

# Reproductive Cycle of the Endangered Pabda, *Ompok pabda* (Hamilton-Bouchanan, 1822) in Bangladesh

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## Abstract

Successive developmental stages of both male and female gonads and estimation of gonadosomatic index (GSI) of *Ompok pabda* (Hamilton-Bouchanan, 1822) belonging to family Siluridae were investigated over a two year period (November 2005 to October 2006). From the histological analysis, four developmental stages namely; spermatogonia, spermatocytes, spermatids and spermatozoa were identified in testes. Three developmental stages of oocytes: oogenesis (oogonia, early perinucleolus stage and late early perinucleolus stage), vitellogenesis (early vitellogenic oocytes, advanced vitellogenic oocytes and maturation stage) and atretic stages were distinguished in ovaries. Maximum mean gonad weight was recorded in June for both females ( $9.05 \pm 0.72$  g) and males ( $2.39 \pm 0.28$  g). The testes at early development stages between November 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 July. Highest percentage of oogonia in the ovary was recorded in the month of December; while highest percentage 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 July but it was prominent in June. The oocytes did not mature at the same time. Only the matured oocytes ovulated during the spawning period while immature oocytes gained maturation under way of vitellogenesis and released at the second spawning time.

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## Introduction

The *Ompok pabda* (Hamilton-Bouchanan, 1822) is a commercial species with high market value in Bangladesh as well as in other South East Asian countries (Talwar and Jhingran 1991). This is a delicious fish which contains 19.2 g protein, 4.6 g carbohydrate, 2.1 g fat, 1.1 g minerals, 310 mg calcium, 73 g moisture and 114 kcal food energy per 100 g fish (INFS 1977). In the past, this fish was abundantly available in open waters but due to over-exploitation and various ecological changes in its natural habitat, it is now in the verge of extinction. The International Union of Conservation of Nature (IUCN), Bangladesh (1998) listed *O. pabda* as one of the endangered fishes in Bangladesh. The development of artificial breeding technique and larval rearing methods are important tools to develop pond culture techniques of this species. This species may disappear from Bangladesh like *Labeo nandina* unless proper steps are taken to protect the fish from extinction (Chakraborty et al. 2007). The fish attains maturity within a year and normally breeds from May to July (Parameswaran et al. 1970).

Studies on the reproductive biology of any fish is essential for evaluating the commercial potentialities of its stock, life history, cultural practice and actual management of small indigenous fishes (Lagler 1956; Doha and Hye 1970). 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 characterizing the breeding season and in establishing the phenotype characters of fully mature breeders for successful artificial propagation. Therefore, it is very important to assess the yearly breeding cycle. Knowledge of gonadal development and the spawning season of a species allow subsequent studies on spawning frequency of its population, which is important for its management. Little work has been done in such direction and limited literature exists in the sub-continent of India. Some earlier works describe the process of gonadogenesis and gametogenesis for various species such as grass carp, *Ctenopharyngodon idella* (Jensen and Shelton 1983), silver carp, *Hypophthalmichthys molitrix* (Mirza 1983), mola, *Amblypharyngodon mola* (Afroze and Hossain 1990), *Clupea pallasii* (Koya et al. 2002) and *Puntius sarana* (Chakraborty et al. 2007). The present work was undertaken to find out the natural reproductive cycle of both sexes of *O. pabda* based on Gonado-Somatic Index and histological preparations.

## Materials and Methods

The experiment was carried out from 2005 to 2006 at the private hatchery of Sarker Fish Seed Farm, Dhubaura, Mymensingh, Bangladesh. The brood fish were collected from the Nethai River of Dhubaura Upazilla, under the Mymensingh district and released in the ponds of Fish

Seed Farm. The area of each pond was 0.05 ha. The ponds were rectangular and average depth was 0.72 m. A balanced diet of fish meal (20%), mustard oilcake 40%), rice bran (30%), wheat bran (9%) and Vitamin E (1%) were supplied twice a day at the rate of 6-8% of the body weight.. The stocking rate of pabda was 24700 ha<sup>-1</sup>.

### ***Sample collection and preservation***

Fish were seined from the grow-out ponds at 15 days interval throughout the investigation period. At least eight fish were captured at a time and one- hundred and ninety- two live specimen of modhu pabda between the ages one and two years and 71.44±0.95 to 155.45±1.47g 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 using a sharp knife preserved in Bouin's solution for histological study. Anatomical differentiation was interpreted as described by Persov (1972), while histological germ cell included transformation into spermatocytes or oocytes marking the initiation of the first meiosis (Barr 1968). The normal expected population sex ratio was determined from the data of the gonadal sex differentiation study; confirmed histological sex differences were manifested. The sex ratio was tested by Chi-square. The accumulated total number of male and female fish was used to calculate the expected ratio of sexes.

### ***Methods used to determine reproductive periodicity***

#### ***General morphological of gonad***

General feature and structure such as size, shape and colour of male and female gonads were considered.

#### ***Gonado-Somatic Index (GSI)***

This is frequently applied to determine the spawning frequency of fishes and it 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 one ovary was removed. The ova were separated in a physiological saline solution (0.85% NaCl) and spread on a glass slide to measure the diameter of ova oogonia under a microscope using an ocular micrometer. The units of the ocular micrometer were standardized with a stage micrometer for measurement of ova diameter in micrometer (µm).

## Histology

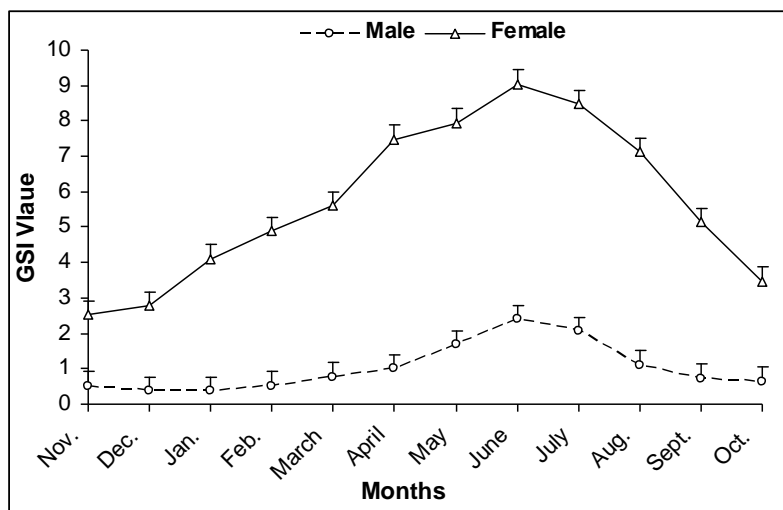
Preserved gonads were processed in an automatic tissue processor using a series of alcohol of increasing concentrations, two changes of xylene and finally molten wax (3 series). Gonads of males and females were collected from November 2005 through October 2006 and were examined histologically to study the different maturational stages (Vitale et al. 2006). Paraffin-embedded tissue was cut using a microtome knife at 4-5 micrometers, and sections were transferred to a water bath at a temperature of 40°C. The sections were placed on a glass slide and kept overnight on a slide drier hot plate at a temperature of 20°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. Maturational stages of oocytes were studied according to Yamamoto and Onozato (1965) and Elorduy-Garay and Ramirez-Luna (1994).

## Results

### Gonado-Somatic Index



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

Seasonal changes in mean GSI values of both males and females of *Modhu pabda* 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 the resting phase. The GSI values for male and female *O. pabda* were found to be from  $0.36 \pm 0.04$  to  $2.39 \pm 0.28$  and  $2.53 \pm 0.05$  to  $9.05 \pm 0.72$  (Fig 1). In the case of male *O. pabda*, it has been found that the weight of the gonad gradually increased from December to June. It increased slowly, 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. But in case of female *O. pabda*, the weight of the gonad gradually increased from November to June. It also enlarged slowly 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 gently from July to November. The samples of the male and female gonads were between  $0.11\pm 0.01$  and  $1.54\pm 0.17$  g, and  $0.58\pm 0.05$  and  $7.74\pm 0.11$  g, respectively.

### **Male Reproductive Cycle**

The first obvious signs of anatomical differentiation were seen in the specimens. The testes (3.5-4.5 cm) are soft and elongated, coiled and nearly flattened structures lying in the body cavity and ventral to the swim bladder and kidney.

It leads postero-ventrally into two vas deferens that unite to form a spermatic duct opening to the exterior through the urogenital aperture. Each testis was 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 centre of the testis.

**Table 1.** Testes characteristics of *Ompok pabda* 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 IV)	3.8-5.8	Loose	Dull white	Lumen of tubules irregular, a gap between germinal epithelium and germ cells which are few and include residual spermatozoa.



**Fig. 2.** A mature testis of *Ompok pabda* (Hamilton-Bouchanan, 1822).

In fish from 16.5 to 19.2 cm TL, the testes varied in length from  $0.41\pm 0.04$  to  $2.39\pm 0.28$  cm. During immature stage, the testes were whitish thread-like structures, very small and translucent, but they became thick, creamy white, highly coiled and opaque when they reach the mature stage. The testis of *O. pabda* consisting of 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). The spent individuals had a loose texture. Based on size, colour, texture and histological differentiations, four stages of testes were recognized as depicted in Table 1.

### ***Histological Observation***

Testes of *O. pabda* 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 *O. pabda* based on the histology of the nuclear and cytoplasmic morphologies (Table 2 and Figs. 3A-3F), which are described as follows:

#### ***1. Spermatogonia***

Spermatogonia proliferated mitotically and were the most abundant 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  $9.8\pm 0.04$   $\mu\text{m}$ . However, under light microscopy no distinction could be made between primary and secondary spermatogonia. Meiosis was initiated in these cells to form primary spermatocytes. The germ cell multiplication becomes more apparent in the testes of fish in the months of November to January (Figs. 3A-3B).

#### ***2. Spermatocytes***

Spermatogonia undergo first maturation division to give rise to secondary spermatocytes. The latter have poorly stainable cytoplasm. The primary and secondary spermatocytes did not show any marked difference in size. The average diameter of primary and secondary

spermatocytes was  $7.4 \pm 0.03 \mu\text{m}$ . The secondary spermatocytes, followed by the second maturation division, gave rise to spermatids. Spermatocytes were also spherical in shape containing a nucleus at the centre and these were the primary spermatocytes. The germ cell maturation becomes more apparent in the testes in the months of February and early March (Fig. 3C). 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).

### 3. Spermatids

The spermatids are small rounded bodies  $4.6 \mu\text{m}$  in diameter. They have a little cytoplasm and most of their volume is occupied by a large nucleus. The average size of the spermatids was  $4.6 \pm 0.02$ . 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.

### 4. Spermatozoa

Finally, the spermatids undergo certain morphological changes to produce spermatozoa. The spermatozoa are crescent shaped structure bearing a short tail. The two ends of the crescent measured  $4.8 \pm 0.02 \mu\text{m}$ , and the widest part in the middle measured  $1.3 \pm 0.01 \mu\text{m}$ . In this stage, spermatozoa started to develop in mid April and was prominent from May to July (Figs. 3E-3F). They appeared as small black-coloured spots during this stage and continued up to August. 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 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 the 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 February, early maturing testes began to develop at 2%. In March, 46% and 45% of the individuals had premature and early maturing testes while the rest were on their way to maturity. In April, most of the individuals had mature and early maturing testes. Only 14% had immature testes. May showed a marked increase in the percentage of maturing fishes (68%). Some immature fishes were also found. From June to October, no immature fishes were observed. In June, the most remarkable increase in percentage (82%) of maturity was recorded and early maturing was recorded at 11%, the rest had spent fishes. Maturity stages in July were more or

less similar as in June. In August, the percentage of mature spent and early maturing fishes were 51%, 45% and 4%, respectively and in September, 85% of spent fishes was recorded. But in October all the individuals were found to be spent.

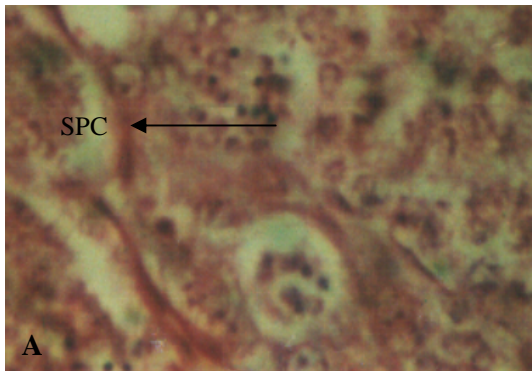
**Table 2.** General organization of germ cells at light microscope level in testes of *Ompok pabda*.

Germ cells	Shape	Size ( $\mu\text{m}$ )	Cell boundary	Cytoplasm	Nucleus	Nucleoli	Chromosomes
Spermatogonia	Spherical	9.8 $\pm$ 0.04	Distinct	Clear	Nuclear wall indistinct	Distinct	Forming a network
Spermatocytes	Spherical	7.4 $\pm$ 0.03	Distinct	Clear	Nuclear wall indistinct	Distinct	Indistinct
Spermatids	Spherical	4.6 $\pm$ 0.02	Distinct	Indistinct	Distinct	Invisible	Condensed
Spermatozoa	Crescent with short tail	L- 4.8 $\pm$ 0.02 W-1.3 $\pm$ 0.01	Indistinct	Indistinct	Indistinct	Indistinct	Indistinct

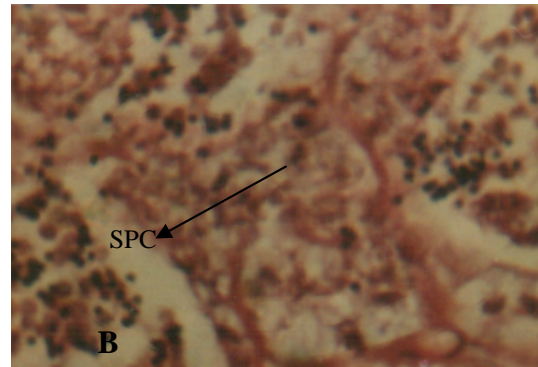
**Table 3.** Ovaries characteristics of *Ompok pabda* at various stages of maturity

Stage of maturity	Testes Characteristics				
	Length group (cm)	Size ( $\mu\text{m}$ )	Texture	Color	Histological examination
Oogenesis	5.1-6.4	9.7	Turgid and folded structure	Grayish and dull	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	7.2-8.0	7.1	Turgid and folded structure	Bright 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	7.5-6.5	4.5	Loose	Grayish	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.

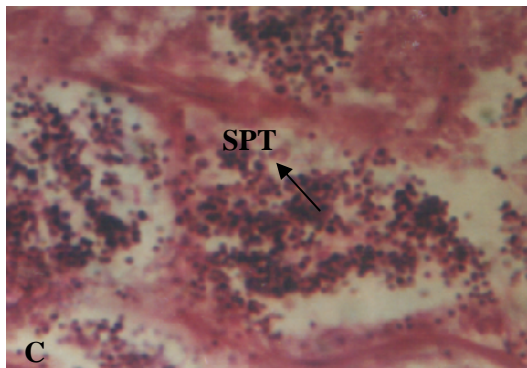




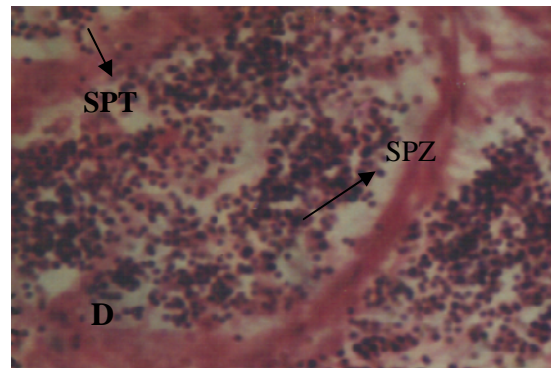
A. T.S. testis with developing increased number of germ cells



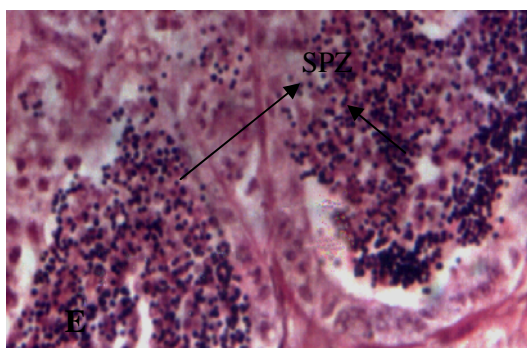
B. T.S. testis increase size dominated by spermatogonia



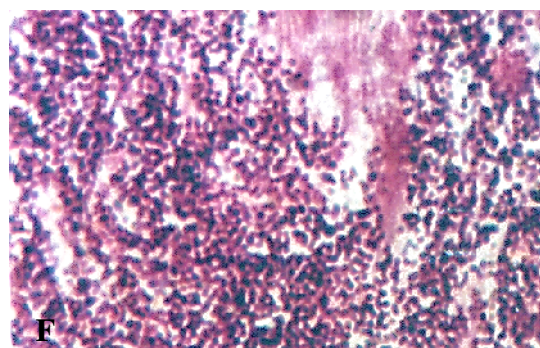
C. T.S. testis increase in size dominated by spermatogonia and spermatocytes



D. T.S. testis increase in size dominated by spermatids 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

**Fig. 3.** Sectional views of the testes of *Ompok pabda* (Hamilton-Bouchanan, 1822) Haematoxyline and eosin ( $\times 200$ ). SPC=Spermatocyte, SPG=Spermatogonia, SPT= Spermatids, SPZ=Spermatozoa

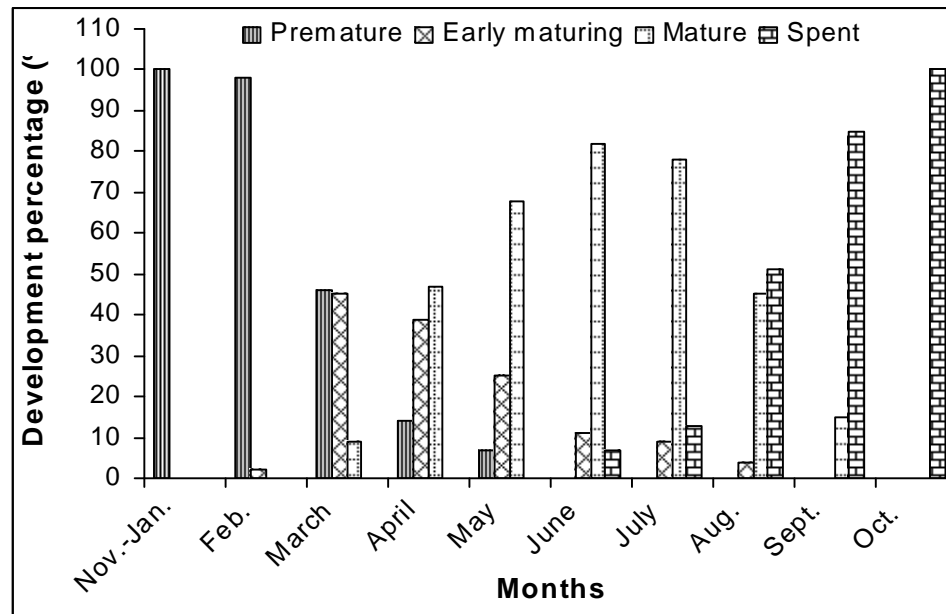


Fig. 4. Percentage occurrence of different maturity stages of male *Ompok pabda*.

### Female Reproductive Cycle

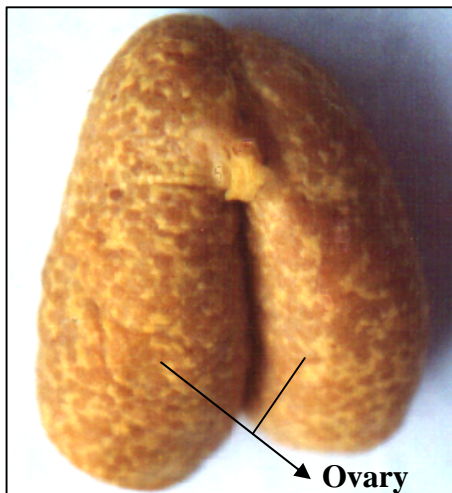


Fig. 5. A pair of mature ovaries of *Ompok pabda* (Hamilton Bouchanan, 1822).

Ovaries of immature fish (14.6 to 20.2 cm TL) were elongated, thin, narrow and slightly flattened structures, colour was grayish and dull. Mature ovaries were thick, bright yellowish and filled with eggs. During the atretic stage, this was grayish and slightly spotted. 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 and the right lobe was longer at the ripe stage. They were covered with an ovarian membrane and numerous ovarian lamellae, protected into ovarian cavity. The ovarian cavity connected the oviducts and the oviducts from each bilateral ovary joined to lead to the genital pore. The ovarian lamellae consisted of

connective tissues 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 May to July, the ovaries became very extended and

occupied the whole cavity. The ovary varied in length from 5.1 to 8.0 cm and the colour of the developing and maturing ovaries of *O. pabda* was gray to bright 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***

Based on the observation of the histological study of the ovary, it was found that oocytes did not develop synchronously and various maturational stages were observed during the breeding season. The following maturational stages of oocytes of *O. pabda* were distinguishable based on their size, appearance of nucleus and nucleolus, and the type of oocytes.

#### ***1. Oogenesis***

The ovary had an outermost epithelial layer. The germinal zone was in the ventrolateral region of the ovary. The oogonial cells were in clusters near the germinal zone which increased in number through repeated mitotic divisions. The mitotic division followed then primary oocytes and finally ova. The developing ova were 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)***

Nests of oogonia proliferated mitotically; they were small round cells characterized by a single conspicuous nucleolus in the nucleus. In the immature ovary, oogonial cells were highly aggregated near the germinal zone. Some oogonia were 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 month of June.

##### ***1.2 Previtellogenic oocytes***

Based on the 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 became located around the periphery of the nucleus. This is the stage of basophilic staining affinity of the dense and homogenous cytoplasm. The follicular layer was not developed in this

stage (Fig. 6A). 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 continued to be basophilic staining. Nucleus with a large number of nucleoli became clearly visible. The follicular cells started to develop around the oocyte, closely connected with the oocyte membrane. In this stage, many oocytes accumulated a small juxtannuclear mass termed 'yolk nucleus' (Fig. 6B). This stage was visible throughout the year but the highest number was observed in the month of February.

## **2. Vitellogenesis**

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

### **2.1 Early vitellogenic oocytes**

This yolk vesicle stage 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. 6C). The zona radiata is formed and becomes visible, staining eosinophylic. 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. This stage was observed in the month of March.

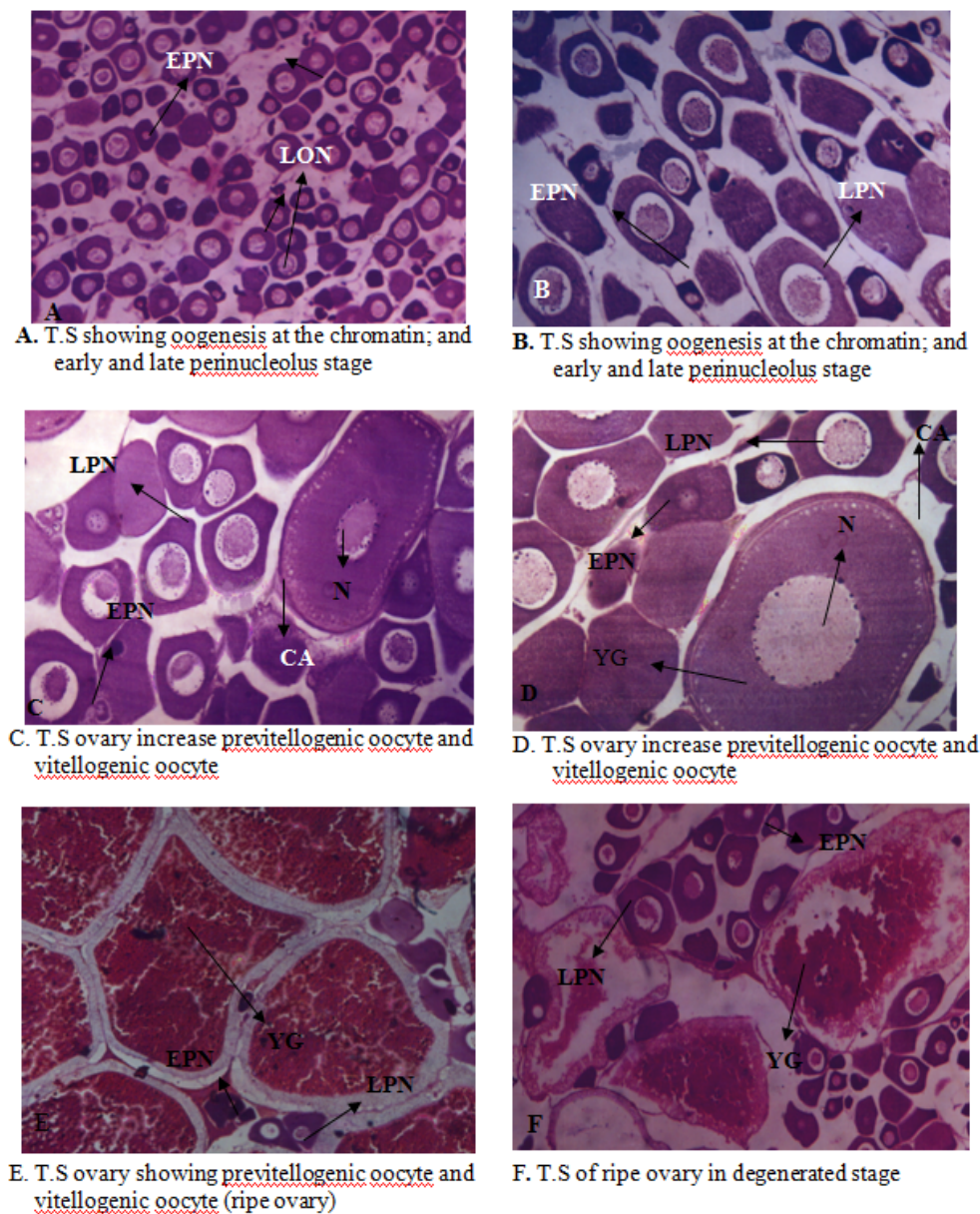
### **2.2 Advanced vitellogenic oocytes**

The oocytes further increased in size, and the number of yolk granules sharply increased. The granules are densely packed and occupy almost the total volume of the oocyte. The oocytes nucleus remains located at the centre of the oocyte. Cortical alveoli were present both at the periphery of the oocyte and in the perinuclear region. The zona radiata with the radial striations become more clearly visible (Fig. 6D). The oocyte is surrounded by a thin, but distinguishable granulose layer and a theca layer more developed and thicker. The yolk granules developed sharply in May to June.



### 2.3 Maturation stage

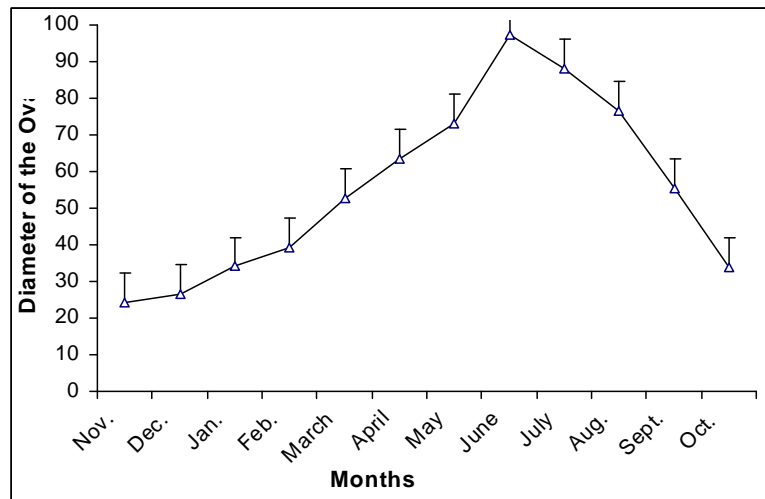
The oocyte became compact full with yolk granules that replaced the cortical alveoli. During this phase vitellogenic oocytes developed further and the nucleus was found to be getting germinal vesicle migration (GVM) towards animal pole by germinal vesicle further broken-down, signaling the attainment of maturational stage (Fig. 6F). The yolk granules increased sharply in number and in size in June and decreased from July to November.



**Fig. 6.** Sectional views of the ovaries of *Ompok pada* (Hamiltlo-Bouchanan, 1822). Haematoxyline and eosin ( $\times 200$ ). CA=Cortical alveoli, N=Nucleus, EPN=Early perinucleolus, LPN=Late perinucleolus, YG=Yolk granule.

### 3. Atretic stage (AO)

Another important structure, the atretic oocyte, was observed in the gonad. The atretic oocytes were classified into three levels, namely: recent atresia, late atresia and mature oocyte atresia. The recent atretic proliferating the smaller-sized oogonia, were arranged in these compact associations. Ovary of *O. pabda* was developed under normal process of oogenesis at the chromatin and perinucleolus developing stages. The diameter of the ova showed increasing trends



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

from November to June ( $24.2 \pm 0.04$  to  $97.5 \pm 0.55 \mu\text{m}$ ) and decreasing trend from July to October ( $88.2 \pm 0.17$  to  $33.8 \pm 0.08 \mu\text{m}$ ), 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 22 to 0%, from November to June, 39 to 1% and 37 to 3% from January to June and an increasing trend 1% to 12% from July to October, 3 to 16% and 4 to 11%, respectively from July to December. The state of cortical alveoli that occupied the ovary was varied at the same trend, 12 to 4% 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 an increasing trend, 8 to 92% from February to June and a decreasing trend 83 to 6% from July to November but it was absent in December and January. The atretic stage of oocyte is found to occur first in July (4%) with an increased rate in number in the month of December (48%) but quite absent in January to June.

Histological sections of ovary of the experimental fish contained highly distinct oogonia at all stages of development throughout the observation period. The percentage of individuals in different stages of maturity was observed during different months computed and depicted in Figure 8. The maturation of oocytes in *O. pabda* 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, other remained in an under developing condition. The developing oocyte proportions indicate that the matured and biggest oocytes ovulated in the spawning period of May to July and other developing oocyte remained under way of vitellogenesis, gained maturation; and released at the second spawning time.

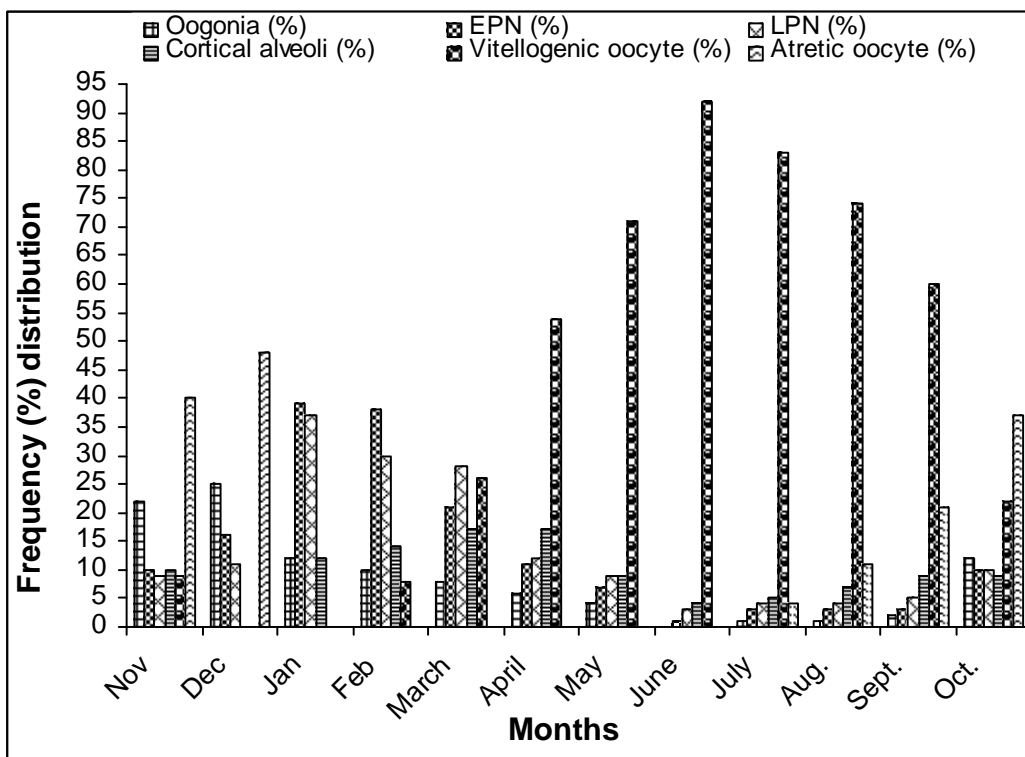


Fig. 8. Average frequency distribution of different developmental stages of oocytes in ovaries of *Ompok pabda* in different months.

### Discussion

The reproductive cycle of *O. pabda* 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. De Vlaming et al. (1982) discussed the utility of GSI as 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 *O. pabda* is highest in June when the fish is found to be mature. The increasing GSI of *O. pabda* suggests that the ovary harbours a certain percentage of yolk laden ripe eggs in June which is similar to the findings of 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 *O. pabda* usually occurs earlier than ovarian development. However, testicular development of salmononids occurs later than ovarian development (Nakamura et al. 1998 and Guraya 1994). The gonad of *O. pabda* developed bilaterally which was similar in the mud eel, *Monopterus albus* (Mei et al. 1993). The development of gonadal maturation began to precede from September onwards which is very similar to *Ompok bimaculatus* (Parameswaran et al. 1970) and *Puntius sarana* (Chakraborty et al. 2007). The development of gonad both in male and female *O. pabda* shows common features such as: 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), white fish, *Caulolatilus princeps* (Elorduy-Garay and Ramirez-Luna 1994), *Pleuronectes flesus* (Jansen et al. 1995), *Puntius gonionotus* (Afroz 1996), *Ompok pabda* (Begum 1997) and *Puntius sarana* (Chakraborty et al. 2007). In the case of male *O. pabda*, 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), Raj puti, *Puntius gonionotus* (Afroz 1996), common carp, *Cyprinus carpio* and crucian carp, *Carasius cuvieri* (Matsumoto et al. 2002) and *Puntius sarana* (Chakraborty et al. 2007).

The diameter of the ova was significantly higher in the month of June where the diameter of the ova decreased in the month of November compared to those of different months, which indicates that the diameter of the ova attained 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 *O. pabda*, 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 the rise in temperature, which agreed with the findings of Khan and Jhingran (1975) in the case of female rohu, *L. rohita*. Spawning was observed from May to July as indicated by the presence of an appreciable number of females in berried condition. May to July formed the major spawning period as evidenced by the presence of the maximum number of berried and spent fishes in these months. The oocytes in *O. pabda* did not mature at the same time. Some of these became fully matured; the others remained in under developing condition. The developing oocyte proportions indicated that the matured and biggest oocytes ovulated in the spawning period of May to July. The developing oocytes remained under way of vitellogenesis and



gained maturation, and released at the second spawning time in the months of July and August which is supported by the findings of Mustafa (1991), CIFRI (1972), Hora and Pillay (1962) and Chakraborty et al. (2007) with mola (*Amblypharyngodon mola*), rohu (*Labeo rohita*), catla (*Catla catla*) and minor carp, *Puntius sarana*.

During the entire period of study except in October to January, maturing as well as mature individuals were observed. *O. pabda* in premature condition were observed to be maximum in November to February, few in February to May and absent from June. Thus, it can be stated that the male breeding period varies from April to August. In October, all the individuals were found to be spent. It can thus be presumed that the period from November to January 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*. *O. pabda* attains maturity within a year and normally breeds from May to August. Parameswaran et al. (1970) reported the breeding season of *O. pabda* to extend from May to August with peak in June and July.

Finally, it can be concluded that identifying the spawning period of *O. pabda* will be very helpful to breeders for induced breeding. Environmental modification and man-made intervention, on 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.

## References

- Afroze, S. and M.A. Hossain. 1990. The reproductive cycle of the freshwater fish *Amblypharyngodon mola* (Ham.) (Cyprinidae). Univ. J. Zool. Rajshahi University, Rajshahi, Bangladesh 9:17-21.
- Afroz, Z. 1996. Comparative study of the gonadal histology and morphology of three different interspecific hybrids of *Puntius gonionotus* (Bleeker). M. Sc. Dissertation, Department of Zoology, Dhaka University, Dhaka, Bangladesh 138 pp.
- Barr, W.A. 1968. Patterns of ovarian activity, pp 163-237. In: E.J. Barrington and C.B. Jorgensen (eds), Perspectives in endocrinology, hormones in the lives of lower vertebrates. Academic press, London and New York, USA.

- Begum, K. 1997. A study on the histological analysis of gonad development, fecundity, embryonic and larval development of *Ompok pabda* (Hamilton-Buchanan 1822). M. Sc. Dissertation, Department of Zoology, Dhaka University, Dhaka, Bangladesh. 108 p.
- Bhatti, M.N. and N.M. Al-Daham. 1978. Annual cyclical changes in the testicular activity of a freshwater teleost, *Barbus luteus* (Heckel), from Shatt-Al Arab, Iraq. J. Fish. Biol., 13:321-326.
- Chakraborty, B.K., Z.A. Mirza, M.I. Miah, M.A.B. Habib and A. Chakraborty. 2007. Reproductive cycle of the endangered sarpunti, *Puntius sarana* (Hamilton, 1822) in Bangladesh. J. Asian Fish. Sci. Manila, Philippines, 20(2): 145-164.
- CIFRI. 1972. Central Inland Fisheries Research Institute Annual Report. Rep. Cent. Inland Fish. Res. Inst., Barrackpore, West Bengal, India. 118 pp.
- De Vlaming, V., G. Grossman and F. Chapman. 1982. On the use of the gonado-somatic index. Com. Biochemis. and Physiol. 73: 31-39.
- Dewan, S. 1973. Investigation into the ecology of fishes of Mymensingh Lake. Ph.D. dissertation. Bangladesh Agril. Uni. Mymensingh, Bangladesh 235 p.
- Doha, S. and M.A. Hye. 1970. Fecundity of Padma River hilsa, *Hilsa ilisha* (Ham.). Pakistan J. Sci., 22 (3-4): 176-178.
- Elorduy-Garay, J.F. and S. Ramirez-Luna. 1994. Gonadal development and spawning of female ocean white fish, *Caulolatilus princeps* in the Bay of La Pazz., B.C.S., Mexico. J. Fish Biol., 44:553-566.
- Guraya, S.S. 1994. Gonadal development and production of gametes in fish. Proc. Nat. Sci. Acad., India, Part B, 60: 15-32.
- Hora, S.L. and T.V.R. Pillay. 1962. Hand book of fish culture in Indo-Pacific fisheries region. FAO Fish. Biol. Tech. Pap., 14: 203 p.
- Humason, G.L., 1972. Animal tissue techniques. W.H. Freeman and Co., San Francisco, CA. 614 p.
- IFNS (Institute of nutrition and Food Science). 1977. Food value of national food stuffs (In Bangla). Inst. Nut. Food Sci. Univ. Dhaka., p 20-26.

- IUCN, Bangladesh. 1998. List of threatened animals of Bangladesh. Paper presented in the Special Workshop on Bangladesh Red Book of Threatened Animals, 22 February 1998, Dhaka, Bangladesh 13 p.
- Jansen, P.A.H., J.G.D. Lambert and H.J.T. Goos. 1995. The annual ovarian cycle the influence of pollution of vitellogenesis in the flounder, *Pleuronectes flesus*. J. Fish. Biol., 47: 509-523.
- Jensen, G.L. and W.L. Shelton. 1983. Gonadal differentiation in relation to sex control of grass carp, *Ctenopharyngodon idella* (Pisces: Cyprinidae). Copeia, 1983: 749-755.
- Jhingran, A.G. and D.N. Verma. 1972. Sexual maturity and spawning of *Gadusia chapra* (Ham.) in Ganga river system. Proc. Nat. Sci. Acad. India, 42(2): 207-224.
- Khan, H.A. and V.G. Jhingran. 1975. Synopsis of biological data data on rohu, *Labeo rohita* (Hamilton, 1822) Central Inland Fisheries Research Institute, Barrackpore, West Bengal, India. 20p.
- Koya, Y., S.K. Soyano, K. Yamamoto, H. Obana and T. Matsubara. 2002. Testicular development and serum profiles of steroid hormones levels in captive male Pacific herring *Clupea pallasii* during their first maturational cycle. Fish. Sci., 68: 1099-1105.
- Lagler, K.F. 1956. "Fresh Water Fishery Biology" Second Ed. W. M. C. Brown Company, Dubuque, Iowa. 541 p.
- Le Cren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in perch. J. Anim. Ecol., 20: 201-219.
- Matsumoto, T., M. Kobayashi, Y. Nihel, T. Kaneko, H. Fukada, K. Hirano, A. Hara and S. Watabe. 2002. Plasma vitellogenin levels in male common carp *Cyprinus carpio* and crucian carp *Carassius cuvieri* of Lake Kasumigaura. Fisheries Science, 68: 1055-1066.
- Mei, W., Y. Cao, R. Li, B. Shi and A. Hu. 1993. A preliminary observation on sex reversal of the mud eel *Monopterus albus*. J. Zhenjiang Shuichan Xueyuan Xuebao, 12: 53-58.
- Mirza, J.A. 1983. Inducement of sex reversal in silver carp. Ph. D. dissertation, Department of Fisheries and Allied Aquacultures, Auburn University of Alabama, USA. 61 p.
- Mustafa, G. 1991. Composite culture and biology of some indigenous fishes of Bangladesh. Ph. D. Dissertation. Faculty of Biological Science, Dhaka University, Dhaka. 299 pp.

- Nakamura, M., T. Kobayashi, X.T. Chang and Y. Nagahama. 1998. Gonadal sex differentiation in teleost fish. *J. Exp. Biol.*, 281: 362-372.
- Persov, G. 1972. Functional liability of the reproductive system of fish. *J. Ichthyol.*, 12: 226-240.
- Parameswaran, S., C. Silvaraj and S. Radhakrishnan. 1970. Observations on the biology induced breeding and cultural possibilities of the cat fish. *Ompok bimaculatus* (Bloch) in ponds. *Proceeding of National Academy of Science, India*, 40 (B), III.
- Talwar, P.K. and A.G. Jhingran. 1991. *Inland fishes of India and adjacent countries*. Oxford and IBH Publishing Co. PVT. Ltd., New Delhi, India. 2: 541 p.
- Vitale F., H. Svedang, M. Cardinale. 2006. Histological analysis invalidates macroscopically determined maturity of the Kattegat cod (*Gadus morhua*). *ICES J. Mar. Sci.*, 63: 485-492.
- Yamamoto, K and H. Onozato. 1965. Electron microscope study on the growing oocyte of the gold fish during the first growth phase. *Memo. Fac. Fish., Hokkaido Univ.*, 13: 79-106.