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Induced breeding of *Clarias batrachus* using "gonopro-fh" in a mini portable hatchery and study on embryonic developmental stages

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Abstract

In this study the breeding of *Clarias batrachus* was carried out from July to September 2023 at Barkatullah University, Bhopal, using Gonopro-FH in a portable mini hatchery setup. Mature brooders (male: 409–545 g, female: 550–605 g) were injected intramuscularly at a 1:1 sex ratio, with carefully measured hormone doses. Fertilized eggs exhibited clear morphological features, and embryonic development was monitored from cleavage to hatching. Key developmental milestones such as the morula, blastula, gastrula, somite formation, heartbeat, and eventual hatching at 22 hours post-fertilization (25.1°C) were recorded in detail. Larval behavior, pigmentation, and organ development were also observed in post-hatching. This study concludes that hormone-induced breeding using Gonopro-FH significantly enhances spawning efficiency, fertilization rate, and seed quality. These findings support the use of synthetic hormones in this fish to achieve predictable breeding cycles, improved seed production, and sustainable aquaculture practices.

Keywords: Induced breeding, Clarias batrachus, Gonopro-FH

1 Introduction

The Asian catfish, *Clarias batrachus*, is a highly valued species in aquaculture due to its resilience, ability to survive in low-oxygen environments, acceptance of pellet feeds, rapid growth, and adaptability

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to captive conditions. Being native to Asia, it remains one of the most suitable species for aquaculture across the region. Its economic significance is notable, as it commands a high market price and is an important food fish. Studies have documented its strong production potential in aquaculture (Thakur & Das, 1986; Areerat, 1987). India, currently the third-largest fish producer and second-largest aquaculture producer globally, contributes approximately 7% to global fish production. Among aquaculture ventures, catfish farming is among the highest-yielding, with global production reaching 250 to 400 tonnes per hectare per crop (Ayyappan et al., 2006). *Clarias batrachus*, commonly known as the walking catfish or "desi magur" in India, is a freshwater, air-breathing species native to Southeast Asia. It has also been introduced in other regions, where it is sometimes considered invasive. The fish earns its nickname from its ability to move over land using its pectoral fins and body in a snake-like motion to search for food or suitable environments (Ramesh et al., 2016). It is especially popular in northeastern India due to its high consumer preference and fetches a higher price compared to carps.

Induced breeding marks a major breakthrough in aquaculture by enabling controlled reproduction in fish. It involves stimulating mature fish using pituitary or synthetic hormones to trigger the timely release of eggs and sperm in captive environments. This technique has significantly increased carp seed production in India from 6,321 million fry in 1985–86 to over 45,000 million fry in recent years. India now ranks second globally in farmed fish production. Aquaculture, the fastest-growing food sector, is expected to supply half of all fish consumed by 2025. Quality seed remains a critical factor in boosting aquaculture productivity. Induced breeding, by manipulating hormonal or environmental conditions, has become essential for producing sufficient quality seed for both artificial ponds and natural water bodies (Panigrahi et al., 2019).

Hormonal spawning techniques influence reproduction by either enhancing or suppressing biological processes. Two main approaches are used: (1) simulating the natural spawning environment, and (2) administering natural or synthetic hormones via injection or feed. Often, both methods are combined. Various hormones have been tested, but current practices highlight two cost-effective and successful approaches: (i) using gonadotropin-releasing hormone (GnRH) analogs with dopamine antagonists, and (ii) injecting purified gonadotropins such as human chorionic gonadotropin (HCG) (Kiran et al., 2016). However, in artificial breeding of *C. batrachus*, the female is stripped of eggs, and the male is sacrificed to collect milt.

Among catfish, *C. batrachus* stands out as a preferred species in Asian aquaculture due to its many advantages. Its robustness and tolerance to poor water conditions allow high-density culture and high yields. Yet, spontaneous breeding in captivity is limited, and dependence on wild seed often unreliable, time-consuming, and costly poses major challenges. Therefore, induced spawning remains the most viable method for ensuring a steady supply of quality seed for the commercial culture of this species.

2 Methodology

1. Study area

The present study was conducted in July-September 2023 at the Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal, M.P., in the hatchery. Breeding trials of *Clarias batrachus* were performed during the monsoon season.

2. Brood tank preparation

Firstly, the tank was collected and wash it with water also wash it with KMnO4 one minute second and then washed it again with water for cleaning purpose in other to prevent the germs or any kind of bacteria.

3. Collection of brooders

It has been collected from Kasturba market. For breeding purpose healthy and sexually mature broods were selected. From the market it was carried out by handling and kept them in FRP tank, it is easy process to carried brooders from market to Department because its air breathing catfish they can survive more than 16hour without water. Also, it was again treated by $KMnO_4$ and keep them for 30 minutes and control from stress or any kind of infection. After that it has been released out into the tank for one week.

4. Scientific identification of brooders

The brood fish for the artificial breeding of *Clarias batrachus* were selected from the fish market. Total 10 brooders were stock in the tank. During breeding season, the sexual dimorphism is fully prominent.

Identification of Male Fish

- **Color:** During the breeding season, the color of mature male *Clarias batrachus* changes to a grayish tone.
- **Body Shape:** Males have a noticeably slimmer belly compared to females, particularly evident during the mating period.
- **Genital Papilla:** The male's genital opening is elongated and conical in shape.
- **Dorsal Fin Spot:** Males can also be identified by the presence of distinctive spots on the dorsal fin.

Identification of Female Fish:

- **Color:** Females become darker in color during the breeding season.
- **Body Shape:** The belly of a female is broader than that of a male, a trait that becomes more pronounced during breeding.
- **Genital Papilla:** In females, the genital papilla is oval and slit-like in appearance.
- **Dorsal Fin Spot:** Females typically lack the dorsal fin spot seen in males.

5. Breeding Tank Setup

Two tanks were used in the setup. An outlet was installed at the center of the breeding tank, which is connected via a pipe to two lower-positioned tanks that act as inlets, allowing water circulation back into the breeding tank.

6. Maturity Size Requirements:

- **Fish Length:** Although this species becomes capable of breeding at around 15 cm, the ideal breeding size is between 25 to 30 cm.
- **Fish Weight:** Fish can reproduce once they reach approximately 80 grams, but the optimal brooder weight is above 100–120 grams.
- **Egg Size:** Females are considered suitable for breeding when their eggs measure between 0.12 to 0.14 cm.

7. Hormone Preparation

Brooders were injected with a hormone mixture prepared using Gonopro-FH and 0.5 ml of distilled water. A 1 ml syringe was used for injection, with the dosage calculated based on the individual fish's length and weight.

8. Injected of brooders

For induced breeding injection is the main part of successful *Clarias batrachus* breeding. First, calculate the body weight of the fish. Then use different types of synthetic hormone with different doses. For induced breeding I used Gonopro- FH injection.For both female and male given two dose, at evening time (1:30 28 Aug 2023) the first dose was given 0.5-0.6 ml/kg body weight for female and for male 0.5ml, at the ratio of 1:1 around 5-6 hours, kept in the breeding tank. It was injected again the 2nd dose was given for female 0.4ml and for male 0.3 ml. Male Magur fish’s body weight is 410gm so, the dose is <0.5 ml. and female fish is 550gm. So, the dose is 0.6 ml (about). The injection injects in the fish in 45º angle.

9. Dosage

General dose of Gonopro-FH is 0.5 ml per body weight of fish. Dose may be varied among species and location.

Female fish 0.3-0.5ml/kg body weight and Male fish 0.1-0.3ml/kg body weight.

10. During injection

Ensure that all equipment, collecting tank, syringe etc should be clean and if possible sterilized. Always handle gently to the fish. Always require quantity of Gonopro-FH withdraw trapped air from the syringe before inject. Hold fish firmly and insert needle firmly at the intra-muscular part or belly behind pelvic fin. Inject spawn Pro carefully.Gently place the fish in to breeding tank of fresh and aerated water.

11. Stripping of female

After 6 hour the female fish was stripped with hand, gently press the belly toward female genital papilla and kept in a tray. If the eggs are white that shows the immature egg. Fully mature eggs look dark brown or brownish green in colour.

12. Dissection of Male

Male have to sacrifice and remove the testis and wash it with distilled water to clean the blood and kept in the same tray and cut the testis into small pecies by scissor and crush it with pistile or hand. And eggs and milt are mixed 30-60 sec with the help of feather or brush and distilled water after mixture then gently spread the eggs in the breeding tank, and start rotated the water in the breeding tank to make the eggs rotated.

3 Results

A. Fertilized Eggs

The fertilized eggs were spherical, sticky, and brownish in color. Before fertilization, the average egg size measured approximately 0.65 ± 0.02 mm, which increased to about 1.01 ± 0.19 mm post-fertilization (refer to Figure 1).

B. Embryonic Development (Cleavage Stage)

Around 35 minutes after fertilization, the blastodisc (or polar cap) formed. The first cleavage occurred 15 minutes later, dividing the blastodisc into two cells or blastomeres. The second cleavage, occurring at a right angle to the first, happened within 55 minutes post-fertilization. The embryo reached the eight-cell stage 10 minutes after that. The fourth cleavage, parallel to the second, took place at 2 hours and 5 minutes, forming 16 cells. Four minutes later, the sixth cleavage resulted in the 32-cell stage. The morula stage was reached 2 hours and 9 minutes after fertilization, and the blastula formed at 3 hours and 17 minutes. It was observed that as cleavage progressed, the size of the blastomeres reduced.

C. Embryo Formation

At 6 hours and 10 minutes post-fertilization, the blastoderm began to spread over the yolk. By 6 hours and 30 minutes, this invasion had progressed significantly, and the eggs reached the late gastrula stage. Gastrulation was fully completed about 40 minutes later.

D. Embryo Differentiation

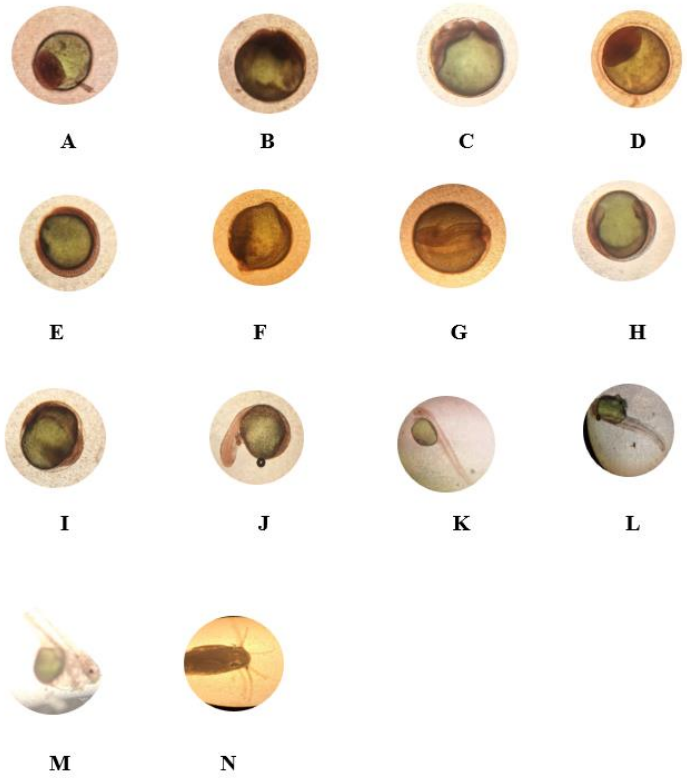
Somite formation began at 7 hours and 10 minutes after fertilization, and more developed somites were observed 13 hours in. The head and tail regions became distinguishable. One hour later, myotomes appeared, and the embryo started resembling a girdle wrapped over the yolk. As the embryo elongated, the tail separated from the yolk, and a heartbeat was detected. By 20 hours post-fertilization, tail movement and general body motion were visible. After an additional 2 hours, the yolk sac had further reduced, and the tip of the tail approached the head. By 21 hours, the embryo was fully developed and ready to hatch.

E. Hatching

At around 22 hours after fertilization, and a water temperature of 25.1°C, the elongated tail tip pushed against the egg's head end, breaking the shell. The head emerged first, followed by the rest of the body as the larva wriggled free from the egg case (see Figure 1).

F. Larval Development

The newly hatched larvae had unpigmented eyes, lacked fin buds, and did not yet have a functional mouth. The anus was located just behind the yolk sac, which protruded near the front end of the larva. Black pigment cells appeared in the fin folds, except at the caudal tip, and were also scattered on the yolk, head, and body. The larvae swam slowly upward before sinking and suspending themselves in an inclined, head-down position in the water column. As development continued, the eyes became fully pigmented and the pectoral fins elongated. The mouth opened, and the esophagus became clearly visible. Pigmentation increased, especially around the tail and caudal peduncle. A few melanophores were present near the posterior gut. The yolk sac was nearly absorbed, leaving only a small remnant. The larvae began slow, deliberate movements with occasional jerks, swimming both near the surface and along the bottom of the water column.



Length and Weight of mature brooders:

At the end of the brood stocking management, it was found that the brooders (Male and Female) grew higher than before collection. Length of the *Clarias batrachus* is below 20-25 cm (both male and female), whereas, weight of the female is 550- 605 g and the male 409-545 g.

TABLE 1: Length and Weight of brooder fish before injection (23-9-2023)

Sex	Length (cm)	Weight (gm)
Female	15.0	550
Male	13.2	410

1. **Doses for female and Male:**

From the study, a ratio of 1:1 were mature fish injected by using GONOPRO-FH hormones, brood fishes were feed in the brood rearing tank by provided artificial diet for good health and full maturation.

TABLE 2: Doses of GONOPRO-FH for Female and Male:

Species name	**Sex**	**No. of Species**	**1st dose (ml/kg nody weight)**	**2nd dose (ml/kg body weight)**	**Hatching time (hour)**
Clarias batrachus	**Female**	**1**	**0.6**	**0.4**	**72-78**
	Male	**1**	**0.5**	**0.3**	

2. **Fertilization rate, Unfertilization rate, Survival rate and Mortality:**

From the experiment, the data has been found that:

TABLE 3: Experiment data of Fertilization rate and Unfertilization rate:

Species name	**Fertilization rate%**	**Unfertilization rate%**
Clarias batrachus	75%	25%

TABLE 4: Experiment data of Survival rate and Mortality:

Species name	**Survival rate%**	**Mortality rate%**
Clarias batrachus	**10%**	**90%**

TABLE 5: Mean of Fertilization rate(%) and Survival rate(%):

a. **Fertilization rate (%)= No. of fertilized egg × 100**

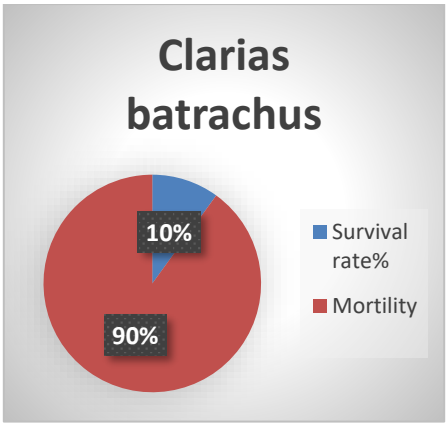
Total no. of egg

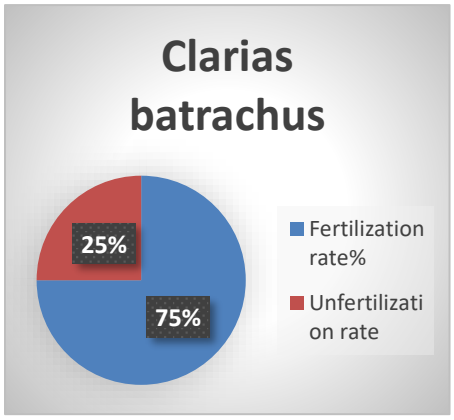
= 75%

b. **Survival rate (%)= No. of survival fry × 100**

Total no. of hatching

= 10%





DISCUSSION

In the current study, GONOPRO-FH was effectively used to induce breeding in *Clarias batrachus*, a commercially important catfish species. The process showed positive stripping responses and led to increased egg production and fertilization rates, although the hatching and survival rates were comparatively lower. The optimal results were obtained when female brooders were injected with a

hormone dose of 1 ml/kg body weight, likely due to the complete maturity of the eggs. Typically, male brooders are sacrificed during the breeding process because they produce very little milt. The sperm is extracted through an incision and mixed with the eggs collected from females by stripping. The hormone dose likely stimulated the female brooders effectively by inducing contraction in the smooth muscles of the gonoduct before ovulation, contributing to the overall breeding success.

The latency period (time between injection and ovulation) ranged from 11 to 23 hours. The fertilized eggs of *Clarias batrachus* were found to be adhesive and demersal (settling at the bottom), similar to those of other catfish species like Mystus montanus, Pangasius sutchi, and Heteropneustes fossilis. In contrast, unfertilized eggs appeared pale and opaque. This adhesive nature of the eggs is an adaptive feature that prevents them from being carried away by water currents and helps ensure proper oxygen availability. In this study, the fertilized eggs appeared yellowish-brown, which is consistent with observations made by Khan and Thakur. It was also noted that the hatching time decreased with increasing water temperature, regardless of the hormone type or dosage used. This observation aligns with findings reported by researchers like Zaki, Abdula, and Herath.

Overall, the induced breeding technique using Gonopro-FH has proven to be a valuable advancement in the culture of *Clarias batrachus*. This method is gaining widespread adoption and is expected to significantly improve the availability and quality of fish seed in the near future. The outcomes of this study will contribute greatly to the production of quality seed, enhancing induced breeding practices and promoting the sustainable culture of this important fish species.

CONCLUSION

This study concludes that induced breeding not only enhances the quality of fish seed but also increases the overall yield and provides better control over the breeding process. It enables the production of desired quantities of spawn at any time and ensures genetically pure offspring of specific cultured fish species. In this research, the synthetic hormone Gonopro-FH proved to be both effective and dependable for the induced breeding of *Clarias batrachus*. This technique can support breeding efforts and contribute to the sustainable cultivation of this important fish species.

Reference

[1] Newaz Sharif, B.M & Abu Zafar, Md (2016). Technology of artificial breeding of catfish species in the hatcheries in Jessore region, Bangladesh. *International Journal of Fisheries and Aquatic studies*. v4(1): pp180-188.

[2] Roy S. & Pal B.C. (1986). Induced breeding of *Heteropneustes fossilis* (Bloch) was studied in laboratory aquaria. *Journal of Fish Biology*. **28(3):** pp 247-254.

[3] Mani, C.V. & Pandey, A.K. (2007). Effect of HCG and WOVA-FH administration on ovarian maturation and spawning of *Heteropneustes fossilis* (Bloch). *Journal of Experimental Zoology, India*. **10(2):** pp317-319.

[4] Chaturvedi, C.S. & Pandey, A.K (2013). Induced breeding and hatching of freshwater catfish *Heteropneustes fossilis* in indoor hatchery of Lucknow, India. *Journal of Experimental Zoology, India*. **16(1):** pp 173-177.

[5] Mollah M.F.A & Amin M.R. (2008). Induced breeding of the riverine catfish *Rita rita* using Carp pituitary gland (PG) extract. *Journal of the Bangladesh Agricultural University*. **6(2):** pp 361-366.

[6] Yadav A.K. & Mishra R.K.(2011). Induced spawning of Asian catfish, *Clarias batrachus*, with different Doses of sGnRH-Based Drugs. *Journal of Expiremental Zoology, India*. **14:** pp 199-202.

[7] Sudhir Raizada & Pandey A.K. (2012). Breeding of *Clarias batrachus* in Low- Saline water under controlled condition in Rohtak (Haryana). *Journal of Expiremental Zoology, India*. **15:** pp 379-382.

[8] Elsadig Arbab Hagar Talib & Zuheir Nour EI Dayem Mahmud (2017). Breeding practices and rearing response of *Clarias batrachus* under hatchery condition. *Journal of Agriculture and Enviromental Management*. **6(5):** pp 089-094.

[9] Goswami P.K. & Devaraj M. (1992). Breeding age and growth of the freshwater shark Wallago attu from the dhir beel of the Brahmaputra basin, Assam, India. *Journal of the Indian Fisheries Association*. **22:** pp 13-20.

[10] SK. Mustafizur Rahman & Md. Ahasan Habib (2011). Embryonic development of *Clarias batrachus* under the influence of aeration and water flow. *Ecoprint an International Journal of Ecology*. **18:** pp 25-31.

[11] Sarkar U.K. & Prashant D.K (2005). Captive breeding of climbing perch *Anabas testudineus* (Bloch,1792) with WOVA-FH conservation and aquaculture. *Aquaculture Research*. **36:** pp 941-945.

[12] Singh M.K & Sonowal S. (2022). Captive breeding of the stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) found in Brahmaputra river, Assam, India using inducing agent ovasis and its early embryogenesis. *Egyptian Journal of Aquatic Biology and Fisheries.* **26(5):** pp 161-173.

[13] Giri A. & Saxena S. (2023). Guideline for breeding of an excellent medicinal fish *Clarias batrachus* (Magur) using synthetic hormone. *International journal of creative research thought*.**11(7):** pp 2320-2882.

[14] Sharma K. & Yadava N.K. (2010). Effect of different doses of ovatide on the breeding performance of *Clarias batrachus.Livestock Research for Rural Development.***22(4).**

[15] Siddiqui M.N. & Habib M.A. (2011).Embryonic development of *Clarias batrachus* under the influence of aeration and water flow. *Ecoprint*,**18:** pp 25-31.

[16] Olumuji O.K. & Mustapha MK. (2012).Induced breeding of African mud catfish, *Clarias gariepinus* (Burchell 1822), using different doses of normal saline diluted ovaprim. *Journal Aquaculture Research Development*,**3:4.**

[17] Panday A.K. & Chaturvedi C.S. (2012).Successful induced breeding of *Clarias batrachus* by ovaprim administration under agro- climatic conditions of Lucknow.*National Journal of Life Science*.**9(2):** pp 163-167.

[18] Kiran B.R. & Murthy K.S. (2013). Review on induced breeding of catfishes, murrels and climbing perches in India. *Advance in Applied Science Research.***4(4):** pp 310-323.

[19] Shukla V.K & Singh R.K. (2013). Captive breeding and larval rearing of endangered Ompok bimaculatus under controlled condition at Raipur, Chhattisgarh (INDIA). *Biochem. Cell. Arch.*,**13(1):** pp 133-136.

[20] Dhara K. & Saha N.C. (2013). Controlled breeding of Asian catfish *Clarias batrachus* using pituitary gland extracts and ovaprim at different temperatures, latency periods and their early development. *Journal Aquaculture Research Development*.**4(4).**

[21] Rashid M. & Ahamad T., (2014). Induced breeding of grass carp (*Ctenopharyngodon Idella)* and Silver carp (*Hypophthalmichthys molitrix)* using ovatide as synthetic hormone at national fish seed farm Manasbal, Kashmir, J&K. *Fisheries and Aquaculture Journal*.**5(4).**

[22] Ali M.M & Faruq O. (2016). Technology of artificial breeding of catfish species in the hatcheries in Jessore region, Bangladesh. *International Journal of Fisheries and Aquatic Studies*.**4(1):** pp 180-188.

[23] Gupta S. (2015). Overview on feeding and breeding biology of Mystus tengra, A Freshwater catfish of Indian sub- continent. *World Journal of Fish and Marine Science.***7(3):** pp 195-197.

[24] More P.R. & Pathan T.S. (2016). Induced spawning, fecundity, fertilization rate and hatching rate of Indian major carp *Catla catla* by using synthetic hormones and carp pituitary extract. *Middle-East Journal of Scientific Research.***24(12):** pp 3805-3811.

[25] Singh Kohli, M.P. & Vidyarthi, S. (1990). Induced breeding, embryonic and larval development in *Heterropneustes fossilis* (**Bloch,** 1794). *Journal of the Indian Fisheries Association.***20:** pp 15-19.

[26] Dhara K. & Das U.N. (2018). Breeding performance of the walking catfish *Clarias batrachus* (Linnaeus, 1758) using synthetic inducing agent, Alpa-FH with special reference of morphological deformities in their early stages of life. *Journal of Entomology and Zoology Studies.***6(6):** pp 957-963.

[27] Kiran B.R. & Somashekar D.S. (2016). A review on Induced breeding of stinging catfish, *Heteropneustes fossilis* in India. *International Journal of Multidisciplinary Research and Modern Education.***2(1):** pp 2454-6119.

[28] Doctolero J.S. & Sotero D.S. (2022). Induced spawning of African catfish (*Clarias gariepinus*) using goat (Capra aegagrushircus) and pig (Scrofa domesticus) pituitary extracts. *Journal of Fisheries.* **10(3):** pp 103204.

[29] Ramesh I & Kiran B.R. (2016). Food and feeding habits of Catfish *Clarias batrachus* (Linn) in Bhadravathi Area, Karnataka. *International Journal of Research in Environmental Science.***2(4):**pp 56-59.

[30] Tan-Fermin J.D. & Fermin A.C. (2008). Breeding and seed production of the Asian catfish *Clarias macrocephalus* (Gunther). *Aquaculture* Extension Manual No.40.

[31] Ali M.M & Asif A.A (2016). Technology of artificial breeding of catfish species in the hatcheries in Jessore Region, Bangladesh. *International Journal of Fisheries and Aquatic Studies*.**4(1):** pp 180-188.

[32] Lopamudra Panigrahi (2019). Studies on induced breeding of some carps for seed production in fish seed hatchery of boudh. *Journal of Emerging Technologies and Innovation Researc*.**6(4).**