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Research Article

Dose Selection for Induced Breeding and Larval Development of Indigenous Ornamental Fish *Puntius chola* (Hamilton, 1822)

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ABSTRACT

Puntius chola (Hamilton, 1822) is a small freshwater indigenous fish with food and ornamental value belonging to the family Cyprinidae found in Pakistan, India, Nepal, Bangladesh, Myanmar, and Sri Lanka. Due to the selective exotic species and the culture of Indian big carp, the species have gradually diminished in India. Evaluating *P. chola*'s optimum selection, induced breeding, embryonic, and larval development is the goal of the current study. The optimum dose was selected through the trial-and-error method applied 5 doses (0.25 ml/kg, 0.50 ml/kg, 1 ml/kg, 1.5 ml/kg, 2 ml/kg body weight) of synthetic hormone, ovatide to both sexes. The study reveals that the optimum dose of synthetic hormone ovatide @ 1.5 ml/kg body weight for females and males is effective for induced breeding of *Puntius chola*. At the optimum dose, fecundity, fertilization, and hatching rates were 106308±3075, 79.28±0.589%, and 78.03±0.495%, respectively. The physicochemical parameters of water have been enlisted for proper induced breeding. The outcomes of this research will enable P. chola to have a more decorative design and assist in its protection by encouraging it to reproduce and survive on its own in the wild. Additionally, the study will aid in community members' economic development.

Keywords: Optimum dose, Induced breeding, *Puntius chola*, Ornamental, Conservation

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INTRODUCTION

Ornamental fish are calm, beautiful fish that are kept as pets in small aquariums or backyard swimming pools so that their beauty can be appreciated. Because of their color, shape, and behavior, ornamental fish are often referred to as "living jewels" (Das et al., 2005). The number of people who keep ornamental fish is growing every day because it is a great way to start a business and make money. Aquaculture's fastest-growing branch could be ornamental fish breeding and rearing and the ornamental fish trade. Even though indigenous ornamental fish contribute less to the ornamental fish trade generally, they have the potential to generate indigenous ornamental fish. Breeding and aquarium technology improvements have given the ornamental fish industry a replacement dimension. India has a huge market opportunity in the production of ornamental fish, which are much sought after both domestically and internationally (Elamparithy, 1996). *Puntius* has become popular as a freshwater aquarium fish due to their striking coloration; several species are traded internationally as ornamentals (Collins et al., 2012). *Puntius chola* (Hamilton, 1822), also known as the "swamp barb" is a freshwater indigenous ornamental fish species belonging to the family Cyprinidae. It is distributed in Bangladesh, India, Pakistan, Nepal, Myanmar, and Si Lanka (Pethiyagoda, 1991), where it can be found in lakes, rivers, streams, ponds, ditches, and inundated water bodies.

Induced breeding is a technique that uses artificial stimulation to breed economically valu-

able fish that do not usually reproduce in captivity. By injecting pituitary hormone or similar synthetic hormone, ripe fish breeders can be encouraged to breed in captivity using the procedure known as "induced breeding" (Mohapatra et al., 2016). The stimulation encourages the release of sperm and eggs on time. Udit et al. (2014) reported 0.3 ml for 232-240 g females and 0.2 ml for 180 g males for induced breeding and larval development on *Puntius sarana*, with male and female ratios of 2:1 and 1:2. Sarma S. (2015) studied the induced breeding of *Pethia gelius* where they applied Ovaprim 0.5-0.4 ml/kg (F), 0.4-0.3 ml/kg (M) with a sex ratio of 2: 1 in the Bihar. Motilan *et al.* (2014) applied WOVA-FH, 0.2-0.4 ml/kg body weight of both males and females with a sex ratio of 2:1 for induced breeding of *Pethia manipurensis*. Saha & Saha (2010) reported some biological aspects of *P. chola* (Hamilton, 1822) but did not work on induced breeding. Sit *et al*. (2020) studied only the diversity of Puntius species but did not study on induced breeding of these species. Vincent and Thomas (2008) observed courtship behavior and nuptial coloration during the breeding of the *P. chola*. There was no clear study on egg release and larval development of *P. chola*. It is clear from the literature already in existence that P. chola from Asia in general and India in particular have not yet been the focus of a thorough investigation of induced breeding. Due to the scarcity and significance of small indigenous fish *P. chola* (Hamilton,1822), we worked on induced breeding to increase the species' availability. Therefore, the present study obtained management of captive broodstock, dose selection for artificial breeding, and larval development of *P. chola* (Hamilton,1822)

MATERIAL AND METHODS

Brooder's collection

The collection of ornamental fish has been carried out covering the period commencing from April 2020 to March 2022 from different ponds and rivers of the Paschim Medinipur district of India using a gill net and a cast net and brought to the ornamental fish rearing and breeding center at Raja Narendra Lal Khan Women's College (A), Midnapore (Figure 1a). Before the brooders were put in their new homes for further research, their length and weight were measured and written down (Figure 1c-d).

Acclimatization and feeding of the brooders

The brooders were acclimated in customized aquarium tanks constructed of glass that featured aquatic plants and artificial hideouts (Figure 1 b). The rearing tanks were sanitized with a 5 percent KMnO4 solution before the fish were introduced. During the acclimatization period, the fish were fed live feed such as mosquito larvae, phytoplankton, zooplankton, and high-protein commercial feed at 4–5 $g.kg^{-1}$ body weight daily. The brooders were fed commercial feed containing 47% protein, 5 % crude fat, 10 % moisture, 17 % ash content, 1 % phosphorous, vitamin A (2,500 IU/kg), vitamin D (2,500 IU/kg), vitamin E (2,000 IU/kg), and ascorbic acid (510 mg/kg).

Hormone injection

During the present study, brooders (6 - 8 g) were injected by using the synthetic hormone ovatide (M/s. Hemmo Pharma, Mumbai) at 5 doses (0.25 g/kg, 0.5 g/kg, 1g/kg, 1.5 g/kg, 2 g/kg) to optimize the ideal dose in both sexes of *P. chola*. Using a 1ml graduated syringe, appropriate doses were administered intramuscularly at a 45° angle on each pair's dorsal side of the caudal peduncle (Figure 1 e). Use a breeding hapa (a mosquito net made for breeding purposes) for each set (Figure 1 f). An emerged portion of the hapa measures 4 feet x 2 feet x 2 feet in surface volume.

Fecundity

After the release of the eggs, the eggs are counted across an area of one square foot on the surface area of hapa and then multiplied by the total surface area of an emerged portion of hapa. Fecundity is measured by the following formula

$$\text{Fecundity} = \frac{\text{Number of egg in one squre feet net} \times \text{Total squre feet of surface area}}{\text{Weight of fish}} \times 1000$$

Hatching rate and fertilization rate

Inside clear egg cells, fertilized eggs had intact nuclei. A sample of approximately 100 eggs was tanked at random in a glass petri dish to determine the fertilization rate of eggs. The rate of fertilization is calculated using the formula below.

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

Spent fish were collected from each breeding hapa after spawning. After that, the fertilized eggs were placed in hatching trays and left to hatch. The hatching rate is calculated using the formula below.

$$\text{Hatching rate} = \frac{\text{Number of spawn}}{\text{total number of fertilized eggs}} \times 100$$

Embryonic and larval development observed

For further research, samples of eggs were taken before fertilization and after fertilization at 10-minute intervals. The embryonic stage takes place within the chorion and culminates in hatching. When the larva can ingest food from outside its body, the larval stage, which is defined by the yolk sac's nutritional contribution, comes to an end. The post-larval stage begins as soon as the yolk sac is absorbed and is characterized by self-feeding. With the help of a light microscope (Olympus CX31), the development of embryos, larvae, and spawn was watched, as well as how long each stage lasted and how it changed in shape (Figure 1 k-l).

Water quality measure

The water quality in the brood, spawning, and larval rearing tanks was regulated at an optimal throughout the experiment, as required for *P. chola*. In accordance with APHA guidelines, the water parameters were monitored weekly during rearing using water analyzer 371 (Systronics). Some of the physical and chemical parameters that were measured were the surface temperature, water temperature, precipitation, pH, dissolved oxygen, hardness, and alkalinity (Figure 1i).

Care for New-borns

The yolk was reduced after 70 - 75 hours of hatching, and then the new glass aquarium used for spawn developed. In general,

Figure 1. Some working photographs; a-Specimen's collection, b-Broder fish in an aquarium, c-Measurement of length, d-Measurement of Weight, e-Hormone injection, f- Breeding hapa, g-Colour changes after injecting hormone, h- Egg on a net of hapa, i- Egg on a petri dish, j-Water parameter analysis, k-egg under the microscope, l-observed embryonic development.

conditions should be similar to those in the main aquarium. The aquarium should not be over-filtered to the point that the fry is being drawn in by the pump. They give them diluted yolk (chicken) for the initial three days. Then they use the dust of dry commercial tubifex, which has crude protein (58 %), crude fiber (12%), crude fat (7 %), crude ash (5%), and moisture for their better growth and development.

Data analysis:
Finally, data were analyzed with the help of Microsoft Excel 2019 and the SPSS-2021 software system.

RESULTS AND DISCUSSION

Optimum Dose selection: To find the best dose for induced breeding, mature brooders (6 -7 g) were using the artificial hormone ovatide at five different doses. The changes in brooders in respect of applied doses are represented in Table 1.

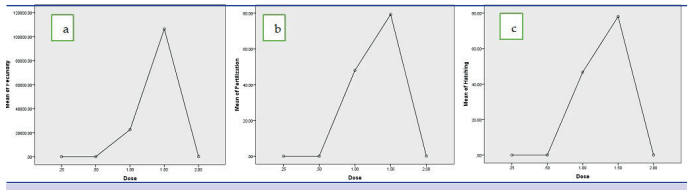

Figure 2. Dose wise Fecundity, Fertilization rate, and Hatching rate.

Breeding behavior
Active spawning was observed hours after the injecting dose. The breeding behavior is enlisted in Table 1.

Water quality
Water quality metrics are important in captive breeding because they duplicate water quality as closely as possible to the natural environment. This makes breeding much easier. In all of the experiments during rearing and breeding time, the parameters of water were shown in Table 3.

Embryo and Larva: The characteristics of the following embryonic developmental phases were observed and noted (Figure 3-5)

Morula
In the morula stage, cleavage begins after 30 minutes of fertilization. The cleavage furrow of cleavage gradually narrows to the animal pole of the cytoplasm. A huge number of cells formed a clump at the animal pole after multiple consecutive cleavages. 'Morula' is the name given to this stage of embryonic development (Figure 3 a).

Blastula
After the morula stage, the growing embryo is separated into multiple cells and organized into a layer called the blastoderm. The blastodisc, or extra cell division, is what causes the blastoderm to gradually develop into a vast number of layers. A gap called a blastocoel develops between the yolk and the blastoderm at this stage. This stage is known as the 'blastula' (Fig. 3 b).

Gastrula
During the early gastrula stage, the blastoderm began invading and spreading in a thin layer over the yolk. This is the start of the gastrulation process. During the mid-gastrula stage, a ring-like structure formed (Figure 3 c). The ring is known as a "germinal ring". The blastoderm covers 80% of the yolk in the late gastrula stage. The embryonic shield could be seen plainly. The rudiment of optics was abundantly obvious.

Organogenesis
The embryo was stretched due to organogenesis. The heartbeat was heard and the head and tail ends could be distinguished from the yolk. The yolk sac could be seen plainly. In this period, the rudiments of many body organs were developed (Figure 3 d).

Embryo in C-Shape
The embryo lengthened and gradually transformed into a head and tail, resulting in a C-shaped embryo. The body took on the shape of a C. Here, between the tail and the head, the yolk was connected. The growth of myotomes has been noticed. The embryo began to move on its own (Figure- 3 e).

Twitching
The tail was freed from the yolk at this point. The yolk sac was only found in the cranium. Myotomes grew in numbers. The embryo grew active and began to twitch continuously (Figure 3 f).

Hatching of the embryo
After completing embryonic growth, the twitching movement became more pronounced, and finally, the embryo was released

Table 1. Dose selection and observation during experimental breeding of *P. chola*.

Set	Dose (ml/kg)	Sex ratio (M: F)	W	Observation	R	F	S	I	Fr (%)	H (%)
1	0.25	02:01	M-6; F-7	No Change	No egg	-	-	-	-	-
2	0.5	02:01	M-7; F-8	Body color change	No egg	-	-	-	-	-
3	1	02:01	M-6; F-7	Aggressive behavior Body color change	Egg release	22447± 1331.19	15 - 17	8	47.97 ± 0.685	46.70 ± 0.39
4	1.5	02:01	M-6; F-7	Aggressive behavior Body color change Aggressive behavior	Egg release	106308± 3075.04	15 - 17	8	79.28 ± 0.589	78.03 ± 0.495
5	2	02:01	M-7; F-8	Dead within Half an hour	No egg	-	-	-	-	-

W=Weight (g); F=Fecundity (Egg/Kg); S=Spawning time (hr); I=Incubation period; Fr=Fertilization rate; H=Hatching rate

Table 2. ANOVA test.

		Sum of Squares	df	Mean Square	F	Sig.
Fecundity	Between Groups	42448578024.400	4	10612144506.100	4725.756	.001
	Within Groups	44911941.600	20	2245597.080		
	Total	42493489966.000	24			
Fertilization	Between Groups	26744.841	4	6686.210	40874.253	.001
	Within Groups	3.272	20	.164		
	Total	26748.113	24			
Hatching	Between Groups	25795.399	4	6448.850	81076.813	.001
	Within Groups	1.591	20	.080		
	Total	25796.990	24			

Table 3. Physico-chemical parameters in water.

Optimum range		
Parameter	**Aquarium**	**Hapa**
Temperature (°C)	26.3±5.22	27±3.2°C
pH	7.6±.84	7.6±9.1
Ammonia (ppm)	0.1±.002	0.03-±0.01
Nitrate (ppm)	80±2.5	80±2.8
Nitrite (ppm)	0.25±0.014	0.3±0.12
DO (ppm)	6.0±.94	6±2.5

within 15–17 hours with a temperature of 27 ± 3.2 ℃ (Figure-3g). The forceful rotation of a fully formed embryo shattered the egg-shell, allowing the larva to emerge. The length of the hatched larva was 0.17 mm and gradually developed to 0.29 mm (Figure 4). The yolk absorbent fry was plucked and put in a stocking tank for continued growth. In 18 days, the fry had developed to a size of 12 to 15 mm (Figure 5).

Sarma (2015) studied the induced breeding of *Pethia gelius* (Hamilton, 1822) where they applied Ovaprim 0.5-0.4 ml/kg (F), 0.4-0.3 ml/kg (M) with a sex ratio of 2: 1 in the Bihar. Motilan et al. (2014) applied WOVA-FH, 0.2-0.4 ml/kg body weight of both males and females with a sex ratio of 2:1 for induced breeding of *Pethia manipurensis* (Menon, Rema Devi & Vishwanath 2000). In this study, ovatide doses of 0.25 ml/kg, 0.5 ml/kg, 1 ml/kg, 1.5 ml/kg, and 2 ml/kg body weight were administered to both male and female *P. chola* with a ratio of 2:1 during the pre-monsoon season. Here, observed only 1.5 ml/kg ovatide was the optimum dose for induced breeding of *P. chola* because this dose was responsible for high fecundity (106308 ± 3075 kg/body weight), spawning, and hatching. *P. chola* required a higher concentration of a synthetic hormone than other species to reproduce.

Vincent & Thomas (2008) observed courtship behavior and nuptial coloration during induced breeding of the *P. chola*; during the present study, the same observation was shown. The significance of the effects of ovatide on fecundity, fertilization, and hatching was observed through the ANOVA with a 0.05 level of significance (Table 2). The dose-wise fecundity, fertilization, and hatching rates

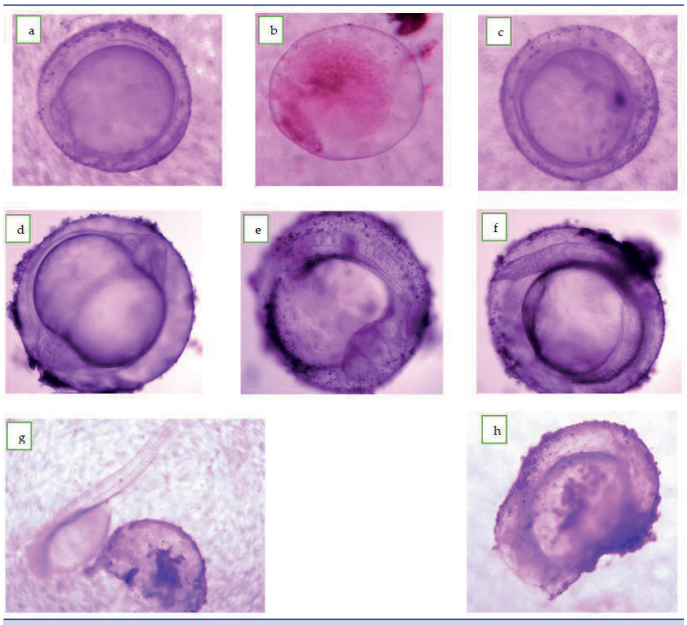

Figure 3. Embryonic development of *Puntius chola*; a-Morula, b-Blastula, c-Gastrula, d-Organogenesis, e-C-shaped larva, f-twitching, g-Hatching, h-Egg brust

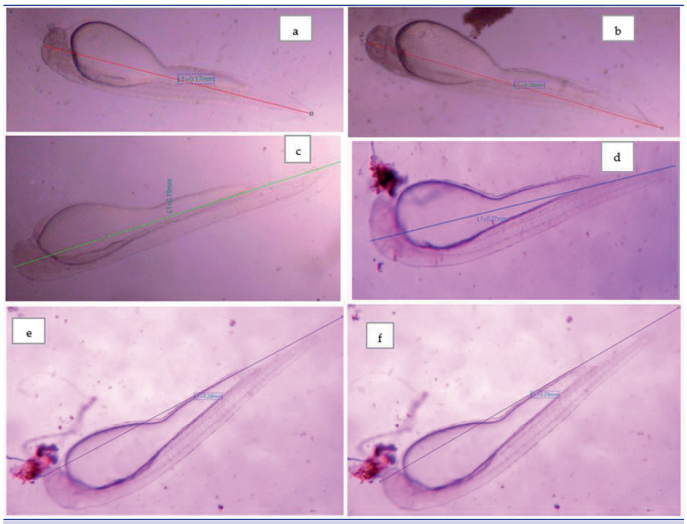

Figure 4. Larval development; a-0.17 mm, b-0.18mm, c-0.19mm, d-0.27mm, e-0.28mm, f-0.29 mm.

have fluctuated; only 1.5 ml/kg ovatide shows a high rate of fecundity, fertilization, and hatching; 1 ml/kgn is very low and the other three doses are not effective for breeding (Figure 2).

In this study, the average fertilization rate (%) was 79.28 ± 0.589, and the hatching rate (%) was 78.03 ± 0.495 through the optimum dose. The hatching and fertilization rate were almost identical to those in the major carp, where Hossain *et al.* (2007) achieved those eggs to early fry (spawn) and could achieve up to 80 % survival (Chaudhuri *et al.*, 1984). In this study, the incubation of the egg lasted 8 hours at 27 ± 3.2℃, and the observed hatching period was between 10 - 11 hours. According to the results of the

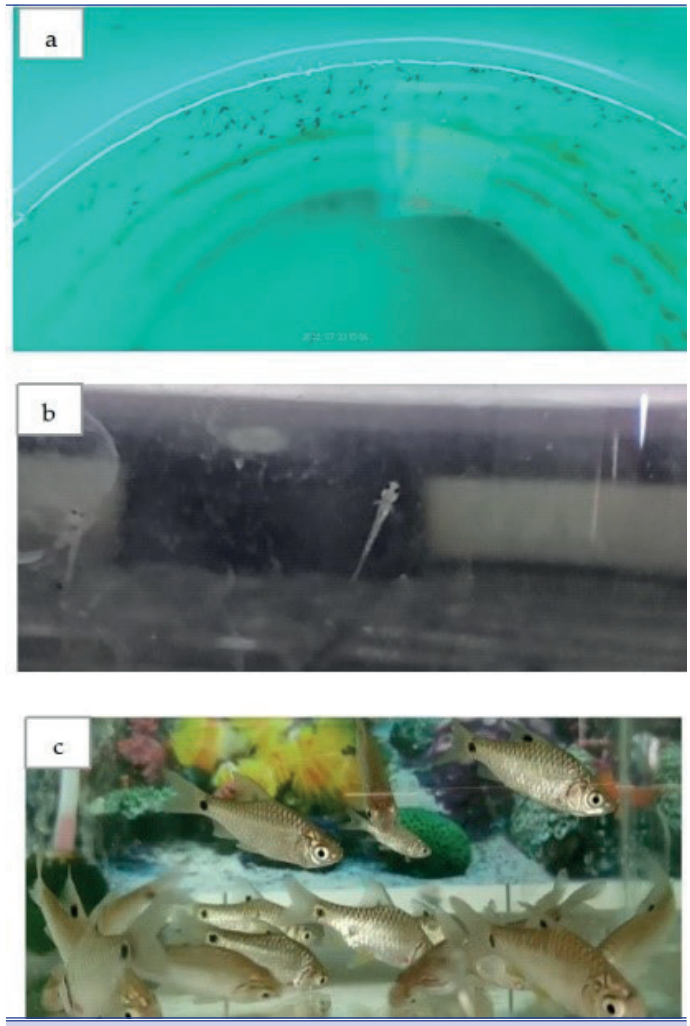

Figure 5. Spawn (a), fry (b), and fingerling (c) of *P. chola.*

current study, fertilized eggs were initially translucent and turned creamy as embryonic development progressed. The size of the fertilized eggs ranged from 0.09 to 0.1 mm. The unfertilized eggs were opaque and white, whereas the fertilized ones were transparent. Common carp's swollen, fertilized eggs range in size from 1.5 to 2.5 mm (Woynarovich et al., 1984). Mumtazuddin et al.(1982) found that the quantity and rate of growth of carp spawn are significantly influenced by the availability of high-quality living food species, especially zooplankton. Early spawn was removed from the hatching tank and stockpiled in a well-prepared tank for continued rearing in the current investigation. The fries were given chicken yolk that had been diluted with water. From day 5 to day 10, the fry was fed newly hatched Artemia nauplii, which is important for fish growth and survival. After 10 to 15 days, it will be able to feed on natural as well as Pilate's artificial feed. Freshly hatched *P. chola* larvae were between 0.16 mm and 0.17 mm in size, compared to 4.8 to 5.0 mm for common carp (Woynarovich et al., 1984). At a water temperature of 27 ± 3.2 °C in the present work, hatchlings of *Puntius chola* reduced the yolk in 65 to 70 hours. However, at temperatures between 24 and 31 °C, the Indian major carp's yolk absorption duration was 3 to 4 days (Woynarovich et al., 1984). The findings show that the length

of time it takes for the yolk to absorb depends on the water's temperature as well as the number and size of the yolk sacs. The yolk sac of P. chola was depleted more quickly because it was smaller compared to that of Indian major carp.

CONCLUSION

The maximum spawning, egg production, and hatching rates in *P. chola* are achieved at an optimum dose of 1.5 ml/kg (ovatide) of body weight. For commercial seed production and the restoration and conservation of species, the male and female responses to a single dose of ovatide are crucial.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Committee Approval: Ethical clearance from IAEC, Approval no. 18/IAEC (05)/RNLKWC/2019, dated 27/07/2019

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