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Evaluation of Spawning Induction of African Catfish (*Clarias gariepinus*) by Heteroplastic Hypophysation

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ABSTRACT
The study was conducted at Ziway Fisheries Resource Research Center situated 160km south of Addis Ababa in the mid rift valley of Ethiopia. In the present experiment, specimens of *Clarias gariepinus* both male and female fishes having body weight in the range of 0.5-2.0 kg were collected from Lake Ziway and stocked in concrete ponds having size of 7x5x1 m provided with a water flow and replacement system. The brood stocks were fed with pellet feed prepared from Noug cake and wheat bran (2:1) at 3 % of their body weight. The whole pituitary glands were removed from the donor fishes (Cat fish, Carp, and Nile tilapia) and preserved in absolute alcohol (97 %). Pituitary glands were homogenized in a tissue grinder (mortar and pestle) with 2ml physiological salt solution (9gm salt in 1 lit of water) and refrigerated until use. After conditioning for a period of fishes of average weight 1kg were selected for injection. The pituitary solution was drawn and gravid females were injected intramuscularly at the dose of 3 mg/kg body weight. The fishes were then kept in spawning tanks and removed after 13.5h of latency period to check the condition. The fully ripened females were stripped to collect the eggs in a shallow bowl and fertilized with milt collected from the male. Wet fertilization was performed with saline solution and water. The fertilized eggs were incubated at 23 ˚C. While incubating the egg samples were drawn and the rate of fertilization was determined. Hatching occurred within 33hrs and hatchability rate (%) was determined by counting the active hatchlings. Those fishes injected with catfish and carp pituitary extract resulted in 76.93% and 80.53% fertility rate, and 45.30% and 42.93% hatchability rate respectively. The average spawning fecundity for fishes treated with catfish and carp pituitary extract were 6993 and 54633 respectively. However, the same dose of Nile Tilapia pituitary extract gave no spawning response. Even though the results on the fertility rate and hatchability were not statistically significant (P>0.05), catfish pituitary extract was equally potent and effective as that of carp pituitary extract to the recipient female catfish.

Keywords: *Clarias gariepinus*, spawning induction, spawning fecundity, stripping.

1. Introduction
Many hormones are involved in the control of maturation and spawning of fishes especially those produced by hypothalamus; the small peptide hormone (Releasing hormone), Gonadotropine hormone which pass into the blood to reach the gonads [7].
Synthetic hormones have been used to induce maturation, spawning and ovulation by using GnRH Analog with Dopamine Antagonist. Leutinizing hormone releasing hormone (LHRH) is the name of a mammalian hormone that has been employed successfully to induce the reproductive hormonal cascade. Synthetic analogues of LHRH, referred to as LHRHa, are found to be far more effective. Because they are purer and are not rapidly metabolized by fish, they remain active for longer periods. Although a trend exist to apply alternative synthetic hormone substances to induce spawning in catfish and other cultured fishes, hypophysation still remains the most common technique [20]. This method is still widely employed particularly for the fish which are economically very essential but do not spawn in confined waters of aquaculture like African catfish and carps [4,8,10,16]. Houssay [15] first demonstrated the effectiveness of crude pituitary extract for induced breeding. *Clarias gariepinus* has been considered as an ideal species for the development of aquaculture in Africa [5,18,13]. The fish has great economic importance next to Nile tilapia in Ethiopia. In nature this fish does not spawn year round, the spawning season of African catfish in the countries to North and south of the equator last from June till September and from November till February respectively [18]. This fish does not spawn in captivity spontaneously since the environmental factors like increase in water level and inundation of shallow areas do not occur at fish farms.

Several researchers have shown that pituitary hypophysation is effective in inducing the spawning of *Clarias gariepinus.* In Ethiopia there is a big research gap in good quality seed production by using induced breeding techniques for acceleration of aquaculture development. Collection of seeds of this species from the natural environment for culture practices is very difficult due to the photophobic behavior [16]. Moreover in the natural habitat *Claris gariepinus* do not spawn year round and hence fry production is required for regular supply to the farmers. In order to achieve production of healthy, uniform size fish seeds for stocking, induced breeding methods are followed in conjunctions with many environmental cues and physiological state of the fish. The present study was carried out mainly to evaluate the efficacy of heteroplastic hypophysation in the maturation of ovary, spawning, fertilization, hatching rate and viability of larvae of *Clarias gariepinus.*

2. Materials and Methods

The study was conducted at Ziway Fisheries Resource Research Center (ZFRRC), Oromia Regional state, Ethiopia center during 2011December to February 2012.The site is situated in the mid Ethiopian rift valley at 7.919ºN and 37.727ºE at an elevation of 1638 m.a.s.l. By virtue of its location, the center is characterized by warm climate with annual mean temperature of 27 ºC and precipitation of 688 mm. The brood fishes *used* for the experiment were collected from Lake Ziway. Matured females and males ranging from 0.5-2 kg were collected and stocked in concrete ponds having size of 7x5x1 m provided with a water flow and replacement system. The Brood stocks were fed with pellet feed prepared from Noug cake and wheat bran (2:1) at the rate of 3 % of their body weight for a period of one and half months for conditioning as well as to check their maturity level.

The choice of brood fish for hypophysation was based on morphological structures. Ripe females were easily identified by their distended round soft abdomen, reddish vent and appearance of few eggs upon slight pressure on the abdomen. Males were not injected with pituitary extract since viable sperm are obtained from their excised testes without any hormonal treatment. The well matured and properly selected recipient fish were weighed before injection and prior to stripping for determination of egg yield.

Pituitary glands of catfish, common carp and Nile tilapia have been removed and preserved in absolute alcohol (97%) in refrigerator [1]. The pituitary extracts were prepared with 2ml physiological salt solution (9gm salt in 1 lit of water).The pituitary solution was drawn in to a hypodermic syringe (5 ml volume) and injected intramuscularly into gravid females at the dose of 3 mg/kg body weight. Hypophysation stimulates vigorous activity and aggressive behavior and the injected females were placed individually in holding tanks to prevent them injuring one another or jumping out of the containers. When the estimated latency time (13.5 h) has elapsed, the female was examined to check the condition of the ova. The matured and conditioned gravid females were stripped to collect the ova into plastic bowls. Before stripping the females, the required number of males were sacrificed to remove the testes. The milt samples collected from the donor males was poured into each subsample of eggs. Wet fertilization was performed by adding 5ml saline water followed by sufficient amount of chlorine free water [21]. The water and egg mass were mixed gently with a feather for 5 minutes. The fertilized eggs were incubated at an average temperature of 23ºC in plastic incubation trays supplied with a continuous slow flow of water. After a certain period of incubation before hatching sub samples of eggs (1gm) kept on separate bowls were examined to assess the fertility rate (%) and hatchability rate (%) as determined from counting the number of active larvae.

The experiment was based on a completely randomized design (CRD) consisting of three treatments and four replications. The treatments were the pituitary donors such as: *Clarias gariepinus, Oreochromis niloticus, and Cyprinus carpio;* whereas the replicates were the recipient four gravid female catfish under each treatment groups. Therefore, a total of twelve gravid female brood stocks of approximately equal size were selected as experimental fish.

Prior to hatching, three sample plots were determined on the screen, and the number of fertilized and unfertilized eggs was counted physically for each fish. The fertilized eggs were green, transparent and flattened whereas the unfertilized ones were whitish in color and thick. The FR% was computed based on the formula [12].

$$FR\% = \frac{\text{No. of fertilized eggs} \quad X\ 100}{\text{Total No. of ova collected}}$$

The hatchability rate was calculated based on the formula [17]

$$HR\% = \frac{\text{Number of hatched eggs} \quad X100}{\text{Total No. of eggs incubated}}$$

Data obtained from the experiment work were statistically analyzed by using SAS 9(SAS Institute, 2002) statistical package to produce descriptive information.

The data were pooled for each treatment and compared by two sample t-test analysis to determine the significance difference (P= 0.05).

3. Results

The spawning fecundity of *C. gariepinus* injected with catfish pituitary extract varied from 49575 to 96120. The mean fecundity was (69939). The fecundity of those injected with carp pituitary extract was maximum 54568 (Table 1) and there was not much variation between the replica and hence. Those fishes induced with carp pituitary extract showed mean egg yield as 54633. Recipient fish induced with *O. niloticus* pituitary extract in the third treatment group, no egg yield was observed since there was no spawning.

Table 1: Spawning fecundity (Eggs/ female)

Replication	T1	T2	T3
1	49575	59280	-
2	96120	51135	-
3	68060	53550	-
4	66000	54568	-
Average	69939	54633	-

T1=catfish, T2=carp, T3=Tilapia, P (> 0.05)

The rate of fertilization was higher (80.53%) for carp pituitary extract injected female *C. gariepinus* than with catfish pituitary homogenate (76.93%) (Table 2). The lowest fertility rate (74.1%) was observed for catfish pituitary recipient. The analysis of

variance test of fertilization percentage indicated no significant difference (P>0.05) for both treatment groups.

Table 2: Fertilization rate (%)

Replication	Treatment 1	Treatment2
1	75.6	79.9
2	74.1	83
3	78	77
4	80	82.2
Average	76.93	80.53

T1=catfish, T2=carp, T3=Tilapia, P (>0.05)

The highest hatchability value 53.9% was recorded for eggs collected from cat fish pituitary extract recipients. The hatchability rate was comparatively less for carp pituitary recipient eggs.

Table 3: Hatchability rate (%)

Replication	T1	T2	T3
1	43.4	49	-
2	43.9	41.7	-
3	53.9	42	-
4	40.0	39	-
Average	45.3	42.925	-

T1=catfish, T2=carp, T3=Tilapia, P (>0.05)

4. Discussion

Hypophysation of African cat fish has been proved to be the most effective means of spawning induction for the commercial catfish farmers in Africa [1]. The successful induction of spawning of *Clarias gariepinus* by using both heteroplastic and homoplastic pituitary extract has been reported earlier [9]. A similar successful attempt was made with *C. lazera* (Cuvier and Valenciennes) by administering 3-5mg/kg pituitary gland extract to 500-800gm fish [14]. Injection of 10-20mg/kg carp and catfish pituitary extracts showed enhanced breeding response for 500-1000gm catfish which lead to a series of studies for the evaluation of spawning induction in *C. gariepinus* by heteroplastic hypophysation [2].

The present experiment was sought to evaluate induced maturation and spawning performance by heteroplastic hypophysation in the African catfish. In this study the spawners were injected with catfish pituitary homogenate solution yield high number of spawned eggs (69939) than those injected with carp pituitary extract (54633). However, the difference was not statistically significant for the four treatments (P>0.05). In a similar study, using *C. gariepinus* pituitary extract 76, 500 eggs as spawning fecundity was reported earlier [3, 9]. The yield of eggs in the present study was comparatively less than the above reports. In the earlier studies they selected hatchery reared and small size (1kg) brood stocks. In the present study the brooders (1 -5kg) were collected from their natural breeding ground. With cat fish pituitary extracts (80%) fertility rate for C. *gariepinus* [10] was obtained by induced spawning. However by using carp pituitary extract (at a dose of 3mg/ kg body weight) for induced breeding of *C. batrachus* reported 85% fertility rate [19].

Through homoplastic hypophysation of *C. gariepinus* 80% hatchability rate was recorded [2]. The hatching rate was less [11, 14] (65%) for *C. lazera* with carp pituitary extract as inducing material. There is considerable variation on the hatchability rates of the two donor species as observed in the present study. It is inferred that hatchability of the incubated eggs not only depends on the potency of the hormones injected but also on the incubation facilities, temperature as well as other water quality parameters.

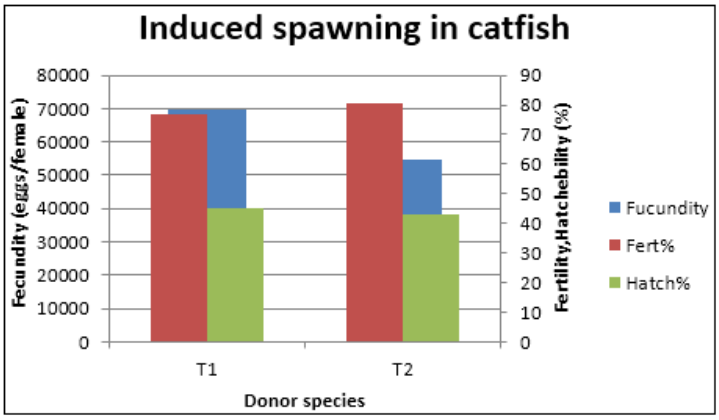

Fig 1: Spawning fecundity, fertility rate, and hatchability of treatments

Carp and cat fish pituitary extract were successful in the induction of spawning in the matured *C. gariepinus females* whereas O. *niloticus* pituitary extract was not successful. Further, investigations are required to find out the usefulness of tilapia pituitary extract for inducing maturation and spawning in catfish. However, hypophysation of *C. gariepinus* using either carp pituitary or the same species would appear to be the most viable option for the commercial catfish culture in Ethiopia.

5. Conclusion

Even though the experiment was carried out with limited facility the result is promising and would serve as base line data for further studies.

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