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Assessment of the Effects of Some Reproductive Hormones on Aspects of Reproductive Biology of *Clarias gariepinus* and *Heterobranchus longifilis*

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Abstract

Protein food sources are highly recommended for a healthy living and fish is one of the most recommended protein sources. This research was aimed at enhancing fish seed propagation and the effects of four fish reproductive hormones (Ovaprim, Carp pituitary extract, human chorionic gonadotropin, and Deoxy-corticosterone acetate) on some aspects of reproductive biology of *Heterobranchus longifilis* and *Clarias gariepinus bought from Itu head-bridge, Akwa Ibom state.* Sixty mature broodstocks (both males and females) of each species weighing 2.0 kg were used in this study. The experimental procedure was made up of four experimental groups (treatments) with three replicates including control. Each group was administered with the appropriate dosage of the respective reproductive hormone. The mean relative fecundity in both species using ovaprim and human chorionic gonadotropin showed no significant difference, while carp pituitary extract showed significant difference ($p < 0.05$) in both species. The mean egg hatchability values in the species administered with ovaprim, human chorionic gonadotropin and carp pituitary extract were similar ($p > 0.05$). Mean values were significantly different ($p < 0.05$) from those administered with Deoxy-corticosterone acetate. The mean fry survival rates in both species were not significantly different ($p > 0.05$) with human chorionic gonadotropin, carp pituitary extract and ovaprim, but showed significant difference ($p < 0.05$), except Deoxy-corticosterone acetate. Female brooders in the control tanks showed no result throughout the study. Treatments with the hormones yielded better result, except in the case of Deoxy-corticosterone acetate. It could be concluded that reproductive hormones, especially ovaprim and carp pituitary are better for breeding *H. longifilis* and *C. gariepinus*. The results from the study are crucial in encouraging fish seed propagation through the application of artificial hormones.

Keywords: Propagation; hormones; pituitary; treatment; broodstocks; gonadotropin.
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Introduction

The culture of clariid catfish had been dependent on annual catch of fingerlings from the wild, but nowadays, they can be bred in captivity through hormonal inducement (hypophysation) (Orji and Uyon, 2006 and Cacot et al., 2002). Though aquatic bio-resources are finite but renewable, efforts must be geared towards increasing its production through improved resource conservation and effective aquaculture practices.

The uncertain government policy on fish production coupled with the increasing demand for fish in Nigeria have increased awareness in aquaculture. This to a large extent has enhanced the provision of high-quality animal protein, income and employment to the citizenry (Delince et al., 1997). Fish culture in Nigeria is dominated by the culture of catfish species of the family Clariidae (Adewumi and Olaleye, 2011). This family consists of the genera *Clarias* and *Heterobranchus*. According to Dada and Madu (1996), these species are very important to the sustainability of the aquaculture industry in Nigeria.

The success in cultivating any organism in a large scale for human consumption and socio-economic development demands that the resource should be renewable and affordable. Several authors have demonstrated the use of crude pituitary extract for induced breeding of different species and contributed greatly towards availability of seeds (Ndimele and Owodeine 2012; Sahoo et al. 2005, Tahghi et al., 2010; Oguntuase and Adebayo, 2014; Charo and Oirere (2020). However, this success story is punctured by the problem of choice of hormone, since it is dependent on the type of hormone (Nwokoye et al., 2007). The implication of this phenomenon is that catfish seeds could still be scarce and limited for stocking fish farms leading to low yield of table fish. Therefore, the eventual hope of Nigerians producing enough fish from aquaculture to compliment capture fisheries to achieve self-sufficiency in fish production and for export could still not be realized.

Commercially, various hormones have been prepared and marketed for the purpose of fish breeding. These include, Crude Pituitary Extract (CPE), Deoxycorticosterone Acetate (DOCA), Human Chorionic Gonadotropin (HCG), Ovaprin and Ovatide, Nwokoye et al., 2007; Taghi et al., 2010).

This work is aimed at evaluating the effects of four fish reproductive hormones and to determine the best ovulating agent (hormone) for artificial breeding of the clarids, (*Heterobranchus longifilis* and *Clarias gariepinus*) as it affects fecundity, fertility, hatchability and fry survival.

Materials and Methods

Study Area

The study was carried out at the fisheries unit of United Nation Development Project (UNDP) – assisted Multipurpose Co-operative Society Limited, Uyo in Akwa Ibom State.

Broodstocks Procurement and Maintenance

The broodstocks consisting of 30 males and 30 females of both *Heterobranchus longifilis* and *Clarias gariepinus*, weighing 2.0kg were removed from the brooder ponds and stocked in concrete tanks for a period of 3 weeks for acclimatization before inducement for this study. The broodstocks were chosen based on their morphological characteristics as documented by (Ayinla et al., 1994). The selected fishes were weighed individually, and the different weights recorded.

The fishes were later stocked individually in plastic troughs of 25 L capacity each.

Experimental Design

The experimental design for the study was a Completely Randomized Design (CRD) with four treatments consisting of Ovaprim, Human Chorionic Ganodotropin (HCG), Crude Pituitary Extract (CPE) and Deoxy-corticosterone Acetate (DOCA) for each species. Each treatment had three replicates with control containing one female and two male broodstocks (Uyon and Orji, (2006).

Preparation of the Spawning Materials

Prior to the commencement of breeding activities, broodstock ponds and hatchery tanks used for the study were thoroughly washed and disinfected, shredded polythene bags which served as kakabans, which served as spawning mats were also disinfected and rinsed before used.

Preparation of Hormones and Injection of Brooders

The quantity of the hormones (Carp Pituitary Extract (CPE), Human Chorionic Gonadotropin (HCG), Ovaprim and Deoxy-Corticosterone Acetate (DOCA) used was commensurate with the weight of brooders to be injected. The hormones were purchased from the market. Each brooder weighed 2.0 kg.

Control

The broodstocks used as control were injected with calculated dose of 0.6 % saline solution (Adigen et al., 1993).

Administration of Hormones

The female broodstocks (30) were brought out for hormonal administration when 90 % of their population were gravid and were approximately uniform in weight being 2.0 kg each. The fishes were divided into five groups corresponding to the four hormones used and the control, and later starved 24 hours before the exercise and anesthetized prior to injection as described by Wontz and Smith (1998). Each female broodstock in a particular group was weighed with top-loading balance (Setra, BL 310) before the administration of the specific hormone. A 2 ml syringe with its standard hypodermal needle was used to administer the correct dosage of the hormone to the female broodstock. Dosages used were, Ovaprim - 0.5 ml/kg, Crude Pituitary extract - 4mg/kg, Human chorionic gonadotropin - 12,000 I.Ukg and Deoxycorticosterone acetate - 50 mg/kg body weight (Obi and Popoola, 1994, Ufodike and et al., 1996 Orji and Uyon, 2006). The injection was done intramuscularly near the base of the caudal peduncle. The injected part was rubbed gently with the thumb for a few seconds to aid even distribution of the hormone, while slowly retracting the needle. After the administration of the hormone, the female broodstocks in each group were returned to their holding tanks where they spent their latency period of 11 hours at 28^{o}C. The entire exercise lasted for forty-eight hours (48 hours). Doses were calculated and determined based on the body weight and concentration of the hormone.

Extraction of Milt

The selection of the ripe male broodstocks was based on the appearance of elongated genital papillae with reddish tip in *Clarias gariepinus* and a dark tip in *Heterobranchus longifilis* respectively. The selected males were tranquilized according to Orji and Uyon, (2006) and placed dorsally on a wet mat. The male broodstocks were sacrificed, testes removed, dissected and the milt collected in two (2) separate labeled plastic bottles according to species. The milt obtained was stored in a refrigerator at 4^{o}C.

Stripping/Fertilization Exercise

At the end of 11 hours latency period, each female broodstock in each group was carefully netted out, weighed, and held with a wet towel. The eggs were stripped out into dry plastic bowls according to Ufodike and Madu, (2001). Dry fertilization method of Tan-Fermin and Emata (1993) was adopted in this study. The eggs collected from each female broodstock were gently stirred with a pre-ethanol washed quill feather to obtain a complete homogenized egg mass. Equal sample of 50 g of the homogenized eggs was taken out from each female broodstock for fertilization in a small bowl individually in both species. 1ml of the stored milt of *Heterobranchus longifilis* and *Clarias gariepinus* was used in fertilizing each egg sample in the respective species. The mixture (milt and eggs) was stirred with the quill feather for a few seconds. To enhance the activation of the spermatozoa, addition of small quantity of fresh water (2 – 5 ml) was added out to the mixture. Calibrated syringe was used.

Incubation/Egg Hatchability

After the fertilization, the fertilized eggs were spread on a matrix of prepared spawning mats (kakabans), placed in hatchery tanks containing fresh water. The fertilized eggs were incubated until hatching occur. From each sample of eggs fertilized, a subsample of 200 eggs were collected randomly and incubated alongside the main sample. This was used for the determination of egg hatchability according to Obi and Popoola (1994). During the incubation period, water quality parameters (Dissolved Oxygen, temperature, and pH) were monitored and maintained at optimal levels. Eggs were incubated for 72 hrs.

Egg Fertility

In each replicate, after eight hours post-fertilization, a sub-sample of 200 eggs was randomly siphoned out. The number of fertilized and unfertilized eggs were noted and recorded. Percentage fertility of the female brooders for the two species was determined based on the samples obtained according to De Kimpe and Micha (1994).

Survivability

After hatching and determination of hatching rate, the unhatched eggs were siphoned from the spawning troughs, to ensure the survival of

the hatched ones and the water medium was partially removed with care. The observations of the set up were done on daily basis after hatching about 4 weeks to ascertain the rate of survival through the estimation of the dead and live ones (Oguntuase and Adebayo, 2014).

Data Analysis

(a) **Relative fecundity:** This was calculated through the number of eggs per unit of weight and is commonly used as an index of fecundity Hogendoom and Visamans (1980).

(b) **Egg fertility:** This was calculated according to De Kimpe and Uicha (1974), as fertility (%) = $\frac{N-n}{N} \times \frac{100}{1}$
Where N = total No. of eggs striped.
n = total No. of eggs unfertilized.

(c) **Egg hatchability:** This was calculated according to Obi and Popoola (1994).
Hatchability (%) = $\frac{\text{No.of hatched eggs}}{\text{No.of fertilized eggs}} \times \frac{100}{1}$

(d) **Fry survival rate:** This was calculated according to Obi and Popoola (1994).

(e) **Survival rate** (%) = $\frac{\text{No. of survivors}}{\text{No. of fry stocked}} \times \frac{100}{1}$

Statistical Analysis

Arcsine transformation of the percentage was carried out using arcsine table to convert the relative data to nominal data. The results were subjected to analysis of variance (ANOVA) to determine significant differences among means. Mean separation was conducted using LSD in mixed procedure mode of SAS.

Results

Water Quality Parameters

Table 1 shows the mean physico-chemical parameters of water in the experimental tanks. The broodstock and control experimental tanks recorded the highest temperature of 27.80 ^{O}C; the larval rearing tank recorded the highest concentration of Dissolved Oxygen (DO) of 10.95 mg/l, while broodstock and control tanks recorded the highest concentration of pH of 7.45 respectively.

Relative Fecundity

Tables 2 and 3, show the mean relative fecundity of *C. gariepinus* treated with four different reproductive hormones and the mean relative fecundity of *H. longifilis* treated with four different reproductive hormones, respectively. The result shows that the highest value of relative fecundity was obtained for *H. longifilis* with Ovaprim and was followed by human chorionic gonadotropin for *C. gariepinus*. The relative fecundity obtained for crude pituitary extract on the two species was similar ($p > 0.05$) and below what were obtained for Ovaprim and human chorionic gonadotropin. The control showed no positive result on the two studied species.

Table 1: Mean physico-chemical parameters of water in the experimental tanks

Tanks	Temp. (^{0}C)	DO (mg/l)	pH
Broodstock	27.80 ± 1.81	9.50 ± 0.90	7.45 ± 0.26
Incubation	27.20 ± 0.50	10.00 ± 0.72	7.30 ± 0.12
Larval rearing	28.35 ± 0.19	10.95 ± 0.07	7.30 ± 0.45
Control	27.80 ± 1.81	9.50 ± 0.90	7.45 ± 0.26

Table 2: Mean relative fecundity of *Clarias gariepinus* treated with four different reproductive hormones

Observation	Fish species	Hormone	Mean value
1	*C. gariepinus*	HCG	145.667^{a}
2	*C. gariepinus*	OVA	141.000^{a}
3	*C. gariepinus*	CPE	80.500^{b}
4	*C. gariepinus*	DOCA	22.667^{c}
5	Control	Saline solution	0.00

means with same superscripts are not significantly different (P > 0.05)

Table 3: Mean relative fecundity of *Heterobranchus longifilis* treated with four different reproductive hormones

Observation	Fish species	Hormone	Mean value
1	*H. longifilis*	OVA	148.167[a]
2	*H. longifilis*	HCG	136.033[a]
3	*H. longifilis*	CPE	85.167[b]
4	*H. longifilis*	DOCA	10.500[c]
5	Control	Saline solution	0[d]

means with same superscripts are not significantly different (P > 0.01)

Egg Fertility

Tables 4 and 5 show the mean egg fertility rates of *C. gariepinus* treated with four different reproductive hormones and the mean egg fertility rates or *H. longifilis* treated with four different reproductive hormones, respectively. The highest fertility rate was obtained in both species with the treatment of Ovaprim hormones. Deoxy-corticosterone acetate showed the poorest egg fertility rate with a zero value in both species. The control broodstock did not show any value.

Egg Hatchability

Tables 6 and 7, show the mean egg hatchability rates of *C. gariepinus* and *H. longifilis* respectively. The result indicated that the eggs obtained from both species and induced with Deoxycorticorrtrone acetate did not hatch. The egg hatchability observed from both species showed superior performance with Ovaprim. The control broodstocks showed a zero value.

Table 4: Mean egg fertility rates of *Clarias gariepinus* treated with four different reproductive hormones

Observation	Fish species	Hormone	Mean value
1	*C. gariepinus*	OVA	61.4903[a]
2	*C. gariepinus*	HCG	44.8602[ab]
3	*C. gariepinus*	CPE	49.4389[b]
4	*C. gariepinus*	DOCA	0[c]
5	Control	Saline solution	0[c]

means with the same superscripts are not significantly different (p > 0.05)

Table 5: Mean egg fertility rates of *Heterobranchus longifilis* treated with four different reproductive hormones

Observation	Fish species	Hormone	Mean value
1	*H. longifilis*	OVA	61.2450[a]
2	*H. longifilis*	HCG	55.5936[a]
3	*H. longifilis*	CPE	51.3253[b]
4	*H. longifilis*	DOCA	0[c]
5	Control	Saline solution	0[c]

means with the same superscripts are not significantly different (p > 0.05)

Table 6: Mean egg hatchability rates of *C. gariepinus* treated with four different reproductive hormones

Observation	Fish species	Hormone	Mean value
1	*C. gariepinus*	OVA	66.6818[a]
2	*C. gariepinus*	HCG	66.2304[a]
3	*C. gariepinus*	CPE	62.4761[b]
4	*C. gariepinus*	DOCA	0[c]
5	Control	Saline solution	0[c]

means with the same superscripts are not significantly different (p > 0.05)

Table 7: Mean egg hatchability rates of *H. longifilis* treated with four different reproductive hormones

Observation	Fish species	Hormone	Mean value
1	*H. longifilis*	OVA	70.9180[a]
2	*H. longifilis*	HCG	66.9975[b]
3	*H. longifilis*	CPE	65.9646[b]
4	*H. longifilis*	DOCA	0[c]
5	Control	Saline solution	0[c]

means with the same superscripts are not significantly different (p > 0.05)

Table 8: Mean survival rates of *Clarias gariepinus* treated with four different reproductive hormones

Observation	Fish species	Hormone	Mean value
1	*C. gariepinus*	HCG	61.84[a]
2	*C. gariepinus*	CPE	60.00[a]
3	*C. gariepinus*	OVA	60.00[a]
4	*C. gariepinus*	DOCA	0.00[b]
5	Control	Saline solution	0.00[b]

means with the same superscripts are not significantly different (p > 0.05)

Table 9: Mean survival rates of *Heterobranchus longifilis* treated with four different reproductive hormones

Observation	Fish species	Hormone	Mean value
1	*H. longifilis*	OVA	61.00[a]
2	*H. longifilis*	CPE	60.84[a]
3	*H. longifilis*	HCG	59.84[a]
4	*H. longifilis*	DOCA	0.00[b]
5	Control	Saline solution	0.00[b]

means with the same superscripts are not significantly different (p > 0.05)

Table 10: Mean final weights gained with the mean post-spawning weight of *C. gariepinus*

Hormone	$\bar{x}$ pre-spawning wt (g)	$\bar{x}$ pre-spawning wt (g)	$\bar{x}$ final wt. gained (g)	$\bar{x}$ wt. gained (g)
OVA	1998.67	1787.00	2055.67	258.67
HCG	1999.00	1790.67	2037.34	246.67
CPE	1998.33	1883.33	2123.33	240.00
DOCA	2001.00	2000.00	2300.33	300.00
Control	2000.00	2000.00	2300.33	300.00

Table 11: Mean final weights gained with the mean post-spawning weight of *H. longifilis*

Hormone	$\bar{x}$ pre-spawning wt (g)	$\bar{x}$ pre-spawning wt (g)	$\bar{x}$ final wt. gained (g)	$\bar{x}$ wt. gained (g)
OVA	1998.33	1786.67	2063.34	276.67
HCG	1995.00	1806.67	2092.34	291.67
CPE	2000.33	1878.67	2153.67	275.00
DOCA	2002.00	1987.00	2325.33	338.33
Control	2000.00	2000.00	2364.00	364.00

Fry Survival Rates

Tables 8 and 9 show the mean survival rates of *C. gariepinus* and *H. longifilis* respectively, both species treated with deoxycorticosterone acetate hormones gave a zero result of survival rates. Survival rate was highest for *C. gariepinus* treated with HCG, followed by *Heterobranchus longifilis* treated with Ovaprim. The control broodstock shows a zero rate of survival.

Spawning Recrudescence

Table 10 and 11 show mean final weight gained with mean post-spawning weight of *C. gariepinus* and that of *H. longifilis* respectively. These indicated that the hormonal treatments had no adverse effect on the growth and spawning recrudescence of both female breeders of both species after the study period.

Discussion

Nowadays, induced fish breeding has been successfully reported on various fish species by many authors (Sahoo et al., 2005, Gbemiso and Nwokoye et al., 2007; Ataguiba et al., 2009; Taghi et al., 2010; Owodeine and Ndimele, 2011, Adebayo, 2014). The physico-chemical parameters of water in the various tanks used in this study were satisfactory for the culture of the fishes (Uyon, 2013). The good environmental conditions coupled with adequate handling of the brood fish accounted for low level of mortality during the study (Orji and Uyon, 2006). The four fish reproductive hormones used in this study did not prompt the same

responses as ovulating agents in the two species of fish (Adebiyi et al, 2013).

Ovaprim gave the best result of relative fecundity (148.167) in *Heterobranchus longifilis*, while human chorionic gonadotropin (HCG) gave the highest relative fecundity (145.667) in *Clarias gariepinus*. Though the performances of these two hormones in the two species were actually not significant at 0.05 level of probability ($P > 0.05$), they were effective and more productive to what was obtained with crude pituitary extract in both species. This confirms the works of Lengendre (1986); Hogendoom and Visamans (1988), Adebiyi et al., 2013. This result confirms that Ovaprin and Human chorionic gonadotropin (HCG) may be the hormones of choice in the economic propagation of these fish species, and further supports the works of Madu and Offor, (2004), and Adebiyi et al., (2013). The low viability of carp pituitary extract could be due to its poor storage (Orji and Uyo, 2006, Uyon, 2013) and method of application (Delince et al., 1998 and Uyon, 2013). The poor performance of Deoxy-corticosterone acetate in the ovulation process of the studied species could be due to poor storage (Orji and Uyon, 2006) and other handling factors (Hoga et al, 2018).

The fertility of eggs obtained from the administration of the four respective hormones did not indicate any bias between the studied species. The fertility rate with Ovaprim hormone induced eggs was the highest, followed by that of Human Chorionic gonadotropin and carp pituitary extracts. This was in consonance with report of Legendre (Legendre, 1996) and Uyon (2006). No viability of eggs treated with Deoxy-corticosterone was observed and this could probably be due to source of purchase and handling, which could affect its potency (Uyon, 2006).

All the three reproductive hormones (Ovaprim, human chorionic gonadotropin and crude pituitary extract), except deoxy-corticosterone acetate (DOCA) did not affect the survival of the fry [14-16]. This could imply that the hormone had no residual effect once the embryos had fully developed and hatched (Orji and Uyon, 2006, Hoga et al., 2018). In this case, it could be the rearing conditions and management that would determine the survival of the hatchlings [1]. The near uniformity of the fry survival rate and the satisfactory physico-chemical conditions in this study indicate a good management procedure (de Graaf et al., 1995) in this study.

After six (6) months post-stripping, the study showed that the fish reproductive hormones used as treatments had no adverse effect on the fish thus affirming the work of Adigun *et al.,* (1983). All female spent broodstocks in the study regained their normal growth and gravid characteristics. Deoxy-corticosterone acetate and saline solutions treatments in both species showed the highest growth and exhibited early signs of gravidity. This could be due to the zero effects of deoxycorticosterone acetate and saline solution of the control treatments on the fish. Hence, their female breeders recovered fast and showed signs of early gravidity (Orji and Uyon, 2006).

Conclusion

The study showed that reproductive hormones have no adverse effects on fish species fertility and recommends some commercial hormones for use specially to enhance aquaculture. The production of fish seeds could be guaranteed where potent fish hormones are available. This must be in the correct quantity and quality and at affordable cost which will allow the low-income farmers to afford. The potential of the four fish reproductive hormones as used in this study, has yielded that three reproductive hormones could be successfully employed in the boosting of reproductive performance for fish seed propagation. The result from this study is a crucial tool in encouraging fish seed propagation using artificial hormones. The study recommends further research on possible ways of substituting these hormones with organic materials to minimize cost on farmers.

Competing Interests

Authors have declared that no competing interests exist.

References

Adigun NO, Anthony AD, Barker JC. Induced Spawning of African Catfish, *Clarias lazera* using

Acetone-Dried Carp Pituitary, Deoxycorticosterone Acetate and Human Chorionic Gonadotropin. In: 3rd Annual Conference of the Fisheries Society of Nigeria (FISON), 22nd – 25th February, 1983, Maiduguri, Nigeria. 1983:108-110.

Ayinla OA. Proceedings of the Fish Seed Propagation Course conducted by African Regional Aquaculture Centre (ARAC), Rivers State, P.H. 14th - 28th August. 1991:25-39.

Cacot P, Legendre M, Dan TQ, Tung T, Thanliem P, Marijouls C, Lazard J. Induced Ovulation of *Pangasius bocurti* with a Progressive HCG Treatment. Aqua. 2002;213:199-206.

Dada AA, Madu CT. Comparative growth and survival of fry of *Clarias gariepinus (B), Clarias anguillaris, Heterobranchus bidorsalis (G), Herobranchus* and *Clarias* Hybrids under outdoor nursery management system. in: 13th Annual conference of Fisheries Society of Nigeria (FISON), 3rd-8th Nov. 1996, New Bussa, Nigeria. 1996:198-203.

De Kimpe P, Micha SC. First guidelines for the culture of *Clarias lazera* in central Africa. Aqua. 1974;4: 227-248.

Delince GA, Campell D, Janssen JA, Kutty MN. Seed production on overview: Lecture presented at African Regional Aquaculture Centre (ARAC) for the Senior Aquaculturists, ARAC/NIOMR, Port Harcourt, Rivers State, Nigeria, ARAC/87/WP/13. 1987:114.

Hoga CA, Almeida FL, Reyes FGR. A review on the use of hormones in fish farming: Analytical methods to determine their residues. Journ. of Food Sci. 2018;16(1):234-239.

Legendre M. Seasonal changes in sexual maturity and fecundity and HCG-induced breeding in the catfish, H. Laquaculture en milieu langunaire, Doc. Scie. Cent. Rech. O*ceanogr*. Abidjan, 1986;14(2):97-107.

Nwuba IA, Aguigwo JN. Studies on the effects of different food items on the survival of hatchlings of *Clarias anguillaris*. Journ. of Aqua. Sci. 2003;17(2):121-124.

Obi A, Popoola O. Effect of pituitary extracts of *O, niloticus, C. carpoi and C. gariepinus* on induced spawning of *C. gariepinus,* Journ. of Aqua. Sci. 1994;9:19-12.

Oguntuase BG, Adebayo OT. Sperm Quality and Reproductive Performance of male *Clarias gariepinus* induced with synthetic hormones (Ovatide & Ovaprim). Intern. Journ. of Fish. and Aqua. 2014;6(1):345-356.

Orji RCA, Uyon PP. Effects of latency period on aspects of the reproductive biology of *heterobranchus longifilis.* Ani. Pro. Res. Adv. 2006;2(4):221-226.

Orji RCA. Fish breeding technology in Nigeria. Issues in Tropical Animal Sciences. 2000;1(1): 220-228.

Tan-Fermin JD, Emata AC. Induced spawning by LHRHa and Pimozide in the fresh water. Asian Catfish, *Clarias macrocephalus,* Journ. of Appl. Ichth. 1993;9:89-96.

Ufodike EBC, Ede EAB, Anthony AD. Effect of intramuscular dose levels of DOCA and CPE on fecundity of *Clarias lazera,* Appl. Fish. and Hydro. 1986;1(1):17-20.

Uyon PP. Evaluation of Effects of four Fish Reproductive Hormones on some Aspects of Reproductive Biology of *Heterobranchus longifilis* and *Clarias garepinus*. Ph.D. Dissertation, Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike. 2013:247.