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Original Article

Study on the reproductive biology of Vietnamese climbing perch (*Anabas testudineus*, Bloch)

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Abstract

A one year study was conducted from January-December 2014 to investigate the reproductive biology of Vietnamese Climbing Perch in earthen pond. The female fish elucidates rounded, swollen abdomen and reddish pelvic fin while the male fish also possess a reddish pelvis fins but generally smaller, narrower. The surfaces of male's pectoral were little rough during the breeding season. The Gonado-somatic index (GSI) value of female *Anabus testudineus* was ranged from 12.71±0.73 (July) to 1.13±0.10 (October) while hepato-somatic index (HSI) value of female was ranged from 4.37±0.64 (January) to 1.58±0.19 (July). The GSI and HSI were inversely related with each other. The fecundity was counted from the month of April to July by using gravimetric method where absolute fecundity during April was 16832.80± 673.34 and during July was 46186.14±2219.15. The relationship between gonad weight and fecundity, body weight and fecundity showed strong positive relation with regression co-efficient of 0.9603 and 0.9265 respectively which indicate that with the increase of body and gonad weight, fecundity was also increased. Microscopic observation of matured female ovary during June to July revealed group asynchronous development. Microscopic observation on matured testes found spermatocytes, spermatids and spermatozoa stages during the month of June and July.

Key words: Ovary, fecundity, GSI, HSI, *Anabas testudineus*

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INTRODUCTION

Climbing perch *Anabas testudineus*, locally known as Koi, is an indigenous air breathing freshwater species in Bangladesh. This fish is suitable for cultivation and is highly recommended for its supreme nourishing quality and prolong freshness even out of water (Potongkam, 1972). It is a predator, carnivore (Pandey *et al.,* 1992) or an insectivore fish (Ahyaudin, 1992). Flesh of *A. testudineus* is rich in iron and copper that support hemoglobin synthesis (Saha, 1971; Sarma *et al.*, 2010) and it has high quality poly-unsaturated fats and many essential amino acids (Kohinoor *et al*., 1991). In recent times, because of higher value and market demand this species prone to getting nearly extinction. This might also be due to environmental changes and over fishing (Sverdrup, 2002; Das *et al.,* 2009).The breeding technology of native koi (*A. testudineus*) had successfully been developed in Bangladesh (Kohinoor *et al*., 1991) but its slow growth and small size does not favor sustainable production per unit area in a culture system (Kohinoor *et al.,* 1991). To overcome this situation, it has been introduced from Thailand in 2002 (BFRI, 2006) which is fast growing species; however, it does not taste like native one. But reproductive biology of Vietnamese Koihas yet to be studied in perspective of local environmental condition of Bangladesh. For this reason, knowledge on reproductive biology is very important for development of brood stock as well as for captive breeding in this local habitat. Knowledge on reproductive events is necessary to obtain information about the size and age of sexual maturity, spawning season and oocytes development (Coward and Bromage, 1998; Bromage *et al.*, 2001; Shabanipur and Hossayni, 2010; Kabir *et al*., 2012). By taking all above into consideration the present study was set out to address some biological aspects of Vietnamese Koi. Information on the different biological parameters like body morphometrics and

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biometrics, HSI, GSI, fecundity are important parameters representing details reproductive status and breeding peaks of fish (Ferdausi *et al.,* 2015; Hossain *et al*., 2015; Ferdausi *et al.,* 2015), which provide key information for aquaculture and fisheries management (Mian *et al.,* 2017) and explain human intervention in aquatic system (Muhammad *et. al.,* 2016).

MATERIALS AND METHODS

Sample collection and processing

The one year study was conducted in the wet laboratory of Fish Biology and Genetics Department, Sylhet Agricultural University, Sylhet. Live fish of more or less same size and age group were collected from the local fish market of Sylhet and were stocked in the experimental pond of Sylhet Agricultural University. Then fish were reared in the pond for research purpose up to December, 2014. For experiment, six fish species of male and female were collected monthly and brought to the laboratory to measure the total length, body weight, gonad weight, gonadal length and liver weight of individual fish.

For gonad collection and sexing, fish were sacrificed and dissected carefully. Gonads were isolated and weighed by using a sensitive portable electronic balance (XS Analytical105). Then the gonads were divided into three sub samples as anterior middle and posterior (range between 1g to 0.95g) and then tagged the samples with markers which kept in Bouin's fixative for 20 minutes for further histological process.

The GSI was determined by the following formula (Alam and Pathak, 2010):

$$\text{GSI (\%)} = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

HSI was calculated by using the formula (Cek and Yilmaz 2009):

$$\text{HSI (\%)} = \frac{\text{Total live weight}}{\text{Whole body weight}} \times 100$$

The ovaries were diluted in Gilson fluid and shaken uniformly to separate the oocytes. Followed by separation of oocytes from the ovary, the sample was put into a Petri-dish and washed with running tape water. Then they were counted by naked eyes with the help of a needle. Total numbers of oocytes (N were calculated using the following formula;

$$N == \frac{Wt}{Ws} \times Ns \text{ (Bagenal 1978)}$$

Where,

W_t= Total weight of ovaries; W_s = Weight of subsample; N_s =Number of oocytes in the subsample.

After following proper histological processes the slides were observed under stereomicroscope (Optika Microscope, Italy) which was connected to computer with a viewer (Magnus viewer). The viewer was also equipped with a camera. By the help of these devices numerous photographs of gonads were snapped at different magnification (10x and 40x). Linear relationship and correlation coefficient (r) between total length and fecundity, body weight and fecundity, gonad weight and fecundity were analyzed in Microsoft Excel.

RESULTS AND DISCUSSION

Gonado Somatic Index (GSI) and Hepato Somatic Index (HSI)

The GSI indicates gonadal development and maturity of fish. It increases with the maturation of fish declining thereafter (Parameswarn *et al.,* 1974). This species has extended breeding period following the peak between April to August (Morioka *et al.,* 2009). The maximum GSI value for ovary were observed in July (12.71±0.73) and the lowest value (1.13±0.10) in the month of October. The GSI value started to increase gradually from January and reach a maximum value in June and July finally, reaching to its peak in July. Later in the month of August the GSI value of female ovary declined sharply which continued up to October (1.13±0.10) shown in Fig 1. So the peak spawning season of this species is from June to July in Bangladesh. On the other hand, the minimum mean values of HSI were found to be 1.58±0.19 in July while maximum mean value was 4.37±0.64 found in January shown in Fig1. The GSI and HSI value in the present study were found to be inversely related with each other which reveal liberation of energy from liver into the ovary. The decline in liver weight continued until the spawning finished which then started to deposit vitellogenin as a preparation for the next spawning. Similar results were obtained by Ghaedi *et al.* (2012) in case of captive reared *C. striatus*and Mian *et al.* (2017) in case of lentic *Channa punctatus*.

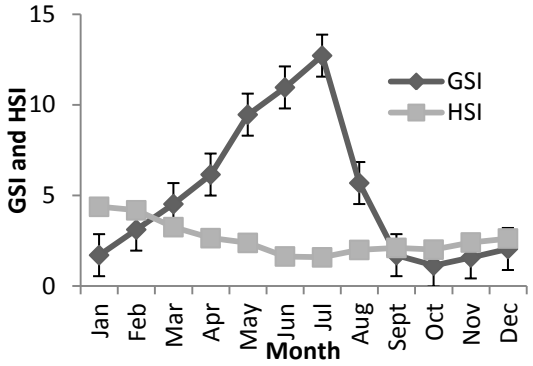

Figure 1: Comparative study on GSI and HSI of female *A .testudineus*

Fecundity estimation

Fecundity in the female varies accordance with species, age of individual, body length, weight and environmental condition. Fecundity has linear relation with length, weight and eggs (Ghafari and Jamili, 2010; Lawson, 2011). The absolute fecundity was estimated from 42 randomly collected fish whose body length varied from 14.35±0.51cm to 17.27±0.43cm, body weight from 66.80±2.03g to 89.00±1.90g and ovary weight from 4.11±0.24g to 11.33±0.84g. The absolute fecundity was found to vary from 16832.80±673.34 in April and 46186.14±2219.15 in July. Banarjee and Prasad (1974) reported that fecundity of Koi ranged from 4588 to 34993 when fish size was 84-100.2 g. Chanchal *et al*. (1978) found fecundity to be between 3481to 42564 in the fish sample of 9.0 to 53.1g weight. This result also coincides with the findings of Ulka (2011) for *A. testudineus* and Mustafa *et al.* (1980) for *Nandusnandus*.

Table I: Body length, body weight, ovary weight and fecundity of *A .testudineus*

Month	Mean total length (cm)*	Mean body weight (g)*	Mean ovary weight (g)*	Mean fecundity*
April	14.35±0.51	66.80±2.03	4.11±0.24	16832.80± 673.34
May	14.98±0.46	75.29±1.25	7.13±0.71	28808.33± 866.85
June	16.97±0.25	81.14±0.50	8.90±0.32	41057.62± 1534.87
July	17.27±0.43	89.00±1.90	11.33±0.84	46186.14± 2219.15

*Mean ± standard deviation

Relationship between fecundity and body weight

The scattered diagram of log of body weight and log of fecundity suggested that there is a linear relationship between the two variables demonstrated in Fig 2. The relationship between average body weight and fecundity gave a correlation coefficient of 0.9265 and regression intercept of 2.2201. The arithmetic equation of fecundity against body weight gave the following result:

Log Y = 3.5507, Log X – 2.2201;

Where, r^2=0.9265, Y = Fecundity, X = Body weight.

The fecundity is correlated with the body weight and comprised a correlation coefficient of 0.9265, which means approximately 92% of changes in fish fecundity is explained by fish body weight (Fig. 2).It manifested a gradual increase of fecundity with increase in total body weight. The fish with increase in body weight tend to have greater the number of oocytes in ovary. Similar findings were concluded by Marimuthu *et al.* (2009) for *A.testudineus.*

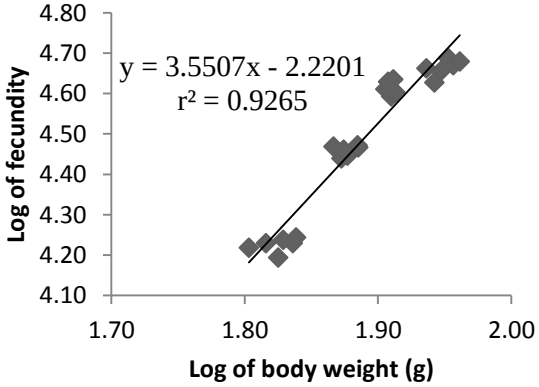

Figure 2: Relationship between log of fecundity and log of body weight

Relationship between fecundity and ovary weight

The scattered diagram of log of ovary weight and log of fecundity suggested that there

is a linear relationship between the two variables as clarified in Fig 3. The arithmetic equation of fecundity against ovary weight gave the following result:

Log Y= 1.0158 Log X+3.6102;

Where, r^2=0.9603, Y = Fecundity, X = Ovary weight

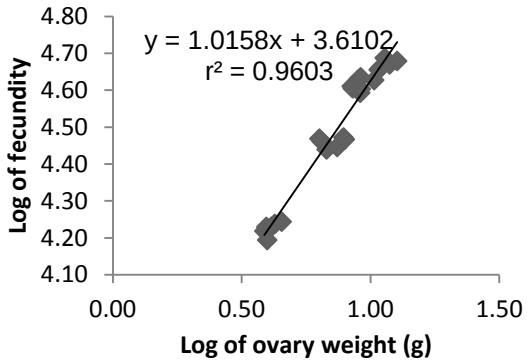

Figure 3: Relationship between log of fecundity and log of ovary weight

The above figure pointed out that the coefficient of correlation between the numbers of eggs with ovary weight is positive as the value of r^2 is 0. 9603. This implies 96% changes in fecundity can be explained by gonadal weight. The fecundity was found to be linear with ovary weight which indicates the number of eggs increases with the increase of ovary weight. Very similar conditions were recorded by Marimuthu *et al*. (2009) and Afroz *et al.* (1999) through their study of *A. testudineus and G. chapra.*

Microscopic observation of female and male gonads

Microscopic observation of ovary of female *A. testudineus* is shown in Fig. 4. Oocyte developments in majority of fish are categorized into five to eight distinctive stages (Reidel *et al.,* 2010; Pham *et al.,* 2011).

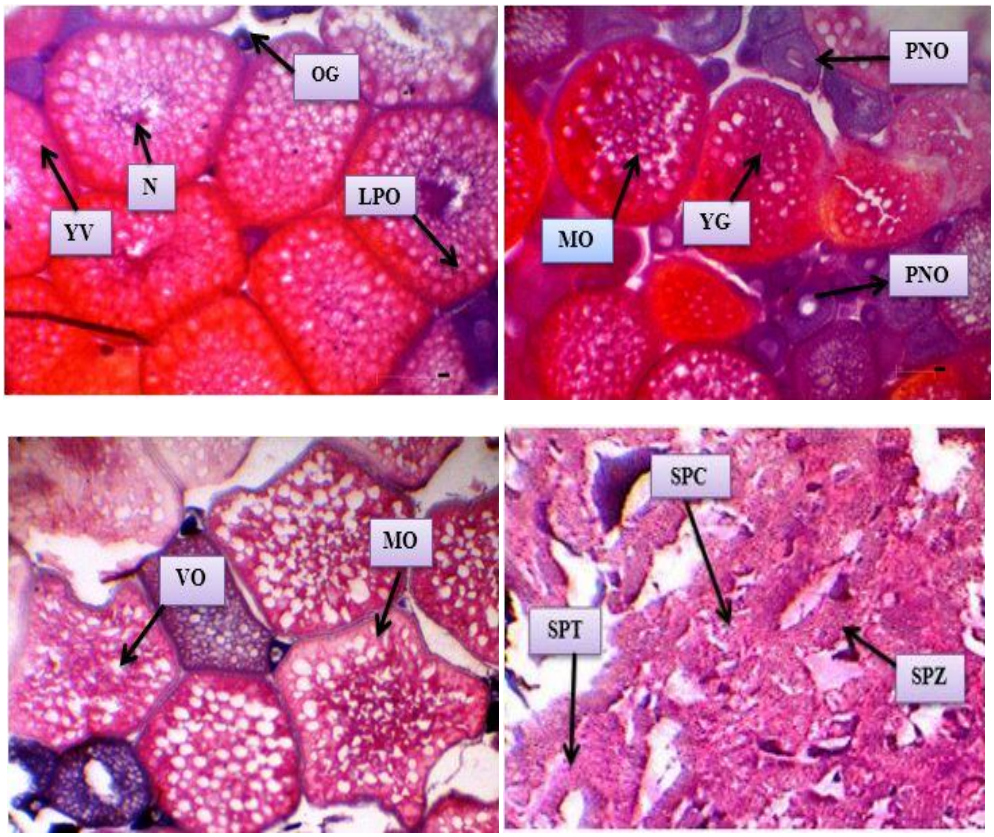

Figure 4 Microscopic observation a) early yolk granule oocyte in theovary in june b) early yolk granule stages of oocytes in the ovary in early july c) late yolk granule stages of ovary in late july d) Mature stage of testis of male in june and july (H&E x 10)

In the present study on histological analysis of female *A. testudineus*, it was observed that vitellogenic oocyte with yolk vesicles increased in number and size. Maturing stage with perinucleolar and previtellogenic oocytes were noticed during July. The mature stage with primary, secondary and tertiary oocytes were found in their early phase and ovary with migratory nucleus at the peripheral region with numerous vacuoles. This indicates that the ovarian development of *A. testudineus* is group asynchronize in nature. These findings are complemented with the outputs of Kabir *et al*. (2012) who worked with *Pangasianodon hypophthalmus*. Similar result was also observed for *C. striatus*. Presence of different development stages in ovary at a time indicates the asynchronous nature of ovary development in this fish (Ghaedi *et. al*., 2013).

Mature stages of testes were studied during July when fish were matured. From the histological study of testis, spermatocyte (SPC), spermatids, spermatozoa (SPZ) it was observed that testes of male *A. testudineus* were rich in spermatids (SPT) and spermatozoa (SPZ) in July (PLATE-IV). This finding indicates peak breeding season of male *A. testudineus* was in July. In a similar study, Alam (2009) observed large amount of SPT, SPZ and small amount of SPC in testis of *O. pabo* during April to July. The findings of present investigation were in agreement with some previous study. Akter (2011) identified SPC, SPT, SPZ and LU in testis of *P. pangasius* where mature stages were abundant mainly during May to October which is partially different with the present study.

CONCLUSION

In the present study of Vietnamese Climbing Perch, the GSI showed higher values during the June to July; therefore, this period may be regarded as the peak spawning season even though normal breeding season may be started before these months. Highest fecundity was found 46186.14± 2219.15 in July. Based on the histological findings of ovary of the species is considered to have group asynchronous nature which indicates long duration required to manipulate with the brood stock in the hatchery.

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