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Reproductive Cycle of the Endangered Sarpunti, *Puntius sarana* (Hamilton, 1822) in Bangladesh

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Abstract

Successive developmental stages of both male and female gonads and estimation of gonado-somatic index (GSI) of *Puntius sarana* (Hamilton 1822) were investigated over a two year period (October 2002 to September 2004). From the histological analysis, four developmental stages such as spermatogonia, spermatocytes, spermatids and spermatozoa were identified in testes. Three developmental stages of oocytes such as ogenesis (oogonia, early perinucleolus stage and late early perinucleolus stage), vitellogenesis (early vitellogenic oocytes, advanced vitellogenic oocytes and maturation stage) and atretic stage were distinguished in ovaries. Maximum mean gonad weight was recorded in June for both females (8.92±0.01 g) and males (4.48±0.21 g). The testes at early development stages between October and March contained mostly spermatogonia, spermatocytes and spermatids, although a small amount of spermatozoa was also found in late March. The mature testes began to develop in early April and were prominent from May to September. Highest percentage of oogonia in the ovary was recorded in the month of November; while the highest percentages of early perinucleolus stage and late perinucleolus stage were recorded in the month of January. Vitellogenesis (matured oocytes) occupied the most areas of the ovaries which were observed from May to mid September but was prominent in June. All oocytes did not mature at the same time. Only the matured oocytes ovulated in the spawning period while immature oocytes gained maturation under way of vitellogenesis and released at the second spawning time in the months of August and September.

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Introduction

The olive barb, *Puntius sarana* (Hamilton 1822) is a commercial barb species with high market value for its high biological value in Bangladesh as well as in other South East Asian countries (Sinha 1975) which prefers shallow waters of floodplains for breeding (Talwar and Jhingran 1991). This fish was abundantly available in our open water systems like rivers, haors, baors, beels and natural depression in the past but due to over exploitation and various ecological changes in its natural habitat; this species is in the verge of extinction. The International Union of Conservation of Nature (IUCN-Bangladesh 1998) enlisted *P. sarana* as one of the critically endangered minor carps in Bangladesh. The development of artificial breeding techniques and larval rearing methods are the tools to develop the pond culture techniques of this species. It is feared that this fish may disappear from Bangladesh like the *Labeo nandina* unless proper steps are urgently taken to protect the fish from extinction. The fish attain maturity within a year and normally breeds from April to mid September (Qasim and Qayyum 1961; Sobhana and Nair 1974; Sinha 1975).

Studies on the reproductive biology of any fish is essential in evaluating the commercial potentialities of its stock, life history, cultural practice and actual management of small indigenous fishes (Lagler 1956; Doha and Hye 1970). The reproductive potential of a population is one of the basic exigencies to designate the individuals of that population in respect to their gonadal conditions (Jhingran and Verma 1972). A histological study helps in detecting the breeding season and in establishing phenotype characters of fully mature breeders for a successful artificial propagation of *P. sarana*. Hence, it is very important to assess the yearly breeding cycle of *P. sarana* to assure success in culture practice. Knowledge of gonadal development and the spawning season of a species allow subsequent studies on spawning frequency of its population, which is very important for its management. Very little works has been done in such direction. Limited literature exists in the subcontinent of India. Some of the earlier works described the process of gonadogenesis and gametogenesis for the *Ctenopharyngodon idella* (Jensen and Shelton 1983) and *Hypophthalmichthys molitrix* (Mirza 1983), *Amblypharyngodon mola* (Afroze and Hossain 1990) and *Clupea pallasii* (Koya et al. 2002). The present work was undertaken to find out the natural reproductive cycle of both sexes of *P. sarana* based on Gonado Somatic Index and histological preparations.

Materials and Methods

The present research work was conducted in the ponds of Fields Research Complex ponds of Bangladesh Agricultural University, Mymensingh between October 2002 and September 2003. The area of each pond was 0.0082 ha. The ponds were rectangular and average depth was 0.77 m. The fishes were fed daily with mustard oil cake (40%), rice bran (30%), wheat bran (20%) and fish meal (10%) at the rate of 4-6% of body weight.

Sample collection and preservation

The fishes were collected from the grow-out ponds at 15 days interval throughout the research period. The fishes were sampled using a seine net. At least 10 fishes were captured and live specimens were brought to the laboratory. Two hundred and forty live specimens of sarpunti between the ages of one and two years and 136.51 to 217.26 g in total weight were sacrificed to evaluate the process of gonadal sex differentiation. A piece of tissue (4 to 5 mm) from the left side of the gonadal region of the sample was excised anatomically using a sharp knife and preserved in Boun's solution for histological study. Anatomical differentiation was interpreted as described by Persov (1972), while histological concerned the process of gonadal germ cell transformation into either recognizable spermatocytes or oocytes marking the initiation of the first meiosis (Barr 1968). The normal expected sex ratio of *P. sarana* population was determined from data of the gonadal sex differentiation study; confirmed histological sex differences were manifested. Chi-square test was performed to test the sex ratio of *P. sarana* population. The accumulated total number of male and female fish was used to calculate the expected ratio of sexes.

Methods for determining the reproductive periodicity of Puntius sarana

General morphology of gonad: General feature and structure as well as size, shape and color of male and female gonads of the experimental fish were considered after sample collection and preservation.

Gonado Somatic Index (GSI): GSI is frequently applied to determine the spawning frequency of fishes and crustaceans and was calculated according to the formula (Lagler 1956):

$$GSI = (\text{Gonad weight}/\text{Total weight}) \times 100$$

Diameter of ova: A small representative part from the anterior, posterior and middle portions of the same were removed separately. The ova was separated in a physiological saline solution (0.85% NaCl) and spread on a glass slide to measure the diameter of ova under a microscope with an ocular micrometer. The units of the ocular micrometer were standardized with a stage micrometer for the measurement of ova diameter in micrometer (μm).

Histology: The preserved gonads were taken out in a perforated plastic holder, which was covered by perforated steel plates. Cleaning, infiltration and dehydration processes were carried out in an automatic tissue processor using a series of alcohol of increasing concentrations, two changes of xylene and finally molted wax (3 series). Gonads of male and female fish collected from October 2002 through September 2003 were examined histologically to study the different maturational stages of spermatogenesis and oogenesis and their morphology (Vitale et al. 2006). Gonadal sections were examined and monthly developmental variations were observed. Paraffin embedded blocks were cut using a microtome knife at 4-5 micrometers and leaving the sections to a water bath at a temperature of 40^{o}C. The sections were placed on a glass slide and kept over night on a slide drier hot plate at a temperature of 20^{o}C. Then the sections were stained routinely with haematoxyline and eosin (Humason 1972).

Microscopic examination of the gonadal tissue: These sections were mounted on the glass slide with Canada balsam and covered by cover slips. The prepared sections were studied under a compound microscope (SWIFT M 4000-D) and photographic records were collected.

Results

Gonado Somatic Index

Seasonal changes in mean GSI values of both males and females of local sarpunti are presented in figure 1. The mean GSI of the fish tends to increase as the fish reaches maturity and after spawning, it declines and the minimum GSI was recorded during resting phase. In the case of male *P. sarana*, it was found that the weight of the gonad gradually increased from January to June. It slowly increased stage by stage until it reached its maximum value in June. Highest GSI value was found in the month of May and the GSI values began to fall gradually from June to January. But in the

case of female *P. sarana*, the weight of the gonad gradually increased from January to June. It slowly increased stage by stage until it reached its maximum value in June. Highest GSI value was found in the month of June and the GSI values began to fall gradually from July to December. The GSI values for male and female *P. sarana* were found to be from 1.53±0.32 to 2.67±0.12 and 2.89±0.04 to 4.12±0.52 as presented in figure 1.

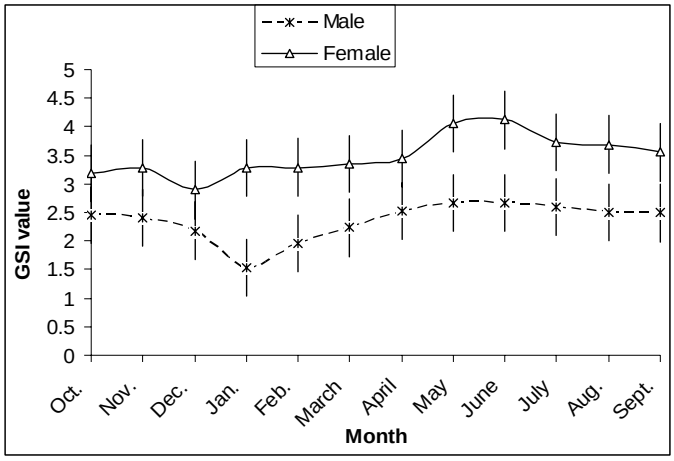


Fig. 1. Monthly mean value of gonado-somatic index (GSI) of male and female *Puntius sarana*

Male reproductive cycle

The first obvious signs of anatomical differentiation were seen in the specimens. The testes are soft and elongated structures lying in the body cavity and ventral to the swim bladder. It leads posterio-ventrally into two vas deferens that unite to form a spermatic duct opening to the exterior through the urogenital aperture. Each testis is attached to the dorsal body wall by the connective tissue, mesorchium and composed of numerous thin walled seminiferous lobules. Within the lobules, cells in various stages of spermatogenesis appeared in discrete nests of cells, each nest consisting of equally developed cells. Again, the lobules containing more advanced germ cells lie towards the center of the testis.

The testes varied in length from 3.5 to 7.2 cm. The testes were found to be in different colors in the development of different stages. At the mature stage, the testes were opaque and creamy white in color. The testes of *P. sarana* having two equal lobes were cylindrical and folded structure but not branched. Testes of maturing and mature individuals had a turgid texture and folded structure (Fig. 2), but the spent individuals had

a loose texture. Based on size, color, texture and histological differentiations, four stages of testes were recognized as depicted in table 1.

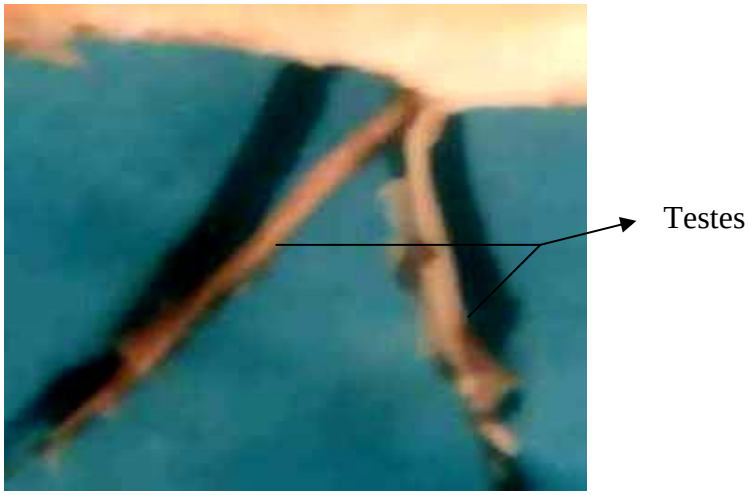


Fig. 2. A pair of mature testes of *Puntius sarana* (Hamilton, 1822).

Histological Observation

Testes of *P. sarana* are made up of a large number of seminiferous tubules of varying sizes held together by a connective tissue. A transverse section of a tubule clearly shows the lumen bounded by a germinative zone and the germ cells in various stages of development. Four distinct stages of spermatogenesis were distinguishable in the male gonads of the experimental fish based on the histology of the nuclear and cytoplasmic morphologies (Figs. 3A-F and Table 2)**,** which are described as follows:

1. Spermatogonia

Spermatogonia are the first group of cells to appear during the process of spermatogenesis and hence are most populous near the germinative zone of maturing or mature testes. These are spherical and basophilic structures with a network of chromatin material and nucleoli but distinct nuclear wall. Their average diameter was 10.6 µm. However under light microscopy no distinction could be made between primary and secondary spermatogonia. These cells undergo further mitotic divisions to form primary spermatocytes. The germ cell multiplication becomes more apparent in the testes of fish in the months of October to January (Figs. 3A-B).

Table 1. Testes characteristics of *Puntius sarana* at various stages of maturity

Stage of maturity	Testes Characteristics			
	Length group (cm)	Texture	Color	Histological examination
Premature stage (Stage I)	3.5-4.5	Turgid	White	Tubule diameter short, compactly packet, mostly with spermatogonia; wall of tubule thick.
Early maturing (Stage II)	4.6-5.4	Turgid and folded structure	Opaque and creamy whitish	Tubule diameter large, spermatocytes and spermatids are dominant, spermatogonia near the germinal epithelium only; wall of tubule thin.
Mature (Stage III)	5.5-7.2	Turgid and folded structure	creamy whitish	Tubule diameter very large filled with spermatids spermatozoa.
Spent (Stage 1V)	3.8-5.8	Loose	Dull white	Lumen of tubules irregular, a gap between germinal epithelium and germ cells are few and include residual spermatozoa.

2. Spermatocytes

Spermatogonia undergo first maturation division to give rise to secondary spermatocytes. The latter have poor stainable cytoplasm. The primary and secondary spermatocytes did not show any marked difference in size. The average diameter of primary and secondary spermatocytes was 7.7 µm. The secondary spermatocytes, followed by the second maturation division, gave rise to spermatids. Spermatocytes were also spherical in shape containing a nucleus in the centre and these were the primary spermatocytes. The germ cell multiplication becomes more apparent in the testes in the months of February and early March (Fig. 3C).

3. Spermatids

The spermatids are small rounded bodies, 4.4 µm in diameter. They have a little cytoplasm and most of their volume is occupied by a large nucleus. The nuclei show uniform condensed chromatin material. During this stage of development, the testes of most fish contained substantial numbers of germ cells, although there were exceptions.

Following active mitosis, it appeared that the spermatogonia underwent a resting stage before undergoing further meiosis and transformation into primary spermatocytes. The testes were dominated by spermatids in the months of March and April (Fig. 3D).

4. Spermatozoa

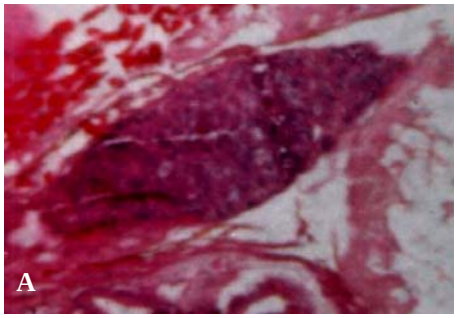
Finally the spermatids undergo certain morphological changes to produce spermatozoa. The spermatozoa are crescent shape structure bearing a short tail. The two ends of the crescent measured 5.0 µm and the widest part at the middle measured 1.4 µm. This stage of spermatozoa started to develop in mid April and prominent in May to September (Figs. 3E and 3F). They appeared as small black-colored spots under the stage where it continued up to mid September. At that time, germ cell development remained unchanged. At this stage the testes had markedly increased in size and similar in appearance to those found in larger fish.

Histological sections of testes of the experimental fish contained highly distinct cysts at all stages of development throughout the period of observation. The percentage of individuals in different stages of maturity observed during different months was computed and depicted in figure 4. In spent testes the germ cell had left the germinal wall of the tubules. The germ cells which included residual spermatids and spermatozoa were scarcely distributed inside the tubules.

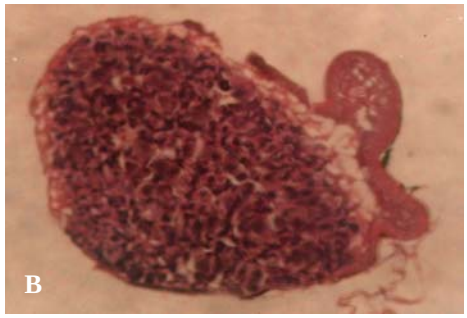
In March, 45% and 48% of the individuals had premature and early maturing testes and the rest were on the way to maturity. In April, most of the individuals had mature and early maturing testes. Only 10% had immature testes. The month of May showed a marked increase in the percentage of maturing fishes (67%). Some immature fishes were also found. From June to September, no immature fishes were observed. In June, the most remarkable increasing percentage (80%) of maturity was recorded and early maturing was recorded at 14%, the rest had spent fishes. Maturity stages in July were more or less similar as in the June. In August, the percentage of mature spent and early maturing fishes were 60%, 35% and 5%, respectively. However, September was equally shared by mature and spent fishes. In September and October all the fishes met were spent.

Female Reproductive Cycle

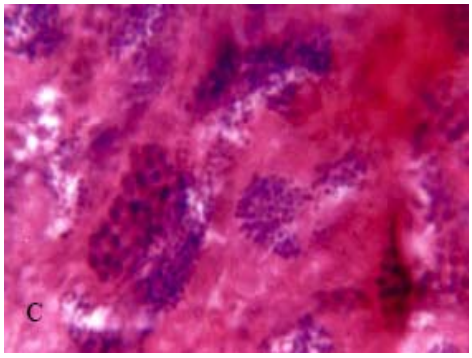
The female reproductive organs were elongated, slightly flattened and semi transparent in appearance. Ovary was internal, usually longitudinal and paired structure. A pair of mesenteries (mesovaria) suspended them dorsolaterally to the body cavity. This paired organ consisted of two ovarian lobes, which were separated by a septum. They were covered with an ovarian membrane and numerous ovarian lamellae, protected into the ovarian cavity. The ovarian cavity was connected to the oviducts and the oviducts from each bilateral ovary were joined leading to the genital pore.



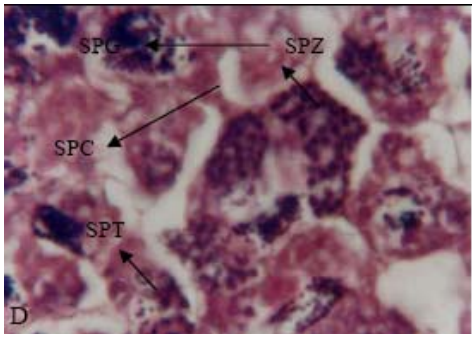
A. T.S. testis with developing increased number of germ cells



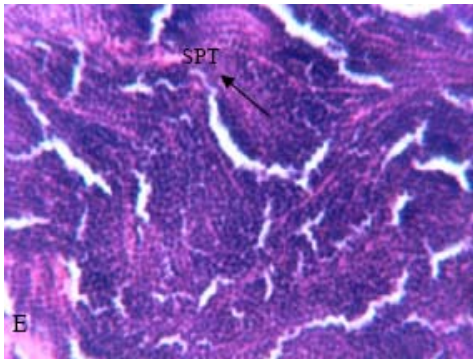
B. T.S. testis increase in size dominated by spermatogonia



C. T.S. testis increase in size dominated by spermatogonia and spermatocytes



D. T.S. testis increase in size dominated by spermatid and with well-formed duct system



E. T.S. testis increase in size dominated by sperm and with well-formed duct system

F. T.S. testis increase in size dominated by sperm and with well-formed duct system

Figs. 3A-F. Sectional views of the testes of *Puntius sarana* (Hamilton, 1822). Haematoxyline and eosin (x200). SPC=Spermatocyte, SPG=Spermatogonia, SPT= Spermatids, SPZ=Spermatozoa

Table 2. General organization of germ cells at light microscope level in testes of *Puntius sarana*

Germ cells	Shape	Size (μm)	Cell boundary	Cytoplasm	Nucleus	Nucleoli	Chromo-somes
Spermatogonia	Spherical	10.6	Distinct	Clear	Nuclear wall indistinct	Distinct	Forming a network
Spermatocytes	Spherical	7.7	Distinct	Clear	Nuclear wall indistinct	Distinct	Indistinct
Spermatids	Spherical	4.8	Distinct	Indistinct	Distinct	Invisible	Condensed
Spermatozoa	Crescent with short tail	L-5.0 W-1.4	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct

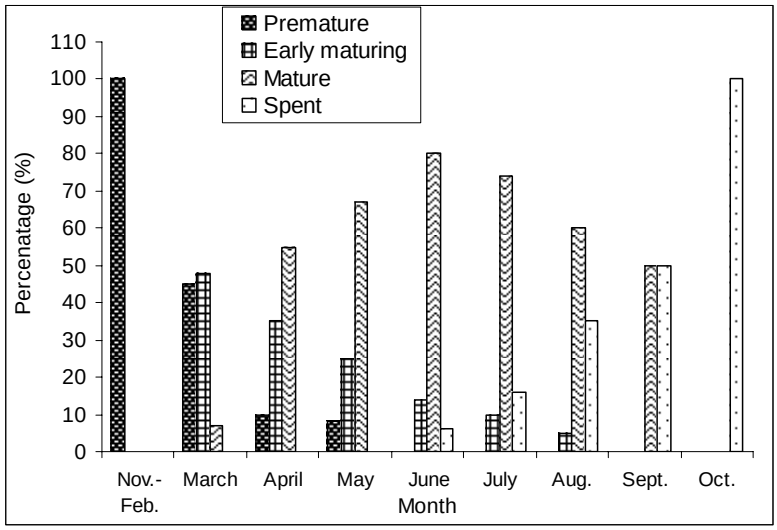


Fig. 4. Percentage occurrence of different maturity stages of male *Puntius sarana*

The ovarian lamellae consisted of a connective tissue lined by germinal epithelium, which contained cell nests of oogonia. Ovarian follicles developed along the lamellae and the vitellogenic oocytes were ovulated into the ovarian cavity. The follicles of vitellogenic and fully mature oocytes consist of an ovary. The middle portion of the two ovarian lobes became broader than both the anterior and posterior regions. During breeding season, especially in mid May to mid September, the ovaries became very

expanded and occupied by the whole cavity. The ovary varied in length from 5.2 to 8.2 cm and the colors of the developing and maturing ovaries of *P. sarana* were creamy, brownish and yellowish, respectively (Fig. 5). Ovary of maturing and mature individuals had a turgid texture and folded structure, whereas those of spent individuals had a loose texture (Table 3).

Maturational stages of oocyte

From the histological study of ovaries, it was observed that oocytes did not develop synchronously and oocytes at various maturational stages were observed in paired ovaries. The following maturational stages of oocytes of *P. sarana* were distinguished based on the size, appearance of nucleus and nucleolus, and the type of oocytes.

1. Oogenesis

The ovary has an outermost epithelial layer. The germinal zone is in the ventrolateral region of the ovary. The oogonial cells are in clusters

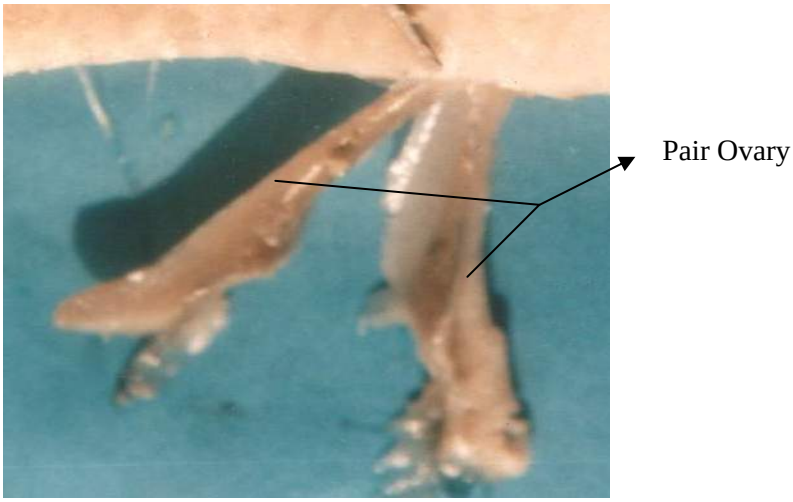


Fig. 5. A pair of mature ovaries of *Puntius sarana* (Hamilton, 1822)

near the germinal zone which increases in number through repeated mitotic divisions. The mitotic division follows then the primary oocytes and finally ova. The developing ova are in radially disposed strings with the immature ones towards the germinal zone and the mature ones towards the periphery.

The distinction of different stages of oocytes was based on the size, their staining affinity, changes in nucleus and the presence and absence of the yolk globules. A brief description of the different stages of oogenesis follows:

1.1 Oogonia (Og)

In this stage, gonadal attachment to the dorsal peritoneum (DP) with oogonia in nests and basophilic oocytes (Oo) were developed. Oogonia were small round cells characterized by a single conspicuous nucleolus in the nucleus. In the immature ovary, oogonal cells were highly aggregated near the germinal zone. Some residual oogonia were also present in the mature ovary. Oogonia developed into previtellogenic oocytes. They appeared to develop as solitary cells. This stage was observed throughout the year except in the months of June and July (Fig. 6A)

Table 3. Ovaries characteristics of *Puntius sarana* at various stages of maturity

Stage of maturity	Testes Characteristics				
	Length group (cm)	Size (µm)	Texture	Color	Histological examination
Oogenesis	5.2-6.8	10.6	Turgid and folded structure	Opaque and creamy white-brownish	Tubule compactly packet, mostly with oogonia; wall of tubule thick. Nucleus increased in size with a large number of nucleoli. Basophilic cells with a dense and homogenous cytoplasm. Follicular cells developed around the oocyte. Zona-radiata was visible.
Vitellogenesis	7.5-8.2	7.7	Turgid and folded structure	Creamy yellowish	Ovaries expanded and occupied by the whole cavity. Oocyte became compact full with yolk granules. Nucleus was small in size with many nucleoli. Oocyte surrounded by the clearly distinguished zona-radiata.
Atretic stage	7.8-7.0	4.8	Loose	Dull brown creamy	Lumen of tubules irregular. Atretic oocytes were due to the reabsorption of non-vitellogenic and partial vitellogenic oocytes and the mature oocyte atresia was due to the reabsorption of mature oocytes.

1.2 Previtellogenic oocytes

Based on size, previtellogenic oocytes were further divided into two groups:

Early perinucleolus stage (EPN)

Concomitant with oocyte growth, the nucleus increased in size and multiple nucleoli were found around the periphery of the nucleus. This is the stage of basophilic cells with a dense and homogenous cytoplasm. The follicular layer was not visible in this stage (Fig. 6B). Early perinucleolus was observed throughout the year but the highest number was observed in the month of January.

Late perinucleolus stage (LPN):

The late perinucleolus stage was distinguished from the early perinucleolus stage by an enlargement of oocyte. Cytoplasm was homogenous and basophilic. Nucleus with a large number of nucleoli was clearly visible. The follicular cells started to develop in this stage around the oocyte, closely connected to the oocyte membrane. In this stage, many oocytes passed an accumulation of a small juxtanuclear mass termed 'yolk nucleus' or balbiani bodies' (Fig. 6C). This stage was observed throughout the year but the highest number was observed in the month of January.

2. Vitellogenesis

This was the synthetic phase of the oocytes in which yolk synthesis took place. Follicle cells also started appearing. This stage was marked by considerable changes in the nucleus, nucleolus and ooplasm, and a brief description of the different stages of vitellogenesis follows:

2.1 Early vitellogenic oocytes

This stage is the yolk vesicle stage which is also known as cortical alveoli stage (CA). Transparent cortical alveoli appeared in this first stage of the secondary growth phase, situated at the periphery of the oocyte. During this stage the alveoli increased in number leaving only a small zone of basophilic cytoplasm around the nucleus in the larger oocytes (Fig. 6D). The zona radiata has become visible. The formation of yolk granules is preceded by micropinocytosis of vitellogenin and fusion of the endocytotic vesicles. The nucleus was solid and central in position. Small round follicular cells appeared around the oocyte. This stage was observed throughout the year but the highest number was observed in April.

2.2 Advanced vitellogenic oocytes

The oocytes further increased in size and the number of yolk granules was sharply increasing. They are densely packed and occupied almost the total volume of the oocyte. The nuclei of the oocyte located at the center of the oocyte. Cortical alveoli was present both at the periphery of the oocyte and in the perinuclear region. The zona radiata with the radial striation became clearly visible (Fig. 6E). The oocyte is surrounded by a thin, but distinguishable granulose layer and a theca layer. The yolk granules developed sharply in June.

2.3 Maturation stage

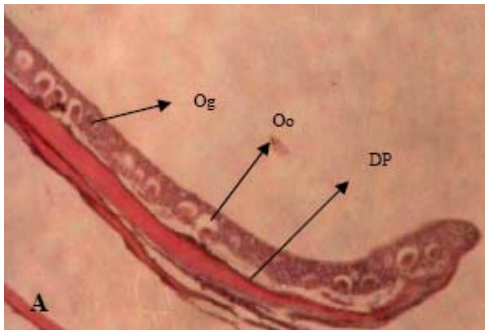
The oocytes became compact full with yolk granules in the cytoplasm and there were no cortical alveoli. During this phase vitellogeneic oocytes developed further and the nucleus was small in size with many nucleoli located at the periphery. The oocyte was surrounded by the clearly distinguished zona-radiata (Figs. 6E and 6F). The yolk granules increased sharply in number and in size in June but decreased from July to November.

3. Atretic stage (AO)

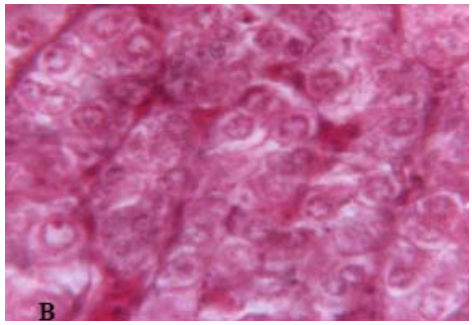
Another important structure was observed in the gonad, namely atretic oocyte. The atretic oocytes were classified at three levels: recent atresia, late atresia and mature oocyte atresia. The recent atretic oocytes were due to the reabsorption of non-vitellogenic and partial vitellogenic oocytes and the mature oocyte atresia was due to the reabsorption of mature oocytes (Fig. 6F). This type of oocyte was formed after the spawning season and it was observed from late July to December.

Development of ovary

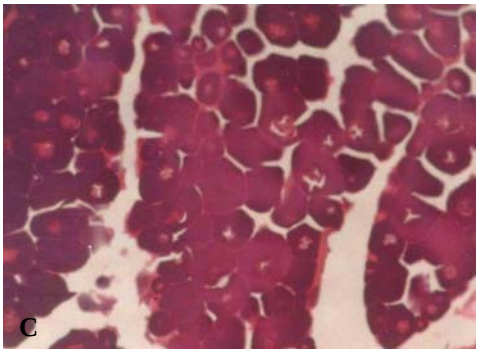
The primary germ cells (PGC) were apparently undergoing increased mitotic activity proliferating the smaller-sized oogonia, which were arranged in these compact associations. Ovary of *P. sarana* was developed under the normal process of oogenesis at the chromatin and perinucleolus developing stages. The diameter of the ova showed increasing trend from November to June (25.2±1.04 to 98.5±0.15 µm) and decreasing trend from July to October (89.4±0.14 to 8.4±0.06 µm), respectively (Fig. 7). The stages of oogonia and perinucleolar, both early and late, of oocytes were present in different proportions in the ovary throughout the year. The proportion of oogonia, EPN and LPN showed a decreasing trend, 20 to 0%, from November to July 39 to 3% and 37 to 2% from January to July and an



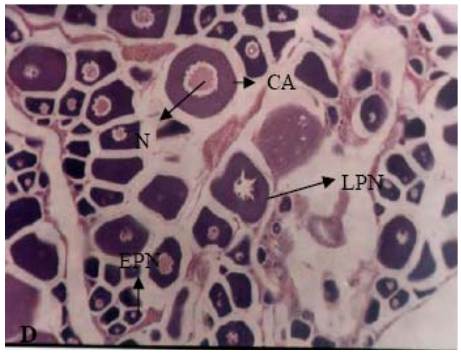
A. T.S ovary showing oogenesis at the chromatin and perinucleolus developing stage



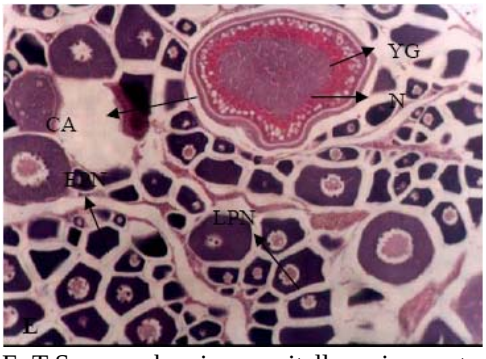
B. T.S showing oogenesis at the chromatin; and early and late perinucleolus stage



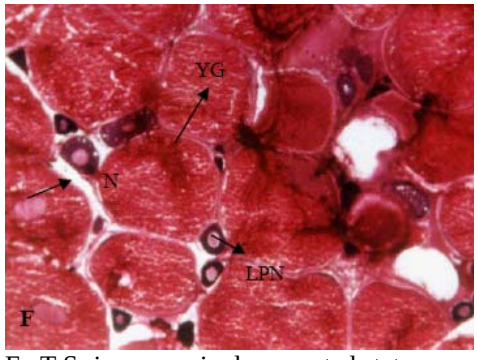
C. T.S ovary with ovigerous lamellae, cytoplasmic oocytes; and early and late perinucleolus stage



D. T.S ovary increase previtellogenic oocyte and vitellogenic oocyte



E. T.S ovary showing previtellogenic oocyte and vitellogenic oocyte



F. T.S ripe ovary in degenerated state

Figs. 6E-F. Sectional views of the ovaries of *Puntius sarana* (Hamilton, 1822). Haematoxyline and eosin (× 200). CA=Cortical alveoli, N=Nucleus, EPN=Early perinucleolus, LPN=Late perinucleolus, YG=Yolk granule

increasing trend 0 to 11% from July to October, 3 to 27% and 5 to17%, respectively from August to December. The state of cortical alveoli occupied in the ovary was varied at the same trend 12 to 3% from January to June and 5 to 10% from July to November like oogonia but it was absent in December. The pattern of vitellogenic oocyte distribution showed increasing trends 8 to 90% from February to June and decreasing trend 86 to 6% from July to November but it was absent in December and January. The atretic stage of oocyte occured first in July (3%) with an increase rate in number in the month of December (40%) but quite absent in January to June.

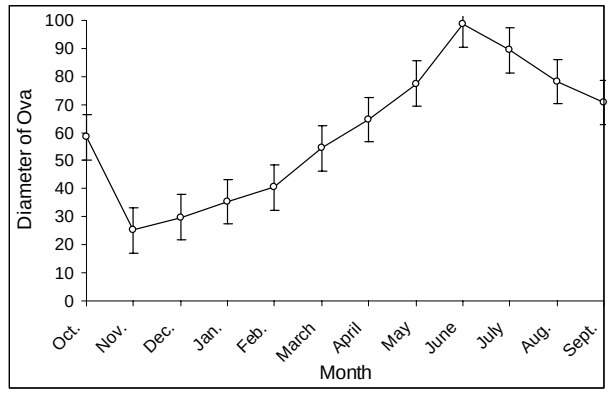


Fig. 7. Average diameter of the ova of *Puntius sarana* showed increasing and decreasing trends in different months.

Histological sections of ovary of the experimental fish contained highly distinct oogonia at all stages of development throughout the period of observation. The percentage of individuals in different stages of maturity observed during different months was computed and depicted in figure 8. The maturation of oocytes in *P. sarana* was found to be asynchronous to partially synchronous because all the oocytes matured at different times. Some of these became fully matured; on the other hand, some remained under developing condition. The developing oocyte proportions indicate that the matured and biggest oocytes are ovulated in the spawning period of

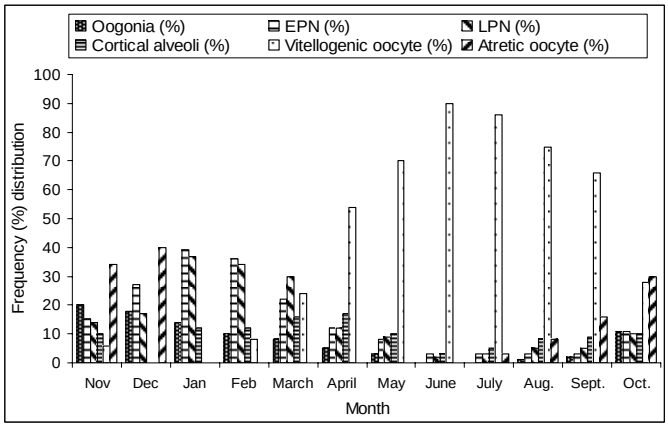


Fig. 8. Average frequency distribution of different developmental stages of oocytes in ovaries of *Puntius sarana* in different months

May to mid September and other developing oocytes remained under way of vitellogenesis, gained maturation; and released at the second spawning time in the months of August and September.

Discussion

The reproductive cycle of indigenous *P. sarana* was examined to observe the pattern and timing of growth phase and maturation stages of germ cells in the gonads of male and female individuals. The GSI is usually established for males and females alike, although the development of gametes is not reflected in this index in an identical way in both sexes. De Vlaming et al. (1982) discussed the utility of GSI as an indicator of the reproductive activity of a stock. The GSI increases with the maturation of fish, being maximum during the period of peak maturity and declining abruptly thereafter, when the fish become spent (Le Cren 1951). The monthly change of GSI reflects the ovarian activity of fish. The results of the present experiment indicated that the GSI of *P. sarana* is highest in June when the fish is found to be mature. The increasing GSI of *P. sarana* suggests that the ovary harbours percentage of yolk laden ripe eggs in June which is similar to Dewan (1973). He found the spawning period of chela (*Chela phulo*) between June and September.

The reproductive potential of a population is one of the basic exigencies to designate the individuals of that population in respect to their gonadal conditions (Jhingran and Verma 1972). In this experiment, it is observed that testicular development of *P. sarana* usually occurs earlier than ovarian development. But testicular development of salmononids occurs later than ovarian development (Nakamura et al. 1998; Guraya 1994). The gonad of *P. sarana* developed bilaterally which was similar in the mud eel, *Monopterus albus* (Mei et al. 1993). The development of gonad both in male and female was studied and three stages of oocyte maturation were identified such as: oogenesis (oogonia, early perinucleolus stage and late early perinucleolus stage), vitellogenesis (early vitellogenic oocytes, advanced vitellogenic oocytes and maturation stage) and atretic stage. These stages of oocyte development are similar to that of gold fish (Yamamoto and Onozato 1965) and white fish, *Caulolatilus princes* (Elorduy-Garay and Ramirez-Luna 1994), *Ompok pabda* (Begum 1997) and *Puntius gonionotus* (Afroz 1996) and somewhat similar to that of *Pleuronectes flesus* investigated by Jansen et al. (1995). In the case of male *P. sarana*, four stages of spermatogenesis, such as spermatogonia (Primary and Secondary), spermatocyte (Primary and Secondary), spermatid and spermatozoa were observed which was very much similar to those found in *Barbus luteus* (Bhatti and Al-Daham 1978), *Puntius gonionotus* (Afroz 1996), and common carp, *Cyprinus carpio* and crucian carp, *Carasius cuvieri* (Matsumoto et al. 2002).

The diameter of the ova was significantly higher in the month of June where the diameter of the ova was decreased in the month of October compared to those of different months, which indicates that the diameter of the ova attained its highest in the peak spawning season. The ovaries contained oocytes only in early developmental stages up to March are small in size which mostly consists of oogonia, early and late perinucleolus stage, cortical alveoli and yolk granule stage. In the breeding season of *P. sarana*, the female possessed gonads that contained exclusively vitellogenic oocytes. Similar sequence of oogenesis was also noted in *Amblypharyngodon mola*, *Barbus stigma* and *Chela phulo* (Dewan 1973). A significant percentage of vitellogenesis (54%) was observed from April onward, climaxing in June. This indicates that ovary maturation is closely correlated with a rise in temperature, which is agreed by Khan and Jhingran (1975) in the case of female rohu, *L. rohita*. Spawning was observed from May to mid September as indicated by the presence of an appreciable number of female berried condition. June to August formed the major spawning period as evidence by the presence of the maximum number of berried and spent fishes in these months. The oocytes in *P. sarana* did not mature at the same time. Some of these became fully matured; on the other hand the others remained under developing condition. The developing oocyte proportions indicate that the matured and biggest oocytes are ovulated in the spawning period of May to mid September. The developing oocytes remained under way of vitellogenesis and gained maturation, and released at the second spawning time in the months of August and September which is supported by the findings of Mustafa (1991), CIFRI (1972) and Hora and Pillay (1962) with mola (*Amblypharyngodon mola*), rohu (*Labeo rohita*) and catla (*Catla catla*).

During the entire period of study except from October to February, maturing as well as mature individuals were observed. *P. sarana* in premature condition were observed maximum from November to February, a few in March and April, and altogether absent from May onwards. Thus, it can be stated that the male breeding period varies from March to September. In October, all the individuals were found to be spent. It can thus be presumed that the period from November to February is the resting period for male individuals. Temperature is closely correlated to mature testis which is also agreed by Khan and Jhingran (1975) in the case of male rohu, *L. rohita*. *P. sarana* attains maturity within a year and normally breeds from late April to mid September. Qasim and Qayyum (1961) reported the breeding season of *P. sarana* to extend from late June to early September with peak in July and August.

Finally, it can be concluded that identifying the spawning period of *P. sarana* will be very helpful to breeders for induced breeding. However, because of environmental changes and man-made intervention, spawning grounds of this important fish species have been severely degraded. In this situation, production of quality seeds through application of our findings might be helpful towards the protection of this species from extinction as well as for its rehabilitation.

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