Gonadal development and induced spawning in spontaneously bred Labeo rohita (Ham.)

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ABSTRACT

Mass spontaneous spawning of Catla catla, Labeo rohita, L. calbasu and Cirrhinus mrigala in selected ponds of the Orissa state fish farm at Kausalyaganga was recorded. Of the above four species, spent brood of L. rohita was studied further for subsequent gonadal development and second breeding. GSI (ovary) of spent L. rohita after spontaneous spawning was recorded as 2.04 ± 0.093, whereas the same in non-bred brood of the adjacent pond was recorded as 17.56 ± 1.35. GSI of the spent female increased to 8.26 ± 0.391 after 15 days of spontaneous spawning and the same after 45 days was 14.1 ± 0.2. Histomorphology of ovary on the first day after spontaneous spawning exhibited 74% empty follicles and 26% unspawned oocytes of different stages. After 15 days of spontaneous spawning, the spent ovary showed many newly recruited oogonia, some developing oocytes and few atretic follicles. Ovary after 45 days of spontaneous spawning had mostly maturing and matured oocytes along with some traces of resorbing atretic follicles. The spent brood could be induced bred second time in eco-hatchery system after 60 days. The spontaneously bred L. rohita on its second breeding produced 0.92 x 10^5 spawn per kg body weight, whereas non-bred (control) brood produced 0.71 x 10^5 spawn per kg body weight. The fecundity and fertilization rate in second breeding were higher, 1.2 x 10^5 and 94-96% respectively than 0.91 x 10^5 and 88-90% of the control.

Introduction

Indian major carps breed in rivers once in a year during monsoon months (Hora, 1945; Ibrahim, 1961; Quasim and Qayyum, 1962; Natarajan and Jhingran, 1963). Spontaneous spawning of Indian major carps was also reported in ‘bundhs’ (Ganapati and Chacko, 1954; Saha et al., 1957; Moitra and Sarkar, 1975; Chondar, 1984). Many freshwater fish species including Indian major carps do not breed in confined pond water and need hormonal induction for spawning. Later it is understood that the pituitary gland of these fish fail to release the required quantity of gonadotropin II (GTH II) in confined water which is responsible for non-spawning of the fish in pond ecosystem (Bhattacharya, 1999). Ever since the first induced breeding report of Indian major carps by the administration of carp pituitary extract (Chaudhuri and Alikunhi, 1957), several permutations and combinations, alterations and additions have been made to simplify the hypophysation technique for carp seed
production on a commercial scale (Chaudhuri, 1960; Das and Khan, 1962; Chondar, 1970; Sinha, 1971; Bhowmick and Kowtal, 1973; Kaul and Rishi, 1986 and Khan et al., 1992). The spent brood of Indian major carp after its first induced breeding could be again induced bred for second time in the confined water (Bhowmick et al. 1977). Multiple induced breeding of these species in one breeding season has also been reported (Chondar, 1986; Somashekarappa, et al., 1988; Gupta et al., 1995). Studies on the ovarian development of the Indian major carps during the inter breeding period between two successive induced breeding in the same season are meager (Rath et al. 2002; Mondal, 2002). The present communication describes the gonadal development and induced spawning performance of the spontaneously bred L. rohita for second time in the same season.

**Materials and methods**

**Spontaneous breeding of carps**

Some of the brood-stock pond dykes of state fish farm at Kausalyaganga, Orissa were severely eroded due to cyclone during 1999. During June 2001, barometric depression caused cyclonic weather and heavy downpour and all the six eroded ponds became one unit. The entire brood stock of the Indian major carps (total 821 kg male and 1232 kg female) started breeding spontaneously on 13 June 2001 in the above pond. The ambient physico-chemical parameters of the pond water caused the spontaneous breeding (water temperature: 22.5°C, transparency: 10 cm, pH: 7-8, dissolved oxygen 5-6 ppm, total alkalinity 84 ppm, free carbon-di-oxide 0. 6 ppm). Rath et al. (2005) has reported more details about this spontaneous breeding.

**Experiment on the spent L. rohita for second spawning**

On the following day of spontaneous breeding, the brood stock pond was netted out. GSI of the spent female L. rohita was analyzed and compared with the same of the adjacent non-eroded pond where there was no spontaneous breeding. Cut pieces of ovary were fixed in Bouin's fixative as well as Zenker's fluid and the brain intact with pituitary gland was fixed in Bouin's sublimate for histological studies.

Ten spent female L. rohita were collected, marked with the vital dye (Khan et al., 1988) and released into another pond with equal number of non-spent females (control). The pond was managed according to the standard

### Table 1: Atmospheric temperature and rainfall at Kausalyaganga during Jan-June 2001*

<table>
<thead>
<tr>
<th>Months</th>
<th>Mean Max. Temp.(range) in °C</th>
<th>Mean Min. Temp (range) in °C</th>
<th>Total rainfall in (mm)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>26.7 (28.5-23.5)</td>
<td>12.7 (16.5-11.0)</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>31.4 (33.6-29.5)</td>
<td>18.6 (24.0-12.0)</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>32.8 (37.0-30.0)</td>
<td>22.4 (24-20.5)</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>34.0 (39.5-32.5)</td>
<td>22.8 (27.5-22.8)</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>37.5 (41.5-34.5)</td>
<td>23.8 (28.0-22.0)</td>
<td>021.0</td>
<td>Sporadic rain in 2nd week</td>
</tr>
<tr>
<td>June</td>
<td>32.4 (35.5-28.5)</td>
<td>21.2 (24.0-19.0)</td>
<td>150.6</td>
<td>11,12,13, (June) total rain days contributed 141.4 mm</td>
</tr>
</tbody>
</table>

* Source: Meteorological Unit of CIFA, Kausalyaganga
management practice for multiple carp breeding (Gupta et al., 1995).

Both spent and non-spent brood fishes were sampled after 15 and 45 days of spontaneous breeding to study the changes in GSI, histo-anatomy of ovary and the pituitary gland. Sections of the ovary were stained in eosin and haematoxylin, bromphenol blue and Sudan black B to observe the histo-anatomical changes and development of oocytes. Pituitary sections were stained in Alcian blue, Periodic acid Schiff Orange G (AB -PAS - OG) and Herlant’s tetrachrome (Pearse, 1968).

Four L. rohita females, each from spent and non-spent groups were induced bred separately with equal number of males in an eco-hatchery system, 60 days after the spontaneous breeding. The fishes were injected uniformly with ovaprim (Glaxo India Ltd.) at 0.5 ml per kg body weight of female and 0.2 ml per kg body weight of male. The eggs were incubated in the incubation pool.

Breeding response, fecundity, fertilisation and spawn recovery rates of the second spawning were compared with that of control (Table 3). All the 4 females were induced bred. The spawn recovery per kg body weight was $0.92 \times 10^5$ in second spawning over $0.71 \times 10^5$ of control group. The spawn of second breeding were found as healthy as the control and yolk sac was absorbed within 65-70 h. of hatching. Neither the second bred fish nor the control one exhibited more than 0.5% embryonic deformities.

Histomorphology of ovary of the spontaneously spent fish revealed on the next day of spawning about 74% of empty follicles and 26% of nonspawned vitellogenic and non-vitellogenic oocytes. The unspawned oocytes retained in the ovary were mainly immature non-vitellogenic and vitellogenic oocytes with some atretic follicles. The empty follicles were the space for ovulated oocytes which

**Results**

Study of gonado-somatic index (GSI) of ovary had clearly revealed the gonadal development trend in 45 days following spontaneous breeding. The GSI of spontaneously bred spent fish increased from $2.04 \pm 0.93$ to $14.12 \pm 1.46$, whereas the GSI of the non-bred (control) fish ranged between $17.56 \pm 1.35$ to $16.86 \pm$ during that period (Table 2). The spent fish could be induced bred after two months of spontaneous spawning in an eco-hatchery system. The breeding performance such as spawning response, spawning fecundity, fertilisation and spawn recovery rates of the second spawning were compared with that of control (Table 3). All the 4 females were induced bred. The spawn recovery per kg body weight was $0.92 \times 10^5$ in second spawning over $0.71 \times 10^5$ of control group. The spawn of second breeding were found as healthy as the control and yolk sac was absorbed within 65-70 h. of hatching. Neither the second bred fish nor the control one exhibited more than 0.5% embryonic deformities.

**Table 2:** Gonado-somatic index of spent and control of L. rohita female after spontaneous spawning up to six weeks

<table>
<thead>
<tr>
<th>Mean 1st day after spawning</th>
<th>15 days after spawning</th>
<th>45 days after spawning</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spent Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.04 ± 0.093</td>
<td>17.56 ± 1.35</td>
<td>8.26 ± 0.391</td>
</tr>
<tr>
<td>18.23 ± 1.46</td>
<td>16.86 ± 1.83</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Second spawning performance of L. rohita after 60 days of spontaneous spawning

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of female</th>
<th>Weight of female</th>
<th>Total egg production X10^5</th>
<th>Egg / kg Body Wt. X10^5</th>
<th>Fertilisation % (range)</th>
<th>Total spawn production X10^5</th>
<th>Spawn / kg Body Wt. X10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt.</td>
<td>4</td>
<td>5.7 kg.</td>
<td>6.84</td>
<td>1.22</td>
<td>94-96</td>
<td>5.24</td>
<td>0.92</td>
</tr>
<tr>
<td>Cont.</td>
<td>4</td>
<td>6.3 kg.</td>
<td>5.73</td>
<td>0.91</td>
<td>88-90</td>
<td>4.47</td>
<td>0.74</td>
</tr>
</tbody>
</table>
retained the folded follicular epithelial layer after spawning (Fig. 1). Ovary after 15 days of spawning was characterised by newly recruited oocytes, a few non-spawned oocytes in different stages of development and almost absorbed empty follicles. Some non-spawned oocytes also attained the atretic phase during this period. Sporadically distributed remnants of empty follicles were seldom found (Fig. 2). Ovary after 45 days of spontaneous breeding was found with full of matured oocytes and very few developing oocytes along with some atretic follicles in advanced stages (Fig. 3). The cyanophilic cells of proximal pars distalis (PPD) of the pituitary gland were found positive to alcian blue and anilin blue in AB-PAS-OG and Herlant tetrachrome respectively. The PPD of spontaneously bred L. rohita showed degranulated and hypertrophied cyanophils (Fig. 4), whereas the same was found full with cyanophlic granules on 45th day of sampling (Fig. 5).

**Discussion**

Mookherjee et al. (1944) and Ganapati and Alikunhi (1949) found that carp spawned in shallow water. Hora (1945) opined that heavy monsoon catchment is capable of inundating vast shallow areas which form the breeding grounds of the fish and believed to be the primary factor for spontaneous spawning. Khan (1959) observed that sudden rise of water level during monsoon caused spontaneous spawning in the natural water bodies. Saha et al. (1957) recorded that low total alkalinity (80-90 ppm) and low temperature (28-29°C) stimulate carps for spontaneous spawning.

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**Fig. 1.** T. S. of ovary of spontaneously bred L. rohita showing the unspawned vitellogenic oocytes (arrow head), non-vitellogenic oocytes (arrow) and empty follicles (EF) in folded condition (H and E X 150)

**Fig. 2.** T. S. of ovary after 15 days of spontaneous spawning showing recruitment of new oogonia (arrow), last remnant of empty follicles (EF) and developing oocytes of different stages (H and E X 150)

**Fig. 3.** T. S. of ovary after 45 days of spontaneous spawning showing matured oocytes (MO), last remnant of some atretic follicles (arrow). Arrow heads show very few non-vitellogenic oocytes (H and E X 150)
breeding. In the present context, few physico-chemical factors are believed to simulate gonad maturation similar to the observation made by earlier workers and the carps bred spontaneously in a confined pond. Badapanda et al. (1982) described such a spontaneous breeding highly oxygenated water, stimulated the fish for spontaneous breeding. The above statement was found true in the present study also.

Sathyanesan (1994) in his review on neuroendocrinology of reproduction in teleostean fishes has explained the receptors of the external stimuli in hypothalamus and their pathway for the act of spawning. Generally, Indian major carps do not breed spontaneously in confined ponds. It is recently understood that inadequate secretion of GTH II by the pituitary gland of the respective brood is the cause for non-breeding in pond water (Lin and Peter, 1996 and Bhattacharya, 1999). The cyanophilic cells of PPD in pituitary are considered as gonadotropins. The degranulation of PPD cyanophilic cells in the spontaneous breeding may be one of the evidences for secretion of gonadotropin by the environmental stimuli as discussed by Sathyanesan (1994). This observation also supports that of Lal (1964) who observed the pituitary PPD cyanophils of L. rohita during spawning period in riverine condition. Further, the accumulation of cyanophilic granules in these cyanophils, as studied after 45 days of spontaneous spawning again corresponds to the maturity status of the gonad. Such accumulation of cyanophilic granules was found during the peak period of gonadal maturation in carps (Sen, 1972; Jose and Sathyanesan, 1977; Das and Singh, 1990).

The GSI dropped to 2.45 on the next day of spontaneous breeding, which subsequently increased to 14.1 on the 45th day after spontaneous spawning. Bhatnagar (1972) and Rao and Rao (1972) reported that GSI can also indicate the maturity status and breeding period of Indian major carps. They found that GSI above 15 indicates

![Fig. 4. Section passing through the pituitary gland of spontaneously bred L. rohita showing the PPD cyanophils. Arrow indicates the degranulated cyanophils and arrow head indicates some of the hypertrophied cyanophils (AB-PAS-OG X 400)](image1)

![Fig. 5. Section passing through the pituitary gland of L. rohita after 45 days of spontaneous breeding showing the PPD cyanophils filled with cyanophilic granules (AB-PAS-OG X 400)](image2)
the spawning period of rohu in nature. The ovary of spontaneously bred spent L. rohita retained few unspawned vitellogenic and nonvitellogenic oocytes along with huge number of empty follicles. Recruitment of new oogonia, their development and maturation observed during the present study support the histoanatomical observations made by Rath et al. (2002) during interspawning period in multiple induced breeding of IMC in the same season. Bhowmick et al. (1977) reported double breeding of pond reared induced bred IMC. Chondar (1986) induced bred the pond reared IMC for three times. Gupta et al. (1995) could breed C. catla four times. The double breeding of spontaneously bred L. rohita observed during the present study revealed the possibility of breeding IMC in nature more than once in a season.

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