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BREEDING BIOLOGY OF FRESHWATER GOBY *GLOSSOGOBIUS GIURIS* USING GONADOSOMATIC INDEX AND GONADAL HISTOLOGY

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Abstract

The breeding season of fishes plays an important role in fish domestication and conservation of fish diversity. Freshwater goby, *Glossogobius giuris* is an important food fish of Bangladesh. Therefore, a study was performed to determine the breeding biology through assessing the gonadosomatic index (I_G), fecundity and histology of gonad of *G. giuris*. A total of 1371 females and 1051 male fishes were collected from the Belai beel of Gazipur district. Significantly ($P<0.05$) higher values of mean I_G were observed during March and from June to August for both females and males. The highest mean fecundity was recorded in August and the lowest was recorded in May. The smallest mean oocyte length was 0.037±0.005 mm in May and the largest was 0.071±0.001 mm in August. The early and late perinucleolar stages of oocytes were observed from January to March. The yolk vesicle stage was observed during April to May and yolk granule stage was observed during March and from June to August. These results revealed that *G. giuris* might breed in March and from June to August.

Keywords: Fecundity, breeding season, oocyte, yolk granule stage.

Introduction

The freshwater gobi, *G. giuris*, one of the small indigenous species locally known as bele or baila, belongs to the order of Perciformes under the Family of Gobiidae. Generally, this fish is available in the freshwater of Bangladesh and other countries of the Indian subcontinent (Hossain *et al*., 2012) and South-east Asia, Indo-west Pacifict to Indo China, Africa, Central Australia and East Indies (Larsen and Britz, 2012). This fish is very delicious and of high demand, having high nutritive value (Hossain *et al*., 1999; Islam and Joadder, 2005). Recently, this species has drawn attention of the fishery scientists as a potential aquaculture candidate because of its high market value and outstanding taste. Inclusion of this small fish in carp polyculture may play significant role in the enhancement of fish production (Roos *et al*., 2007). Consequently, *G. giuris* may be cultured in captive condition with carps. On the other hand, this fish is a prolific breeder (Islam, 2004) like tilapia, may have more than one breeding peak. The pond culture of the species could be popularized through the development of induced breeding of this fish in the hatcheries by determining the breeding season.

Very few researches were carried out on *G. giuris* such as on the ecology, the biology, biochemical composition, fishing, harvesting (Islam, 2004); the induced breeding (Islam

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and Mollah, 2013; Islam *et al*., 2014); gonado-somatic index and fecundity (Hossain, 2014; Roy *et al*., 2014), food and feeding habit (Achakzai *et al*., 2015) and histology of gonad (Jahan *et al*., 2015). Knowledge on the gonadal development and the spawning season of a species are necessary for the determination of the breeding frequency. The histology of gonads denotes the different stages of maturation, and is useful to determine the spawning season of fishes. The abundance of this species is decreasing day by day, possibly because of natural and human induced phenomenon (Hossain, 2014). As a result, it is essential to establish the induced breeding technique so that it could be cultured in controlled environments determining its breeding biology and breeding season. Therefore, considering the above essential points, this study was conducted by assessing I_G, fecundity, and gonadal development stages for determining the breeding season of this fish.

Materials and Methods

Collection of the fish samples and assessment of length and weight

The experiment was conducted from January to December 2015. A total number of 2,422 samples of both sexes of fish were collected monthly from the Belai beel of Joydebpur upazilla of Gazipur district. The samples were put into icebox and brought to the laboratory. The total length of fish was measured, using a centimeter scale (cm), from the tip of the head to the tip of the caudal fin. The weight of the specimens was recorded to the nearest gram (g) with a portable electric balance (Digital Balance OHAUS PA214). Thereafter, the samples were preserved in 70% ethanol.

Calculation of Gonadosomatic Index ($I_{G)}$

I_G is only used to show the degree of development of the gonads for both male and female fish. The gonads of fish were collected through scissor and weighed by a portable electric balance. The I_G of the samples were measured according to the following method

$$I_G = (\text{Gonad weight} / \text{Body weight}) \times 100$$

Fecundity estimation

Fecundity of *G. giuris* was estimated according to Lagler (1956). The gravimetric method was applied to determine the fecundity because the eggs of these fish were relatively small and irregular in shape. In this method, scissors were used to separate the entire of 956 ovaries. Then the ovaries were cleansed up by removing the external connective tissues and blotting paper was used to absorb the moisture of the ovaries. The ovaries of *G. giuris* were weighed by a portable electric balance. For calculating fecundity, the ovarian parts were collected accurately from the different portions of each ovarian lobe including anterior, middle and posterior portions. The numbers of eggs (N) were counted from each portion of ovarian lobe and fecundity (F) was determined by the following formula:

$$F = N \times \text{Gonad weight (g)} / \text{Sample weight (g)}$$

Determination of the oocyte diameter of *G. giuris*

The oocytes of *G. giuris* were irregular in shape. They were oval shape in immature stage, but in mature stage it became elongated. As the oocyte shape was elongated so the length of oocyte was measured by graticule eyepiece micrometer.

Histology of gonads

The different maturation stages of gonads of the sample fish following were observed in the histological process. A total of 50 ovaries and 50 testes were separated very carefully from the female and male samples, respectively. Then these gonads were cleansed up and weighed by a portable electric balance. For further investigation, 10% buffered formalin solution was used to preserve the gonads in the vials. Small pieces of testicular tissue from 50 male samples and small parts of ovaries of 50 females were removed from the mid region of the testes and ovaries and then fixed in Bouin's solution for 6h and subsequently processed for histology. A perforated plastic holder with perforated steel plates was used to preserve these gonads. An automatic tissue processor (Automatic Tissue Processor Leica, TP1020) was used to perform the histological process including cleaning, infiltration and dehydration. In this histological process, a series of alcohol of increasing concentrations and finally through molten wax (three series) were applied. Thereafter the samples were taken out from the automatic tissue processor and embedded in paraffin for preparing blocks. Then these blocks were trimmed and sections of 4 to 5 µm size were carefully made by a microtome knife (Microtome machine– Leica Model RM2125RTS). Then these sections were kept into a water bath (Paraffin Bath - Leica HI 1210) at 40ºC for cleaning as well as placed on glass slides and kept on a drier hotplate at 20ºC for the whole night. Xylene was used to clear these sections and then these sections were rehydrated with alcoholic series (three times). According to the schedule, haematoxylin and eosin stains were applied to stain these sections (Humason, 1972). After staining, Canada balsam was used to mount these sections and then covered by a cover slip. The prepared slides were kept on the clean platform to hold the cover slips permanently. Then these slides were examined under a compound microscope (Human Scope Classic Max-Planck-Ring-2165205 Wiesbadan, Germany) to observe the monthly developmental variations of the different stages of gonads.

Statistical analysis

Statistix 10.0 was followed to determine the significance ($P<0.05$) level of only I_G.

Results and Discussion

Total length and weight of *G. giuris*

The total lengths of female fishes were ranged from 3.62±0.20 to 21.49±1.09 cm for a breeding cycle. The highest total length was 21.49±1.09 cm in August and the lowest length was 3.62±0.20 cm in January (Table 1). The highest weight was 113.17±11.58 g in August and the lowest weight was 8.33±0.37 g in January (Table 1). On the other hand, a total of 1,051 male fish's total length, weight and gonad weight were measured (Table 2). The highest total length was 21.33±1.05 cm in September and the lowest length was 4.22±0.20 cm in January as shown in Table 2. The highest weight of the samples was 106.13±6.84 g in August and the lowest weight was 9.76±0.35 g in January (Table 2).

Table 1. Mean and standard deviation (±SD) of length, body weight and gonad weight in female *G. giuris*

Months	No. of fish examined	Total length (cm)	Body weight (g)	Gonad weight (g)
January	102	3.62±0.20	8.33±0.37	0.03±0.001
February	108	9.48±0.59	11.68±1.43	0.08±0.01
March	84	11.01±1.41	19.76±1.80	0.83±0.24
April	93	12.34±0.55	38.01±3.02	0.73±0.08
May	96	13.37±0.94	45.91±4.04	1.31±0.78
June	101	17.03±1.33	77.06±8.53	3.32±1.04
July	96	19.78±1.20	99.11±11.49	5.17±1.60
August	101	21.49±1.09	113.17±11.58	6.43±1.19
September	287	20.33±1.05	101.19±10.39	1.95±0.08
October	96	19.00±0.58	45.67±1.86	1.34±0.95
November	102	20.70±1.16	115.25±17.02	1.23±0.42
December	105	20.4 ±0.65	105.42 ±9.58	0.79 ±0.46

Table 2. Mean and standard deviation (±SD) of length, body weight and gonad weight in male *G. giuris*

Months	No. of fish examined	Total length (cm)	Body weight (g)	Gonad weight (g)
January	50	4.22±0.20	9.76±0.35	0.04±0.001
February	75	8.41±0.59	11.54±1.42	0.07±0.01
March	51	10.01±1.41	59.60±1.85	0.63±0.14
April	110	12.70±0.55	36.01±3.08	0.43±0.18
May	89	11.37±0.94	35.71±4.04	0.51±0.15
June	92	16.03±1.33	71.06±7.53	0.72±0.14
July	72	18.78±1.20	89.61±11.53	0.99±0.50
August	132	20.49±1.09	103.27±11.55	1.03±0.19
September	104	21.33±1.05	85.09±11.21	0.65±0.08
October	101	20.2±0.98	57.8±7.24	0.196±0.02
November	90	20.35±0.56	106.13±6.84	0.12±0.05
December	85	19.32 ± 1.01	69.23 ± 12.34	0.08 ± 0.01

I_G and fecundity of *G. giuris*

Significantly ($P< 0.05$) higher of mean for I_G values were found in March and from June to August than other months. (Fig.1a). Moreover, significantly higher values of mean for I_G of males were observed from March to August (Fig. 1b). The I_G values of fish increased in every month from April to August. The I_G is an indicator of the breeding season of the teleosts (De Vlaming, 1972). There was a sudden rise of I_G value in March and a spectacular rise in these values from June to August. The higher values of mean I_G were observed in March and from June to August significantly and the lowest value was in January in female (Fig. 1a) and the higher values of mean I_G were observed in March and from June to August and the lowest value was observed in December in male (Fig. 1b), indicating that *G. giuris* might breed in March and from June to August. These findings agreed with the findings of Hossain (2014), who reported that *G. giuris* spawned for several months and this also indicated the prolonged breeding season, which was extended from March to November. However, a little contradiction was found with the findings of Rao and Rao (2007), who found the prolonged breeding season of this species from August to January with the peak in the month of September. This species is a prolific breeder that breeds throughout the year with a peak in August (Islam, 2004). On the other hand, Islam and Mollah (2013) observed the highest I_G value of this species in April in captive condition. This slight variation might be due to the environmental variation of the habitat. Miah and Dewan (1984) stated three spawning peaks in case of *Sarotherodon nilotica.* Rheman *et al*. (2002) found two spawning peaks in *Liza parsia*. Recently, Jahan *et al*. (2015) reported that the breeding season of *G. giuris* extended from April to June which is a little contradiction with the present findings. This might be due to various reasons including habitat and environmental factors. This is might be due to its evolutionary adaptation to facilitate breeding process and for thus at the time of breeding season fish don't take any food or less amount of food but after the spawning period fish start to take higher amount of food for recover, growth

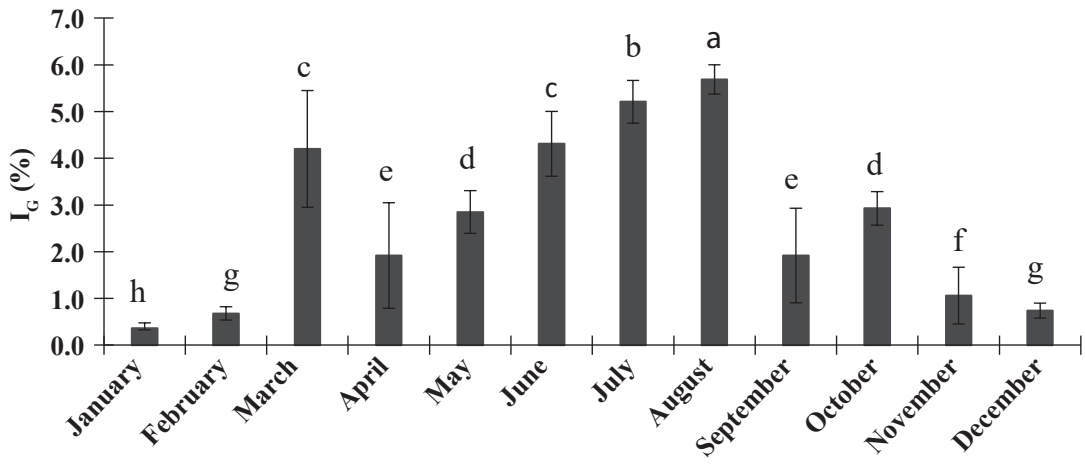

Fig. 1a. Monthly mean I_G (%) of female *G. giuris.*

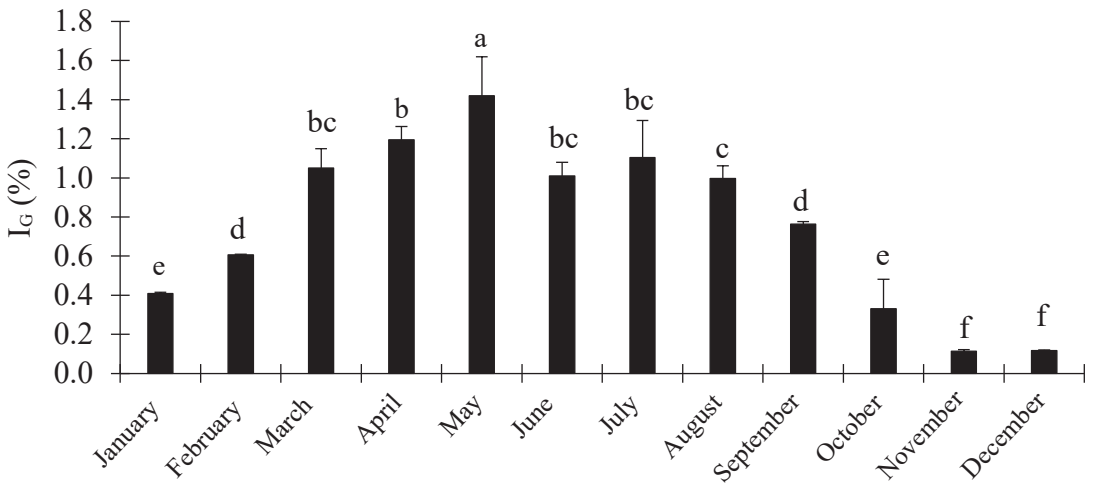

Fig. 1b. Monthly mean I_G (%) of male *G. giuris.*

and finally for reproduction in next breeding season.

The fecundity was estimated from 478 randomly collected female fish samples. The fecundity was observed in March and from May to August (Table 3). The highest fecundity of *G. giuris* was recorded in August and the lowest fecundity value was recorded in May. Moreover, the peak fecundity values were recorded in March and from June to August (Table 3) which were consistent with the previous findings I_G values (Fig.1a), indicating that *G. giuris* might breed in two breeding seasons. This study was firmly agreed with the findings of Dan (1977), Rheman *et al*. (2002) and Begum *et al*. (2010). During the study, it was observed that the ovaries of *G. giuris* contained different numbers of eggs with different shapes like jackfruit seeds and oval. This might be due to the variations of food intake by the individual. This variation is common in fish and has been reported by many researchers (Rao and Rao, 2007; Sulistiono, 2012; Hossain, 2014; Roy *et al*., 2014). The present study also indicated that *G. giuris* belonging to the same size group had varying number of eggs in their ovaries. The higher fecundity was found in March and from June to August indicating that *G. giuris* breeds a few months in a year. The similar findings were also observed by Begum *et al*. (2010) in *Liza parsia* and Roy *et al.* (2014) in *G. giuris*.

Oocyte length of *G. giuris*

Length (mm) of the oocyte was measured from female *G. giuris* by graticule eyepiece micrometer in March, May, June, July and August and are shown in Fig. 2. In immature stage the oocyte was almost oval shaped, but in mature stage the oocyte was elongated. In mature condition the physical feature of the oocyte was like jackfruit seed. However, histologically the oocyte shows leg foot like. The smallest mean oocyte length was recorded 0.037±0.005 mm in May and the largest mean oocyte length was 0.071±0.001 mm in August as shown in Table 4.

Table 3. Mean and standard deviation (±SD) of total length, body weight, ovary weight and fecundity of female *G. giuris*

Month	Total length (cm)	Body weight (g)	Ovary weight (g)	Fecundity
January	3.62±0.20	8.33±0.37	0.03±0.001	-
February	9.48±0.59	11.68±1.43	0.08±0.01	-
March	11.01±1.41	19.76±1.80	4.83±0.24	21219.3±2686.87
April	12.34±0.55	38.01±3.02	0.73±0.08	-
May	13.37±0.94	45.91±4.04	1.31±0.78	50240±72.1981
June	17.03±1.33	77.06±8.53	3.32±1.04	81943.2±14789.14
July	19.78±1.20	99.11±11.49	5.17±1.60	107716.4±12213.08
August	21.49±1.09	113.17±11.58	6.43±1.19	118331.3±8563.51
September	20.33±1.05	101.19±10.39	1.95±0.08	-
October	19.00±0.58	45.67±1.86	1.34±0.95	-
November	20.70±1.16	115.25±17.02	1.23±0.42	-
December	20.4 ±0.65	105.42 ±9.58	0.79 ±0.46	-

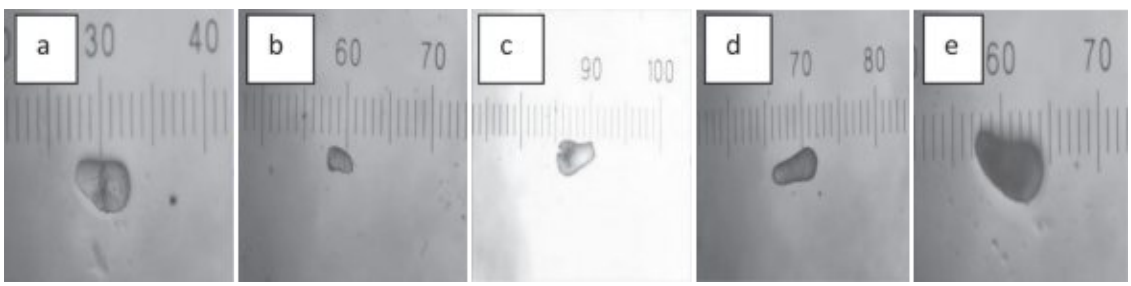

Fig. 2. Length (mm) of oocyte of *G. giuris* collected in March (a), May (b), June (c), July (d) and August (e).

In mature condition the smallest mean oocyte length was recorded in May and the largest mean oocyte length was found in August (Table 4). Moreover, the higher mean oocyte length was found in March and from June to August indicating that *G. giuris* might have more than one spawning season which was consistent with Jahan *et al.* (2015). Moreover, Kovacic (2007) also reported that the presence of three sizes of oocytes in ripe ovaries indicated that the striped gobi could spawn at least twice during the breeding season and further mentioned among gobi species fecundity and egg size generally depended on species size which has supported the present study.

The histology of the ovary

The diameter of the early perinucleolar oocytes could be easily identified by the peripheral arrangement of a large number of small nucleoli on the inner side of the nuclear membrane. Their shapes varied, mostly irregular, probably depending upon the stress imposed on them by expanding oocytes around them (Fig. 3a). This stage was observed in January and February from 25 ovaries. The late perinucleolar oocyte's nucleus was enlarged and appeared to become more regular in shape (Fig. 3b). This stage was observed in March from 33 ovaries. The **y**olk vesicle stage was characterized by the formation of the

Table 4. Mean ovary weight and mean oocyte diameter and standard deviation (±SD) of different developmental stages of gonads of female *G. giuris*

Month	Developmental stages	Ovary weight (g)	Oocyte length (mm)
January	Early perinucleolar	0.03±0.001	-
February	Early perinucleolar	0.08±0.01	-
March	Late yolk granule	4.83±0.24	0.0515±0.003
April	Late perinucleolar	0.73±0.08	-
May	Yolk vesicle	1.31±0.78	0.037±0.005
June	Early yolk granule	3.32±1.04	0.049±0.003
July	Early yolk granule	5.17±1.60	0.062±0.002
August	Late yolk granule	6.43±1.19	0.071±0.001
September	Spent phase	1.95±0.08	-
October	Spent phase	1.34±0.95	-
November	Chromatin Nucleolar stage	1.23±1.42	-
December	Chromatin Nucleolar stage	0.79 ±1.46	-

yolk vesicles in the periphery of the ooplasm (Fig. 3c). Initially they formed as a single row which appeared colourless when the slides were stained with haematoxyline and eosin. These yolk vesicles developed initially as minute bodies but gradually increased in size and number to form several irregular peripheral rows, but in some cases, they also appeared elsewhere in the nucleus. This stage was observed in April and May from 15 ovaries. The final stage of vitellogenesis and oocyte development was characterized by the formation of the yolk granules. The yolk granules formed only in oocytes with fully developed yolk vesicles. The yolk granules first appeared close to the zona radiates (Fig. 3g). This stage was observed during June and July from 12 ovaries. At a later stage, the whole oocyte was filled with the yolk granules (Fig. 3e and 3f). In the late yolk granule stage**,** the diameter of the oocytes increased simultaneously with the advancement of the yolk granule stage. In some cases, the yolk granules appeared to coalesce to form larger tightly packed granules (Fig. 3e and 3f). This stage was observed in the months of March and August from 32 ovaries of *G. giuris* by histological process. The spent phase was observed during the month of September and October (Fig. 3g) from 11 ovaries of *G. giuris* process. The chromatin nucleolar stage was found during the month of November and December (Fig. 3h). In this present study, ovaries carried immature eggs during January, February and April and mature eggs in May and ripe eggs in March as well as extended from June to August (Fig. 3) which implies that the species has a long spawning period and may have two spawning peaks. Jahan *et al.* (2015) conducted the histological study of ovary of *G. giuris* for the determination of breeding season and they showed that the breeding season of this species was restricted from April to June. However, they did not find more breeding seasons rather than from April to June. Actually gonadal development depends on hormonal signal as well as this hormonal secretion might be hampered through different chemicals in water body including pesticides and insecticides. However, spawning season had two peaks one is in March and the other from June to October (Hossain, 2014),

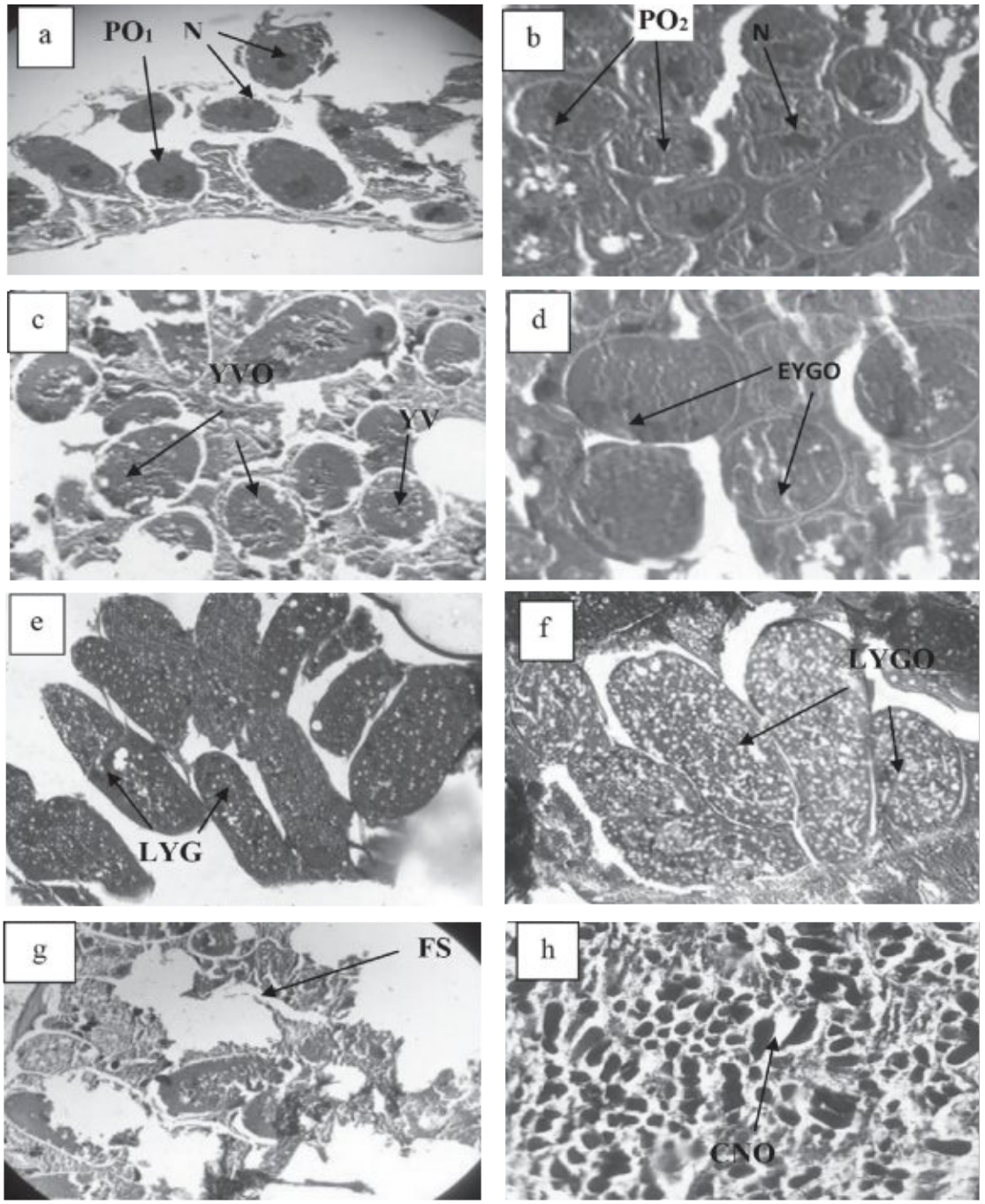

Fig. 3. Different development stages of oocytes of *G. giuris*. (a) Early perinucleolar stage (PO_1) in January and February; (b) Late perinucleolar stage (PO_2) in April;(c) Yolk-vesicle stage (YVO) in May; (d) Early yolk-granule stage (EYGO) in June and July; (e) Late yolk-granule stage (LYGO) in March; (f) Late yolk-granule stage (LYGO) in August; (g) Spent phase in September and October; (h) Chromatin Nucleolar stage (CNO) in November and December. N= Nucleus; N= Nucleus with nucleoli (Nu); H&E×200.

which was consistent with the present study. Moreover the breeding season of *Rita rita* was extended from June to July (Khan, 1934; Das, 1964; Rahman and Mollah, 2013).

Developmental stages of testicular germ cell

Spermatogonia (SPG) are the primary stage of spermatogenesis had thin, pale cytoplasm and a nucleus with a prominent basophilic nucleolus. These cells are large. It was observed spherical in shape from 16 small pieces of testicular tissue of *G. giuris* (Fig. 4a). Spermatocytes (SC) were spherical in shape containing a nucleus in centre (Fig. 4b). These were smaller in size than the spermatogonia. This stage was observed from 27 small pieces of testicular tissue. Spermatids (ST) were spherical shape observed from 19 pieces of testicular tissue in which nucleus was not clearly seen from its dark appearance under the microscope (Fig. 4c). These cells were found in clusters in the lumen of the lobules.

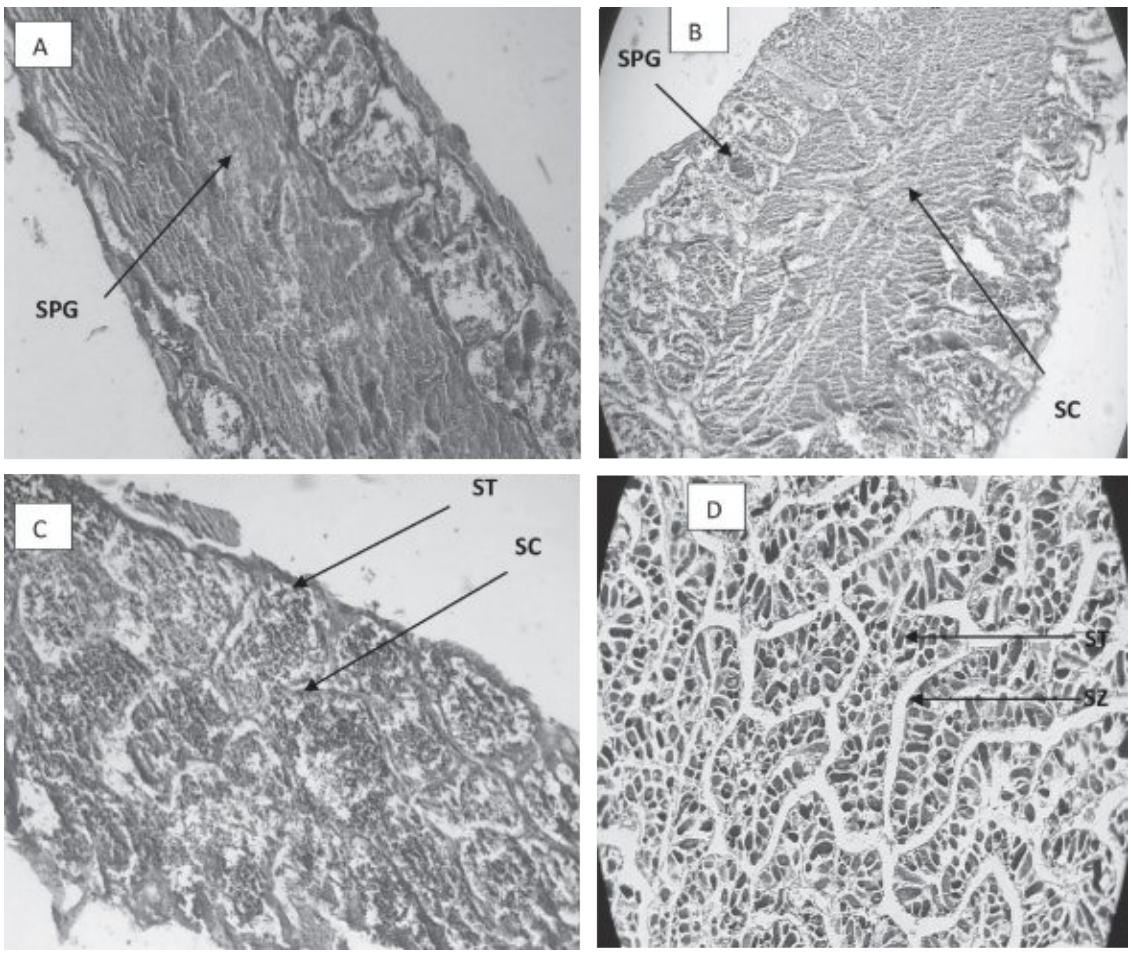

Fig. 4. The maturing testis with (a) spermatogonia (SPG) stage in March (b) Spermatocytes (SC) and SPG in June (c) Spermatids (ST) in July and (d) ST and spermatozoa in August of *G. giuris* during the spawning season. H & E×200.

Spermatozoa (SZ) were the smallest cells of all germ cells in testis observed from 16 small pieces of testicular tissue (Fig.4d).

Cyclical changes in testicular activity had been documented for a number of fish species (Sulistyo *et al*., 2000; Santos *et al*., 2001). The present study revealed the four stages of spermatogenesis for male (Fig. 4) showing more or less similar to the findings of Mollah (1988) for *C. macrocephalus* and Guraya (1994) for salmonids. Again, no standardization histological observation had been done on testes of *G. giuris.* The histological study of testes in this study showed that the testes of *G. giuris* were rich in ST and SZ (Fig. 4). This finding indicated the breeding season of male *G. giuris* and is indicative of the fact that the male fishes get mature and ready to spawn during the entire breeding season. Siddiqua *et al*. (2000) studied the testicular development *O. pabda* and identified four germ cells stages of testes including SPG, SC, ST and SZ.

The natural breeding cycle of various fishes differs from region to region. The breeding cycle of *Clarias* starts in July and ends in September in Egypt (Nawar andYoakin, 1963), and the same is for *C. lazera* in Central African Republic (Micha, 1975). Finally, the results of the present study suggest that *G. giuris* breeds in March and from June to August and have a prolonged spawning season.

Conclusion

The breeding biology of freshwater goby was assessed by analyzing of GSI and gonad histology. This result revealed that *G. giuris* has a prolonged breeding season which extends in March and from June to August. Findings of this study might be useful for sustainable fishery management and aquaculture in Bangladesh. Further research should be carried out to have clear information on biology of *G. giuris* for its induced breeding artificially as well as discovering hormonal signaling pathway of this species so that this species could be saved from extinction in the future.

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