E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2025; 13(1): 213-222

www.fisheriesjournal.com
Received: 19-12-2024
Accepted: 25-01-2025

Akhand Pratap Singh
Research Scholar, St. Andrew's College, Gorakhpur, Uttar Pradesh, India

M Anto Claver
Professor, St. Andrew's College, Gorakhpur, Uttar Pradesh, India

C Vijayakumar
Professor, St. Andrew's College, Gorakhpur, Uttar Pradesh, India

Corresponding Author:
Akhand Pratap Singh
Research Scholar, St. Andrew's College, Gorakhpur, Uttar Pradesh, India

Captive breeding of the catfish, *Pangasius pangasius* by using one natural and two analogous synthetic hormones, Ovasis and Ovatide

Akhand Pratap Singh, M Anto Claver and C Vijayakumar

DOI: https://www.doi.org/10.22271/fish.2025.v13.i1c.3038

Abstract
The performance of various inducing agents in induced breeding of *Pangasius pangasius*, the catfish, is compared in this study. Three set of brooders were used for treatment of three hormones, during the disquisition. Pituitary gland extract (PGE) was given at a dose of 5 mg/ kg body weight for males and female in low (0.2 mg), medium (0.4 ml) and high (0.6 ml) in three triplicates and same for other two analogues synthetic hormones. In comparison to PGE and ovasis - convinced individualities, breeding success was shown to be advanced in ovatide - treated individualities in this study in all orders, including latency period, ovulation rate, fertilization rate, hatching rate, and incubation length. The latency period was 15 hours for PGE and ovasis given brooders and within 10 hours for ovatide doses individualities. Likewise, the current study showed that using ovatide at a rate of 0.6 ml/ kg body weight of female fishes is more effective than using other breeding hormones in terms of ovulation, fertilization, and hatching rates. The current study's findings will help hatchery directors in overseeing convinced parentage.

Keywords: *Pangasius pangasius*, threatened fishes, induced spawning, pituitary gland extract, ovatide, ovatide, ovulation rate, fertilization rate, hatching rate, survival rate

Introduction
In order to meet the demand and also to reduce pressure on natural resources for survival of the species, the ex-situ breeding has been considered a useful tool in the conservation of threatened species. Induced breeding was started in 1934 for the first time in Brazil and the fish pituitary glands were injected to induce ovulation. Later, this technique is known as hypo-physation. In India, first attempt of induced breeding was made by Khan, 1938 on *Cirrhinus mrigala.* Later Ramaswamy and Sunderaraj (1956, 1957) reported successful breeding of *Heteropneustes fossilis* and *Clarius batrachus* respectively by hormone injection. Induced breeding is a method whereby using pituitary hormone or any other synthetic hormone ripe fish breeders are induced to breed in captivity (Kumar *et al*., 2018) [8]. Recently a new drug, Ovaprim manufactured by Syndel lab. Canada has been used several workers for induced spawning with great success. The drug contain analogue of Salmon gonadotropin releasing hormone and dopamine antagonist, domperidone. It can be used safely (Brzuska, 2006) [1].

In the present study we used three different hormones, previously PGE and WOVA-FH used for captive breeding of *P. pangasius* (Chaudhary, 2022) [3] their observation has lots of inadequate results. Hence it was undertaken. Furthermore, to expand the aquaculture of *H. fossilis* and *P. pangasius*, knowledge of early larval development less and only few studies have been made (Nesa, *et al*., 2017) [9] in the UP part of India. Therefore, present study was conducted.

Methods & Materials (Fish culture and Pond maintenance)
The study took place in between July 2020 to August 2023 at Aquaculture lab, Department of Zoology St. Andrew's College. A total 58 *Pangasius pangasius* were collected (32 from Rapti and 26 from Ghaghra). All fish were kept in cement pond (10×8×5 m^3). They were fed mixed food diet 5% their body weight with 35% of crude protein and cleared chicken intestine twice a day at 10 am and 4 pm. During the experimental period, following physical parameters

was investigated: nature of the day (Sunny/ rainy/windy), color of water, temperature, ph, DO and alkalinity. In both, July 2022 and 2023 we choose healthy and mature brooders for experiments due to the favorable natural climatic factor. Male and female were placed in different ponds.

Sexual dimorphism

S. N.	Characters	Male	Female
1	Body shape and color	Body slender, more translucent and less pigmented and smaller than male	Body robust and pigmented large in body size.
2	Abdomen	Normal and not bulky	Abdomen bulging, elastic and soft
3	Genital opening	With a pinkish color and protruding genital opening	Muscular genital opening with pink color during breeding season
4	Vent	Normal vent	Prominent reddish vent
5	Putting pressure on abdomen	On slight pressure above the vent on the abdomen milky white fluid (milt) runs out through the vent	Pressure slight yellowish discharge or few ova may come out through the vent
6	Pectoral fin	Dorsal surface slightly rough	Dorsal surface smooth

Pituitary gland

The carp pituitary gland was collected from Medha fish hatchery in Maharajganj U.P. it was removed from skull of sexually maturated carp fish. Each PG was preserved in alcohol immediately after collection and stores it at cool place. Before injection PG extract prepared.

Ovasis and Ovatide

They are highly active and ready to use inject able solution intramuscularly below dorsal fin containing a synthetic peptide, analogous to Gonadotropin Releasing Hormone (GnRH) with dopamine antagonist.

Experimental design

The breeding design is shown in Table 1. Each group treated with hormonal injection in triplicate and fourth set was treated with saline water in the ratio of 1:2 (Male: female). The hapa for *P. pangasius* was made out with muslin cloth in cement tank (1×3×2) and allow flowing of water through it. To stimulate natural induced breeding environmental conditions fine gravels and aquatic plants like water hyacinth (*Echornia craspis),* spirogyra etc. were introduced into the tank, which was kept in the research lab for hiding purposes. A gentle flow of water is provided over the breeding unit**.**

Breeding

Immediately after administering the hormone injections at 6.00 pm evening, the breeding pairs were transferred to the breeding hapa. Courtship behavior like- pair swimming smoothly chasing of females by the males before spawning and the brooders spawned next day morning within 10.00-18.30 hr after injection, the low dosage individual spawned after 20.00-21.00 hr after injection and the control fishes (saline water) did not spawn even after 3 days. The fishes were allowed to spawn completely about 4-5 hr after breeding started. Later, they were removed, and eggs were weighed for count their number. Once the breeding is over, the spawners were given bath in 1% saline solution for 15-20 minutes, followed by freshwater bath and fishes were then maintained for future use. The average range of water quality parameters during study period: Temperature- 29-32 ºC, Dissolved oxygen- 4.6-7.5 mg/l, pH- 7.1-7.3 and Salinity- 1.01-1.03%

Fig 1: Intramuscular hormonal injection in *P. pangasius*

Fig 2: Breeding unit in cement tank and plastic trough

Egg counting and observation

Total number of eggs laid can easily be calculated by counting the number of eggs / gms (Chonder,):

Ovulation rate =No. of eggs per gram × Wt. of total egg (gm)

spawned by female fish

Eggs collected from each breeding compartment and the percentage of fertilization was estimated by examining a sample of at least 100 eggs from each breeding set and the percentage of fertilization rate was calculated (Chonder,)

$$\text{Percentage of fertilization} - \frac{\text{Number of fertilized eggs}}{\text{Numbers of eggs in a sample}} \times 100$$

Thereafter, the brooders fishes were checked individually for the extent of spawning by gently pressing the belly and if some eggs oozed out they were classified as partially spawned while others whom eggs were not oozed out classified as no response category. Now, eggs were treated with 2 ppm concentration of malachite green (by dipping the eggs to prevent bacterial, viral and other infection) and transferred them to the hatching unit (provided the system with sufficient oxygen by spraying and circulating water that were maintained by PVC pipe). Quality of eggs, no. of eggs, fertilization rate, time taken for hatching and viability of embryo and larval development stages were examined.

Hatchery operation and feeding

The submerged eggs were collected from the floor of breeding hapa and directly transferred to the hatchery unit. Dull, unfertilized eggs were separated from transparent, living ones and percentage of living embryo calculated by taking sample from the hatching unit (Figure 13) with the help of wide mouth pipette and 100 of these were placed on Petri dish, and developing embryo counted under magnifying lens (2x), flow of water maintained 0.6 l/min. for rotation of eggs.

$$\text{Hatching rate} = \frac{\text{Number of eggs hatched}}{\text{Total no. of fertilized eggs}} \times 100$$

Fig 3: Hatching unit

After 8 hrs water flow was increased to 1.5 l/min for the removal of undeveloped larva through outlet with water flow and the hatched larva goes towards the bottom and walls of hatchery unit and covered with thick mesh to minimize light and by which yolk absorption had taken place, larvae were fed with dried tubifex larva (Figure 14) (3-15 days), chopped chicken liver (16-25 days) and larval development monitored for 20 days and then fish were transferred to the cement culturing tank (2m×1m×1m) and fed with formulated feed. Formulated feed provide them well growth and also reduce cost of captive breeding.

Studies on developmental biology

The eggs were collected from hatchery unit every hour for the first 24 hr and every 8 hour for the next three days. From the 4th day onwards sample were collected at every 16 hr interval and in each sample minimum 6 larvae were taken. The sample of eggs observed as per Bruton method. The embryonic development was studied through microscopically observations by using binocular microscope (under 40x) and photographs were taken.

Statistical analysis

The effect of different doses of Pituitary extract, Ovasis and Ovatide on the latency period, egg number, fertilization rate, incubation period, hatching percentage, survival at first feeding, survival at fry and fingerlings were analyzed by Mean ± SD and ANOVA (Turkey test). The statistical analysis of the data was carried out by using Microsoft Excel 2012).

Captive breeding and development studies of *Pangasius pangasius*

In present study, one natural (PGE) with two dosages and two analogues synthetic hormones (Ovasis and Ovatide) with single dosage**,** were used for induced breeding of *Pangasius pangasius* in breeding hapa made up of muslin cloth (cement tank) while embryonic study take place in plastic trough. During the study, male fish ranged from 44-48 cm in total length (1649-1879 gm in body weight) while female fishes ranged from 45-49 cm in total length (1760-2095 gm in to body weight) of the brooders were selected for experiment. After 8 to 16 hrs of injection, male move actively around female. They started nudge with its snout at the ventral region of female and at this time brooders are chosen for striping by applying gently pushed in the abdomen region.

Fig 4: Striped female eggs by applying gently pushed in in the abdomen region

Fig 5: Striping of male milt by applying gently pushed the abdomen region

With the help of soft brush, sperm and ova are mixed to ensure fertilization. Water added while swirling to firm the eggs and washed the same for 3 to 4 times. Before transferring hatchery unit, the eggs treated with malachite green (3-4 gm/lit. water) and in the hatchery unit containing water also treated with potassium permanganate (5-9 gm/lit.). Then, the fertilized eggs slowly fall down to the bottom of the plastic trough. It was black and greenish in color. They were collected by siphoning while unfertilized eggs were seen white or opaque which floating above.

Table 1: Induced spawning of *Pangasius pangasius* by using three different hormones:

Hormones	Dosage level (per kg of body weight)	Female length (cm)	Female wt (gm)	Male length (cm)	Male wt (gm)	Spawning	Hatching
Pituitary extract (Two injections at one hr interval)	Low 1.0 mg + 2.0 mg	45.2±1.92	1760.24±155.36	44.3±1.37	1649.34±156.35	Partial	Normal
	Medium 1.0 mg+ 4.0 mg	47.4±2.10	1956.84±137.26	48.1±2.46	1856.49±152.36	Complete	
	High 1.0 mg+ 6.0 mg	49.1±1.32	2095.59±89.35	44.6±3.46	1759.34±145.34	Complete	
Ovasis (Single dosage)	Low 0.2 ml	46.6±3.12	1867.15±155.34	44.2±2.01	1654.20±128.37	Partial	
	Medium 0.4 ml	47.1±1.23	1895.49±142.03	44.6±1.09	1650.12±138.78	Complete	
	High 0.6 ml	44.7±1.89	1769.25±128.37	44.0±1.95	1568.18±142.71	Complete	
Ovatide (Single dosage)	Low 0.2 ml	48.1±2.46	2040.75±155.36	48.3±2.03	1879.94±148.59	Partial	
	Medium 0.4 ml	48.4±3.18	2068.48±148.54	48.1±3.45	1874.84±167.22	Complete	
	High 0.6 ml	47.7±2.01	1987.14±139.34	43.9±2.75	1721.24±170.35	Complete	
Control (Saline water)	0.6 ml	47.2±1.65	1967.24±145.67	47.8±2.07	1815.54±167.34	No response	

Result

Latency period is maximum in low dosages of PGE (18.61±0.60) while it is minimum in high dosages of ovatide (8.2±0.25). Number of eggs laid is minimum in low dosages of PGE (235.33±25.16) while maximum in high dosages of ovatide (46,647.67±251.66). Fertilization rate minimum in low dosages of PGE (57±1.73), maximum in high dosages of ovasis (78.33±0.57) and ovatide (81.33±1.15). Incubation period is maximum in low PGE (30.53±0.49) and minimum in high dosages of ovasis (18.65±0.48) and ovatide (17.91±0.43). Hatching period is minimum in low dosage of PGE (35.33±0.57) and maximum in high dosage of ovatide (70.66±0.57). Survival at first feeding stage is minimum in low dosage of PGE (42.33±0.57) and maximum in high dosage of ovatide (81.33±0.57), Survival at fry minimum in low dosage of PGE (35.33±0.57) whereas it was maximum in high dosage of ovatide (72.33±0.57) while Survival at fingerlings stages is minimum in low dosages of PGE (46.63±0.57) and maximum in high dosages of ovasis (78.66±0.57) and ovatide (82.66±0.57).

Hence, in present study declare that high dosage of ovatide is best for captive breeding of *Pangasius pangasius*. (Table 2) (Graph 2 a - h).

Table 2 a-h: Summary of Captive breeding of *P. pangasius* using various hormones (Mean± SD). Values followed by same alphabets are statistically not significant (Turkey test).

a Latency period (hrs)

Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	18.61±0.60b	16.63±0.4b	14.01±0.40ab
Ovasis	16.22±1.01b	14.05±0.42ab	10.47±0.07a
Ovatide	14.26±0.22ab	11.27±0.19ab	8.2±0.25a

b. Number of eggs

Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	235.33±25.16aA	23,075.33±288.67b	37,779±360.55cA
Ovasis	1,100±100.00 aB	28,242.33±173.20bA	42,289±251.57cA
Ovatide	1,716.66±28.86aB	30,260.67±288.67bA	46,647.67±251.66dA

c. Fertilization rate (%)

Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	57±1.73a	62.66±1.52a	70.66±0.57a
Ovasis	64.33±0.57a	70.33±0.57a	78.33±0.57a
Ovatide	68.33±0.57a	75.33±0.57a	81.33±1.1a

d. Incubation period (hrs)

Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	30.53±0.49b	27.56±0.42ab	23.98±0.46ab
Ovasis	23.8±0.43ab	21.5±0.43ab	18.65±0.48a
Ovatide	22.6±0.36ab	20.1±0.17sb	17.91±0.43a

e. Hatching (%)

Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	35.33±0.57a	47.33±1.15ab	52.66±0.57aA
Ovasis	45.33±0.57ab	57.66±0.57aA	65.33±0.57aB
Ovatide	47.66±0.57ab	60.66±0.57aA	70.66±0.57aB

f. Survival at 1st feeding (%)

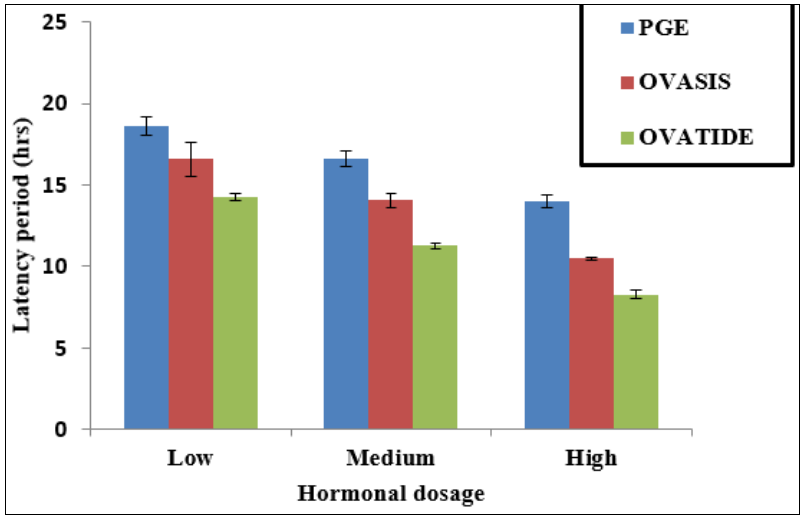
Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	42.33±0.57a	52.33±0.57ab	69.66±0.57aB
Ovasis	48.66±1.15a	58.66±0.57ab	75.33±0.57A
Ovatide	62.33±0.57saB	67.66±0.57aB	81.33±1.15A

g. Survival at fry stage (%)

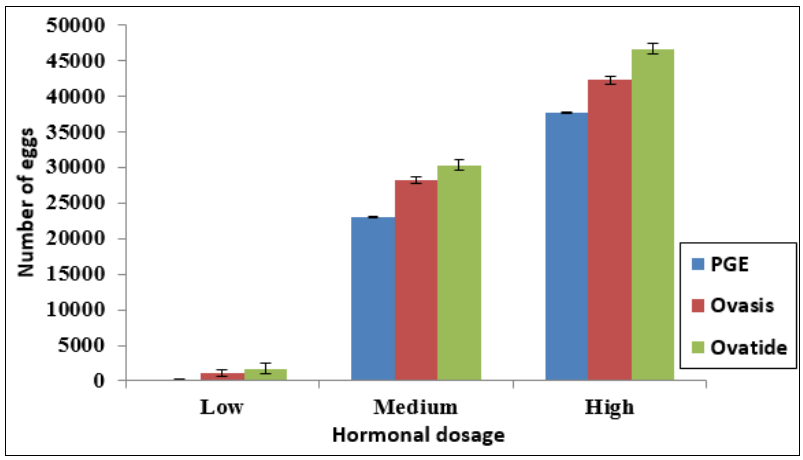
Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	35.33±0.57a	47.33±0.57aB	64.33±0.57Ba
Ovasis	39.66±0.57a	51.66±0.57Ab	68.66±0.57Ba
Ovatide	41.33±0.57aB	58.66±0.57Ab	72.33±0.57Ba

h. Survival at fingerlings stage (%)

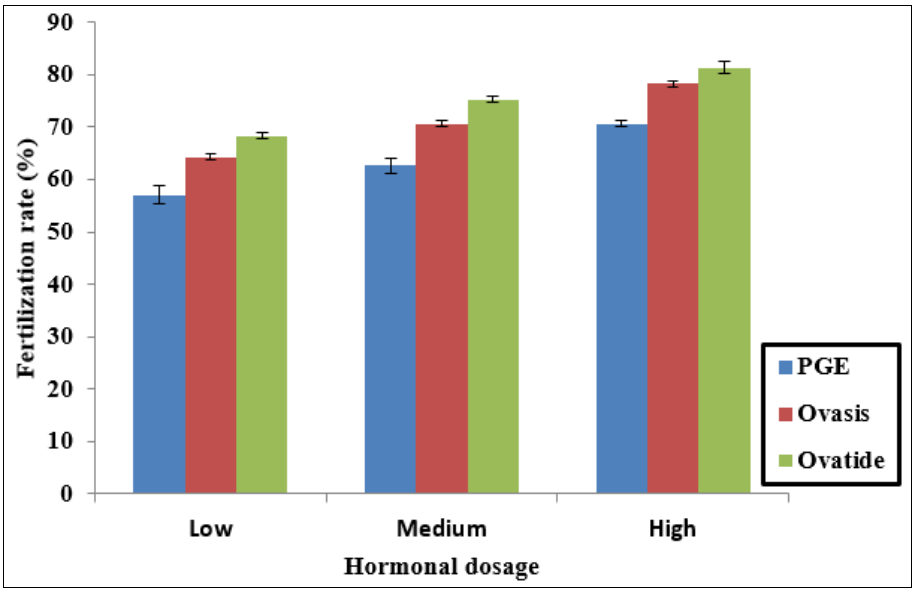
Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	46.63±0.57a	65.66±0.57aB	70.66±0.57Ab
Ovasis	51.33±0.57a	71.33±0.5aB7	78.66±0.57Ab
Ovatide	58.66±0.57a	73.66±0.57aB	82.66±0.57Ab



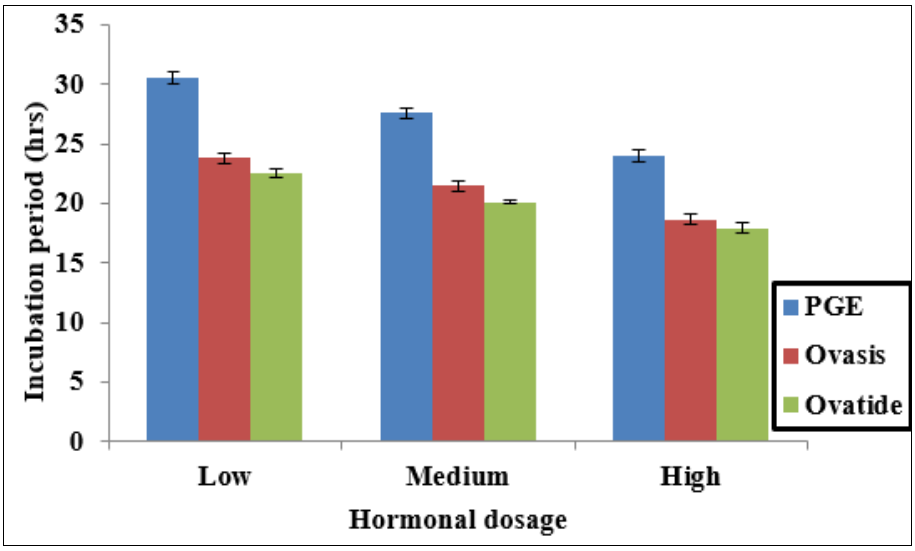
Graph 1a: Effect of various hormones on latency period (hrs) of *P. pangasius* (vertical bar indicate SD)



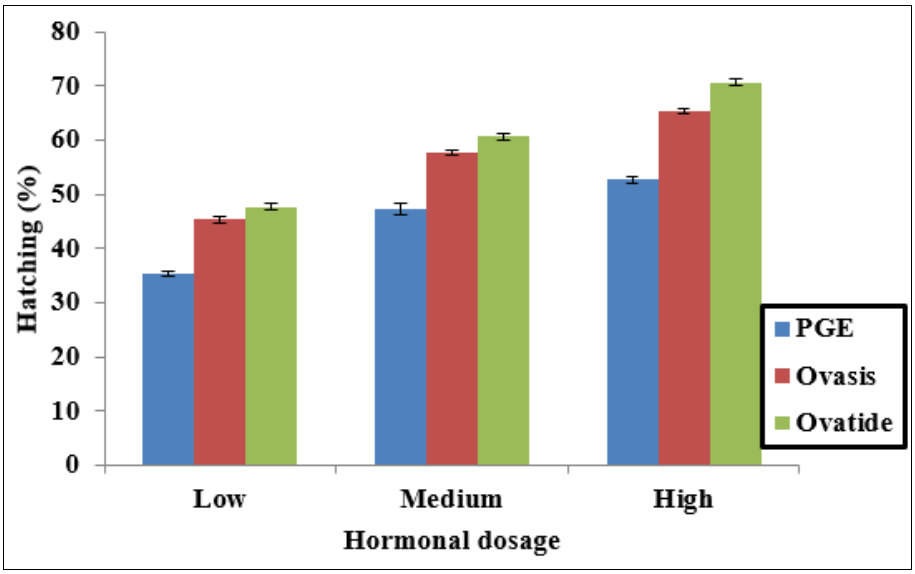
Graph 1b: Effect of various hormones on number of eggs of *P. pangasius* (vertical bar indicate SD)



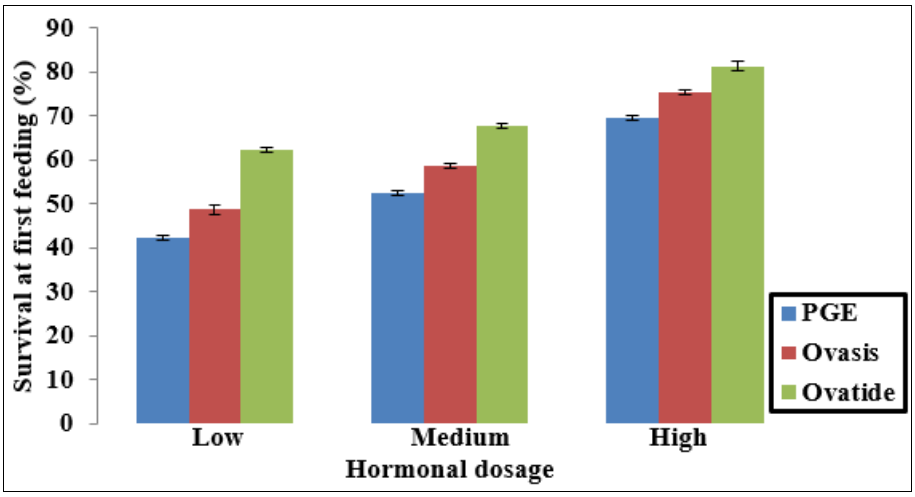
Graph 1c: Effect of various hormones on fertilization of eggs (%) of *P. pangasius* (vertical bar indicate SD)



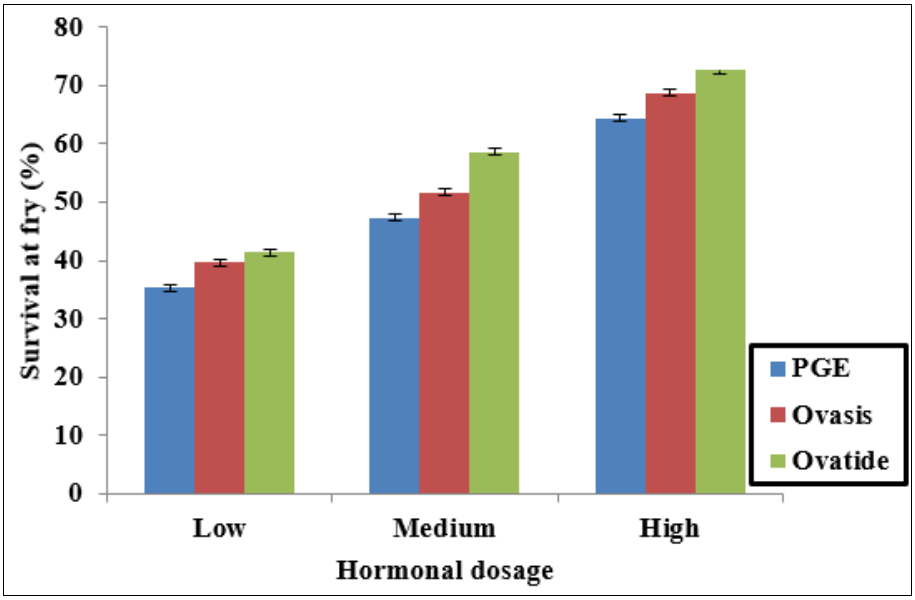
Graph 1d: Effect of various hormones on incubation period (hrs) of *P. pangasius* (vertical bar indicate SD)



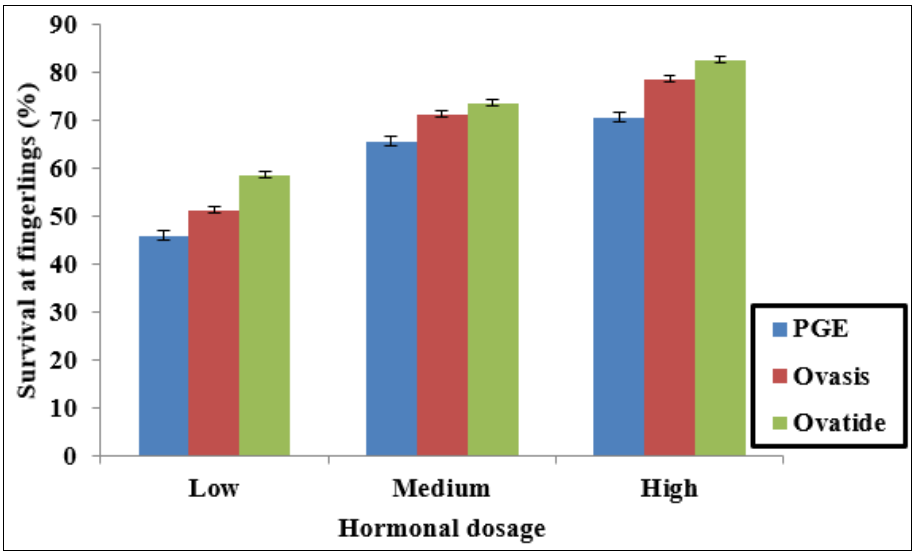
Graph 1e: Effect of various hormones on egg hatchability (%) of *P. pangasius* (vertical bar indicate SD)



Graph 1f: Survival of *P. pangasius* at 1st feeding stage (%) from day 4 to 6 after hatching (vertical bar indicate SD)



Graph 1 g: Percent survival of *P. pangasius* fry showed by various hormones (vertical bar indicate SD)



Graph 1h: Percent survival of *P. pangasius* fingerlings showed by various hormones (vertical bar indicate SD)

Developmental biology of *Pangasius pangasius*

Striped eggs were transparent, spherical, adhesive, and whitish in color (diameter 1.08-1.26 mm). The egg membrane was separated from the rest of the egg by a small peri-vitelline space.

a) Unfertilized eggs: Looks dull, opaque, white and nucleus disintegrated within one hour (1.01-1.1) (Image 21 a).

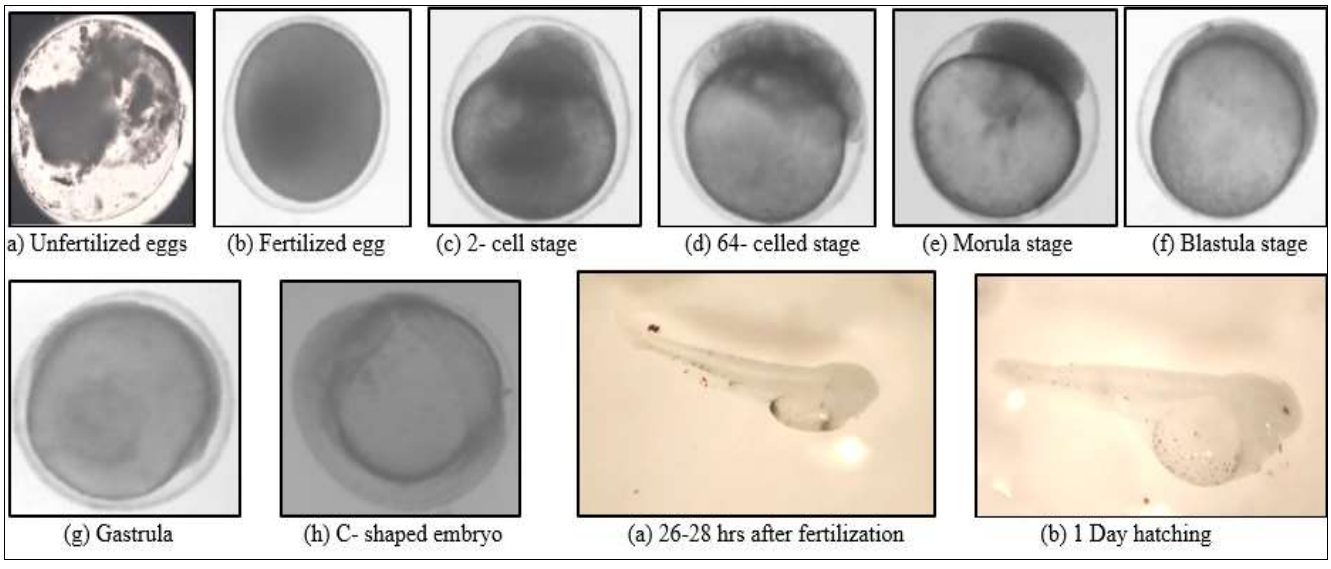
b) Fertilized egg: Round, clear, adhesive in nature and

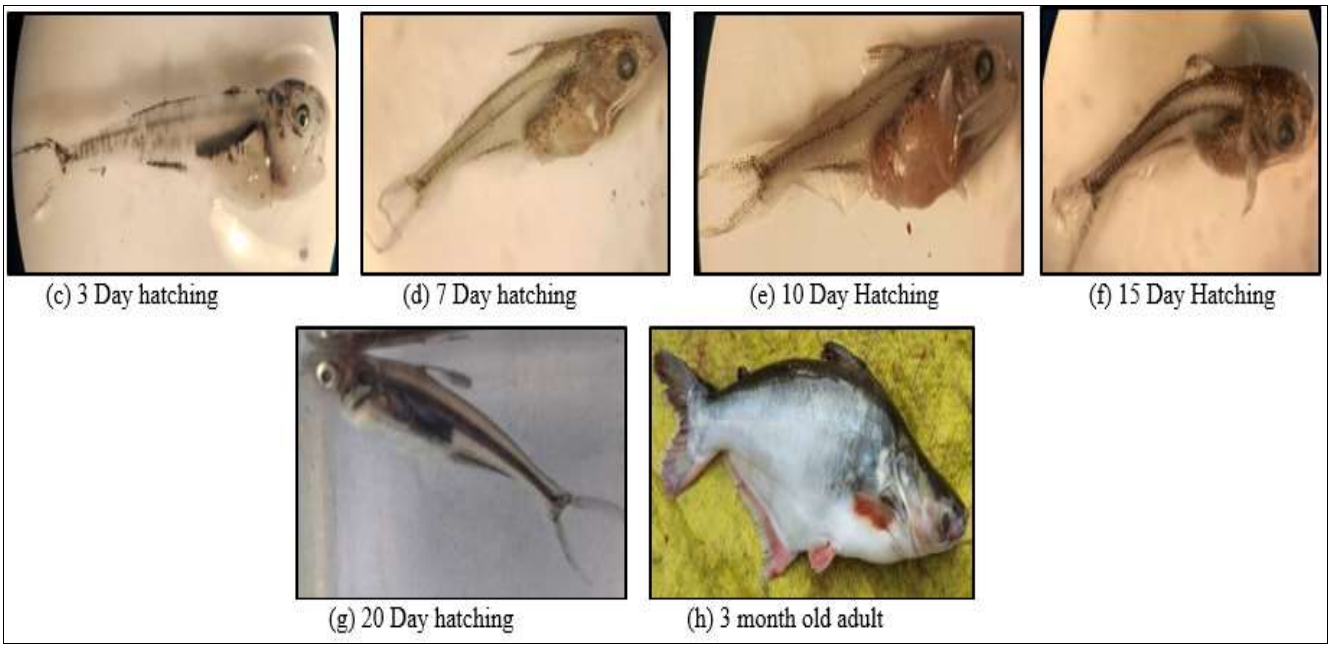
reddish spot on one pole (1.2-1.5 mm) (Image 21 b).

c) **2- cell stage (00.50-01.02hrs):** At the animal pole, an outgrowth blastoderm grew over the vitelline sphere which is clear and free of yolk and completely separated the egg membrane and blastodisc divide into two celled (Image 21 c).
d) **64- celled stage (02.36-02.40 hrs):** The blastoderms at these stages were unequal and reduced in size. Blastoderm also overlapped and their boundaries slightly visible (Image 21 d).
e) **Morula stage (03.50-04.25 hrs):** Due to continual division, blastodermal cells were very tiny and gave animal poles a floral appearance and lost their boundaries. They appeared compact and look like marigold flower at animal pole. (Image 21 e)
f) **Blastula (05.20-06.30 hrs):** The blastomeres are challenging to distinguish. Over the yolk sphere, the blastoderm was squeezed and covered less than half of the animal pole (Image 21 f)
g) **Gastrula (07.35-08.40 hrs embryos):** Over the yolk sphere, a thick layer of blastoderm or the genital ring took up 60% of the space and embryonic shield in the middle that extends into the germ ring. Its broad end, which would later form the embryo's copal, was noted **(**Image 21 g**).**
h) **C- shaped embryo (09.40-10.55 hrs embryo):** At this stage head and tail end was recognized. After another 6-7 hrs from this stage differentiation of cephalic region, optic vesicle, and tail region seen and the embryo look like mini fish encircled yolk.(Image 21 h). Twitching movement was observed at 21.50-23.05 hrs of post fertilization. The embryo was fully formed and shows clear differentiation between head and tail region. Head was attached with yolk while tail free. Beating of tail take place in this stage just before hatching and the larva started hatching at 26.00-28.00 hrs.

Larval development

a) **26-28 hrs after fertilization:** Larvae that had just emerged from the egg were slim, straight, translucent, progressively tapering towards the tail, and capable of free swimming. The tail lengths were 20% of the overall length. Larvae measured 3.5±0.01 mm in size. The yolk sac was spherical, compact, light colored and slightly transparent. Head is jointed to the yolk sac as a result, seen served in the anterior part. The mouth and mouth cleft indistinguishable. There was no discernible back apparent eye (Image 22 a).
b) **1 Day hatching:** After hatching, mouth was not immediately apparent. The tail gets bulkier. The mouth stayed open for the first day after hatching, and as the larvae grows older, the space between the gradually shrunk. The eyes are tiny and resemble a black dot on the front of the head. Larvae measured 4.5±0.5 mm in length. Barbells show a slight elevation below the lower jaw (Image 22 b).
c) **3 Day hatching:** Larvae were fully grown at this stage. The yolk sac lacks a spherical shape, has been completely absorbed, and has thinned out to form conspicuous pectoral and pelvic fin fold. There was noticeable mouth cleft. Operculum and pigmented dark eyes were both clearly appeared. Barbells on the upper jaw were also readily apparent. Pectoral, dorsal and pelvic fins were present but not particularly noticeable. Length of larvae was 6.0 ± 0.3 mm (Image 22 c).
d) **7 Day hatching:** The upper jaw was somewhat longer than lower jaw, and lips have fully formed. Mouth is still open, but the snout appears round. The barbells on the lower jaw were longer than upper jaw. It was easy to see the air bladder. The eyes were prominent which 0.2 mm in diameter was. The number of heads with pigmentation spot. The alimentary canal coils up to vent and length of larvae became 7.5±0.5 mm (Image 22 d)
e) **10 Day Hatching:** Length became 10.8±2.00 mm and post anal length 4.8 mm. Dorsal, pectoral, anal and caudal fin were clearly seen. Larvae frequent movement on surface and phenomena of aerial breathing was observed. (Image 22 e).
f) **15 Day Hatching:** Length became 14.2±0.2 mm and post anal length 7.2 mm. Larvae showing active swim and voracious feeding behavior. Prominent black spot was seen mostly on dorsal body surface (Image 22 f). Larvae look like adult.
g) **20 Day Hatching:** At this stage length became 18.6±0.9 mm and post anal length were 13.7 mm. Pigment appear of cell over the body of the juvenile, organogenesis was completed and juvenile were morphologically similar to the adult expect for their color pattern (Image 22 g).





Discussion

In the current study, each female was reported to have produced between 44,000-48,000 sticky eggs in a single spawn. This number is all time high (Chaudhary, 2022) [3] and again fertility rate too at Gorakhpur water. Diameter of unfertilized egg ranged from1.01-1.3 mm and fertilized egg 1.3-1.4 mm which is similar to the study of Foresekhan *et al* (2015) [4]. Latency period (after injecting high dosages of ovatide) is 8.30-10.30 hrs. It started for spawning which is similar to Sah *et al*. (2018) [12] observations. In the present study we obtained Fertilization rates (82%) and hatching rate (77%) when injecting high dosage of ovatide. Similar, fertilization rates (85.20%) but very low hatching rate (48.20%) of *P. pangasius* observed using ovasis (Sah *et al*, 2018) [12]. The mode of cleavage pattern recorded in the present study was similar to other cat fish species such as *Pangasius sutchi* (Islam, 2005) [6]. In present study, the hatching time is 26 to 28 hrs at 29-32 ºC but previous study its about 24 to 25 hrs at 27-28 ºC (Singh *et al*, 2015) [16] while incubation period is 18 hrs when injected to high dosages of ovatide which is less than previous study (Singh et el, 2018) [13]. The morula stage was reached between 03.50 and 04.25 hrs, blastula stage is 05.20-06.30 hrs, and gastrula stage is 07.35-08.40 hrs after fertilization. These durations were almost similar to the values of another cat fish, *Ompak bimaculatus* (Vijayakumar *et al*.,). Just before hatching the embryo of *P. pangasius* showed twisting movements 21.50-23.05 and similar behavior reported in *P. sultchi* (Islam, 2005) [6].

In the present study complete yolk sac absorption was observed on 3rd day and similar event occurred on the 3rd day in other study also (Ogunji *et al*, 1999) [10].

Acknowledgments

Author is largely thankful to Mrs. R.N Samuel, Head, Department of Zoology, St. Andrew's College, Gorakhpur and the Prof. C.O. Samuel, Principle and Secretary of St. Andrew's council operation for furnishing necessary laboratory installations. I'm also obliged to native fisherman of these tributaries for their co-operation during entire study period.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of the present paper.

Reference

1. Brzuska E. Artificial propagation of female Hungarian strain 7 carp (Cyprinus carpio) after treatment with carp pituitary homogenate, Ovopel or Dagin. Czech J Anim Sci. 2006;13:87-96.
2. Chondar SL. Induced carp breeding. 3rd ed. New Delhi: CBS Publishers and Distributors; c1994. p. 142.
3. Chaudhary. Induced breeding and rearing of Pangas (*Pangasius pangasius*) using synthetic hormone Wava-FH at Shree Sanjay fish and fry production centre, Santnagar village (Siraha), Nepal. 2022.
4. Ferosekhan S, Sahoo SK, Giri SS, Saha A, Paramanik M. Embryonic and larval development of yellowtail catfish, *Pangasius pangasius*. J Aquac Res Dev. 2015;6:6.
5. Brutun. Have fishes had their chips? The dilemma of threatened fishes. Environ Biol Fishes. 1995;43:1-27.
6. Islam A. Thai Pangus (Pangasius sutchi) flower embryonic and larval development. 2005;47:1-6.
7. Khan MHK, Mollah MFA. Gonadal histology of *Pangasius pangasius* (Hamilton). Bangladesh J Fish. 1938;21(2):22-73.
8. Kumar P, Biswas G, Ghpshal TK, Lailasam M, Vijayan KK. Embryonic and larval development of brackish water catfish Mystus guilo induced breeding with HCG and consequent larval rearing. 2018;13706.
9. Nesa NU, Ahemed SSM. Breeding performance, embryonic and larval development of stinging catfish, Heteropneustes fossilis in Bangladesh. J Environ Sci Nat Resour. 2017;10(1):141-148.
10. Ogunji JO, Rahe RE. Larval development of the African catfish Heterobranchus longifilis VAL, 1840 (Teleostei; Claridae) and its larval behavior. J Aquac Tropics. 1999;14:11-25.
11. Ramasawamy LS, Sundaraj B. Inducing spawning in the Indian catfish. Science. 1956;123:1080.
12. Sah U, Wagle SK, Mehta SN, Mukhiya YK. Preliminary observations on breeding and fry rearing of pangas (Pangasius hypophthalmus) in eastern Terai region of Nepal. IJFAR. 2018;3(3):14-16.

13. Datta SN, Singh A, Jassal G, Pandey A. A study on induced breeding, embryonic and larval development of Pangasius hypophthalmus in semi-arid agro-climate. J Environ Biol. 2018;39:671-678.
14. The IUCN. The IUCN Red List of Threatened Species. Version 2020-1-4. 2020.
15. Vijayakumar C, Sridhar S, Haniffa MA. Low cost breeding and hatching techniques of the catfish (Heteropneustes fossilis) for small-scale farmers. Naga. 1998;21:15-17.
16. Singh JS. Microbes: the chief ecological engineers in reinstating equilibrium in degraded ecosystems. Agriculture, ecosystems & environment. 2015 May 1;203:80-82.