



The Effect of Habitat Manipulation on Early Gonad Maturation of *Channa striata* in Captive Condition

Dushyant Damle¹, Rajesh Kumar², B. Ahilan¹, Bindu R. Pillai², P. Chidambaram¹,
Pragyan Paramita Swain², Jackson Debbarma², J.K. Sundaray²

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ABSTRACT

Background: With remarkable growth in aquaculture, the need for system diversification and species diversification has been demanded by the producers to gain fruitful output in terms of profit. Striped murrel, *Channa striata* has been identified as a suitable species for modern aquaculture systems in terms of species diversification due to their fast growth rate, air-breathing nature, higher market price and consumer acceptance. The major bottlenecks in seed production of murrel are captive maturation and short spawning window. To overcome these problems, an attempt was made to achieve early gonadal maturity of striped murrel in captive conditions by intervening in the brooder rearing habitat.

Methods: In this regard, four habitats were tested in duplicates, namely T1 - Tank with clean reservoir water without macrophytes and soil base, T2 - Tanks provided with floating (Water hyacinth, *Eichhornia sp*) and submerged (Waterthyme, *Hydrilla sp*) aquatic macrophytes without any soil base, T3- Tanks provided with only soil base (15-20 cm) without any macrophytes and T₄ - Tanks provided with both soil base and macrophytes.

Result: At the end of the three-months rearing phase, the fishes provided with macrophytes and soil base (T4) resulted in early maturation characterized by an egg size of around 1.29 ± 0.21 mm and the majority of late granular stage oocytes and spermatozoa in ovary and testes, respectively. The results concluded that providing a bottom soil base of 15-20 cm and covering the 20-30% water spread area using aquatic macrophytes in the brood rearing tank helped in the gonadal maturation of striped murrel during pre-monsoon in captive conditions.

Key words: Early maturation, Gonadal maturation, Habitat manipulation, Pre-monsoon.

INTRODUCTION

Striped murrel, *Channa striata* (Bloch, 1793), is an important snakehead fish with high market demand and value and its popularity as major cultivable species is growing day by day (Qin and Fast, 1997; Kumar *et al.*, 2011). It has emerged as one of the major species contributing to aquaculture in Asian countries (Vidhayanon *et al.*, 1997; Kumar *et al.*, 2012). It is geographically distributed in South Asia (India, Pakistan, Bangladesh, Sri Lanka, Nepal) and Southeast Asian countries (Myanmar, Vietnam, Thailand, Philippines, Malaysia, Indonesia, Cambodia, Laos and Southern China) and they serve as an important food fish in the native distribution region (Courtenay and Williams, 2004; Kumar *et al.*, 2014; De Silva *et al.*, 2015; Kumari *et al.*, 2018). The *Channa striata* fish flesh is considered best quality due to its taste, muscle quality and medicative value (Yaakov and Ali, 1992; Jais *et al.*, 1994; Qin and Fast, 1998; Baie and Sheikh, 2000; Kumar *et al.*, 2011; Laila *et al.* 2011). Sahu *et al.*, 2012a, 2012b; Kumar *et al.*, 2022). The *Channa striata* is a good source of antioxidants and it is also a rich source of protein which is around 25.2 g of protein per 100 g of fish flesh (Hidayat and Amelia, 2020).

At present, the global capture production of *C. striata* is stagnant and its aquaculture production is continuously increasing, indicating their ample scope for culture in their native regions. In India, the *C. striata* production is mainly from capture fisheries and its aquaculture is mainly

¹Tamil Nadu Dr. J. Jayalalitha, Fisheries University, Dr. M.G.R Fisheries College and Research Institute, Ponneri-601 204, Tamil Nadu, India.

²ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, Odisha, India.

Corresponding Author: Rajesh Kumar, ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, Odisha, India. Email: rajeshcife@gmail.com

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dependent upon natural seed collection from the wild (Kumar *et al.*, 2017). The aquaculture is mainly dependent on fish seed availability (Damle *et al.*, 2010; Damle and Chari, 2011) which should be continuous with sufficient supply in the required amount. Hatchery seed production technologies have been standardized at ICAR-CIFA, Bhubaneswar and the establishment of commercial hatcheries has recently begun in the country with the technical support from the institute. The low fecundity is one of the major reasons for scaling up the striped murrel seed production therefore the

commercial hatcheries require a large number of broodstock to produce more seed which requires colossal infrastructure and investment. The striped murre is monsoon breeder (June-August) with an abridged peak spawning season. Therefore, an effort was needed to develop the brooders to mature before the peak monsoon in captive conditions. In this experiment, an attempt was made to develop the striped murre broodstock in concrete tanks with habitat manipulation for maturation and breeding during the pre-monsoon period.

MATERIALS AND METHODS

Fish and husbandry

The experiment was carried out in the Air-breathing Fish Unit, ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, India. The adult fishes were collected from the culture pond of ICAR-CIFA in January 2020. The collected fishes were examined for external injuries or health conditions. The healthy fishes were acclimatized in hatchery conditions for two weeks after disinfection with 5-10 ppm KMnO_4 solution for 20 seconds. During the acclimatization period, the fishes were fed with a commercial pellet diet twice a day at the rate of 3% BW/day. After that, the fishes were stocked in randomly assigned experimental tanks. The experiment was conducted under the natural light regime throughout the experiment duration.

Experimental design

The experiment of habitat manipulation was conducted from February to May 2020. Prior to stocking experimental fishes, the experimental tanks were washed properly and disinfected with KMnO_4 @ 25 ppm and NaCl @ 5% and dried under sun. The experimental tanks were filled with reservoir freshwater and covered with nylon nets to prevent the escapement of fish. Fishes with the weight range of 400-600 g were stocked in eight randomly assigned concrete tanks (5×3×1m) after the dip with KMnO_4 (5-10 ppm) and common salt (1.5-2%) in February 2020. The four habitats were tested in duplicates such as T1- Tank with clean reservoir water without macrophytes and soil base, T2- Tanks provided with floating (Water hyacinth, *Eichhornia sp*) and submerged (Waterthyme, *Hydrilla sp*) aquatic macrophytes without any soil base, T3- Tanks provided with only soil base (15-20 cm) without any macrophytes and T4- Tanks provided with both soil base and macrophytes. The aquatic macrophytes were provided to cover about 20-30% of the water spread area. Each tank was stocked with an equal number of fishes (16 fishes per tank with 1:1 sex ratio) of approximately similar sizes.

Feed and feeding intervention

Quality feed and feeding management are of utmost importance during broodstock development. In the present experiment, fishes were fed with live and wet feed at 3% of body weight per day. The daily ration was divided into thrice meals (0800; 1230; 1630) consisting of small indigenous

forage fishes (Puntius Sp., Tilapia fry, Chanda Sp.) and prawns @1%, sardines @ 1% and squids @ 1%. Sardines were chopped directly into small pieces and were fed to fish. The squids were bought from the local fish market in Bhubaneswar. Before feeding, the internal skeletons were removed from small-sized squids and fed directly, whereas, in large-sized squids, the internal skeletons were removed before chopping into pieces.

Management of experimental tank

About 10-20% water was replaced everyday and therefore complete replacement in a week. The excreta and left-over feeds were siphoned every day to keep water quality in good condition. Liming was applied every week @ 200kg/ha and fishes were examined fortnightly during complete water exchange for any possible infection. Fishes were disinfected with KMnO_4 @ 5 ppm and common salt @ 1% for 30 seconds after every examination. The excess macrophytes spread over 20 to 30 % of the water area were removed every fifteen days otherwise, it hampers the movement and feed intake of the brooders.

Assessment of brooders maturity

The gonadal maturity status of brood fish was assessed by cannulation method using the catheter. The fishes were anesthetized using clove oil before collecting the gonadal tissue. The collected gonadal tissues were examined for their maturity status under a trinocular microscope (Olympus, CX 31) attached with the digital camera.

Histological studies of gonads

Histological studies of gonads were done as per (Lohrmann and Von Brand, 2005). Gonad samples of brooders stocked in different habitats were collected and stored in Davidson's fixative. The samples were transferred from Davidson's fixative to the 70% ethanol solution for further storage. The stored sample was used for the histological section by following standard histology steps such as fixing, cassetting, processing, embedding (in paraffin wax), microtome sectioning (sections of 5 μm were cut using a microtome and fixed on slides), staining (hematoxylin and eosin), microscopy with Radical RxLr-5 Nexocope microscope and photography with ProCAM. Based on the histological observations, the maturity statuses of the brooders in the different treatment groups were determined.

Water quality parameters of experimental tanks

The water quality parameters like dissolved oxygen, ammonia, alkalinity, pH and hardness were analysed as per the standard methods for examining water and wastewater (APHA, 1992).

RESULTS AND DISCUSSION

Physico-chemical parameters of water

The physicochemical parameters of water such as temperature ($^{\circ}\text{C}$), pH, dissolved Oxygen (mg L^{-1}), Total hardness (mg L^{-1}), total alkalinity (mg L^{-1}), ammonia (mg L^{-1}),

Nitrite-N (mg L^{-1}), Nitrate-N (mg L^{-1}), were recorded. The observed water quality parameters were as follows: temperature ($28.5^{\circ}\text{C} - 30^{\circ}\text{C}$), pH (7.3-7.8), dissolved oxygen ($5.02\text{-}6.35 \text{ mg L}^{-1}$), alkalinity ($104 - 136 \text{ mg L}^{-1}$ as CaCO_3), total hardness ($102\text{-}114 \text{ mg L}^{-1}$ as CaCO_3), Water conductivity ($0.194\text{-}0.36 \text{ S/m}$), Phosphate level ($0.0075\text{-}0.85 \text{ mg L}^{-1}$), nitrite-N ($0.21\text{-}0.87 \text{ mg L}^{-1}$), nitrate-N ($0.007\text{-}0.87 \text{ mg L}^{-1}$) and ammonia ($0.04\text{-}0.08 \text{ mg L}^{-1}$) during the experimental period of 120 days.

Egg size and fish maturity

The mean egg sizes of the broodstock fishes from different treatment groups were presented in Table 1. The average egg size of treatment T1 (Provided with only reservoir water without any soil base and macrophyte) was found smallest among all treatments, measured about $0.68 \pm 0.03 \text{ mm}$. The average diameters of eggs were measured around $0.80 \pm 0.03 \text{ mm}$ and $0.95 \pm 0.71 \text{ mm}$ in T2 and T3, respectively. Egg size in T4 was observed to be the largest among all the treatments and recorded to be $1.29 \pm 0.21 \text{ mm}$. The pictures of immature and mature egg are represented in Plate 1.

Histological observations of male and female brood fish

The gonad histological sections of the brooders raised in different habitats are presented in Fig 1 and 2. The ovarian stages of fishes from different treatment groups were varied considerably (Fig 1). In the treatment T1 (without any soil base or macrophyte), the ovary was found at maturing stage characterized by the presence of chromatin nucleolar oocyte and early peri nucleolar stages. Most oocytes in T2 were of the late peri nucleolar stage, but the fishes were still maturing. Yolk vesicle stage is clearly visible in the matured oocytes of ovary of the brooder fishes from treatment T3 and Granular stage was also visible in many matured oocytes of the same sets of ovary. The fishes from the T4

were almost in the spawning stage, where most of the cells were in late granular stage. Apart from the ovary, the histology of testes of the experimental fishes was also showed significant differences among different treatment groups (Fig 2). In T1, testis cells were mostly found in the spermatogonia stage, whereas in T2, the spermatocytes were visible along with the spermatogonia. In the T3 and T4, the testis cells had both spermatids and spermatozoa in abundance and especially the histo-morphology of T4 indicates the spermeation stage.

Habitat plays a vital role in the successful spawning of fishes and the captive environment, often resulting in inhibition of reproductive maturation. Thus, the availability of quality and desirable spawning habitat is the key to reproduction success in fishes (Taylor *et al.*, 2019). Striped murrel brooders usually prefer to stay in a muddy area with heavy macrophytes (Kumar *et al.*, 2021a). However, rearing striped murrel brooders in the weed-infested earthen ponds also causes hardships during the collection due to their hiding behavior under the mud. This increased handling stress on striped murrel brooders often results in poor breeding response upon successive induced breeding trials. Thus, mimicking natural conditions in the confined tank environment is an effective strategy for speeding up the reproductive maturation of the fishes in the captive seed production process. Bottom substrate and aquatic vegetation are proven to be affecting the maturation synchronization of the brooder in captive breeding (Zohar, 1989; Yaron, 1995; Bohlen, 2003; Migaud *et al.*, 2013 and 2018). Considering the effect of habitat, the various manipulations on the tank environment were tried to achieve early maturation in striped murrel. The maturing stages were analysed through external morphological characters, egg size and histological studies.

During the present study, the striped murrel brooders were fed with a nutritious diet to favour the early maturation process in captivity (Kumar and Mohanty, 2018) and the bulk of the dietary protein share comes from fishmeal (Siddaih *et al.*, 2022 and 2023). Small indigenous fishes were packed with healthful nutrients like omega -3 and omega-6 fatty acid (12.2 to 27.2%), fat (>4%), protein (>16%), minerals (calcium, iron and manganese,

Table 1: Egg size of broodstock fishes in treatments with different habitat.

Treatment	Egg diameter (mm)
T1	$0.68 \pm 0.03\text{c}$
T2	$0.80 \pm 0.03\text{b}$
T3	$0.95 \pm 0.71\text{b}$
T4	$1.29 \pm 0.21\text{a}$

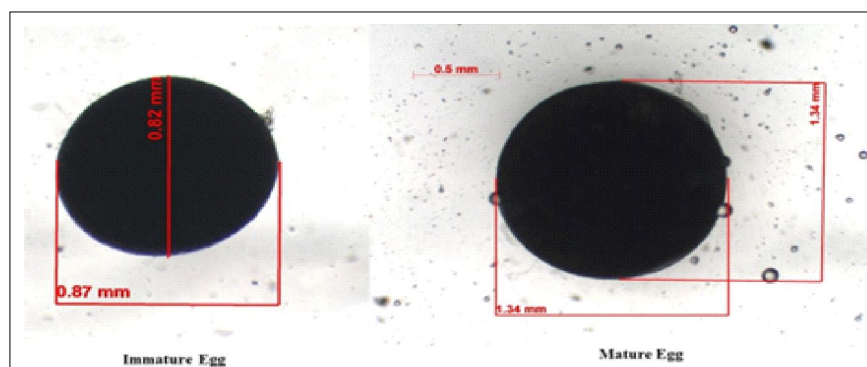


Plate 1: Fish egg from habitat experimental tank.

phosphorous) and vitamin A, D, E and K (Mohanty *et al.*, 2019). In addition to their nutritive value, feeding the brooders with live fishes often helps maintain the predatory and chasing behaviour of the striped murrel brooders. Sardines are a rich source of protein, omega 3 fatty acid, vitamin B12, minerals (Nisa and Asadullah, 2011; Shaji and Hindumathy, 2013; Mohanty *et al.*, 2019;). Squids are a rich source of protein and contain a good amount of omega-3 polyunsaturated fatty acid (Steffens, 1997; Atayeter and Ercoşkun, 2011).

In this study, different habitats were tested for early maturation. Habitat, which resembles their natural environment (soil base majorly having clay loam soil type with macrophytes), had a more positive impact on the gonadal maturation of striped murrel brooders. The more or less similar effects of the environment were suggested by (Zohar and Mylonas, 2001). The size of an egg is a key tool

for assessing the gonadal maturity of the fishes. In striped murels, the ova of above 1000 µm diameter is considered mature and indicates the readiness of spawning (Kilambi, 1986; Kumar *et al.*, 2021b). Accordingly, in the present study, the eggs size of fully matured striped murrel brooder fish was ranged from 1.29 ± 0.21 mm to 1.38 ± 0.006 mm. Apart from that, the size of the egg also has a strong impact on the hatchability and vitality of the offspring produced, as studied by (Furuita *et al.*, 2003; Biswas *et al.*, 2005; Furuita *et al.*, 2009; Sarmiento *et al.*, 2018; Kumar *et al.*, 2021b).

Dopamine a potential neurotransmitter produced in the brain that inhibits the action of GnRH in the pituitary of fish (Peter *et al.*, 1988). The increased breeding performance in the soil-based and macrophytes provided tanks of the present experiment is probably because of the elimination or reduction of that maturation inhibiting compounds in fishes. Apart from that, the environmental microbiome of

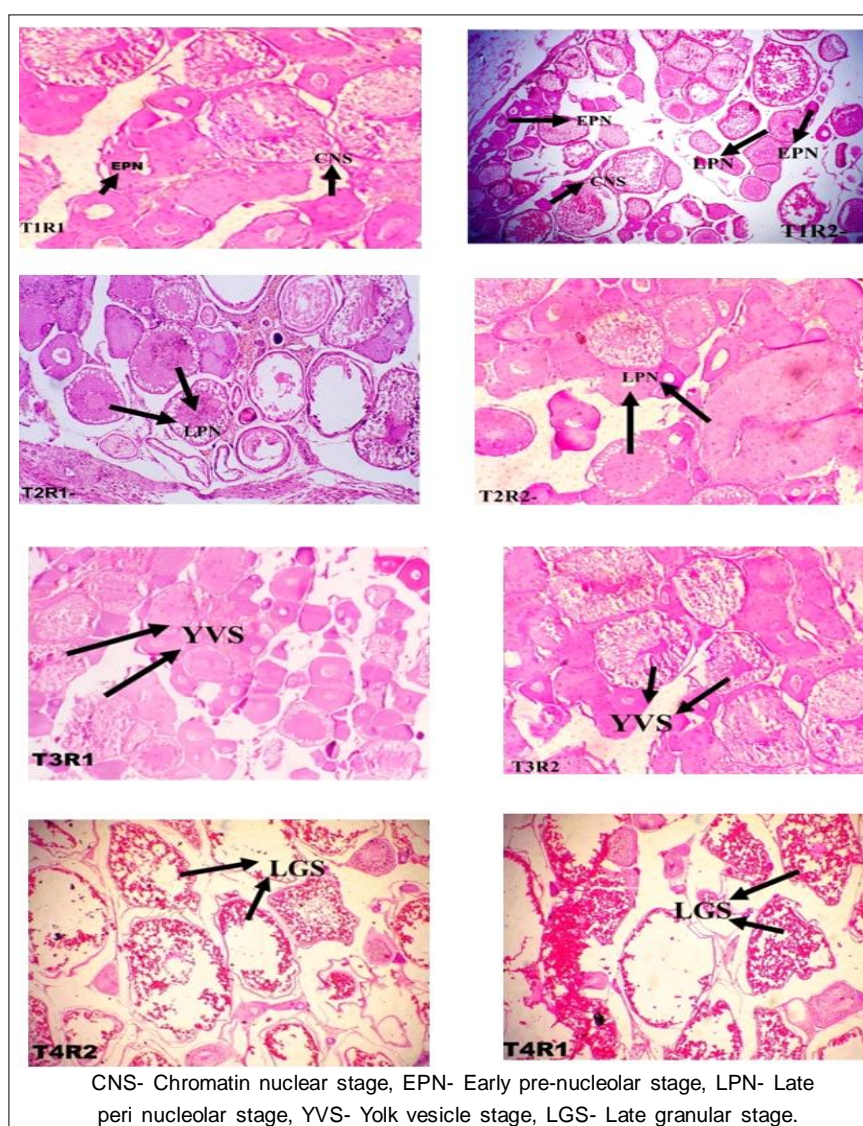


Fig 1: Haematoxylin- Eosin-stained ovary sections from treatment with different habitat.

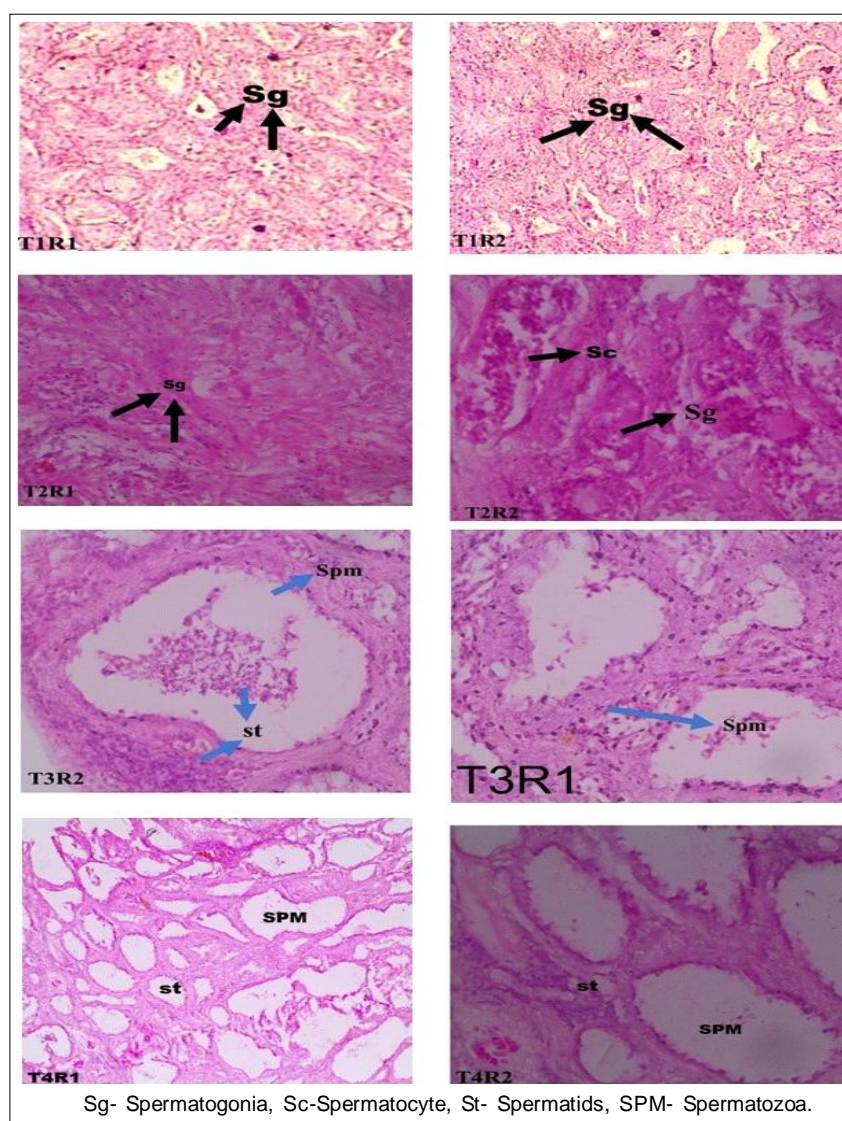


Fig 2: Haematoxylin- Eosin-stained testis sections from treatment with different habitat.

the rearing tank is enriched when provided with the bottom soil layer. These naturally correlated microbiomes influence the gut microbiome of the fishes, which is suspected of having a connection with the brain and might eventually alter the behavior pattern of fish (Gershon and Margolis, 2021). The consecutive positive changes in the reproductive endocrinology and physiology of the experimental fishes might be the reason for the early maturity in the fishes reared in captivity.

Histological studies of T3 and T4 indicated that the male and female fishes were fully mature and in the spawning stage. In the mature ovary, the cells were blended with the yolk vesicle and yolk granules, whereas yolk granules and late granules were observed in spawning ovaries. In the case of matured testes, the spermatid and spermatozoan cells were abundant. A similar observation has also been recorded in striped murrel by various authors (Irmawati *et al.*, 2019; Milton *et al.*, 2018; Boonkusol *et al.*, 2020). In the

present study, the brooders from the T1 and T2 were in maturing stage, T3 and T4 were in matured and spermeation stage, respectively.

CONCLUSION

In conclusion, the results suggested that the inclusion of soil base (15-20 cm) and aquatic macrophytes (20-30%) in the brood rearing concrete tanks resulted in pre-monsoon gonadal maturation. This was evidenced from the bigger ova size, majority of oocytes in late granular stage in the ovary and mature spermatozoa in the testes in the fishes reared in tanks having soil base and aquatic macrophytes.

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Declaration of competing interest

The authors declared that they have no known conflict of interest to this work.

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