

## HOMESTEAD ARTIFICIAL PROPAGATION, GROWTH AND MORPHOMETRIC CHARACTERISTICS OF THE AFRICAN CATFISH (*Clarias gariepinus*, PISCES: CLARIIDAE)

<sup>1</sup>UDE, Emmanuel Fame, <sup>1</sup>UGWU, Lawrence Linus Chukwuma and <sup>2</sup>MGBENKA, Bernard Obialo  
Department of Animal Production and Fisheries Management, Ebonyi State University, PMB 053, Abakaliki, Ebonyi State, Nigeria

<sup>2</sup>Department of Zoology, Fish Nutrition and Aquaculture Unit, University of Nigeria, Nsukka, Nigeria

Corresponding Author: Dr. Ugwu, L. L. C., Department of Animal Production and Fisheries Management, Ebonyi State University, Abakaliki, Nigeria. Email: [dozlin@yahoo.com](mailto:dozlin@yahoo.com) Phone: +2348037508462

### ABSTRACT

Thirty (30), eighteen months old gravid females ( $458.20 \pm 2.256$ ) of the African catfish, *Clarias gariepinus* were injected intramuscularly with different doses (0.00, 10.00, 30.00, 50.00 and 70.00  $\mu\text{g Kg}^{-1}$ ) of luteinizing hormone releasing hormone analog (LHRHa) at a water temperature of  $25 \pm 1.00^\circ\text{C}$ . Fifteen (15) mature males ( $453.97 \pm 2.13\text{g}$ ) received half the dose given to the females and they provided the milt (spermatozoa) used in the artificial fertilization of ovulated eggs from females. The hatched fry were randomly allotted to 15 indoor concrete tanks ( $0.70 \times 1.50 \times 0.50\text{m}$ ), arranged in 5 rows with 3 replicates per row ( $5 \times 3$ ) and allowed to stay for 10 days. Twelve (12) concrete tanks ( $8.00 \times 4.00 \times 1.00\text{m}$ ) were used outdoor for the feeding of the advanced fry on formulated ration (CP = 38%) at 5% body weight per day for 7 days. The results of the artificial inducement of the catfish with different doses of LHRHa ( $10\text{-}70 \mu\text{g Kg}^{-1}$ ) indicate that there were significant variations in the percent ovulation ( $P < 0.01$ ), spawn weight ( $P < 0.01$ ), percent fertilization and survival ( $P < 0.05$ ) of the fish. The mean body weight, head diameter, standard and total body lengths of the fry also varied significantly among the different hormonal doses ( $P < 0.05$ ). These results signified that different doses of LHRHa affected the growth and morphometric indices of *C. gariepinus* fry.

**Keywords:** *Clarias gariepinus*, Luteinizing hormone, Artificial Inducement

### INTRODUCTION

Scarcity of fingerlings from the wild to stock existing ponds in tropical Africa, and the growing aquaculture industry have stimulated the propagation of culturable warm water fish species. Reports on induced spawning of fish using different hormonal materials (Hogendoorn and Vismans, 1980; Young *et al.*, 1989; Ayson, 1991) are available. Successful trials have also been reported with carp pituitary (Janseen, 1985), human chorionic gonadotropin (HCG) (Legendre, 1986), progesterone, and luteinizing hormone releasing hormone analog (LHRHa) (Richter *et al.*, 1987; Solar *et al.*, 1990).

Advances made in aquaculture include the understanding and application of scientific knowledge relating to piscine reproduction (Harvey *et al.*, 1993; Donaldson and Devlin, 1996; Donaldson, 2000, 2001; Lee and Donaldson, 2001; Zohar and Mylonas, 2001). The various contributions have enabled the sophistication of biochemical, physiological, endocrine and genetic technologies for the optimization of reproductive processes in cultured finfish. Endocrine techniques for the induction of ovulation and spermiation have advanced from the use of pituitary extracts to the use of gonadotropin releasing hormone (Gn RH) with or without dopamine antagonists (Donaldson, 2003). There have been advances in the

methods of the administration of the hormones by injection, by implantation, and more recently by dietary administration.

In many developing countries of the world, the application of sophisticated techniques to piscine reproduction is not much. In Nigeria, Nwadukwe (1993) used locally available frog pituitary extract to spawn the African catfish (*Heterobranchius longifilis*) Mustafa *et al.* (1984) spawned the Asian catfish (*Heteropneustes fossilis*, Bloch) with the pituitary extract from the Indian frog (*Rana tigrina*, Daudin). Semi-natural or hormone induced propagation of *Clarias gariepinus* in ponds/tanks has not proved to be a reliable method for mass propagation of fry (Delince *et al.*, 1987). Artificial propagation under controlled hatchery conditions has been adopted for the mass production of fry and fingerlings. The deliberate spawning of large numbers of tilapia became important in recent time due to the advances made in hybridization, genetic selection and the need to meet the growing demands of extension work (Delince *et al.*, 1987).

The technicality involved in the artificial spawning of *C. gariepinus*, and the cost of constructing modern hatcheries has greatly hindered the mass propagation of this species in Nigeria. The present study was conducted to determine the effect of different hormonal concentrations of LHRHa on growth

and morphometric characteristics of *C. gariepinus* fry. The aim was to provide information on the use of simple realizable techniques to achieve artificial propagation of the species and to keep pace with the current advances in piscine reproduction by using the LHRH analog.

## MATERIALS AND METHODS

**Collection of *C. gariepinus* Broodfish:** Thirty, eighteen months old gravid females ( $458.20 \pm 2.25$ ) and fifteen matured males ( $453.97 \pm 2.13$  g) of *C. gariepinus* were purchased from a private fish farmer at Ihiala, Anambra State, Nigeria. Identification of the individual brood fish was done following the method described by Reed *et al.* (1967). Selection of broodfish was based on ovarian biopsy of the oocytes as described by Legendre (1986). The selected fish were given 2 ppm potassium permanganate, prophylactic-tic treatment and stocked according to gender in two outdoor concrete tanks (1.80 x 1.20 x 0.80 m). Feeding was carried out twice daily at 3 % biomass for 14 days with locally formulated diet (CP = 38 %). The juveniles of *Oreochromis niloticus* (L.) were stocked in each tank to serve as natural food for *C. gariepinus*. In readiness for artificial spawning, the female broodfish (30) were scooped out of the concrete tanks in the evening and randomly introduced in 15 plastic containers (25 l) at 2 fish per container. The male broodfish (15) were left in the tank till the next morning when milt preparation was necessary.

**Arrangement of Water Holding Facilities:** Ten litres of dechlorinated tap water were introduced into 15 plastic containers (25 l) in 5 rows on elevated platforms in the mini-hatchery of the Department of Animal Production and Fisheries Management, Ebonyi State University, Abakaliki, Nigeria. Each plastic container was stocked with 2 female broodfish of *C. gariepinus* and left for 6 hours before hormone injection. Fifteen indoor concrete tanks (0.70 x 1.50 x 0.50 m) were washed and disinfected with 0.02 ppm malachite green (fungicide). Thirty centimeters of water were put in each tank from a 500 litre plastic water tank, suspended on 1.00 m high table. Water was sprayed onto the concrete tanks with perforated plastic hoses (0.050 cm diameter) from a height of 0.05m. Water was drained from the tanks through turndown pipes installed to regulate water volume.

**Preparation of Milt:** One male fish per a pair of female fish was killed, dissected, and the milt sac removed one hour prior to artificial spawning. The sac was cut open with a sharp razor blade and the milt washed into a vial with 0.90% saline solution. For this study, 12 vials with milt were prepared to cater for fish spawned with triplicates of 4 doses (10, 30, 50 and 70

$\mu\text{g Kg}^{-1}$ ) of luteinizing hormone releasing hormone analog (LHRHa), i.e.  $4 \times 3 =$ . Fish under the control experiment were also injected in triplicates with physiological solution (0.90 % saline).

**Artificial Spawning:** All the 30 gravid females were weighed ( $458 \pm 2.25$  g) 6 hours before the commencement of hormone injection at 8 pm. Six fish specimens (2 fish/container) from each row of triplicate plastic containers were injected with 2 ml of LHRHa with concentrations 10, 30, 50 and 70; while the fish in the control (6) were injected in triplicates with 0.90 % physiological (saline) solution. Similarly, 3 male fish (1 fish/container) for each row of triplicate plastic containers were injected with half dose (1 ml) of the hormone. In all cases, infection was intramuscularly just below the dorsal fin. Water temperature ( $25 \pm 1.0^{\circ}\text{C}$ ) was measured with a Celsius thermometer. All the induced fish were covered with wooden boards and left for 11 hours.

One induced female fish per plastic container was dissected to recover the ovaries and estimate the percent ovulation (i.e. the percentage of the oocytes in the ovary that were ovulated after injection). Stripping and fertilization commenced at 7 am the next day in accordance with the method described by Hogendoorn and Vismans (1980). The remaining 3 concrete tanks earlier prepared for the study were left empty since no eggs were stripped of the control fish injected with 0.90 % saline solution.

The fertilized eggs ( $59.80 \pm 1.50\text{g}$ ) from each hormone treatment were scooped with a plastic spoon and sparsely spread on strands of polyethylene fibres (*kakabans*) submerged in water contained in 12 indoor concrete tanks (0.70 x 1.50 x 0.50m). The now sticky fertilized eggs were left to incubate for 27 hours at  $25 \pm 1.0^{\circ}\text{C}$ .

Hatching commenced after the incubation period. Dead whitish eggs were siphoned out to avoid bacterial and fungal infection. The *kakabans* were then removed. The sac fry were retained in the same concrete tank for 5 days for the yolk sacs to be completely reabsorbed. Mixed zooplankton obtained with No. 35 (10 mm) bolt silk plankton net were fed to the fry, 6 times daily for 10 days. The fry were subsequently transferred to 12 outdoor nursery tanks adequately protected with mosquito-mesh nets. Feeding was by the use of a mixture of palm kernel cake, groundnut cake, brewer's waste and ground crayfish; at 5 % body weight per day for 7 days.

Records of the percent fertilization and hatching, fish standard and total body lengths, as well as head diameter were taken for each hormone treatment. The data obtained were statistically analyzed using analysis of variance (Steel and Torrie, 1980).

**Table 1: The results of artificial propagation of *C. gariepinus* hypophyzed with different doses of luteinizing hormone releasing hormone (LHRH) analog**

Experimental parameters	Luteinizing Hormone Releasing Hormone (LHRH) analog dosages ( $\mu\text{g Kg}^{-1}$ )							
	10.00	3.00	50.00	70.00	Control 0.00	Overall Mean	S.E $\pm$	Significant difference
Number of gravid females	6.00	6.00	6.00	6.00	6.00	6.00	-	-
Number of sexual mature males	3.00	3.00	3.00	3.00	3.00	3.00	-	-
Female broodfish weight (g)	455.00	460.00	458.00	456.00	460.00	458.20	2.25	n.s.
Weight of mature male (g)	450.50	456.40	460.20	452.40	450.36	453.97	2.13	n.s.
Spawn weight (g)	58.00	61.00	66.00	72.00	-	59.80	1.50	**
% Ovulation (Ov) (or % spawning)	68.00	73.00	82.00	85.00	-	77.00	-	**
% Fertilization (% surviving embryos 10h after fertilization)	71.00	72.00	77.00	78.00	-	75.00	-	*
% Hatching (HT)	56.00	65.00	73.00	75.00	-	67.00	-	**
% Survival (SV) (17 days old fry)	68.00	70.00	75.00	86.00	-	75.00	-	**

S.E $\pm$  = standard error, \* = significant at 5%, \*\* = significant at 1%, ns = not significantly different at 5%. LHRH analog has chemical structure of: L-Pyroglutamyl-L-Histidyl-L-Tryptophyl-L-Seryl-L-Tyrosyl-D-Alanyl-L-Leucyl-L-Arginyl-L-Proline Ethyl amide supplied by ELISCO SCIENTIFIC EQUIPMENT LIMITED ENUGU, NIGERIA.

**Table 2: The results of growth and morphometric parameters of *C. gariepinus* fry hypophyzed with different doses of LHRHa<sup>1</sup>**

Parameters	LHRHa dosage in $\mu\text{Kg}^{-1}$				Control 0.00	Overall mean	Significant level (P = 0.05)
	10.00	30.00	50.00	70.00			
Mean Body weight (MBW)	15.20 $\pm$ 0.08	15.95 $\pm$ 0.70	6.30 $\pm$ 0.93	16.50 $\pm$ 0.70	-	15.99 $\pm$ 0.78	*
Standard body length (SBL)	5.60 $\pm$ 0.20	6.70 $\pm$ 0.04	6.75 $\pm$ 0.40	6.90 $\pm$ 0.40	-	7.91 $\pm$ 0.16	*
Total body length (TBL)	7.40 $\pm$ 0.13	7.90 $\pm$ 0.16	8.06 $\pm$ 0.16	8.26 $\pm$ 0.18	-	7.91 $\pm$ 0.16	*
Head diameter (HD)	0.80 $\pm$ 0.02	1.75 $\pm$ 0.04	1.76 $\pm$ 0.03	1.85 $\pm$ 0.04	-	1.24 $\pm$ 0.03	**

LHRHa = luteinizing hormone releasing hormone analog with a chemical structure of: L-Pyroglutamyl-L-Histidyl-L-Tryptophyl-L-Seryl-L-tyrosyl-D-Alanyl-L-of: L-Pyroglutamyl-L-Histidyl-L-Tryptophyl-L-Seryl-L-Tyrosyl-D-Alanyl-L-Leucyl-L-Arginyl-L-Proline Ethyl Amide; supplied by ELISCO SCIENTIFIC EQUIPMENT LIMITED, ENUGU, Nigeria. \* = significant at 5%. \*\* = significant at 1%.

**Determination of Parameters:** The spawn weight was determined by estimating the mean weight of eggs used to achieve percent (%) fertilization. The percent (%) ovulation was estimated from the weight of eggs released as a percentage of the total weight of the vary. The percent fertilization was estimated from the surviving embryos 10 hours after fertilization. The

percent (%) hatching was the number of hatched fry relative to the fertilized eggs; while the percent (%) survival was the number of surviving fry after 17 days of feeding with mixed zooplankton and artificial diets. The fish standard and total lengths, as well as head diameter, were measured with a metre rule fixed on a fingerling table.

## RESULTS

The results of the homestead artificial propagation of *C. gariepinus* using different doses of luteinizing hormone releasing hormone analog (LHRHa) are shown in Table 1. Oocyte maturation and ovulation occurred in all females hypophyzed with 10, 30, 50 and 70  $\mu\text{g Kg}^{-1}$  LHRHa within 11 hours of latency period, at a temperature of  $25^{\circ} \pm 1.0^{\circ}\text{C}$ . No spawning was observed in any of the control sets that received saline injection. During stripping, the oocytes were extruded at the slightest pressure and they appeared transparent. When a few oocytes were placed in a Petri dish containing little water and examined under light, the cytoplasm appeared shifted to the periphery. In all hormonal treatments, dead eggs appeared whitish and opaque within 8 to 10 hours of fertilization. The eggs on the *kakabans* hatched after 27 hours of incubation at a temperature of  $25^{\circ}\text{C}$ . The fry later aggregated at the dark corners of the tanks.

The range values of the mean weight of the female broodfish were 455.00g for fish induced with 10  $\mu\text{g Kg}^{-1}$  LHRHa to 460.00g for fish induced with 30  $\mu\text{g Kg}^{-1}$  and the control (0.00  $\mu\text{g Kg}^{-1}$ ) (Table 1). Similarly, the male broodfish ranged from 450.50g for fish induced with half dose of 10  $\mu\text{g Kg}^{-1}$  LHRHa to 460.20g for fish induced with half dose of 50  $\mu\text{g Kg}^{-1}$  LHRHa. The range values of the spawn weight (SW) of eggs in the induced female *C. gariepinus* were 58.00g (10  $\mu\text{g Kg}^{-1}$  LHRHa) to 72.00g (70  $\mu\text{g Kg}^{-1}$  LHRHa). These values varied significantly among the different hormonal treatments ( $P < 0.001$ ) (Table 1).

The range values of the percent ovulation (OV) also varied significantly as the hormonal dosage increased from 10  $\mu\text{g Kg}^{-1}$  to 70  $\mu\text{g Kg}^{-1}$  ( $P < 0.01$ ) (Table 1). The percent ovulation ranged from 68% (10  $\mu\text{g Kg}^{-1}$  LHRHa) to 85% (70  $\mu\text{g Kg}^{-1}$  LHRHa) and the percent values increased with increasing LHRHa dosage. The percent fertilization (FT) of the eggs ranged from 71% (10  $\mu\text{g Kg}^{-1}$  LHRHa) to 78% (70  $\mu\text{g Kg}^{-1}$  LHRHa). There was also a significant difference in the values of percent fertilization as the hormonal dosage increased ( $P < 0.05$ ). Both the percent hatching (HT) and percent survival (SV) of the fry (Table 1) followed the same pattern of increase as demonstrated by SW, OV and FT above. Both parameters (i.e. HT and SV) varied significantly with increase in hormonal dosage ( $P < 0.01$ ). Fish under the control experiment, and injected with 0.90% physiological (saline) solution remained dormant to the artificial propagation technique applied and hence did not provide values for SW, OV, FT, HT and SV.

The growth and morphometric parameters of *C. gariepinus* fry namely: mean body weights (MBW), standard body length (SBL), total body length (TBL) and head diameter (HD) are shown in Table 2. MBW, SBL and TBL varied significantly as LHRHa dosage increased from 10  $\mu\text{g Kg}^{-1}$  to 70  $\mu\text{g Kg}^{-1}$  ( $P < 0.05$ ) (Table 2); while HD was significantly different at 1% ( $P <$

0.01). Generally, the growth and morphometric parameters considered in this study indicated that the values for MBW ( $15.20 \pm 0.80\text{g}$ ), SBL ( $5.60 \pm 0.20\text{cm}$ ), TBL ( $7.40 \pm 0.13\text{G}$ ) and HD ( $0.80 \pm 0.20\text{cm}$ ) were least when the fish were induced with 10  $\mu\text{g Kg}^{-1}$  LHRHa (Table 2). These values increased progressively up to the hormonal dosage of 70  $\mu\text{g Kg}^{-1}$  LHRHa which recorded the highest values for MBW, SBL, TBL and HD (Table 2).

## DISCUSSION

While Thalathiah *et al.* (1988) reported a 30% to 60% spawning in *Leptobarbus hoevenii* treated with a combination of 50 to 300 IU of human chorionic gonadotropin (HCG) and carp pituitary extract; Saidin (1986) reported a 50% to 70% spawning in catfish, *Clarias macrocephalus* treated with 4000 IU. HCG. A 68% to 85% spawning was recorded in this study for *C. gariepinus* induced with luteinizing hormone releasing hormone analog (LHRHa), with the best dosage for growth and morphometric characteristics recorded at 70.00  $\mu\text{g Kg}^{-1}$ . The difference between the percent spawning in this study and those recorded for other species by Saidin (1986) and Thalathiah (1988) could be due to generic/species differences and type of hormone applied.

The increase in percent fertilization (FT) of *C. gariepinus* eggs as LHRHa increased from 10.00 to 70.00  $\mu\text{g Kg}^{-1}$  (Table 1) in this study was consistent with the increase in FT recorded by Nwadukwe (1993) for *H. longifilis* as the dosage of frog pituitary extract applied increased from 30 to 300  $\text{mg } \mu\text{g Kg}^{-1}$ . The range values of FT for *C. gariepinus* in this study (71.00 - 78.00 %), with an overall mean of 75.00 %, compared closely with the range values (59.00-86.00%), with an overall mean of 73.00%, recorded by Nwadukwe (1993) for *H. bidosalis*. Similarly, the study recorded a mean percent hatching of 67.00 % for *C. gariepinus* eggs and this value is comparable with the 63.00% percent hatching for *H. longifilis* eggs (Nwadukwe, 1993).

It was evident from this study that the choice of LHRHa to stimulate spawning (77 %), fertilization (75 %) and hatching (73 %) in *C. gariepinus* superceded the results of earlier workers (i.e. Thalathiah *et al.*, 1988; Saidin, 1986). Hence, the present results are consistent with Donaldson (2003) report that the use of aqueous extracts of either fresh piscine pituitaries, or acetone-dried piscine powder, or HCG has been superceded by the use of LHRH and GnRH analogs. The range of LHRHa dosage suggested by Donaldson (2003) was 5.00 - 100.00  $\mu\text{g Kg}^{-1}$  and this covered the range of 10.00-70.00  $\mu\text{g Kg}^{-1}$  used in this study.

It was observed that the inducement of *C. gariepinus* with LHRHa ranging between 10.00 to 70.00  $\mu\text{g Kg}^{-1}$  resulted in progressive increases in SW, FT, HT and SV (Table 1). This implied that in order to obtain reasonable results from spawn weight (SW), %

fertilization (FT), and % hatching (HT) of the African catfish (*C. gariepinus*) broodfish, up to 70.00 µg Kg<sup>-1</sup> LHRHa should be applied. This same deduction is applicable to the growth and morphometric characteristics of the *C. gariepinus* fry when MBW, SBL, TBL and HD are considered (Table 2).

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