EFFICIENCY OF FOUR REPRODUCTIVE HORMONES IN THE ARTIFICIAL PROPAGATION OF *Heterotisniloticus*(Cuvier, 1829)

ABSTRACT

The efficiency of four different hormonal preparations (*Heterotisniloticus* pituitary extract, ovaprim, *Clarias gariepinus* pituitary extract and carp pituitary extract) on latency period, fecundity, fertilization, hatching, and larval survival in artificial propagation of *Heterotisniloticus* was investigated. Total of twelve females and twelve males earthen pond raised, 24months old broods were used. The females were divided into four groups. Each group wasinduced to spawn with the same hormone. Sperm from one male was used to fertilize eggs strippedfrom individual females. The broods were injected with four different hormonal preparations; Treatment 1,*Heterotisniloticus* pituitary extract (HnPE); Treatment 2, ovaprim (Ova); Treatment 3, *Clarias gariepinus* pituitary extract (CgPE) and Treatment 4, CPE: all replicated thrice in a completely randomized design. The results of the experiment were subjected to analysis of variance (ANOVA) and all of them received equal treatment. Latency period was significant ($p<0.05$) among the treatments, as the period lasted for 10hrs, 9hrs, 12hrs and 11hrs for Trt1, Trt2, Trt3 and Trt4 respectively at a temperature of 28^{o}C. Percentage egg fertilization among treatments was significant ($p<0.05$) with the highest value of 89.66% and 89.33% recorded with HnPE and Ovaprim respectively, and lowest result of 67.33% recorded with CgPE. Percentage egg hatchability among treatments was significant ($p<0.05$) with the highest value of 84.16% and 80.66% recorded with Ovaprim and HnPE respectively, while the lowest result of 57.33% and 59.50% was recorded with CgPE and CPE respectively. Percentage larval survival among treatments was significant ($p<0.05$) the survival was highest with eggs hatched with Ovaprim (68.66%) closely followed with CPE (66.66%) and HnPE (63.16%) while the lowest result of 47.33% and 49.50% was recorded with CgPE and HnPE respectively. The results obtained, indicates the possibility of achieving the effectiveness of controlled reproduction in *Heterotisniloticus* studied, by applying the appropriate type of hormonal stimulation. At the end of this research, there were disparities in the efficiency of the four hormonal preparations and it was evident though one (ovaprim) gave the highest reproductive indices in fecundity, fertilization, hatchability and larval survival as it can be easily accessible though expensive in affirmation with the work of Asangusung and Uka (2016) than other one's but on the average, ovaprim, *Heterotisniloticus* pituitary extract and CPE could successfully be depended upon for commercial propagation of *Heterotisniloticus*.

Keywords: Hormones, latency period, fecundity, fertilization, hatchability, survival, *Heterotisniloticus*.

INTRODUCTION

Hormones are "chemical messengers in the body of living organisms" also defined as chemicals that are produced in an organ or gland and then carried by the blood to another part of the body where they produce a special effect for which they were designed. The term hormone is derived from a Greek word meaning "to excite". The use of synthetics and natural hormones brings about quick ovulation, high percentage of hatched fish, though synthetic hormones give higher yield than the natural hormones (Asangusung and Uka, 2016).

The successful large-scale production of any organism for human consumption demands, that the resources be easily renewable. It is clearly disadvantageous to cultivate any organism when the supply of the young cannot be easily replenished (Asangusung and Uka, 2016). The high demand for fish fingerlings in the phenomenal growing aquaculture industry has stimulated the need for artificial propagation of cultural warm water fishes, as 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, 2016).

The African bonytongue *"Heterotisniloticus"* belongs to the family – Arapaidae, order – Osteoglossidae, generally called "Bonytongue" and locally named as "Ecomog fish", with a maximum size of 100cm in its standard length (SL) and a maximum weight of 10.2kg. This fish is a pelagic fresh water dweller considered as mud feeders**,** which serves a purpose in aquaculture and for commercial seed also, as it is normally found in the tropics of 25-30 and 18 -22 in the Nile (Asangusung, and Uka, 2016). The male has anal and urogenital openings just anterior to the anal fin whereas the female has a genital orifice separate from the urinary opening. Family characteristics also include: a scale-less head, a large mouth usually turned upwards, pointed pectorals and small pelvic fins, a small or reduced caudal and a mosaic-like pattern of large bony scales (Adite*et al*., 2006; Froese and Pauly, 2012).

The present study was initiated to evaluate the efficiencies of these hormonal preparations; *Heterotisniloticus* pituitary extract, ovaprim, *Clarias gariepinus* pituitary extract and C.P.E, as hatchery propagation of *Heterotisniloticus*has not been in existence in Nigeria before now, because of some factors. Therefore, most hatchery users find it difficult to domesticate (commercialize) the existence of this species, during breeding. More often than not, the choice in the use of these hormonal preparations among many farmers in Nigeria is almost entirely based only on convenience and availability. There was therefore, need to match the commercially available hormonal preparations with efficiency of their usage for profitable decision and rewarding recommendations in choice of hormonal preparations; to evaluate the best ovulating agent (hormone) on *Heterotisniloticus* as it affects their: latency period, fecundity, fertilization, hatchability and larval survival that will help in setting pace for the production of this species; since their natural reproductive habitats (nursery grounds) are gradually going into extinction via destruction of mangroves swamps in Nigeria (in the quest for wealth). Acknowledging the fact that, these mangrove swamps and floodplains in fresh water environment: serves as their breeding and nursery ground (Froese and Pauly, 2012).

MATERIALS AND METHODS

Earthen pond raised 24 months old gravid broods were selected. All brood fish were selected by external morphological characteristics, using the method of Asangusung and Uka (2016). Twelve (12) males and females with average weight of 2.20kg were selected. Spawning containers of fifty (50) liters capacity with flow through system were used. The containers were labeled; A1, A2, A3; B1, B2, B3; C1, C2, C3 and D1, D2, D3 for treatments and replicates. The hormonal agents were injected intramuscularly into the dorsal muscles above the lateral lines and below the anterior part of the dorsal fin of each brood fish, as no scale was injured. The selected gravid females were injected with 5.0mg/kg *Heterotisniloticus* pituitary extract, 0.5ml/kg ovaprim, 5.0mg/kg *Clarias gariepinus* extract and 6.0mg/kg C.P.E in Treatment A, B, C and D respectively.

Each treatment was replicated thrice, as the temperature of water in the plastic basin (holding each female brood fish) were recorded using a thermometer (fisher brand) to determine the latency period (with reference to injection and stripping period). The injected fish were returned separately into their respective 50 liters aquaria. The ovulated female were carefully caught with a hand net and held tightly by two persons using wet towel. The main operator, held the spawners head with one other hand from the anterior to the pectoral fin onto the genital papilla with a slight pressure. The second operator held the tail of the female fish, as the hormone treatment caused the ovulated eggs to flow out easily (in a thick-jet form) from the genital papilla, and the eggs were collected into a dry plastic. At completion of stripping, only few eggs were ejected and some blood together with translucent eggs appeared, which was a sign that all or nearly all the ovulated eggs has been released. Sperm from each matured males were used to

fertilize eggs stripped from each replicate. 1g of the ovulated eggs were used from each female and fertilized for spawning (Asangusung and Uka, 2016). Milt was squeezed over the eggs and stirred. The two sex products were mixed with plastic spoon, as 0.9% saline solution was added and further agitated. The process from stripping to incubation took two minutes to be accomplished.

The fertilized eggs were spread into the already prepared incubation tanks containing forty (40) liters water for incubation, in a single layer to avoid suffocation and death of the eggs that were under others; and were equipped with water flow-through facilities. Water parameters were monitored, temperature was measured with centigrade thermometer, pH was monitored using Hanna pH meter (model pH-009 111) and optimum oxygen level (model EUTECH DO 600) was maintained with water flow through system.Incubation was carefully done to ensure that the eggs were properly deposited on artificially made kakaban (mosquito nets and plastic floaters). The percent fertilization was estimated from the surviving embryos ten (10) hours after fertilization and the fertilized eggs were counted physically. Few hours after fertilization, the dead and unviable eggs, which turned whitish, were carefully siphoned out to prevent infections and subsequently mortality of the fertilized eggs, and the unhatched eggs were counted physically.

After determining the hatching rate, the unhatched eggs were siphoned out of the spawning bowls in order to ensure the survival of the hatched ones. After their yolk sac absorption, one hundred (100) larvae per replicate were carefully siphoned, stocked and fed with Skretting 0.1mm (68% CP), as water was partially changed with utmost care via a flow through system. Mortality rate was observed daily, while the rate of survival was counted on daily bases, as early mortalities sets in within 14days of feeding; thus the rate of survival was counted. The following parameters were calculated:

Fecundity was estimated as follows (Asangusung and Uka, 2016)
Fecundity (Total no. of eggs stripped per fish) = 00.00g
Fertilization in each experiment was calculated as follows (Asangusung and Uka, 2016)

$$\% \text{ Fertilization} = \frac{\text{Number of fertilized eggs}}{\text{Total no. of eggs incubated}} \times 100$$

Hatchability in each experiment was calculated as follows (Asangusung and Uka, 2016)

$$\% \text{ Hatchability} = \frac{\text{Number of hatchlings}}{\text{Total no. of eggs incubated}} \times 100$$

Success in survival in each experiment was calculated as follows (Asangusung and Uka, 2016)

$$\% \text{ Survival} = \frac{\text{No. of fry after 14days feeding with coppens (0.1mm)}}{\text{No of larvae stocked after yolk sac absorption}} \times 100$$

Statistical analysis
Data collected were compared using a one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) to determine significant differences among means ($P< 0.05$).

RESULTS AND DISCUSSIONS

The effect of the four hormonal preparations, on the latency period, fecundity, fertilization, hatchability and larval survival of*Heterotisniloticus* fish was investigated. Latency period was significant ($p<0.05$) for *Heterotisniloticus* pituitary extract, ovaprim, *Clarias gariepinus* pituitary extract and carp pituitary extract (C.P.E) with 10hrs, 9hrs, 12hrs, and 11hrs respectively at a temperature of 28^{o}C (Table 1). These results are traceable to the work of Uka *et al.* (2019) which they observed that *C, gariepinus* exhibited latency period within 9-12hours at 27-28^{o}C; and Asangusng*et al.* (2018) also obtained their best results with a mean temperature of 27^{o}C; together with Asangusung and Uka (2016) work, which they succeeded in attaining 9hours, 10hours, and 11hours of oocyte maturation and ovulation for *Heterobranchuslongifilis*; with ovaprim, ovulin and C.P.E, respectively within the same temperature range.

Moreover, the effectiveness varied as the differences in time taken to achieve the attained oocyte maturation in the *Heterotisniloticus* were dependent on the type of hormones used, this agrees with the report of Asangusung and Uka (2016) on the efficiency of reproductive hormones on *Heterobranchuslongifilis*. The differences in the period of reaching their spawning maturation by the females used between groups stimulated with HnPE, Ov, CgPE and CPE can be as a result of their compositions of the preparation, especially for the two synthetic hormones used (Asangusung*et. al,* 2018).

The four fish reproductive hormones did not bring about the same response in the females in terms of egg production (expressed as fecundity). The fecundity of the females treated with different hormones were significantly difference ($p<0.05$) as the highest fecundity value was obtained from *Heterotisniloticus* induced with Ovaprim (47.00g) against other hormones that recorded 30.33g, 30.00g and 29.33g for HnPE, CgPE and CPE, respectively as shown in Table 1 with an average weight of 2.20kg each. The difference in ovulation time resulting from treatment with different GnRH analogues has been recorded in other fishes in Carp (Asangusung and Uka, 2016). Crandell *et al*., (1995), Uka *et al.*, (2019) and Asangusung*et al.*, (2018) confirmed that the ripening of the ovary after injection depends on the type of hormones used to introduce the female fish.

Hatching occurred exactly 26hours 25mins, 28hours 20mins, 27hours 18mins, 24hours 50mins for *Heterotisniloticus* induced with HnPE, Ovaprim, *Clarias gariepinus* and CPE respectively (Table 1). This is affirming the work of Orji and Uyon (2006), Asangusung and Uka (2016), and Asangusung*et al.*, (2018) that inducing *Heterobranchuslongifilis* is successful with the inducement of ovaprim as ovulating agent at dosage level of 0.5ml/kg body weight. This result implies that ovaprim hormone may be the hormone of choice in the successful propagation of *Heterotisniloticus*, since it was able to enhance ovulation greatly compared to other hormones used but this result could vary with the sources and batch of the hormone which might affect its viability or potency. This aligns the works of other researchers which stipulates that the use of synthetics and natural hormones brings about quick ovulation, high percentage of hatched fish, though synthetic hormones give higher yield than the natural hormones (Richter and Van den Hurk, 1996) as some of these hormonal materials (natural and synthetic) include cHCG (Eyo, 1997; 1998), HCG (Eyo, 2002); clomiphene citrate (Aguigwo, 1991), pituitary extract (Janssen, 1985; Haniffa*et al*., 2000) and Ovaprim (Manosroi*et al*., 2004; Abol- Munafi*et al*., 2006). Secondly, when the hormone is used as a single knockout dose, as in this study or as split doses as recommended by Asangusung and Uka (2016) together with Asangusung, *et al.*, (2018), the result may probably be affected.

Table 1: Latency period and mean reproductive efficiencies of four hormonal agents in artificial propagation of *Heterotisniloticus*.

Parameters		***H.n* PE**	**Ovaprim**	***C.g* PE**	**C.P.E**
Latency Period	Duration to spawning (hrs.) at 28^{o}C	10	9	12	11
Fecundity	Mean wt. of eggs produced (g)	30.33	47.00	30.00	29.33
Mean of total eggs produced		25,780	39,950	25,500	24,930
Average wt. of 3 female parents (kg)		2.20	2.20	2.30	2.20
Relative fecundity (g^{-1})		13.79	21.36	13.04	13.33
Hatching Commencement Time (hrs)		26:25	28:20	27:18	24:50
Termination Time (hrs)		36:05	38:05	39:10	38:25
Duration of hatching (hrs)		09:40	09:45	11:52	13:35

The fertilization of eggs obtained from the administration of the four fish reproductive hormones was highly significant ($p<0.05$). The fertilization rate from HnPE induced fish gave the highest result with 89.66% followed by ovaprim with 89.33% (Table 2). The lowest egg fertilization was recorded from the administration of CgPE and CPE as 67.33% and 69.83% respectively (Table 2).The hatchability rate of eggs obtained from the administration of four fish reproductive hormones was highly significant ($p<0.05$). The fertilized eggs demonstrated high hatchability values, as the highest hatchability rate was recorded with *Heterostisniloticus* induced with ovaprim (84.16%) followed by *Heterotisniloticus* induced with HnPE (80.66%) while the lowest hatchability rate was recorded with *Heterotisniloticus* induced with CgPE with value of 59.50% (Table 2).The survival of larvae from eggs obtained with the administration of four fish reproductive hormones were highly significant ($p<0.05$). The *Heterotisniloticus* induced with ovaprim had the highest value of survival with 68.66%; closely followed with *Heterotisniloticus* induced with HnPE (63.16%); while the second runner up was *Heterotisniloticus*induced with CPE with a survival rate of 66.66% (Table 2).

Generally, it was observed and physically affirmed that the highly performed tanks with highest rate in fertilization, hatchability, and larval survival were attainable with *Heterotisniloticus* induced with HnPE (Control), ovaprim and CPE may be due to the tolerable physico-chemical qualities of the culture water (Aliu and Obasogie, 2006 and Asangusung, *et. al.*, 2018). However, the percentage fertilization (89.33%), hatchability (84.16%) and survival rate (68.66%) induced with ovaprim were significantly ($p<0.05$) higher than all other results. The survival of fry's was not highly significant ($p<0.05$) this may imply that the hormones induced may not had have a residual effect once the embryos had fully developed and hatched out as it is generally said and affirmed by advanced researches that the effects of hormones inducement does not exceed the pre vitellogenic stage, thus, after complete maturation of

oocyte and ovulation process (Asangusung, *et. al.*, 2018) but the rearing conditions and water management that will greatly determine the survival of the hatchlings, as this also allied with the reports of Nlewadim*et al.* (2004). For the purpose of information on *Heterotisniloticus* eggs size, the eggs stripped were measured but there was no significant difference in their sizes based on different hormones inducement weighing 0.82 – 0.88mg and diameter 2.2 – 2.8mm; affirming the reports of Moreau (1982), recording an average size of 2.5 – 3mm and fecundity 2,700 – 27,500eggs, with maturity stage of tw**o** (2) years. This brings awareness for more research to be carried out on egg development of this species.

Table 2: Effects of hormonal agents on Egg Fertilization, Hatchability, Larval survival and Egg sizes in *Heterotisniloticus.*

Parameters	H.n PE	Ovaprim	C.g PE	C.P.E	Test
Fertilization	89.66^{a}	89.33^{b}	74.16^{d}	80.00^{c}	*
Hatchability	80.66^{b}	84.16^{a}	66.66^{d}	73.16^{c}	*
Larval survival	63.16^{c}	68.66^{a}	53.33^{d}	66.66^{b}	*
Egg size (mg)	0.86	0.84	0.88	0.86	ns

The average water quality parameter recorded during the study, showed no significant differences ($p>0.05$) amongst the water parameter during the experimental period. Number of environmental factors such as temperature, pH, dissolved oxygen and TDS; play decisive roles in ovulation, as temperature is of vital importance. 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 26-28^{o}C, dissolved oxygen range of 7-9mg/l and a pH of 6-7 was recorded throughout the experiment as this conforms with the result recorded with Uka *et al*. (2019) in the effects of different synthetic hormonal preparation on fertilization and hatching in *C. gariepinus* and also validates the work of Asangusung and Uka (2016), they observed *H. longifilis* exhibiting latency period with the best results at 25^{o}C. The pH of 6.00 to 8.00 was within normal range for culture fishes (Asangusung and Uka, 2016). The pH obtained, in this study were lower than the pH obtained by Asangusung*et al*. (2018). The temperatures obtained in this study are slightly higher than the temperature obtained by Asangusung*et al*. (2018), and that of Asangusung and Uka (2016). The essence of measuring water quality in this research work was to ensure that we do not introduce variables other than the ones to be evaluated.

Table 3 Mean water quality recorded during hatchery propagation of *Heterotisniloticus* with different hormonal agents.

Type of Tank	Temp. (^{o}C)	DO (mg/l)	pH	TDS (pp/m)
Broodstock	28	9.65	7.45	187
Incubation	27	7.50	6.75	146
Fertilization stage	26	9.50	7.85	186
Hatching stage	28	8.20	7.00	206
Larval rearing	26	7.00	5.90	210
Statistic Test	ns	Ns	ns	ns

Water quality results recorded during fertilization indicates no significant differences in dissolved oxygen and pH in all levels irrespective of the different hormone treatments; as this can be attributed to

the fact that there is no respiration of any kind, in the system that would warrant any depletion in oxygen abundance within the 0–8hours before hatching commences. Furthermore, the non significant appearance within the system during fertilization can be attributed to the active process of a flow through system that perhaps didn't allow the disintegration of some eggs that were not welled placed in the kakaban to accumulate the incubation water system to be altered with the presence of ammonia.

CONCLUSION

The results obtained, indicates the possibility of achieving the effectiveness of controlled reproduction in *Heterotisniloticus* studied, by applying the appropriate type of hormonal stimulation. At the end of this research, there were disparities in the efficiency of the four (4) hormonal preparations and it was evident though one (ovaprim) gave the highest reproductive indices in fecundity, fertilization, hatchability and larval survival as it can be easily accessible though expensive in affirmation with the work of Asangusung and Uka (2016) than other one's but on the average, ovaprim, *Heterotisniloticus* pituitary extract and CPE could successfully be depended upon for commercial propagation of *Heterotisniloticus*.

No wonder Bake and Sadiku (2005) described a decline in the population density of *H. niloticus* from Oyun reservoir, Nigeria, over a two-year period (January 2002-December 2003), however their records indicated a decline of the species from similar reservoirs in Nigeria, showing that the species is threatened in this environment, due to general environmental degradation including oil spillages, pollution and most importantly; destruction of mangrove swamps, this species has lost an estimated 60% of its previous breeding and nursery habitat in Nigeria.

This is because, aquaculture in Nigeria is essentially focused on catfish and tilapia with few other species including *H. niloticus* gaining little interest among fish farmers in southern Nigeria. The few culturists who culture *H. niloticus* depend on the artisanal fishermen and the wild for the juvenile and broodstocks. Information on its propagation using hormone induced techniques was lacking hence the need for increased stock propagation through induced breeding, with the recommended hormones to enhance fry and juvenile availability for farmers to use and more production of this species to be facilitated.

RECOMMENDATIONS

Based on the results of this study, ovaprim, *Heterotisniloticus* pituitary extract and CPE are recommended for hatchery propagation of *Heterotisniloticus* (seed) production.

Secondly, further investigation should be conducted on the effective split doses and single knockout doses of ovaprim, *Heterotisniloticus* pituitary extract and CPE together with other hormones.

Thirdly, more research work should be carried out on this species in order to avoid the species moving into extinction via over exploitation by our fishermen without commercializing the stock for pond culture other than accessing it from the wild completely when compared to our African catfishes and Tilapia.

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