INTRODUCTION

Studies on reproductive cycle of fish are of great importance in maintenance of healthy fish population in any water system. Moreover, it has been well established that in most of teleosts the ovaries undergo fairly regular changes. But the variation was observed in different fish species under different environmental conditions. Because gonadal development in fish is affected by environmental conditions i.e. temperature, photoperiod, dissolved oxygen, water current etc (Gadakar, 2014). To get a good quality seed, it is desirable to have indepth knowledge on the reproductive biology of fish, so that the exact timing of spawning can be determined. Availability of such information, to a great extent can help a fish farmer to conduct a successful venture induced breeding (Agarwal et al., 2001 and Gogoi et al., 2013).

G. gotyla gotyla is one of the important hill stream fish of J&K, India and in our neighboring country Nepal a major proportion of their population depends upon this for fish protein. Though a lot of work on reproductive cycle of different fishes inhabiting plain as well hilly area of J&K has been done by different workers (Malhotra, 1965; Jyoti, 1972; Gupta, 1980; Singh, 2009 and Vohra, 2011) but there has been no report related to reproductive cycle of G. gotyla gotyla so far. Therefore presently an attempt has been made to generate information related to ovarian development and reproductive cycle of G. gotyla gotyla from Jhajjar stream, J&K, India.

MATERIALS AND METHODS

Live specimens of G. gotyla gotyla were collected monthly from the Jhajjar stream, a tributary of river Tawi for one year. Length and weight of each individual fish was recorded and after that ovaries were carefully removed, excessive moisture was blotted and quickly weighed on an electronic balance. To make permanent slides of ovaries routine methodology was adopted and for that ovaries were firstly fixed in bouin’s fixative for 24 hours [freshly prepared from saturated solution of picric acid (75%) to which formalin (20%) and acetic acid (5%) was added at the time of use]. After treatment in bouin’s fixative, ovaries were then washed, dehydrated and embedded in paraffin histo wax (54-56ºC). 5-7 μm transverse sections of ovaries were cut with the help of microtome and were stained using haematoxylin-Eosin stain. Different phases of gonadal cycle were identified by observing the prepared slides under microscope.

Detailed examination of the histology of the ovaries was attempted by measuring the mean diameter of different stage oocyte by taking the average of the horizontal and vertical diameters of oocyte whereas percentage distribution of different stage oocytes was calculated by studying ovarian microslides at different focal points. Gonadosomatic index (GSI) was calculated by using formula weight of gonads (gms)/weight of fish (gms) x 100.

RESULTS AND DISCUSSION

Morphology of Ovaries

The ovaries of G. gotyla gotyla are paired (Fig. 1-A and 1-B), sac like structure which lie ventral to kidneys but dorsal to alimentary canal in the body cavity. They are attached to the...
The shape, size and colour of ovaries of fish, *G. gotyla gotyla* undergo considerable changes during different stages of maturity. The ovaries usually are transparent and translucent during early stages of maturity but turn opaque and get studded with yolky ova as fish matured (Table 1).

**Histology**

The wall of ovary is composed of an outer peritoneal membrane overlying tunica albuginea. The innermost layer is the germinal epithelium which projects into the ovocoel in the form of lamellae (Fig. 1). A large number of oogonia and oocytes in various stages of development can be seen studded along ovigerous lamellae (Fig. 1). These oogonia before transforming into an oocyte undergoes various changes pertaining to cytoplasm and its inclusions like vesicles, vitellogenesis and egg membranes. Based on changes an oocyte undergoes during its development into mature ovum, following stages have been identified.

**Oocyte stage-I/chromatin nucleolar stage**

These are the smallest oocytes observed along the ovigerous lamellae and range in diameter from $0.0231 \pm 0.02534$mm to $0.0592 \pm 0.01324$mm (Table 2 and Fig. 1). They were nearly spherical in form with transparent cytoplasm. The nucleus, which is centrally placed, has a nucleolus (either centric or acentric in position) besides a network of chromatin threads.
Oocyte stage-II/perinucleolar stage
Oocytes of this stage grow further in size and now vary from 0.0533 ± 0.02310mm to 0.2010 ± 0.01540mm in diameter (Table 2). During this stage nucleus also enlarges proportionally. The nucleoli observe an increase in their number and get distributed adjacent to nuclear membrane (Fig. 1). With the advancement of growth, ooplasm further increases and becomes much greater in volume than nucleus. These oocytes now seem to be surrounded by flattened follicular cells (representing the follicular epithelium).

Oocyte stage-III/early yolk vesicle stage
Oocytes of this stage are characterized mainly by the first appearance of minute vacuoles in the cortical area of cytoplasm, termed as yolk vesicle (Fig. 2). All such oocytes have wavy nuclear membrane in which nucleoli were seen to occupy pockets of waves (Fig. 3). A few oocytes even exhibited the nucleoli piercing through undulating membrane to enter into cytoplasm (Fig. 2 and 3). Follicular layer now is better developed and a thin vitelline membrane internal to follicular epithelium has been noticed in some oocytes of this stage. Diameter of the oocytes of this stage ranged between 0.1150 ± 0.03125mm to 0.3530 ± 0.06425mm (Table 2).

Oocyte stage-IV/late yolk vesicle stage
During this stage, cortical alveoli show random movement/distribution in the ooplasm that further increases in number and size. The undulation of nuclear membrane becomes clear and more and more nucleoli are seen extruding in ooplasm (Fig. 4). All the oocytes now showed tremendous growth and ranged from 0.2500 ± 0.03520mm to 0.4428 ± 0.02764mm in diameter (Table 2).

Oocyte stage-V/early yolk stage
During this stage yolk granules were seen to be deposited in yolk vesicles. Extra vesicular yolk also has been observed to be deposited in the ooplasm. These extra vesicle granules were observed to form large spherical yolk globules filling almost entire ooplasm. All these oocytes observed an increase in size and now measured from 0.3490 ± 0.01046mm to 0.5210 ± 0.03016mm (in diameter). Nucleus of the oocyte did not observe any increase and rather showed signs of dissolution. An indistinct theca was observed to surround the fully developed follicular epithelium (Fig. 5).

Oocyte stage-VI/late yolk stage
During this stage an extensive deposition of yolk globules is observed which further increases with increase in size of oocyte. Yolk now almost fully occupy whole of ooplasm around the nucleus. Rapid accumulation of yolk globules further results in growth of oocyte. Follicular epithelial layer increases in thickness. The thecal layer surrounding the follicular epithelium becomes more visible and vitelline membrane inner to follicle becomes thicker (Fig. 6). Diameter of oocytes of this stage varied from 0.4280 ± 0.02460mm- 0.5910 ± 0.01268mm (Table 2).

Oocyte stage-VII/ripe egg stage
During this stage ovaries were observed to be packed with fully grown yolky oocytes. Nucleus which now becomes indistinct was usually seen to be acentric in position (Figure 7). All these oocytes were observed to be surrounded by well developed egg membranes viz. from inside to outside being represented by vitelline (zona pellucida), follicular epithelium (zona granulosa) and theca. These oocytes ranged in diameter from 0.5710 ± 0.01205mm-0.7500 ± 0.02156mm (Table 2).

Based on the percentage of growing oocytes and nature of ovarian wall on yearly basis, the ovarian cycle has been divided broadly into six phases viz. immature/virgin phase (September-October), early maturing phase (November-January), developing phase (February-March), developed phase (April-May), spawning phase (June-July) and spent/resting phase (August) (Tables 1 and 3).

Immature/virgin phase
This phase of reproductive cycle extends from September to October. During this phase, ovaries are thin, translucent, pale and dirty brown in color, with less vascular supply (Table 1). Histologically the ovaries show the presence of ovigerous lamellae (Fig. 1). The ovigerous lamellae are thin and enclose an inner lumen which is filled with oocytes of stage I and stage II. Most of the oocytes (94.8%) are in stage I (chromatin nuclear stage) of development and rest (5.2%) in stage II (perinucleolar stage) (Table 3). GSI was observed to be 2.80 ± 0.16 in the month of September and 0.82 ± 0.24 in the month of October.

Early maturing phase
This phase which extends from November to January, show ovaries to be slightly thicker, opaque and yellowish in color.
Vascularization is feeble (Table 1). The ovigerous lamellae are greatly swollen during this phase and laden with oocytes of stage I (64.5%), stage II (28.3%) and rest stage III (7.2%) (Table 3). GSI was noted to be 0.40±0.20, 5.56±0.34 and 5.76±0.14 respectively during the month of November, December and January.

Developing phase

This phase extends from February to March. Color of the ovaries during this phase changes to dark yellow. The blood capillaries become inconspicuous because of profuse blood supply (Table). During this phase, the ovaries were observed to be populated by oocytes of stage IV (20.65%), stage V (33.4%) and stage VI (29.45%). Oocytes of stage I and II too are seen but in lesser number (9.1%). Few oocytes of stage VII also make their appearance during this phase (Table 3). The ova are tightly held and the ovary cannot be stripped by applying gentle pressure. An increase in GSI has been recorded during this phase (7.0±0.65 in February and 7.53±0.47 in March).

Developed phase

During this phase which extends from April to May ovaries become deep yellow in color. Vascularization is extensively developed (Table 1). Ovaries now are packed with as high as 85.5% % of stage VII oocytes besides 9.5% stage VI oocytes. Few stage I and II oocytes invariably also can be seen during this phase of ovarian cycle (4.5%) (Table 3). The fish passing through this phase of reproductive cycle have a bulging abdomen (Table 1). Further increment in GSI has been seen in this phase i.e. 8.05±0.54 in the month of April and 9.31±0.05 during May.

Spawning phase

This phase begins from early June and last almost till the end of July. During this phase ovaries are yellowish and turgid due to the presence of a large number of mature ova. Ovaries exhibit rich vascularization and called to be in running phase as ova ooze out from the oviduct with slight pressure on the abdomen (Table 1). Histological sections of the ovaries during June and July show oocytes of stage VII (74.5%), 7.2% of stage VI oocytes beside very few stage I and II along with a number of discharged follicles (Table 3). A decrement in the values of GSI has been recorded (8.68±0.39 in June and 5.53±0.16 in July).

Resting/ Spent phase

Ovaries during the month of August appear thin, flaccid, delicate, slender and dull in color. There is decrease in the volume and weight of ovary (Table 1). Vascularization is reduced (Table 1). Histologically, the ovaries show residual
oocytes as well as discharged follicles and atretic follicles (Table 1). GSI was found to be 3.03 ± 0.84 during this phase of reproductive cycle (August).

Besides presence of oocytes of different stages viz. stage I, stage II, stage III, stage IV, stage V, stage VI and stage VII, the histological sections of ovaries of *Garra gotyla gotyla* also show the presence of atretic oocytes as well as that of corpus luteum. Atretic oocytes were observed during different phases of reproductive cycle whereas discharges/ corpora lutea were observed only during spawning and post spawning (spent phase) phases of reproductive cycle (Fig. 8 and 9).

**DISCUSSION**

In females of *G. gotyla gotyla* ovaries are a pair of sac like structure (Figs.1-A and 1-B) that flank an air bladder within their inner margins. Many workers have earlier also reported similar morphology of ovaries in different teleosts like *Salvelinus fontinalis* (Henderson, 1962), *Schizothorax niger* and *Crossocheilus latius diplocilus* (Iyot, 1972), *Channa punctatus* and *Puntius sorne* (Gupta, 1980), *Tor putitora* (Singh, 2009), *Danio devario* (Hina, 2010), *Esomus danicus* and *Raslora rasbora* (Vohra, 2011) and *Xenentodon cancila* (Subba and Meheta, 2012) but differ from fish like brook lamprey (Okkelberg, 1921), *Botia bardi* (Malhotra, 1965), *Noemachelius kashmiriensis* (Iyot, 1972) and *Schizothorax niger* (Hajam, 2011) where in the female reproductive system has been observed to consist of a single ovary lying mid ventrally and also from *Trichogaster fasciatus* (Jyoti, 1972) where nucleolus extrusion has been observed in stage II of *Yamamoto and Yamazaki (1961)*, *Malhotra* (1960; Malhotra and Rathi, 1970; *Jyot*, 1972; *Malhotra et al*, 1978, 1979, 1980; *Wallace and Selman*, 1981; *Robb*, 1982, *Hatikakoty and Biswas*, 2004; *Agarwal*, 2008; *Hina*, 2010 and *Subba and Meheta*, 2012).


**Table 3: Degree of Ovarian maturation and percentil occurrence of different stages of maturity in *Garra gotyla gotyla*.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Degree of maturation</th>
<th>Months of availability</th>
<th>Percentage of occurrence</th>
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<tr>
<td>I</td>
<td>Immature/Virgin</td>
<td>September-October</td>
<td>Max-I (94.8%) Rest-II (5.2%)</td>
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<tr>
<td>II</td>
<td>Early maturing</td>
<td>November-January</td>
<td>Max-I (64.5%) &amp; II (28.3%) Rest III (7.2%)</td>
</tr>
<tr>
<td>III</td>
<td>Developing</td>
<td>February-March</td>
<td>Max-V (33.4%) &amp; VI (29.45%), 20.65% (IV) &amp; I &amp; II (9.2%) &amp; few VII</td>
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<tr>
<td>IV</td>
<td>Developed/Pre-spawning</td>
<td>April-May</td>
<td>Max-VII (83.5%), Min-VI (9.5%) and few I &amp; II (4.5%)</td>
</tr>
<tr>
<td>V</td>
<td>Spawning</td>
<td>June-July</td>
<td>Max-VII (74.5%), VI- (7.2%), few I &amp; II and Discharged and Atretic follicles</td>
</tr>
<tr>
<td>VI</td>
<td>Resting adult</td>
<td>August</td>
<td>Atretic and Discharged follicles</td>
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</table>

and there are different viewpoints putforth by different workers. Bullough (1939), *Mendoza* (1943), *Dixit* (1956), Tromp-Blom (1959), *Bara* (1960), *Jyot* (1972), *Malhotra et al.* (1978), Raina (1999) postulated that new crop of oogonia originates from the germinal epithelium. On the other hand, according to Wheeler (1924), *Yamamoto* (1956a, b) and Andrew and Pinto (1957) new crop of oogonia develops from the follicular epithelial cells of spent follicles. Dixit (1956) reported their origin from stromal tissue in *Mystus seenghala*. According to some other authors (Mathews, 1938; Belsare, 1962 and Agarwal, 1982) new crop of oogonia arise from the residual oogonia. In present studies fish *G. gotyla gotyla* is in agreement with those who also have (op.cit) held germinal epithelium and ovigerous lamellae to be the seat of origin of new crop.

An oogonium has a large nucleus and a thin layer of ooplasm. Each oogonium undergoes successive maturation divisions and form new generation of oocytes. Before ripening into a mature ovum, a series of cytonuclear changes take place in the oocyte. Based on these changes, the development of an oocyte has been classified into different stages by various workers (Wood, 1930; *Yamamoto*, 1956a, b; *Srivasitava and Rathi*, 1970; *Jyot*, 1972; *Malhotra et al.*, 1978; *Wallace and Selman*, 1981; *Robb*, 1982, *Hatikakoty and Biswas*, 2004; *Agarwal*, 2008; *Hina*, 2010 and *Subba and Meheta*, 2012).

Presently it has been observed that during the different phases of reproductive cycle, the oocytes of fish *G. gotyla gotyla* show considerable change in their shape, size, weight and colour (Table 1). The oocytes were transparent and translucent during early stages of maturity and turned opaque and get studded with ova as they mature. Similar to present findings, *Agarwal* (1982), *Singh* (2009), *Vohra* (2011) and *Subba and Meheta* (2012) also observed such changes in the oocytes of fish they studied. Histologically, the oocytes of fish *G. gotyla gotyla* under present investigation resemble that of other teleost in being cytoplasmic type i.e. covered over by an envelope comprising of tunica albugenia and peritoneal epithelium, which at places extends centripetally forming ovigerous lamellae within the oovocel (Agarwal, 1982; Vohra, 2011 & Gadekar, 2014). Germinal epithelium lying beneath the tunica as well as ovigerous lamellae appears to be the site from where germ cells develop.

Review of literature reveals that mode of origin of new crop of oogonia or germ cells has remained a controversial matter
in *Rita* *Ophiocephalus* and *Cirrhinus*. According to Bose and Bose (1964) and Guraya et al. (1975) the nucleolar extrusion do not play any direct role in vitellogenesis but others reported that extruded nucleoli play a direct role in vitellogenesis (Chaudhary, 1951; Gupta, 1980 and Singh, 2009) and present author also support the workers who claim their direct role in vitellogenesis.

Kapoor (1977) supporting their role in vitellogenesis held the view that the extruded nucleoli bring some information (probably through messenger RNA) from nucleus into ooplasm. Malhotra (1963, 1965) also supporting their role in vitellogenesis stated that the extruded bodies by the process of disintegration and subsequent action with cytoplasm near the egg periphery bring about the process of yolk formation, Malhotra (1965), Jyoti (1972) and Gupta (1980), however, regarded RNA (acting as messenger) to be the seat of feed back principle, which stimulates the pituitary to release FSH, with which is associated onset of vitellogenesis.

Presently oocytes of stage IV are characterized by appearance of yolk vesicles in peripheral part of ooplasm and their centripetal movement to finally occupy entire ooplasm. During stage V, all the three egg membranes viz. theca, follicular epithelium and vitelline membrane are well developed but get comparatively thicker than the oocyte of previous stage followed by centripetal extension of yolk granules during stage VI and peripheral movement of nucleus during stage VII (Agarwal, 2008 and Subha and Mehetra, 2012). Apart from these oocyte stages a large number of corpora lutea were observed during late spawning and spent phase of ovarian cycle. Corpora atretica were however observed during all the different phases of reproductive cycle.

GSI has been used as a model for gonadal development and reproductive efforts in several teleosts (Delahunty and de Vlaming, 1980 and Gadekar, 2014). De Vlaming et al. (1972) while discussing the utility of gonadic indices stated it to be a good indicator of reproductive activity of a stock and also mentioned that it can be used as a reliable indicator of spawning period in fishes. The lowest value of GSI (0.40±0.20) was recorded during November and highest (9.31±0.05) during May. After that a decline has been noted in its value. Therefore only one peak is observed for GSI in *G. gotyla* gotyla. Fall in GSI is used as an indicator of spawning period (June and July) and single peak reflects that *G. gotyla* gotyla is an annual breeder.

Thus the overall pattern of oocytes development in *G. gotyla* gotyla is basically the same as in all other teleosts and follows a cyclic pattern. Growth of oocyte which gets initiated in immature phase and extend upto developed phase when they become fully mature and finally start liberating/oozing during the spawning act. GSI predict that the spawning season extend from June to July.

REFERENCES


reproductive cycle of *Trichogaster* from Jammu (India). Zoological Orientalis. 3(1&2): 41-46.


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