Introduction

Photoperiod and temperature are important environmental factors regulating gonadal development and maturation and other reproductive events in most of seasonally breeding teleosts (Lam, 1983) including cyprinids (Hontela and Stacey, 1990). Long photoperiod and increasing temperature were found to be favourable for gonadal development in Cirrhinus reba (Verghese, 1975), Mystus tengara (Guraya, 1976), Heteropneustes fossilis (Sundararaj and Vasal, 1976), Clarias batrachus (Singh and Singh, 1983) and Cirrhinus mrigala (Singh and Singh, 1984). The spawning of Indian major carps was correlated with the rainfall and lowering of temperature (Sinha et al., 1974).

Normally six spermatogenic stages, i.e., primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatocytes (sperm) are described in the testes of fish (Agarwal, 1996). The morphocytochemical changes in interstitial Leydig cells have been correlated with steroidogenesis in fishes (Guraya, 1976; Kanwar and Sheikhar, 1978). Histological changes in liver showing augmented biosynthetic activities corresponded with testicular growth and maturation and changes in hepatopancreatic cells were suggestive of energy mobilization.
ies in males are meagre (Campbell and Love, 1978; Wooton et al., 1978). In view of lack of similar correlative information in male of L. rohita, the present study was undertaken.

**Materials and methods**

The adult specimens of L. rohita were obtained from the Instructional Fish Farm of the College, Pantnagar. The specimens of L. rohita were stocked in broodstock pond at the rate of 2500 kg/ha and routine package of practice for rearing of broodstock was followed (Singh, 1986). Monthly data of temperature, day-length and rainfall were recorded.

For histological study of testis and liver, samples were collected in the mid of each sampling month from August 1998 to June 1999. Small portions of testis and liver were fixed in Bouin’s solution. Paraffin sections were cut at 5 μm thickness and stained with haematoxylin-eosin for histological observations prior to microphotography.

**Results**

The environmental temperature was maximum in April and minimum in January. The variations in maximum and minimum temperatures were high in March and April and low during November to January. The lower range of water temperature was recorded during November to January. It started increasing from February and attained peak in May after which continued to decrease up to October.

The day-length increased from March to June and decreased from September to December (Table 1). The longest day-length ranged between 12.84 (May) and 13.42 hours (June) while shorter day-length ranged from 11.24 (October) to 10.44 (December) hours. The rainfall occurred regularly during July to October in 1998 and January, May and June in 1999.

The gonadal development was normal in L. rohita in captivity under Tarai conditions. Six spermatogenic stages were observed in testis of L. rohita on the basis of nuclear and cytoplasmic characteristics (Table 2).

**Histological stages in testis of L. rohita during reproductive cycle**

The proliferation of spermatogonia was at initial stage and lobular lumen

---

**Table 1. Meteorological conditions in Tarai region of Uttarakhand during July 1998 to June 1999.**

<table>
<thead>
<tr>
<th>Months</th>
<th>Environmental Temperature (°C)</th>
<th>Water Temperature (°C)</th>
<th>Day length (hrs)</th>
<th>Rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>July</td>
<td>32.26</td>
<td>26.06</td>
<td>27.93</td>
<td>13.15</td>
</tr>
<tr>
<td>August</td>
<td>30.77</td>
<td>25.10</td>
<td>25.95</td>
<td>12.83</td>
</tr>
<tr>
<td>September</td>
<td>31.85</td>
<td>24.27</td>
<td>22.70</td>
<td>12.32</td>
</tr>
<tr>
<td>October</td>
<td>30.24</td>
<td>19.00</td>
<td>19.60</td>
<td>11.24</td>
</tr>
<tr>
<td>November</td>
<td>27.70</td>
<td>12.25</td>
<td>15.98</td>
<td>10.44</td>
</tr>
<tr>
<td>December</td>
<td>22.55</td>
<td>7.17</td>
<td>14.37</td>
<td>10.05</td>
</tr>
<tr>
<td>January</td>
<td>18.08</td>
<td>7.26</td>
<td>13.80</td>
<td>10.25</td>
</tr>
<tr>
<td>February</td>
<td>24.70</td>
<td>9.40</td>
<td>17.25</td>
<td>10.94</td>
</tr>
<tr>
<td>March</td>
<td>30.40</td>
<td>11.55</td>
<td>21.70</td>
<td>11.76</td>
</tr>
<tr>
<td>April</td>
<td>36.83</td>
<td>16.60</td>
<td>24.00</td>
<td>12.45</td>
</tr>
<tr>
<td>May</td>
<td>35.70</td>
<td>23.27</td>
<td>29.00</td>
<td>12.84</td>
</tr>
<tr>
<td>June</td>
<td>34.78</td>
<td>25.13</td>
<td>28.50</td>
<td>13.42</td>
</tr>
</tbody>
</table>
had a few spermatogonial cells during November to December. The scanty presence of spermatocytes in most lobules gave appearance of empty space (Fig. 1). The lobules increased in size and possessed good number of primary and secondary spermatogonial cells but not spermatocytes in January. At the interlobular junctions small inactive interstitial Leydig cells were also visible. Lobular wall remained considerably thick and appeared fibrous due to increased loose connective tissues (Fig. 2). Germinal cysts containing primary and secondary spermatogonia with a few primary and secondary spermatocytes compactly filled the lobular lumen in February (Fig. 3). The size of lobules had increased and a few of them contained good number of spermatids and spermatocytes. The interlobular mass, incorporating large active interstitial Leydig cells with prominent rounded nuclei decreased in March (Fig. 4).

The spermatogenic proliferation was at peak in April and spermatogenic cells were passing through stages of maturation and differentiation (Fig. 5). Increased lobule size and presence of sperm bundles in most of them indicated further increase in spermatogenic activities in May. Large active interstitial Leydig cells

<table>
<thead>
<tr>
<th>Sperm Cell Types</th>
<th>Cytological Characteristics</th>
<th>Reference (Figure number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Primary spermatogonium (sperm mother cell)</td>
<td>Largest among spermatogenic cell types, spherical in shape, present in cystic form, large nucleus with prominent eccentric nucleolus, less cytoplasmic affinity with dyes (i.e. Haemotoxylin - Eosin)</td>
<td>2</td>
</tr>
<tr>
<td>2. Secondary spermatogonium</td>
<td>Produced as a result of mitotic division of primary spermatogonia, smaller, rounded in shape, present in groups, centrally placed nucleus with visible chromatin threads and nucleolus, less cytoplasmic content.</td>
<td>2</td>
</tr>
<tr>
<td>3. Primary spermatocyte</td>
<td>Produced by multiplication of secondary spermatogonia, smaller in size, eccentrically placed nucleus with chromatin threads gathered on one side, forming synaptonemal complex (Agarwal, 1996), nucleolus in invisible condition.</td>
<td>3</td>
</tr>
<tr>
<td>4. Secondary spermatocyte</td>
<td>Produced as a result of reduction division of primary spermatocyte, nucleus with dark clumps of chromatin, nucleolus not visible, less cytoplasmic content.</td>
<td>4</td>
</tr>
<tr>
<td>5. Spermatid</td>
<td>Produced as a result of second meiotic division of the secondary spermatocyte, smaller in size, appeared as compact dotlike structures, deeply stained with haemotoxylin, rounded nucleus, nucleolus absent, cytoplasm scanty.</td>
<td>4</td>
</tr>
<tr>
<td>6. Spermatozoa</td>
<td>Produced as a result of metamorphic changes in spermatids by orientation and reorganization of nucleoplasm and cytoplasm together with the development of flagellum, small, rounded, deeply stained structures present in clusters.</td>
<td>7</td>
</tr>
</tbody>
</table>
Most of the lobules were packed with sperm masses with occasional presence of a few secondary spermatocytes and spermatids during June-July (Fig. 7). The lobules contained moderate number of sparsely distributed sperm along with residual secondary spermatocytes in August. Decreased nuclear size and cytoplasmic content in interstitial Leydig cells, indicative of their inactivation, were observed (Fig. 8).

Residual spermatozoa along with moderate number of spermatids were present in September. Phagocytosis characterized by dissolution of residual sperm and spermatid cells was also evident. The interlobular space containing involuted and inactive interstitial cells increased in September. An increase in number of primary spermatogonial cells was noticed during September-October (Fig. 9).

On the basis of histological observation of testes, the reproductive cycle of male L. rohita could be defined as: 1. Resting phase (November-January); 2. Preparatory phase (February-March); 3. Pre-spawning phase (April-May); 4. Spawning phase (June-August) and 5. Post-spawning / Regression phase (September-October).

Liver histology

Hepatocytes had large nuclei and
more cytoplasmic content and hepatopancreatic cells were small and inactive during resting phase (Fig. 10). Cytoplasmic vacuolization, indicative of activation, was observed in hepatocytes in January (Fig. 11). The cytoplasmic granulation and vacuolization of hepatocytes were observed during early preparatory phase (Fig. 12). Hyperactive and exhaustive hepatocytes with full cytoplasmic vacuolization and nuclei with small nucleoli were observed during later part of this phase (Fig. 13). Cytoplasmic granulation and increased nuclear size of hepatopancreatic cells in March was indicative of their activation. Increased cytoplasmic content and nuclear hypertrophy in both hepatocytes and hepatopancreatic cells were seen in April (Fig. 14) and May (Fig. 15). The activation was more pronounced in May when most of the hepatocytes had enlarged round nuclei with prominent nucleoli and increased cytoplasmic granulation and vacuolization (Fig. 15). During spawning phase hepatocytes as well as hepatopancreatic cells exhibited less biosynthetic activities (Fig. 16). These had less cytoplasmic content and small nuclear size. The inactivation was more pronounced in August (Fig. 17). The hepatocytes remained inactive but hepatopancreatic cells exhibited hypertrophy and hyperactivity marked by increase in cytoplasmic and nuclear size with corresponding vacuolization during
Fig. 5. Testis during initial pre-spawning phase (i.e. April) showing abundance of secondary spermatocytes (➢), spermatids (➢) and also sperm (*) bundles in some lobules. HE X450.

Fig. 6. Terminal pre-spawning phase (i.e. May), the testis of fish exhibiting large sized lobules containing spermatids (➢) and sperm bundles (*). Active interstitial cells could also be seen (➜) within interlobular space. HE X450.

Fig. 7. Initial spawning phase (i.e. June), the testicular lobules filled with huge sperm mass (+) and occasional presence of a few number of secondary spermatocytes (➢) and spermatids (➢). HE X450.

Fig. 8. Testis during terminal spawning phase (i.e. August) showing marked presence of spermatids (➢) and group of other germinal cells (➜) along with sperm (+). HE X450.
Testicular cyclicity in rohu

**Fig. 9.** Testis during post-spawning phase (i.e. October) showing lobules filled with residual number of sperm (*) and spermatids (➢). Phagocytosis of spermatogenic component (➢) could also be visualised. HE X450.

**Fig. 10.** Liver of fish during initial resting phase (i.e. November) exhibiting increased nuclear size and more cytoplasmic content of hepatocytes (➢). Inactive and small hepatopancreatic cells could also be seen (➢). HE X450.

**Fig. 11.** Liver of fish during terminal resting phase (i.e. January) indicating hyperactivity of hepatocytes with drastic increase in nuclear size (➢) and cytoplasmic vacuolization. Hepatopancreatic cells (➢) exhibited no change. HE X450.

**Discussion**

Increased activity in testis of *L. rohita* with increasing day-length and temperature from later part of preparatory phase to commencement of spawning phase was indicative of their intricate relationship. The peak of spermatogenesis during pre-spawning phase and occurrence of spermiogenesis with the onset of monsoon during spawning phase further corroborated the relationship. As the day-length was still long, the lowering of water temperature due to rainfall might have played an important role in acceleration of spermiogenesis during post-spawning phase (Fig. 18).
Fig. 12. Liver of fish during early preparatory phase (i.e. February) showing cytoplasmic granulation and vacuolization of hepatocytes (>) indicating energy mobilization and normal hepatopancreatic cells (➢) with no change. HE X450.

Fig. 13. Liver of fish during terminal preparatory phase (i.e. March) revealing induced hyperactivity of hepatocytes evidenced by small nuclear size (>) and cytoplasmic degranulation. Hepatopancreatic cells (➢) exhibit activation with increased nuclear size as well as cytoplasmic granulation. HE X450.

A positive relationship between increasing temperature and day-length and gonadal development and fall of temperature due to rainfall and upsurge in gonadotropin level during spawning phase has been reported for another Indian major carp, *C. mrigala* (Singh and Singh, 1984).

A few spermatogonial cells occupied lobular lumen during resting phase when other stages were absent. Lobule size had increased with the progression of spermatogenic activities resulting in decrease of inter-lobular space. Primary and secondary spermatocytes filled lobular lumen in preparatory phase. Spermatids appeared in preparatory phase but their domination along with sperms was in spawning phase. Inter-lobular space started increasing with advent of phagocytosis of sperms and spermatids after peak spermatogenic activity. Similar observations have also been made on spermatogenic development and differentiation in a number of teleosts of subtropical region (Bisht, 1974; Nauriyal, 1983; Agarwal, 1996). Increased activity and number of interstitial cells during preparatory to pre-spawning phases was indicative of their role in steroidogenesis and spermatogenic proliferation (Hyder,
Fig. 14. Liver of fish during early pre-spawning phase (i.e. April) showing hypertrophied hepatocytes (>) and hepatopancreatic cells (➢) with increased nuclear size. HE X450.

Fig. 15. Liver of fish during terminal pre-spawning phase (i.e. May) exhibiting hepatic energy mobilization evidenced by extreme stormal vacuolization (➢) and cytoplasmic vacuolization of hepatopancreatic cell (➢). HE X450.

Fig. 16. Liver of fish during early spawning phase (i.e. June) showing hepatocytes (➢) as well as hepatopancreatic cells (➢) with less biosynthetic activity evidenced by smaller nuclear size and homogenous cytoplasm. HE X450.

Fig. 17. Liver of fish during terminal spawning phase (i.e. August) exhibiting extreme inactivity of hepatocytes (➢) and hepatopancreatic cells (➢). HE X450.
Decrease in number, size and activity of interstitial cells during spawning phase were similar to observations of Sathyanesan (1959) and Pandey and Mishra (1981). Increased cell and nuclear size, cytoplasmic granulation and vacuolization of hepatocytes during preparatory and pre-spawning phases showing augmented biosynthetic activities seemed to be correlated with testicular development. Activated hepatopancreatic cells might have played a role in energy mobilization from liver to testis for gametogenesis. The diminished biosynthetic activities characterized by less cytoplasmic content and smaller nuclear size during spawning phase might have been indicative of no apparent role during maturation. Increased hepatic biosynthetic activities during post-spawning phase could be correlated with the resumption of active feeding rather than testicular cycle. In oestrogen treated female freshwater eel, Anguilla anguilla (Oliverseau and Oliverseau, 1979) and red grouper, Epinephelus akaara (Tam et al., 1983) an increase in the hepatosomatic index, enlargement of hepatocytes and their nuclear size and vacuolization in cytoplasm were correlated with increased biosynthetic activities. Seasonal changes in HSI, lipid and water contents in liver observed during the present study correlated with the testicular cyclicity as recorded by Kumar et al., 2001 in L. rohita. It seems that increasing water temperature and day-length are stimulatory for spermatogenic proliferation and testicular development in L. rohita and liver is associated with testicular development, whereas lowering of water temperature by rainfall potentiates terminal events of spermiogenesis during spawning phase.

Acknowledgement

Authors thank the Dean, College of Fishery Sciences, Head, Department of Fishery Biology, and O/I Instructional Fish Farm, for providing facilities for the present work.

References


Testicular cyclicity in rohu


