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Studies on Reproductive Performance of Clarias Batrachus with various Inducing Agents for their Conservation in Marathwada Region, Maharashtra, India

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Abstract: ***In the present research work attempts were made to evaluate the reproductive performance in Clarias batrachus with various inducing agents and latency period. Brood females were administered with different doses of ovatide, ovaprim, pituitary gland extract (PGE) and human chorionic gonadotropin (HCG), optimum response were observed at dose 1.0 ml/kg, 2.0 ml/kg, 120 mg/kg and 4000IU/kg body weight of female respectively. Latency period ranged between 12 to 16 hours. The higher rate of fertilization (%) for ovatide, ovaprim, PG and HCG were observed 95± 2.5, 87±3.1, 84±2.1 and 86±2.5 respectively. While higher rate of hatching (%) was 91.80±4.1, 76±3.0, 72±2.0 and 78±2.0 respectively. Results of the present study clearly indicated that Clarias batrachus spawned in laboratory conditions with various inducing agents.***
Keywords: Clarias batrachus, breeding, ovaprim, ovatide, fertilization, hatching.

I. INTRODUCTION

Asian catfish, Clarias batrachus is very important and popular for its nutritive food value in India and neighboring countries. The Clarias batrachus is one of the most popular food fish of India, Myanmar, Bangladesh, Srilanka and Malaysia (Mookerjee and Mazumder, 1950). C. batrachus is cherished as a medicinal fish and is particularly popular among the pregnant and lactating mothers for medicinal purposes. It is equally used for the convalescent of the patients and malnourished persons because of its high nutritional status (Debnath, 2011). The population of Clarias batrachus species is declining day by day due to drying up of wetlands, use of pesticides in the paddy field, loss of habitat and overfishing particularly in Marathwada region (Jagtap and Kulkarni, 2013). Presently Clarias batrachus becomes rare species due to loss of natural breeding grounds and depletion of natural stocks. However, population of this species has been reduced drastically during recent years due to overfishing, habitat alterations and indiscriminate use of pesticides in preferred natural breeding grounds such as paddy fields and wetlands (Goswami, 2007; Khedkar et. al., 2010).

Clarias batrachus breeds once in a year during June to August in suitable natural environmental conditions but rate of survival is less (Chondor, 1999). Culture of this species has been identified as a national priority owing to its good demand, decline in fishery, hardy nature and aquaculture potential (Sharma et. al., 2010). However the quality of seed produced and breeding performance in captivity cannot be assured due to poor quality of brooders and lack of sufficient knowledge on reproductive traits in wild condition, resulting in over dependency on wild seed for stocking in ponds which is uneconomical and less sustainable (Kiran *et. al.*, 2013).

This species is classified under threatened category (Argungu *et. al.*, 2013). There is a need for conservation and sustainable management of this commercially important fish. Hence to maintain population of *Clarias batrachus* there is need of induced breeding by producing seeds in artificial hatcheries. Induced spawning techniques for *C. batrachus* have been successfully used for seed production by few workers using various natural and synthetic agents like piscine pituitary gland extracts, Human Chorionic Gonadotropin (HCG), Ovaprim, Ovatide etc. (Hossain et. al., 2006, Srivastava et. al., 2012, Sahoo, 2008, Sahoo, 2006). However, the commercially available synthetic inducing hormones in ready made form containing GnRHa and dopamine blocker receptor (Ovaprim, Ovopel and Aquaspawn) are becoming very popular and found to be efficient in successful spawning of fishes (Peter et. al., 1988, Nandeesha et. al., 1990, Cheah and Lee, 2000; Brzuska, 2001).

The breeding performance and egg quality of *C. batrachus* has been reported by the use of different dose of inducing agents with latency combinations in several occasions (Zonneveld et. al. 1988). For induced breeding different synthetic hormones are injected for successful ovulation and collection of eggs.The use of synthetic inducing agents for successful ovulation followed by stripping

in catfish is a common practice and has been studied at several occasions (Manickam and Joy, 1989, Tan-Fermin et. al., 1997, Sahoo et. al., 2003). The production potentiality of this species in aquaculture has been reported (Thakur and Das, 1986). The hardy nature and tolerance to adverse environmental conditions particularly low oxygen levels in water enable its intensive culture with high production rate (Sitasit, 1968).

Several attempts have been made in past to induced bred C. batrachus using various hormones (Rahmatullah et.al., 1983). Captive breeding of Clarias batrachus by using doses of hormones like ovaprim, ovatide, PG and HCG has been found most efficient for the purpose of fish production. Human chorionic gonadotropin (HCG) is one among them and is reported successful in catfish during induced ovulation (Legendre, et. al., 2000). Successful induced breeding of this catfish species has been reported with the use of pituitary extract as an inducing agent (Rao and Ram, 1991; Goswami and Sarma, 1997).

The aim of the present research work was to evaluate the role of different hormones like ovaprim, ovatide, PG and HCG on reproductive performance of Clarias batrachus in laboratory conditions, to standardize the induced breeding technique to increase their population in nature.

II. MATERIALS AND METHODS

Ovaprim - is synthetic analogue of SGnRHa and domperidone ready to use each phial contains 10 ml solution. Ovatide - developed by Hemma Pharma, Mumbai & CIFE, Mumbai. These hormones were purchased from Maharashtra Fish seed production center, Majalgoan, Dist. Beed (M.S.) private fish farm. For breeding experiments each set having 3 pairs. Pituitary gland extract from Indian major craps and human chorionic gonadotropin from local medical store.

1) *Collection of the Brooders:* The breeding experiments were conducted under controlled conditions at research Centre. Adult brood catfishes were collected from Central Institute of Fisheries Education (CIFE), Sub-Center, Kakinada, Andhra Pradesh and transported carefully to research Centre, before breeding season.
2) *Separation of the Brooders:* Brooders were collected, identified and separated from common ponds and stocked in experimental tank. Males were selected on the basis of pointed and reddish genital papilla, while females by a round and reddish papilla, softness of abdomen (Sharma et. al., 2010). Length and weight of brood fishes were recorded. Brood fishes reared in cement tanks. Tanks bottom spread with soil up to 5 cm and then water up to 30 cm from bottom. Tanks fertilized with cow dung and urea for production of natural food. These fishes fed with natural live food as well artificial food.
3) *Preparation and Administration of Hormones:* For PG extract Indian major carps were collected from local fish market. These inducing agents were separately administered each group containing three pairs.
4) Breeding trials were carried out in Aquarium (70x30x40cm) during 2017 to 2018. Before breeding trials, proper gonadal development of females were checked. Brooders were injected intramuscularly and released into aquarium.
5) *Collection of Eggs and Milt:* For milt male fishes were dissected and testes were removed. Testes were cut into many pieces by using sterile and sharp blade and these pieces crushed in mortar and pestle with 0.9 % saline solution. Latency period was followed by stripping method. Females were removed from aquarium for stripping after completion of latency period. Females were stripped after 15 hours of administration of dose. Eggs and sperm were simultaneously collected from brood fishes. Eggs were collected into clean plastic tray and mixed with few drops of sperm suspension by gently and slowly stirring with a soft feather. Sperms allowed for next five minutes to remain with eggs. Excess sperms were washed by water for 2-3 times.
6) *Incubation of Eggs by Using Water flow System:* Eggs were cleanly washed with water and released in plastic tubs (12 cm dia, 6 cm high). During incubation period water temperature of plastic tubs between 25-28 ^{o}C. Six Plastic tubs were arranged on platform stand in a row. Water was supplied from an overhead tank through a common pipe with separate control taps for each tub. Each tub was provided with an outlet at height 4 cm from base of tub. Fertilized eggs provided with continuous flow of water (@ 200 ml/min) and aeration by aerator. Incubation takes place within 30 to 36 hours after egg laying depend upon temperature. Hatchlings were transferred to plastic container for caring and rearing.
7) *Rate of Fertilization and Hatching:* Before counting the eggs, all eggs were collected in known volume of water. A small quantity of egg mass were collected with known volume of water from stripped out eggs in petri dish and calculated total number of eggs by multiplying with total volume of water. The percentage of eggs fertilization was calculated after 1 hour of artificial insemination with the help of observation of eggs by visual method and under microscopes. After incubation of three hours, eggs become translucent which is indication of fertilized eggs and opaque eggs becomes unfertilized. Likewise percentage of hatching was also calculated on the basis of number of eggs hatched from total fertilized eggs. Unfertilized eggs were discarded.

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III. RESULTS AND DISCUSSIONS

Physico-chemical parameters of water were studied and observed ranges temperature 25-28 ^{0}C, pH (7-7.5), DO (5.8 -7.2 mg/lit) and total alkalinity (290-335 mg/lit) during breeding trials.

The spawning response of *Clarias batrachus* at different doses of various inducing agents like ovaprim, ovatide, PG and HCG were given in tables 1, 2, 3 and 4 respectively. The latency period of captive breeding was 12-16 hours. The stripping response at 1.0 ml/kg body weight of ovatide was significant, followed by 0.8 and 0.6 ml/kg body weight. The dose of ovatide at 0.6 ml/kg body weight showed lowest stripping of egg. The latency period was 14±1.5 hours. Fecundity was 2510 ± 250, rate of fertilization 95±4.2%, rate of hatching 91.80±4.1% were recorded during breeding trials. Hatching takes place in 28 to 32 hours and yolk was absorbed in four days. For male broods 0.5 ml/kg body weight (table 1).

Table1. Response of Ovatide on Captive Breeding of C*larias batrachus*

Sr. No.	Weight of brooders (g)		Dose (ml/kg)		Fecundity	Rate of fertilization (%)	Rate of hatching (%)
	Female	Male	Female	Male			
1	110±10	90±15	1.0	0.5	2510±150	95±2.5	91.80±4.1
2	180±20	100±10	0.8	0.4	2225±90	93±3.0	89.00±3.5
3	110±15	80±20	0.6	0.3	2080±80	90±2.2	86.60±2.4

The stripping response at 2.0 ml/kg body weight of ovaprim dose was significant, followed by 1.5 and 1.0 ml/kg body weight. The dose of ovaprim at 1.0 ml/kg body weight showed lowest stripping of egg. The latency period was 15±1.2 hours. Fecundity was 3115±150, rate of fertilization 87±3.1%, rate of hatching 76±3.0% were recorded during breeding trials. Hatching takes place in 27 to 29 hours and yolk was absorbed in four days. Male broods showed highest response at 1.0 ml/kg body weight (table 2).

Table 2. Response of Ovaprim on Captive Breeding of C*larias batrachus*

Sr. No	Weight of brooders(g)		Dose of (mg/kg)		Fecundity	Fertilization (%)	Hatching (%)
	Female	Male	Female	Male			
1	90±10	100±10	2.0	1.0	3115± 150	87±3.1	76±3.0
2	85±15	120± 5	1.5	0.7	2960± 105	84±2.5	73±2.2
3	100±20	130±20	1.0	0.5	2786±90	81±2.1	71±2.0

The stripping response at 120 mg/kg body weight of pituitary gland extract dose was significant, followed by 80 and 40 mg/kg body weight. Zonneveld *et. al.* (1988) were also in opinion that optimum quantity of egg is obtained in right combination of pituitary dose and latency period in catfish. The dose of pituitary gland extract at 40 mg/kg body weight showed lowest stripping of egg. The latency period was 15±1.5 hours. Fecundity was 4867±140, rate of fertilization 84±2.1%, rate of hatching 72±2.0% were recorded during breeding trials. Hatching takes place by 26 to 27 hours and yolk was absorbed in four days. Male broods showed highest response at 60 mg/kg body weight (table 3). Goswami and Sarma (1997) also reported higher egg output while stripping the female at 17h post-injection in combination with 30-50 mg pituitary gland extract dose. Goswami and Sarma (1997) and Sahoo *et. al.* (2008) reported high fertilization of the eggs obtained from 30-50 mg pituitary dose in combination with 17 h latency period.

Table 3. Response of Pituitary Gland Extract on Captive Breeding of C*larias batrachus*

Sr. No	Weight of brooders (g)		Dose of (mg/kg)		Fecundity	Fertilization (%)	Hatching (%)
	Female	Male	Female	Male			
1	110±10	130±20	120	60	4867± 140	84±2.1	72±2.0
2	105±15	140±10	80	40	4045± 200	76±2.5	68±3.2
3	123±15	170±13	40	20	3657± 250	71±3.4	65±2.7

The stripping response at 4000 IU/kg body weight of HCG dose was significant, followed by 3000 and 2000 IU/kg body weight. The dose of HCG at 2000 IU/kg body weight showed lowest stripping of egg. The latency period was 16±1.2 hours. Fecundity was 3420±120, rate of fertilization 86±2.5%, rate of hatching 78±2.0 % were recorded during breeding trials. Hatching takes place by 28 to 30 hours and yolk was absorbed in four days. Male broods showed highest response at 60 mg/kg body weight of PG extract (table 4).

Table 4. Response of HCG on Captive Breeding of C*larias batrachus*

Sr. No.	Weight of brooders (g)		Dose (IU/kg)	Fecundity	Rate of fertilization (%)	Rate of hatching (%)
	Female	Male	Female			
1	225±20	150±12	4000	3420± 120	86±2.5	78±2.0
2	205±15	120±20	3000	2765± 200	78±3.2	73±3.1
3	100±10	112±15	2000	2347± 225	74±1.5	71±2.5

In present research study it is concluded that optimum dose of ovatide, ovaprim, PG extract and HCG for induced breeding of C*larias batrachus* in present condition is 1.0 ml/kg, 2.0 ml/kg, 120 mg/kg and 4000 IU/kg body weight for female respectively. For male optimum dose of ovatide, ovaprim and PG extract is 0.5 ml/kg, 1.0 ml/kg and 60mg/kg body weight for male respectively**.**

IV. CONCLUSION

Results of the present research work clearly indicates successful artificial breeding of C*larias batrachus* is feasible under controlled conditions for seed production and survival. This method of breeding can be useful for fish farmers only by accepting some practical and methodical procedures. This is also helpful for increase their population in nature and fulfill the nutritive fish food demand of increasing population.

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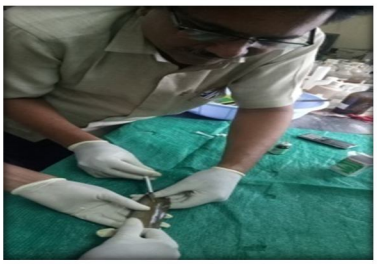
Fig. 1 Hatchery Unit Developed for Induced Breeding of Magur, *Clarias batrachus* in Laboratory


Selection of brooders


Administration of hormone


Stripping method for egg collection


Fertilization of eggs

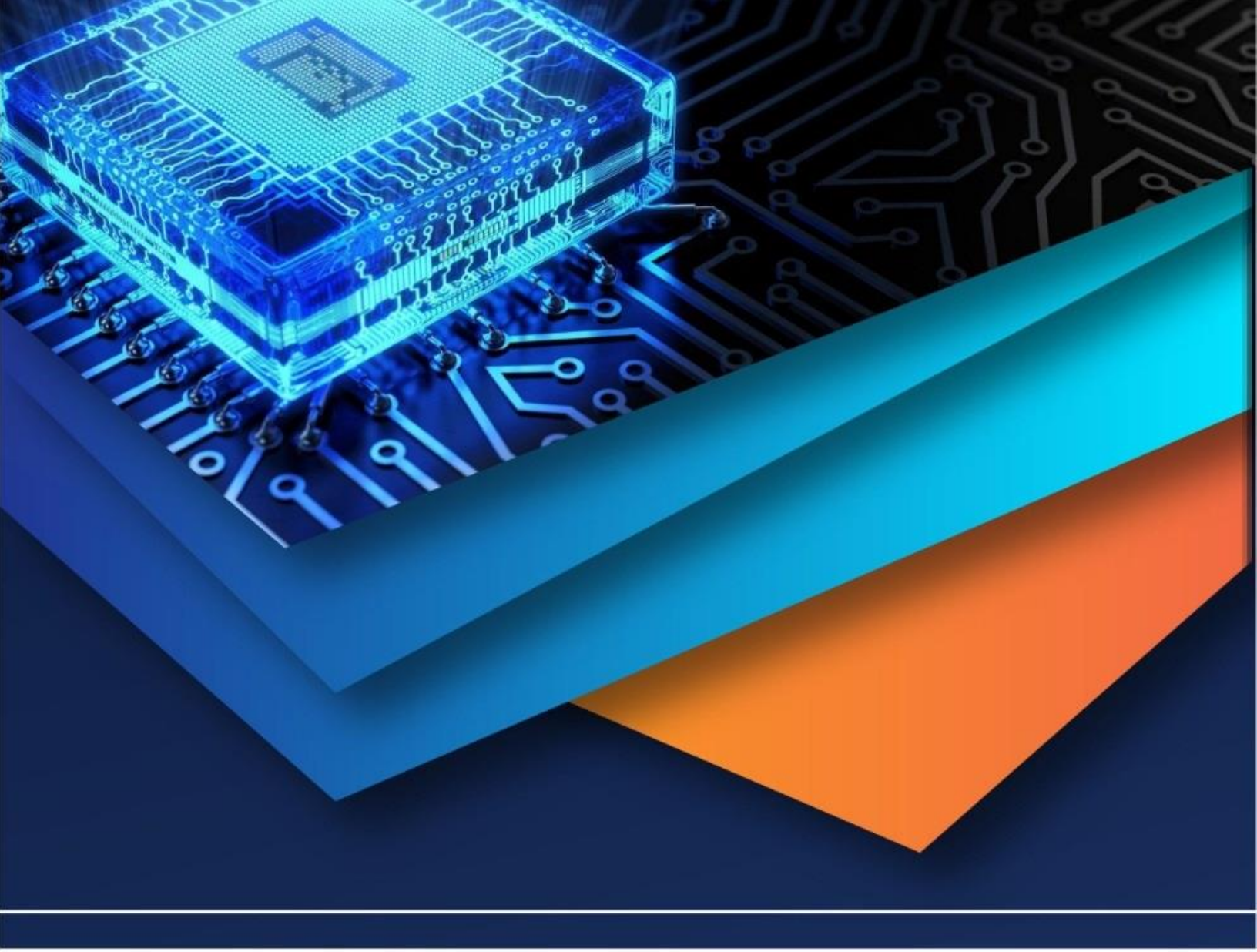

Incubation unit


Hatchlings


Advanced yolk sac fry


Early Fingerlings


Fingerlings



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