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Histological Analysis of Ovarian Development and Sex Ratio of a Catfish, *Ompok pabda*

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Abstract

A histological analysis of ovarian development as well as the spawning pattern and the sex ratio were determined in an endangered freshwater catfish, *Ompok pabda* (Hamilton-Buchanan, 1822). A total of 77 fish samples were randomly collected from the same rearing pond for 9 months at the Freshwater Station, Bangladesh Fisheries Research Institute (BFRI), Mymensingh, Bangladesh. The sex ratio of the 77 sampled fish was 40:37 (male:female) and this difference was not significant ($p>0.05$). From histological analysis, five developmental stages of oocytes were distinguished, namely, (1) oogonia, (2) pre-vitellogenic oocytes including early perinucleolus and late perinucleolus, (3) vitellogenic oocytes including initial vitellogenic oocytes (cortical alveoli) and active vitellogenic oocytes (yolk granules), (4) mature oocytes, and (5) atretic oocytes. The percent distribution of matured oocytes (about 76%), as well as gonado-somatic index (13.73±0.95), were observed to be highest in July, whereas the highest values of both body weight (28.55±2.9 g) and ovary weight (3.85±0.8 g) were recorded in June. Oocytes became larger from April to June (211 µm–295.20 µm) but shrank slightly in July (287 µm). It is evident that *O. pabda* spawns once a year extending from May to July with the peak spawning period occurring in June and July.

Introduction

Ompok pabda (Hamilton-Buchanan, 1822) belongs to the family Siluridae of the order Siluriformes and is commonly known as butter catfish. It is found throughout Afghanistan, Bangladesh, Myanmar, the north-eastern states of India, the Indus plain, and adjoining hill areas of Pakistan (Talwar and Jhingran, 1991). It is a traditional favourite food of the people of Bangladesh where it is locally known as 'madhu pabda', and is found in almost all freshwater resources of Bangladesh. According to a survey of the INFS (Institute of Nutrition and Food Science, 1977), *O. pabda* contains 19.2 g protein, 1.1 g minerals, 4.6 g carbohydrate, 2.1 g fat, 73 g moisture, 310 mg calcium and 114 kcal food energy per 100 g of fish. Unfortunately, it is now considered as being an endangered species (IUCN, 2000). Three species of *Ompok* are found in Bangladesh, namely, *O. pabda, O. bimaculatus* and *O. pabo* of which *O. pabda* has gained most popularity.

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Literature shows that *O. pabda* has received very little attention so far. Although a number of studies have examined the reproductive pattern in some commercially important catfishes (Zaki and Abdula, 1983; Rimmer, 1985; Sudha and Shakuntala, 1989; Khan et al. 1990; Çek and Yilmaz, 2007) as well as in many teleosts (de Vlaming et al. 1982; Awaji and Hanyu, 1987; Matsuyama et al. 1991; Moiseeva and Kukharev, 1992; Palmer et al. 1995; Santos et al. 2005; Al-Absawy, 2010), what little is known of the reproduction and fecundity of *O. pabda* comes exclusively from classifying its ovaries according to their macroscopic features and ova diameter (Hossain et al. 1992), not from histological analyses. These authors (Hossain et al. 1992) used macroscopic features including gonado-somatic index, gonadal length index, colouration of gonads and the ova diameter in *O. pabda* to conclude that spawning occurs in April to August with peak in May to July. However, without examining the histological sections of ovaries under light microscopy the above conclusion remains doubtful. Research by Çek and Yilmaz (2007) on a catfish is comprehensive. They demonstrated that macroscopic observation of the ovaries is inadequate measure of reproductive activity. The basic information on reproductive biology, including fecundity, spawning, gonadal development, fertilization, embryonic and larval development, etc., of a cultivable fish species is of great importance for its successful culture, breeding and proper larval rearing. The culture of the experimental fish species, *O. pabda*, has not gained much attention due to the low availability of their reproductive information. Even though it is well known that histological evaluation of the stage of gonad development is the most reliable means of assessing the reproductive strategy and tactics of a particular fish (West, 1990), there is no knowledge of ovarian histology of *O. pabda*. In view of its economic importance and precarious ecological position, the aim of the study was to analyse the ovarian development of *O. pabda* histologically to define the successive maturational stages of its ovaries so that a clearer understanding of its reproductive development can be gained.

Materials and Methods

Collection and rearing of brood fish

The experimental fish was introduced at the BFRI (Bangladesh Fisheries Research Institute) pond, Mymensingh, Bangladesh by collecting from the natural open water bodies. Healthy fish were reared in brood fish rearing pond and supplementary feed consisting of 40% rice bran, 30% sesame oil cake, and 30% fish meal was used. After full maturation, the spawners were induced by injecting them subcutaneously with pituitary extract.

Induced spawning

The dosage of the pituitary extract was determined on the basis of body weight of the experimental fish and the procedure was based on the study of Akhteruzzaman et al. (1993). At first, the female alone was injected with a stimulated dose of 3 $mg \cdot kg^{-1}$ body weight. Then the male and female breeders were kept in a hapa with a 1:1 ratio. After an interval of 6 hr from the first dose, a second dose of 14-16 $mg \cdot kg^{-1}$ was applied to the female. The male was given a single dose of 6

$mg \cdot kg^{-1}$ at the time of second injection applied to the female, and set of breeders at a rate of 1:1 (male:female) was released into the breeding hapa fixed in a cistern. A large number of progeny of *O. pabda* was produced overnight. After hatching, the fish larvae were transferred to the fish rearing pond.

Collection of samples

A total of 77 fish samples were collected for 9 months from January to September from the same rearing pond. Eight to ten samples were randomly collected each month to determine their sex ratio and the ovaries were analysed to determine different maturational stages of ovarian development histologically.

Total body length and body weight were measured before dissecting each fish. The gonads were carefully removed and all surrounding tissues were removed. After recording the weight and physical features of the gonads, the sex of the fish was recorded and each ovary sample was fixed in Bouin's fluid for 18 hr and then preserved in 70% ethanol for subsequent histological sections. The GSI was calculated by using the following formula (de Vlaming et al. 1982):

$$\text{The Gonado-somatic index (GSI)} = \frac{\text{Gonad weight}}{\text{Total body weight}} \times 100$$

Histology

The preserved ovaries were processed according to standard histological microtechniques (Humason, 1979) with some modifications. After dehydrating the preserved tissues with graded ethanol and clearing with xylene, paraffin was used for impregnation and a section of 5-6 µm was cut into transverse section on a microtome with a blade placed at a 45° angle. Haematoxylin and eosin were used to stain the nucleus and cytoplasm, respectively.

For the histological analysis, the prepared slides were examined at 40 to 400 magnification and all the successive stages of oogenesis were observed over successive months. In the present study, the maturational stages of the oocytes were distinguished on the basis of the size, appearance of the nucleus and nucleolus, and the type of oocytes, according to the studies of Elorduy-Garay and Ramirez-Luna (1994) and Palmer et al. (1995). The diameter of the oocytes and their nuclei were measured in samples of 20 cells of every developmental stage at successive months with an ocular micrometer under the microscope.

Statistical analysis

The sex ratios were analysed by using chi-square goodness-of-fit test. Both Pearson correlation and Spearman's rho were utilised to find out the correlation between the monthly variations of GSI values and the percentage distribution of different maturational oocytes. SPSS 17.0 was used for statistical analysis.

Results

General morphology of the ovary

The female gonad of the studied fish consists of two unequal ovarian lobes suspended dorso-laterally from the gut by a pair of mesenteries, called mesovaria, and the two lobes are attached to each other by a membranous layer. In immature stage, the greyish, dull ovaries are elongated, thin, narrow and slightly flattened. When mature, they become bright yellowish, thick and filled with eggs. At the atretic stage, they are greyish and slightly spotted.

Growth and sex ratio

The mean body weight, length, and gonad weight for females ranged from 8.24±1.56 g to 28.55±2.99 g, 12.24±0.81 cm to 17.70±0.57 cm and 0.04±3.90 g to 3.85±0.88 g, respectively (Table 1). The highest values of body length and weight of female were recorded in June when the maximum mean gonad weight was also observed. Out of the 77 fish samples, the sex ratio was recorded as 40:37 (male:female) with no significant difference which was close to the expected ratio of 1:1 (male:female), χ^2 (1, n=77) = 0.12, p=0.73 (p>0.05).

GSI

The Gonado-somatic index for female sexes increased slowly from January to April, and then increased rapidly to reach a maximum value of 13.73±0.95 (Table 1), after which time it fell sharply in August. From this observation, it can be inferred that *O. pabda* spawns just once a year having a spawning period from May to July, with the peak spawning occurring in June and July. The GSI values were strongly and positively correlated with the percentage of mature oocytes.

Ovarian histology

The histological changes suggest that oocytes did not develop synchronously and oocytes at various maturational stages were observed in paired ovaries. The following maturational stages were distinguished:

Table 1. Mean and standard deviation (±) of total body length, body weight, gonad weight, and GSI at successive months in female *O. pabda*.

Months	No. of fish	Body length (cm)	Body weight (g)	Ovary weight (g)	Mean GSI
January	5	12.24±0.8	8.24±1.5	0.04±3.9	0.57±0.07
February	4	13.05±0.6	9.63±1.2	0.06±4.9	0.66±0.12
March	4	13.28±0.7	10.68±1.2	0.08±0.0	0.84±0.2
April	4	14.47±0.5	12.73±0.9	0.26±0.0	2.09±0.45
May	4	16.08±0.8	19.96±2.8	1.52±1.1	7.73±6.21
June	4	17.70±0.5	28.55±2.9	3.85±0.8	13.49±2.49
July	4	17.38±0.7	21.80±1.7	3.00±0.3	13.73±0.95
August	3	17.53±1.1	11.40±2.5	0.71±0.3	6.1±2.39
September	5	17.42±0.8	12.88±1.6	0.08±0.0	0.68±0.22

A. Oogonia: These were very small round cells characterized by a single conspicuous nucleolus in the nucleus (Fig. 1B). They appeared as solitary cells. This stage was not observed after April.

B. Previtellogenic oocytes

a) Early perinucleolus stage: Concomitant with oocyte growth, the nucleus increased in size, and multiple nucleoli were observed 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 (Fig. 1A).

b) Late perinucleolus stage: This stage was distinguished from the early perinucleolus stage by the enlargement of the oocyte and an increasing number of nucleoli in the nucleus. Nuclei with many nucleoli were clearly visible. The follicular cells started to develop around the oocyte. Many oocytes had accumulated a small juxtanuclear mass, termed ‘yolk nucleus’ or ‘balbiani bodies’ (Fig. 1B). This stage was observed in all ovary specimens from January to September with highest percent distribution in March.

C. Vitellogenic oocytes:

a) Initial vitellogenic oocytes: This stage is the yolk vesicle stage, which is also known as the cortical alveoli stage. It was characterised by the presence of whitish oil-droplets at the periphery of the oocytes. Nuclei had many nucleoli at the periphery, all of which were quite similar in size (Fig. 1C). The zona- radiata was clearly visible.

b) Active vitellogenic oocytes: This stage is also known as the yolk globule or yolk granule stage. At the beginning of this stage, the oocytes had non-homogenous cytoplasm, which denotes the formation of the vitellogenic substance, although yolk granules were not clearly distinguishable. Highly compacted distinct yolk granules developed to the latter end of stage (Fig. 1D). A number of cortical alveoli were present both at the periphery of the oocytes and in the perinuclear region. The size of the nucleus was comparatively small.

D. Mature oocytes: In this stage, oocytes were full of yolk granules in cytoplasm. The nuclei were small with a small number of nucleoli at the periphery. The oocyte was surrounded by the clearly visible zona-radiata (Fig. 1E).

E. Atretic oocytes: Atretic oocytes were observed in August, after the spawning season (Fig. 1F). These were characterized by the presence of folded and collapsed follicular layer. Some oocytes contained small amount of yolk granules and some contained none.

Diameter of cell and nucleus of oocytes

Oocyte diameter was maximal in June (295.20 µm) with the nucleus of 77.0 µm, but the greatest nucleus diameter (81.4 µm) was observed in May (Fig. 2). Minimal size of both oocytes and nucleus were found in January.

Frequency distribution of maturational stages

The frequency distribution of mature oocytes was highest in July and there was no mature oocyte in ovaries between January and April (Fig. 3).

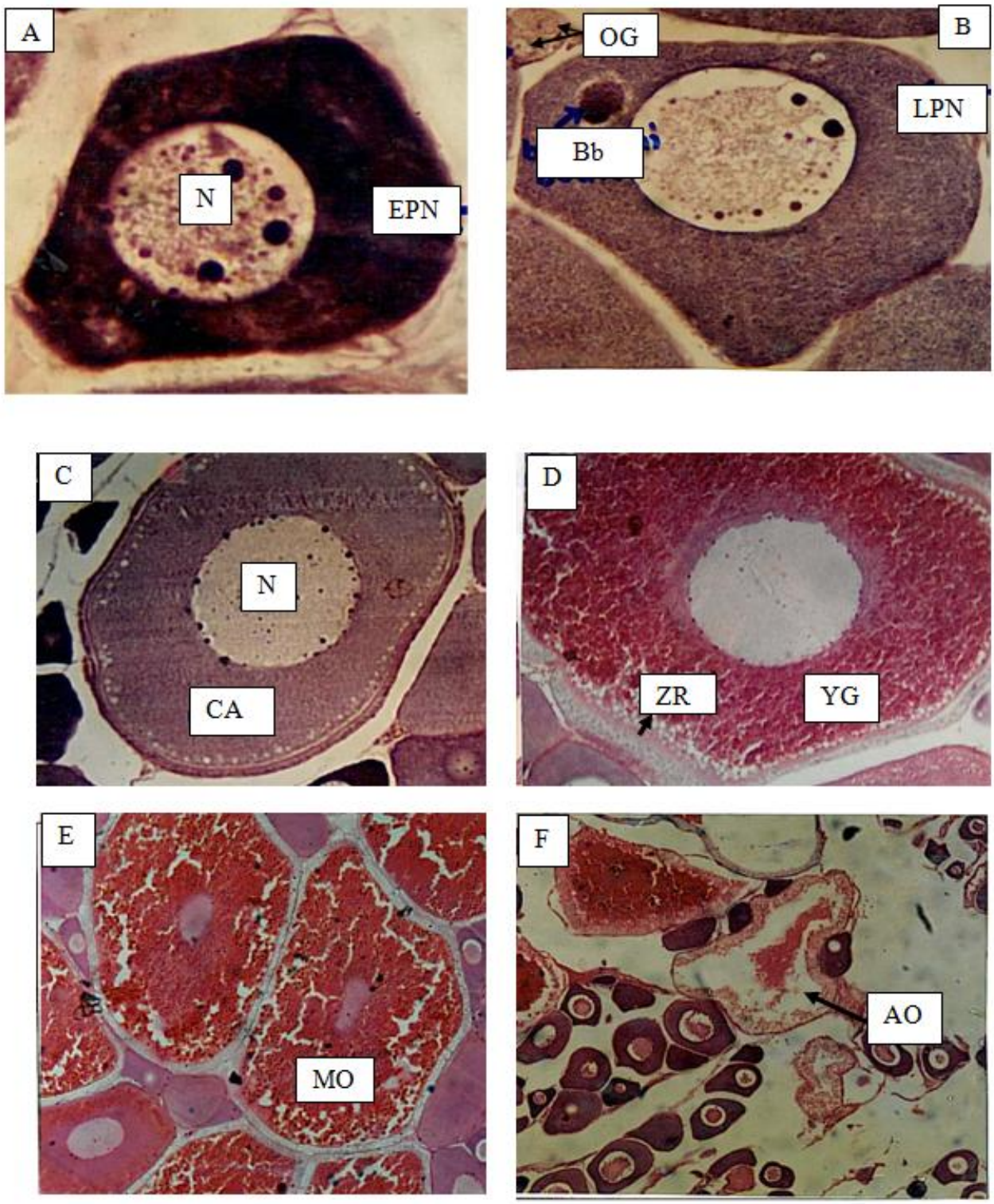

Fig. 1. Different developmental stages of oocytes in *O. pabda*: EPN (A) = Early Perinucleolus Stage; LPN (B) = Late Perinucleolus Stage with balbiani bodies; CA (C) = Cortical Alveoli; YG (D) = Yolk Granules; MO (E) = Mature Oocytes; AO (F) = Atretic Oocytes. Bb= Balbiani bodies, N= Nucleus, OG= Oogonia, ZR= Zona Radiata (A & B- ×400; C & D- ×100; E & F- ×40).

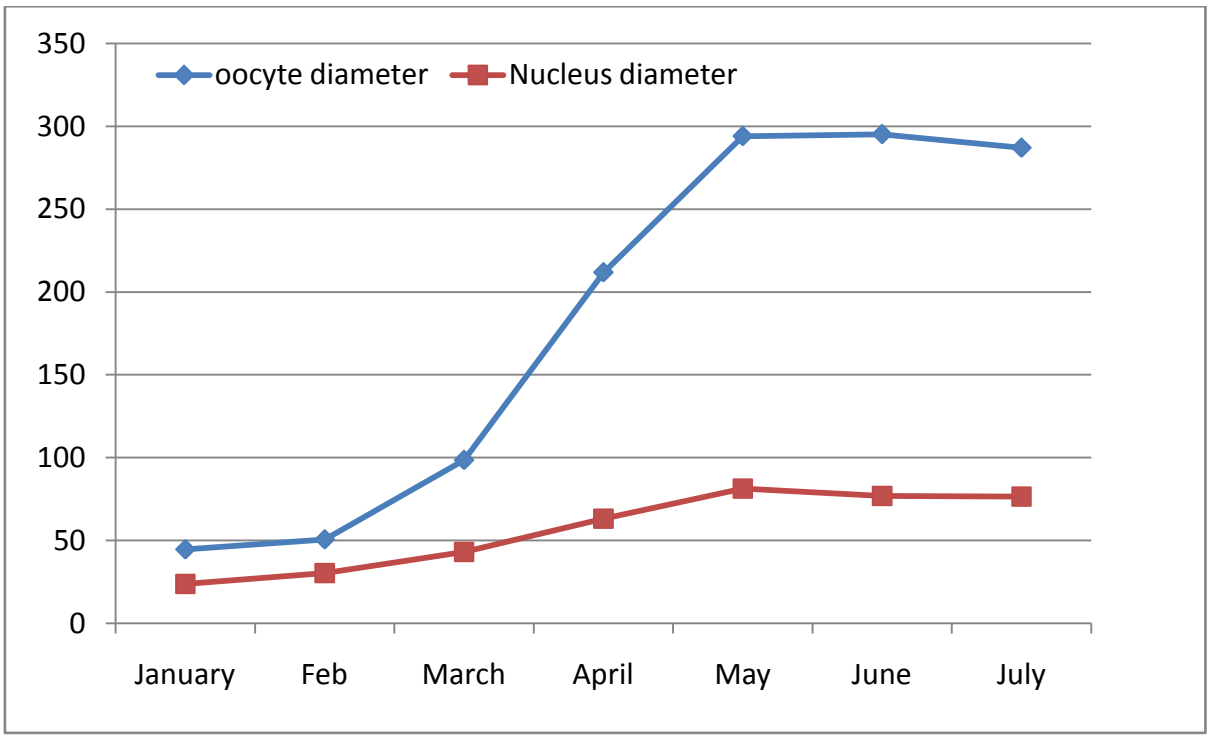

Fig. 2. Mean diameter of oocytes and their nuclei at successive months in *O. pabda*

Ovarian development

Based on the macroscopic features as well as microscopic characteristics, four types of ovaries were recognized in *O. pabda*, namely, immature ovaries, developing ovaries, ripe ovaries and spent ovaries.

A. Immature ovaries: Ovaries that were thin, small, slightly elongated, greyish and dull coloured were considered to be immature ovaries, including oogonia, previtellogenic oocytes and cortical alveoli stage oocytes. Immature ovaries were observed in January and February.

B. Developing ovaries: These gonads had mainly oocytes containing initial vitellogenic oocytes (cortical alveoli stage), fewer of previtellogenic oocytes, and few early active vitellogenic oocytes. The ovarian wall was considerably thickened. Later, the well-developed ovaries contained mainly active vitellogenic oocytes (yolk granule stage), and some mature oocytes. This stage was observed in March and April.

C. Ripe ovaries: The ovaries that contained mature oocytes as the dominant oocytes and also some yolk granule stage oocytes were described as being ripe ovaries though few previtellogenic oocytes or cortical alveoli stage oocytes were present. These ovaries were observed from May to July, but mainly in June and July.

D. Spent ovaries: These contained mainly previtellogenic oocytes and atretic mature oocytes. They were elongated, but irregular. In some, active vitellogenic oocytes and mature oocytes were present in moderate numbers, but the proportion of atretic structures was higher. This stage was observed in August and September.

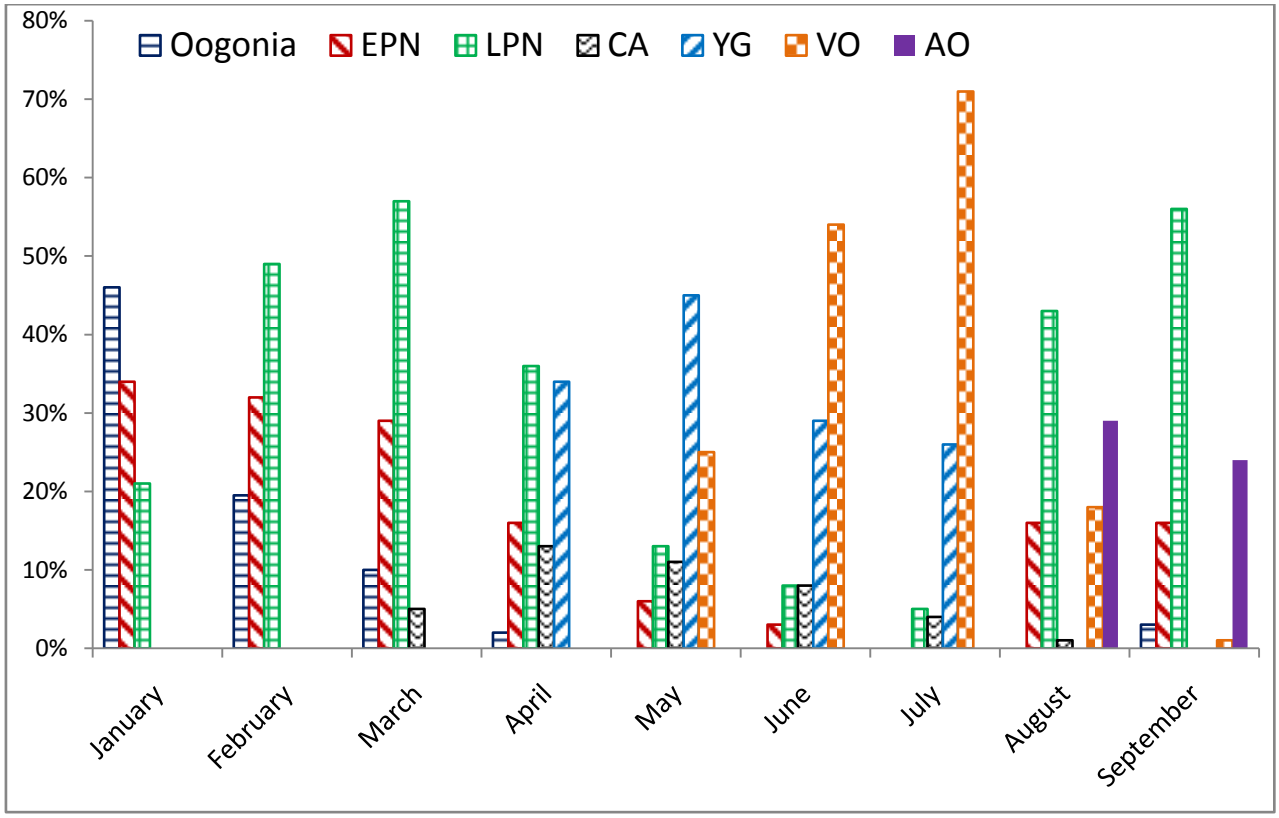

Fig. 3. Frequency (%) distribution of different developmental stages of oocytes in ovaries of *O. pabda* at successive months (EPN= Early Perinucleolus; LPN= Late Perinucleolus; CA= Cortical Alveoli; YG= Yolk Granules; VO= Vitellogenic Oocytes; AO= Atretic Oocytes).

Discussion

To successfully culture a fish, it is important to understand the breeding pattern during the spawning season and this is best done by monitoring the Gonado somatic index (GSI) and by observing the macroscopic and microscopic features of gonadal development. Gonads are often classified on the basis of their macroscopic features, including the size, coloration, and degree of vascularisation. However, it is important to understand that such observations alone may be insufficient to classify the maturational stages of ovaries, because different maturational stages cannot be determined precisely by observing the gonad externally. Similarly, the GSI alone is also insufficient to determine the reproductive maturity because it is related to the gonad weight which does not represent the peak percentage of matured oocytes in the ovary, even though the GSI remains useful as an indicator of the reproductive activity of a stock (de Vlaming et al. 1982). Although maximum oocyte size has been used as a measurement of ovary development in some

studies (West, 1990; Ramsay and Witthames, 1996), the most common practice to determine the spawning season of a species is the establishment of its gonado-somatic index and the histological examination of the gonads (Assem, 2000; 2003; Honji et al. 2006).

The heaviest ovary (3.85 g) and largest ova-diameter (295.20 µm) in *O. pabda* were observed in June, but the highest GSI (13.73) and the maximum frequency distribution (%) of mature oocytes (about 76%) were observed in July which support the GSI as a useful indicator. From the analysis of monthly variations in mean GSI and percentage distribution of different maturational stages of oocytes in ovaries of *O. pabda*, it is evident that the GSI increased progressively with the increased percentage of mature oocytes during the spawning periods. Though the ova-diameter was 211 µm in April, the ovary weight was only 0.26 g and contained no matured oocytes. Depending on the presence of matured oocytes in ovaries, it can be suggested that the spawning season of the experimental fish species extends from May to July with peak in June and July. Interestingly, in another study, the spawning season for the same species was reported to extend from April to August with peak in May to July which was not based on histological examination of ovaries (Hossain et al. 1992). In the present study, no mature oocytes were observed in any ovaries of fish samples collected in April. So, it can be suggested that analysing the ovarian development histologically is better practice than observing them macroscopically to determine the spawning pattern of a fish species, even though histological processes are time-consuming and comparatively costly. It is also suggested that different species of same fish genus may have different period of peak maturity. The highest percentage of matured oocytes was observed in July in the ovaries of *O. pabda* whereas it was found in May in the ovaries of *O. bimaculatus* (Parameswaran et al. 1970).

In *O. pabda*, five maturational stages of oocyte development were distinguished such as: (i) oogonia, (ii) pre-vitellogenic oocytes including early perinucleolus and late perinucleolus, (iii) vitellogenic oocytes including initial vitellogenic oocytes (cortical alveoli) and active vitellogenic oocytes (yolk granules), (iv) mature oocytes, and (v) atretic oocytes. These maturational stages of ovaries are similar or closely similar to that of some other teleosts fish, such as, white fish (Elorduy-Garay and Ramirez-Luna, 1994), *Puntius sarana* (Chakraborty et al. 2007) and sharptooth catfish (Çek and Yilmaz, 2007). Of all maturational stages in ovaries of *O. pabda*, oogonia were the primary phase of oocyte maturation. Cortical alveoli which play an important role in fertilization (Bazzoli and Rizzo, 1990) were the initiative phase of the active vitellogenic oocytes. The immature ovaries were observed in January to February and mature ovaries in May to July and both ovaries were markedly separated from each other by presenting maturing or developing ovaries in fish samples collected in March and April. From the analysis of ovarian development, it is established that female *O. pabda* becomes sexually matured within 1 year with a body length of 16 to 17 cm and body weight of 19 to 22 g which can be used as brood stock for successful seed production.

The ovaries of *O. pabda* can be classified as “group synchronous” which is similar to that of sharptooth catfish, *Clarias gariepinus* (Çek and Yilmaz, 2007). This type of ovaries contains at least

two distinct groups of oocytes at different developmental stages and the fish species spawns once a year and has a short breeding season (reviewed in Nagahama, 1983).

The ovaries of *O. pabda* contain a continuum of different sizes of oocytes which raises the possibility of extending the breeding season, especially given that the active male gametes (spermatozoa) in *O. pabda* develop as early as February (Siddiqua et al. 2000). It has been reported that the ovary weight, and quality and quantity of the oocytes in the ovary of sharptooth catfish, *C. gariepinus* is influenced by the dietary energy levels (Çek and Yilmaz, 2009). If mature oocytes can be developed in February through to March by giving special treatment, improved feed formulation and proper management, the breeding season in this species may be extended for several months. This could be particularly useful in many floodplains in Bangladesh where carp fish culture is not possible but could easily be used for catfish culture.

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