovulation and permeation are induced as a result of the sex steroids that have been produced. However, most fish species will not readily breed in captivity all year round, thus the need for artificial seed production using hormones.

The use of both synthetic and natural hormones brings about quick ovulation and higher percentage of hatched fish, although synthetic hormones have been found to give higher yield than the natural hormones (Krol *et al*., 2006). Administration of gonadotropin releasing hormone analogue, in artificial spawning of fish has been reported to increase levels of plasma sex steroids in female fish to induce ovulation (Zhuo *et al.,* 2011). The author, from his study also showed that Gonadotropin releasing hormone analogue multiple injections potentially accelerated testicular maturation of male yellow catfish. Ovary-prim and Ovatide are some among synthetic hormones imported and used for artificial spawning of fish in Nigeria. Both are synthetic hormone preparations containing salmon gonadotropin releasing hormone analogue and domperidone (SGnRHa + Domperidone) antagonists.

The Giant African Catfish *Heterobranchus bidorsalis* is a hardy specie for aquaculture, it is widely accepted in the tropics and commands good commercial value. However, there is generally dearth of knowledge on the reproductive biology of this species, except for few studies on its haematological and nutritional characteristics, salinity tolerance, digestive enzymes profile and parasite fauna (Fagbenro *et al*., 1991, 2013; Adebayo and Fagbenro 2004). This is probably due to the species' limited availability, breeding constraints of longer timed sexual maturity and short breeding period which is at the peak of rainy season. Though the species has not been listed as endangered, there is risk of extinction because of environmental problems that affect the breeding sites (Honji *et al.* 2009, 2012). Further threats are anthropogenic activities like the construction of dams, riparian habitat destruction, water pollution and fishing (Honji *et al*. 2009, 2012; Olaniyi 2014). Hence the need to conduct more studies on the reproductive behavior of this species in a bid to alleviate the threat of extinction, through artificial propagation should remain a research priority. More so not a lot of research has been done to test the effectiveness and potential of these two hormone on *H. bidorsalis* spawning in semi-arid region of Nigeria.

This research is therefore conducted to investigate the effect of ovary-prim and ovatide hormones on the reproductive index of giant African catfish *H. bidorsalis* in Sokoto, semi-arid region of Nigeria.

MATERIALS AND METHODS

Experimental Location: The experiment was conducted in August 2016 at the Fish Hatchery Unit, of the Department Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto on latitude 13°07'7"N and longitude 05°12'25"E at 275m above sea level (Google Earth, 2011). The Ovary-prim hormone (containing Salmon Gonadotropin Releasing Hormone Analogues and Domperidone) by ZDHF PHARM, China, was used While the Ovatide (containing a synthetic peptide protein that is analogue to naturally occurring gonadotropin releasing hormone (GnRH), and dopamine antagonist) was supplied by Hemmo Pharma, Mumbai, India with the recommended dosage of 0.20ml/kg body weight of catfish.

Broodstock Preparation: Six broodstocks of *H. bidorsalis* (2 males and 4 females) were collected from the Unit's broodstock pond. Identification of sex was based on external morphological characteristics – the observation of protruded and reddish genital papilla in the males, while the females were gravid with a round, soft and bulging abdomen, with pinkish and protruding reddish genital openings – as described by Metwally and Fouad, (2008). The fish samples were each weighed, while the two males weighed as 1600g and 1900g, the females weighted between 3000g to 3600g (Table 1). The hormones were administered according to manufacturer's recommendation, which is 0.5ml per kg of female fish for Ovary-prim and 0.2ml per kg of the male fish for ovatide. The females were given full dosage of the hormone while the males received half the doses administered to the females (Viveen *et al.*, 1985).

Experimental Design: The experimental design consisted of two treatments, based on the different hormones tested (ovary-prim and ovatide), and these were replicated three times in a Completely Randomized Design (CRD). Six plastic bowls of 75litres were used as spawning troughs after they were washed and thoroughly dried. The bowls were filled to about 75% capacity and constantly aerated with aerator pump, while temperature ranged from 24 to 31^{o}C (Table 3). Nets (Spawning mats) were washed and placed inside each of the experimental units. The fish were injected intramuscularly above the lateral line towards the dorsal fin using a graduated hypodermic syringe

of 2ml (Haniffa and Sridhar, 2002), at an angle of approximately 30° in the direction of the head as described by (Viveen *et al*., 1985). The injected spawners were then kept separately in containers to avoid disturbances and self-injuries.

Collection of Milt: The male broodstocks after observing the latency period, were removed from the troughs. They were placed dorsally on a wet towel, and held firmly down to ensure careful removal of the testes using a sharp blade. The abdominal cavity of the fish was dissected and testes were carefully removed from the ventral wall of the abdominal cavity and mopped with clean tissue paper to remove stains of blood. The extracted testes were then incised and squeezed of the milt. The milt was diluted with physiological saline to prepare a sperm suspension, and was afterwards stored in refrigerator.

Stripping of Eggs and Fertilization: After observing the ovulation period (Table 3.1), the female broodfish were removed from the trough and weighed. Each fish was carefully held firmly with a wet towel at both ends by two operators, and the abdomen was then pressed carefully to release the eggs into a dry bowl. Each spent female was carefully weighed and returned into the trough. Content of the testes (milt) was spilled on the eggs for fertilization, the eggs were then poured inside the labeled spawning troughs already containing water for the sperm activation, and the fertilized eggs were left in the spawning troughs for incubation with water temperature between 25 and 27°C. Sub-samples of each treatment were collected using spoon of approximately 1g. The fertilized eggs were then spread on the net in each of the bowls, prepared earlier for this purpose and continuously aerated. After fertilization, the viable and dead eggs were determined. The viable eggs were translucent while the non-viable eggs were white and opaque (Sahoo *et al,* 2005) and these were carefully removed by siphoning. Hatching occurred at about 14 hours, and completed after 16 hours. The percentage hatchability was estimated after two days of hatching and the yolk sac had been absorbed. Water quality parameters such as the Temperature, pH, and Electrical conductivity were monitored with the aid of a pen type pH meter that was fixed with mercury in glass thermometer.

Analytical Procedure: Fecundity, fertilization and hatchability indices were calculated for each treatment to determine the performance (efficacy) of Ovatide and ovary-prim at manufacturer recommended dose levels as follows:

Stripping percentage = $\frac{\text{weight of stripped eggs}}{\text{Body weight}}$ x 100

Brzuska (2003)

The total number of eggs stripped (spawned) was estimated by counting the number of eggs in 1g of egg weight as described by Sahoo *et al.* (2005).

The relative fecundity was calculated as described by Billard (1990) in Fraud *et al.* (2010), as follows:

Relative Fecundity = $\frac{\text{Number of stripped eggs}}{\text{body weight}}$

The mean fertilized eggs in all the replicated bowls was recorded and expressed as percent fertilization per female (Adebayo and Popoola, 2008) as follows:

Fertilization = $\frac{\text{Number of fertilized eggs}}{\text{Total no of egg counted}} \times 100$

Hatchability was determined by direct counting of the number of hatchlings of two days old (Haniffa and Sridhar, 2002) and estimated as follows:

Percentage Hatchability = $\frac{\text{Number of hachlings (two days old)}}{\text{Total no of fertilized egg}} \times 100$

The per cent survival was determined according to the method in Adebiyi *et al* (2013)

Percent survival rate = $\frac{\text{Total number of suvived larvae until c}}{\text{Total number of counted larvae at c}}$ x100

RESULTS AND DISCUSSION

Table 2 presents the results obtained for the various tests conducted. The average latency period under mean temperature of 28°C was 11hours 1 minute for TRT I and 11 hours 6 minutes for TRT II, both of which were statistically similar. The weight of the brood fishes used were higher (Table 1) than the lower limit postulated in Viveen *et al.*(1985), who stated that *Clarias gariepinus* become sexually mature at 200 to 500g, This is also in consonance with the findings of Nwoke *et al*., (2007) who spawned successfully with, *H. bidorsalis* of weight range from 310 to 550g.

Zonnelveld *et al*. (1988) reported that *C. gariepinus* exhibited a latency period in excess up to 15hours, at temperature of 25°C. Olaniyi and Omitogun, (2014) reported that when *H. bidorsalis* broodstock was induced to spawning using ovaprim, ovulation was achieved within the latency period of 14hours at ambient temperature of 27°C. Khan *et al*., (2014) reported 11-12 hours of latency period for giant catfish

(*Sperata seenghala*) at water temperature range of 28-29°C when ovaprim, HcG, LHRH and ovatide were used. These studies unanimously support the findings of this experiment in terms of latency period. The spawning success of *H. bidorsalis* when subjected to ovary-prim and ovatide hormone in inducing ovulation and final maturation of eggs (Table 2) indicates that the broodstock responded well to both hormone treatments. Complete spawning success of ovatide has been reported in several fish species such as African catfish (*Clarias gariepinus*) (Shinkafi and Ilesanmi, 2014), Carp (Thakur and Reddy, 1997), Pabo catfish (*Pabda ompok pabo*) (Mukherjee and Das, 2001), Stinging catfish (*Heteropneustes fossili*), and Snake head murrel (*Channa punctatas*) (Marimuthus *et al.,* 2000; 2007). Likewise ovaprim hormone was successfully reported to induce ovulation on several fish species such as Common carp (*Cyprinidae; Cyprinus carpio*) (Lin *et al*., 1988; Haniffa *et al.,* 2007), Australian eel-tailed catfish (*Neosilurus ater*) (Cheah and Lee, 2000), Red-tailed tinfoil barb (*Barbonymus altus*, formerly *Puntius altus*) and walking catfish (*Clarias batrachus*) (Sahoo *et al*., 2005). Moreover this research provide a rare information on the reproductive performance of *H. bidorsalis* on ovary-prim and ovatide in the semi-arid region of Nigeria. Ovapy-prim (TRT II) was seen to have greater weight of eggs (183.23±51.70), spawning fecundity (109,000±34,261.39), percentage of stripped eggs (6.89±0.91), relative fecundity (39.92±6.73), number of fertilized eggs (80441.67±20991.97) and percentage fertilization (78.00±7.02) than Ovatide (TRT I). There was however no significant difference ($P>0.05$) between both treatments in all of these parameters. The stripping percentage of ovulated eggs of the broodstock body weight, was higher than that recorded in Ipinjolu *et al*., (2013) who record stripping percentage of 11.77% on cross of exotic Dutch *Clarias*, *H. bidorsalis* and *H. longifilis* using ovatide hormone. This could be as a result of the brood fish size and weight used in this experiment which was higher than in the Ipinjolu *et al*., (2013) findings. Fertilized eggs usually develop normally if the incubation

conditions (Temperature, cleanliness, oxygen etc) are adequate (FAO, 2011). These factors were taken care of during the experimental period. This finding is similar to an Ivorian study where African catfish *Heterobranchus longifilis* (*Clariidae*) showed percentage fertilization of 76% when treated with HcG (Legendre *et al.*. 1986). Also, Khan *et al*., (2014) reported a lower percentage fertilization rate of 56% on Giant Catfish (*Sperata seenghala*) with ovatide. Nwoke *et al.,* (2007) however reported higher percentage fertilization rate of 98.31% on *H. bidorsalis* using ovary-prim. The relatively lower percentage fertilization recorded in the present study result might be attributed to asynchrony between maturation and ovulation. Since, blood on the stripped eggs clogs the micro pile, which could result to poor fertilization as reported by (Piper *et al*., 1982). Ovatide was seen to have performed significantly better ($P<0.05$) than ovary-prim in terms of percentage hatchability (Table 2). While 86.67±8.84% was recorded for broodstock subjected to ovatide, 72±3.06% was recorded for broodstock subjected to Ovary-prim. In a similar experiment, Shinkafi and Ilesanmi (2014) recorded the highest hatchability of eggs when 0.2ml of ovatide per kg weight of brood stock was used as compared to other doses. Nwoke *et al.* (2007) recorded hatchability of 91% for *H. bidorsalis* using ovaprim while Khan *et al*. (2014), reported a lower hatchability of 43% for Giant Catfish (*Sperata seenghala*) with ovatide. However, Aluko and Popoola, (2002) reported higher percent hatchability 96.09% in the crosses of *H. longifilis*, While Nwaduke (1993) recorded 40 to 75% hatching success in *Heterobranchus longifilis*.

The percentage survival rate was found to be higher in hatchlings treated with ovatide (69.42±0.39) significant difference ($P < 0.05$) with those treated with ovary-prim (58.21± 0.51).

Environmental and Water Quality Parameters: The results for the environmental and water quality parameters monitored during the experiment include Water temperature, room temperature, hydrogen ion concentration (pH), and electrical conductivity (EC) (Table 3). The highest mean water temperature (28.80±0.25) was recorded in the

evening, while water temperature generally ranged between 30.50°C and 28.10°C. Room temperature generally had similar readings with water temperature, ranging from 26.90°C to 30.70°C. The highest mean room temperature was 28.82±0.30°C, recorded for afternoon. pH mean values ranged from 7.97±0.12 to 7.96±0.29. Throughout the duration of the experiment, the highest pH reading recorded was 8.01 while the least was 7.18, indicating that a slightly alkaline water condition was maintained throughout the experimental duration. Electrical conductivity (EC) also ranged between 451.00 and 569.00, with a maximum mean of 556.71±14.45µs/cm recorded for evening. The water quality parameters measured during this study were within the acceptable range for *H. bidorsalis* in (Table 2) Viveen *et al.,* (1985) and Boyd, (1979) reported that warm water fishes grows best at temperature between 25-32^{0}C, pH value of 6.7 to 8.5 and dissolved oxygen ranged from 5mg/l to 7mg/l. The results obtained in the two treatments was within these averages. The optimum water conditions could easily be attributed to the continuous aeration of water the experimental period.

CONCLUSION

The use of synthetic hormone is a better way of improving catfish production with respect to reproductive performance in aquaculture. The efficacy of these synthetic hormones in the Sokoto semi-arid region of Nigeria was evident on the reproductive performance as tested on *H. bidorsalis*. However, the obtained result clearly indicated that induced spawning with regard to high percentage hatchability and fry survival after one week was obtained from ovatide, this shows that, ovatide was more efficient when compared with ovary-prim. There is need to conduct further and intensive studies on the proper domestication of African giant Catfish *Heterobranchus bidorsalis* in order to have a better understanding of the species specific reproductive behavior in such environment.

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Table 1: Parameters Used in Spawning

Parameter	Treatment I (Ovary-prim)			Treatment II (Ovatide)		
	1	2	3	1	2	3
Weight of brood fish (g)	3000	3150	1600	3000	3600	1900
Hormone dose (ml)	0.5	0.5	0.5	0.2	0.2	0.2
Actual dose (ml)	1.5	1.58	0.8	0.6	0.72	0.38
Time of injection (hrs:min)	11:35	11:38	11:41	12:06	12:07	12:11
Time of stripping (hrs:min)	10:37	10:38	10:41	11:12	11:14	11:16

Table 2: Spawning Performance of *Heterobranchus bidorsalis* on Comparison between Ovary-prim and Ovatide inducing Hormones

Parameters	Treatment I (Ovary-prim)	Treatment II (Ovatide)
Latency period (Hrs.)	11:00	11:06
Average weight of egg spawned (g)	183.23±51.70	180.57±20.56
Spawning fecundity	109,000±34,261.39	104,640±26,160.00
Stripped percentage	6.89±0.91	6.55±0.56
Relative fecundity	39.92±6.73	35.82±4.64
Number of fertilized eggs	80441.67±20991.97	60690.67±22181.31
Percentage fertilization (%)	78.00±7.02	76.00±6.11
Percentage hatchability (%)	72±3.06^{b}	86.67±8.84^{a}
Percentage survival (%)	58.21± 0.51	69.42±0.39^{a}

Table 3: Mean water quality parameter during experimental period

Parameters	Morning	Afternoon	Evening
Water Temperature (°C)			
Mean	28.05±0.81	28.79±0.87	28.80±0.25
Minimum	28.1	28.7	28.3
Maximum	28.7	29.3	30.5
Room Temperature (°C)			
Mean	27.08±0.13	28.82±0.30	28.73±0.36
Minimum	26.9	28.2	27.8
Maximum	27.7	30.6	30.7
pH			

Mean	7.97±0.29	7.92±0.12	7.96±0.12
Minimum	7.21	7.18	7.18
Maximum	7.98	7.98	8.01
Electrical Conductivity (µm/cm)			
Mean	548.64±11.80	550.43±15.92	556.71±14.45
Minimum	463	451	453
Maximum	561	569	557

Google Earth, 2011. Image. Viewer.

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