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Effect of Temperature on Fertilization and Hatchling Rate of *Clarias batrachus* induced with Ovatide

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***Abstract*: Research was conducted on the induced breeding of the Asiatic catfish (*Clarias batrachus*), a species of high medicinal and economic value that is becoming increasingly scarce in the wild. The primary objective was to optimize fertilization and hatching rates by testing various dosages of the synthetic hormone Ovatide across different thermal environments, while also identifying the ideal temperature for hatchling survival. The study involved injecting female broodstock (weighing 132–144g) with Ovatide doses ranging from 0.5 to 2.5 ml/kg of body weight. These trials were carried out at five specific temperatures: 26°C, 27°C, 28°C, 29°C, and 30°C. Optimal Dosage: 1 ml/kg of body weight. Optimal Temperature: 28°C. Peak Results: This combination yielded the highest success rates, achieving 73.52% fertilization and 78.98% hatching.**

***Keywords*: *Clarias batrachus*, ovatide, , inducing agents, fertilization, temperature.**

I. INTRODUCTION

The escalating importance of aquaculture has necessitated technological advancements to secure high-quality fish seed, which remains the foundational requirement for productive fish farming. Modern aquaculture is increasingly dependent on the artificial propagation of preferred cultivable species, as reliance on natural water bodies for seed collection is often insufficient and unpredictable (3). Artificial propagation serves as the most viable method for supplying quality seed to confined environments such as ponds, reservoirs, and lakes (4). To achieve production goals and ensure rapid growth within the shortest possible timeframe, farmers must have access to a consistent supply of juveniles.

In the Indian context, fish serves as the most affordable source of animal protein, representing approximately 40% of total protein intake (1). However, a critical bottleneck hindering the development of the sector is the scarcity of fingerlings of desired species (2). Projections suggest that to reach a production potential of one million tons under semi-intensive management, an annual supply of at least 2 billion fingerlings is required (1).

Current artificial propagation techniques require continuous refinement to ensure the availability of seed throughout the year. The modern fish industry utilizes specialized methods to manipulate reproduction, including the alteration of sexual cycles, induction of advanced or delayed maturation, and artificial fertilization. These advancements rely heavily on understanding reproductive physiology, where environmental and hormonal cues regulate spawning behaviour. Central neurological mechanisms translate environmental stimuli into chemical messengers that activate the reproductive organs, highlighting the critical functional relationship between the hypothalamus, the pituitary gland, and the pineal gland in regulating sexual maturation.

The long-term sustainability of fish farming depends on the availability of high-quality seed to replenish stocks as marketable adults are harvested. The Asian catfish (*Clarias batrachus*) is highly favoured by farmers and consumers due to its significant commercial value. However, wild-sourced seeds are often unreliable due to seasonality, species uncertainty,

disease risks, and limited quantities. Consequently, the present study investigates the controlled breeding of *Clarias batrachus* across varying temperatures using different dosages of Ovatide, utilizing the stripping method to optimize fertilization and hatching success rates.

II. MATERIALS AND METHODS

Test organism

In the present study, healthy and disease free male (average weight 138 ± 7 gm) and female (average weight 140 ± 5 gm) *Clarias batrachus* were used for this experiment. The test fish was selected on the basis of external morphological features. Female fish were identified by the presence of swollen belly with button shaped genital organ. The maturity of female was examined by the size uniformity of the eggs released. Males were identified by their slender body with elongated genital organ having pointed tip. They were collected from the local ponds and were maintained in the laboratory condition for 48 hours prior to breeding operation in the glass aquarium. During acclimatization male and female fishes were kept in separate aquaria and no feed was supplied to them.

Inducing agents

Ovatide is the synthetic inducing agent and new ovulating drug which has been successfully tested by the Central Institute of Fisheries Education (CIFE), Mumbai including some other parts of India under different agro climatic conditions, with varying degree of success. It is readily injectable spawning agent inducing gonadotropic hormone, consisting of GnRH analogue and dopamine antagonist and is also found to be efficient in induced spawning in the fish.

Breeding operations

The experiment of induced spawning was designed with five replicate trials throughout the study period. The breeding experiments were followed at five different temperatures (26°, 27°, 28°, 29° and 30°C). Ovatide was separately administered to different sets of fish. The doses of ovatide were 0.5 , 1.00, 1.5, 2.0 and 2.5 mg/kg bw for female and single dose of 1.00 mg/kg bw for males in each set .Ovatide was administered intramuscularly as separate injections at the same time in left & right sides of the caudal region above the lateral line by hypodermic syringe with a small size needle. After injection, male and female fishes were kept separately in the glass aquarium (water filled).

Eggs and milt were collected by stripping method. Fertilization was done by mixing of eggs and milts (sperms) and gently stirred with a clean feather to hasten the fertilization of eggs. Sperms were allowed to remain with the eggs for 5-10 minutes and then the excess sperms were removed by 3-4 consecutive washes with saline (0.36%) water. The transparent eggs were considered as fertilized ones whereas the opaque eggs were considered as dead eggs. Spawning behavior of hormonally induced fishes was closely observed. After spawning, the fertilized eggs were carefully collected from the breeding tank using a 500 ml beaker and transferred to a glass aquarium containing 15 L of water under gentle aeration. The fertilization rate was calculated by the following formula:

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs}}{\text{Number of total eggs}} \times 100$$

The fertilized eggs were then transferred into a hatching jar and maintained an ambient water temperature and dissolved oxygen through continuous aeration. After 28-40 hours of fertilization, hatchling were started to come out from the egg shell and hatching was completed within another 8 hours.

The rate of hatching was calculated by the following formula:

$$\text{Hatching rate} = \frac{\text{Number of hatchling}}{\text{Number of total egg}} \times 100$$

III. RESULT

The results regarding the fertilization and hatchling percentage of Asian catfish induced with ovatide at various temperatures are mentioned in Table no. 1. The fertilization rate of catfish at 26^0C at different doses of ovatide (0.5ml to 2.5ml) was 44.44%, 49.29%, 52.33%, 50.18% and 41.56% respectively. The highest percentage of fertilization was 52.33% at 1.5 ml

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dose of ovatide. The hatchling percentage was 48.58%, 55.76%, 50.40%, 55.32% and 30.23% respectively. The highest hatchling percentage was observed i.e. 55.76% (1.00ml). The fertilization rate of catfish at 27^{0}C at different doses of ovatide (0.5 to 2.5ml) was 52.30%, 54.39%, 61.32%, 57.00% and 49.59% respectively. The highest fertilization percentage was observed at 1.5 ml dose of ovatide. The hatchling percentage was 55.32%, 60.84%, 66.23%, 56.03% and 50.10% respectively. The highest percentages of hatchlings are observed at 1.5 ml of dose of ovatide.

Likewise, the fertilization rate of catfish at 28^{0}C at different doses of ovatide (0.5 to 2.5ml) was 66.56%, 73.52%, 72.01%, 66.23% and 50.45% respectively. There is significant difference ($P<0.05$) between the fertilization percentages at various doses. The highest fertilization rate was observed at 1.00 ml of ovatide dose. The hatchling percentage was 61.23%, 78.98%, 69.92%, 60.87% and 50.25% respectively. The highest hatchling percentage was observed i.e. 78.98% (1.00ml).

Whereas, the fertilization rate of catfish at 29^{0}C at different doses of ovatide (0.5 to 2.5ml) were 62.56%, 64.13%, 69.27%, 56.89% and 55.71% respectively. The highest fertilization percentage was observed at 1.5ml dose of ovatide. The hatchling percentage was 66.26%, 68.82%, 70.89%, 60.14% and 49.87% respectively. The highest hatchling percentage was observed i.e. 70.00 (1.5 ml). The fertilization rate of catfish at 30^{0}C at different doses of ovatide (0.5 to 2.5ml) were 50.85%, 58.94%, 60.20%, 50.44% and 40.12% respectively and the highest fertilization percentage was observed at 1.5 ml dose of ovatide. The hatchling percentage was 44.17%, 57.59%, 64.56%, 54.45% and 38.88% respectively. The highest hatchling percentage was observed i.e. 64.56% (1.5ml).

The results obtained at various temperature and doses of ovatide, the highest percentage of fertilization and hatchling was observed at 28^{0}C at 1.00ml dose of ovatide at laboratory condition. There is significant ($P<0.05$) difference was observed at all temperatures for fertilization and hatchling percentage.

Table 1: Effect of different water temperatures on fertilization rate and hatchling percentage of *Clarias batrachus* induced with ovatide.

Temperatures (^{0}C)				**26^{0}C**		**27^{0}C**		**28^{0}C**		**29^{0}C**		**30^{0}C**	
SrNo	Gender	Weight of Fish (g)	Dose of Ovatide (ml/kg BW)	Ferti. %	Hatchl. %	Ferti. %	Hatchl. %	Ferti. %	Hatchl. %	Ferti. %	Hatchl. %	Ferti. %	Hatchl. %
1	Male	145±2.96	-	44.44 ±7.40	48.58 ±7.19	52.30 ±4.21	55.32 ±6.02	66.56 ±11.50	61.23 ±9.36	62.56 ±10.57	66.26 ±7.92	50.85 ±4.32	44.17 ±3.97
	Female	144±2.98	0.5										
2	Male	133±3.69	-	49.29 ±6.75	55.76 ±5.54	54.39 ±7.23	60.84 ±4.16	73.52 ±13.38	78.98 ±12.43	64.13 ±6.43	68.82 ±7.69	58.94 ±10.16	57.59 ±5.59
	Female	132±3.56	1.00										
3	Male	142±3.59	-	52.33 ±2.17	50.40 ±8.53	61.32 ±6.26	66.23 ±7.44	72.01 ±5.26	69.92 ±12.39	69.27 ±5.59	70.89 ±7.70	60.20 ±8.63	64.56 ±8.17
	Female	140±5.25	1.5										
4	Male	130±4.29	-	50.18 ±7.69	55.32 ±3.56	57.00 ±5.86	56.03 ±4.28	66.23 ±14.79	60.87 ±14.10	56.89 ±4.95	60.14 ±4.62	50.44 ±5.86	54.45 ±12.26
	Female	134±4.89	2.00										
5	Male	136±3.96	-	41.56 ±8.02	30.23 ±3.67	49.59 ±3.03	50.10 ±4.55	50.45 ±7.73	50.25 ±5.07	55.71 ±9.78	49.87 ±11.24	40.12 ±4.98	38.88 ±7.40
	Female	139±3.27	2.5										

The data are based on the Means (±SD) of five replicates. Mean values bearing different superscript differ significantly ($P<0.05$).

IV. DISCUSSION

In the present work, the different doses of ovatide (0.5-2.5ml) was given to the Asiatic catfish to assess the fertilization and hatchling percentage at different temperatures i.e. 26^{0}C to 30^{0}C. In the present investigation, the fertilization and hatchling rate was maximum at 28^{0}C after the administration of 1.00ml dose of ovatide (Table 1). The quality of water was found suitable with respect to temperature, pH, total alkalinity and dissolved oxygen levels which were recorded in the range of 28^{0}C, 6.9-7.6, 128-136mgL^{-1} and 6.8-7.4 mgL^{-1} respectively during larval rearing period of *Clarias batrachus* (12). The

hatchability at high temperatures was higher (51.9% at 29^0C and 52.2% at 27^0C) than (42.2%) at lower temperature(13). The temperature is an important factor determining egg and larval development as it influences cellular function (11) and metabolic rate (7). Dhara and Saha (2013) (5) observed highest rate of fertilization (77%) and hatchling (65%) in catfish *C. batrachus* at higher dose of ovaprim at 28^0C and also opined that the highest rate of fertilization and hatchling in indoor rearing at 28^0C in all treatment may be due to the fact that this temperature played key role to optimize all physiological activities of hormone injected fish. Shinkafi and Ilesanmi (2014) (9) observed the high fertilization and hatchling percent at 28^0C to 30^0C in African catfish *Clarias gariepinus* induced with ovatide.

High hatchability and survival rate of hatchlings of African catfish at higher concentration of dissolved O_2 were also observed by many workers (8). According to Boyd (1982),(2) the higher temperature decreased the dissolved O_2 concentration by reducing its solubility in water. The findings of present work are in agreement with El-Gamal (2009) recorded the high hatchling mortality at low temperature (20^0C and 24^0C) in *C. carpio.* The low temperature was likely to be cold shock resulting increased stress due to which decreased fertilization and mortality elevated. Small and Bates (2001) (10) noticed the hindered embryonic development below 21^0C in the channel catfish *Ichtalurus punctatus*.

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