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Samarendra Behera
Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences 5, Budherhat Road, Chakgaria, P.O. Panchasayar, Kolkata – 700 094.

Sanjeev Kumar
Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences 5, Budherhat Road, Chakgaria, P.O. Panchasayar, Kolkata – 700 094.

Laishram Monlisa Devi
Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences 5, Budherhat Road, Chakgaria, P.O. Panchasayar, Kolkata – 700 094.

Rinku Gogoi
Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences 5, Budherhat Road, Chakgaria, P.O. Panchasayar, Kolkata – 700 094.

Pushpendra Singh Snegar
Rural Development Officer, Indian Bank Jagraon, Punjab-142026.

Puspendu Smanta
Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences 5, Budherhat Road, Chakgaria, P.O. Panchasayar, Kolkata – 700 094.

Snigdha Baksi
Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences 5, Budherhat Road, Chakgaria, P.O. Panchasayar, Kolkata – 700 094.

Olik Jomang
Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences 5, Budherhat Road, Chakgaria, P.O. Panchasayar, Kolkata – 700 094.

Correspondence
Sanjeev Kumar
Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences 5, Budherhat Road, Chakgaria, P.O. Panchasayar, Kolkata – 700 094.

Determination of Seasonal Cyclicity of Gonad by Studying Its Histology during Pre-Spawning and Spawning Period of *Anabas testudineus* (Bloch) In Natural Environment

Samarendra Behera, Sanjeev Kumar, Laishram Monlisa Devi, Rinku Gogoi, Pushpendra Singh Sengar, Puspendu Samanta, Snigdha Baksi, Olik Jomang

Abstract
The present study attempts to understand the histology of gonads of *Anabas testudineus* (Bloch) collected from natural environment of Mohanpur in West Bengal. From the study of histological characteristics the gonadal cycle of *A. testudineus* was found in preparatory phase and spawning phase. During January to February, tunica albuginea was thick and seminiferous lobules were not dense and less spermatogenic activity was seen. During this period, primary and secondary spermatogonia are visible. Histological observations revealed that testicular and lobular wall were thin. The lobules were filled with spermatozoa and sperm cells. During January and February, more number of immature oocytes stages like stage I, stage II and stage III are observed. This stage was characterised by the formation of minute yolk vesicles (early yolk vesicles) in the peripheral ooplasm. In the oocytes, at late perinucleolus stage the oocytes were observed with increased ooplasm. During March to June, the ovaries were very much enlarged and reached their maximum size and occupied the whole body cavity. In this phase, the ovarian wall became thin and the oocytes of late yolk vesicle stage and early yolk stage were found more in number than the late perinucleolus stage and early yolk vesicle stage. During this period, the oocytes showed an increased in size and ovaries are filled with ripe eggs.

Keywords: *Anabas testudineus,* histology, gonad, spermatogonia and oocytes.

1. Introduction
Inland sector of fish production of the state can be increased further by adopting certain biological management technique. Due to the lack of proper management, some of the freshwater resources are diminished due to the deposition of silt which resulted in the formation of swampy areas which are infested with dense submerged and floating vegetation. So this area can be utilized for the *Anabas* culture. In West Bengal for the development of rural areas fishery sector is a good field to increase the rural economy and poverty alleviation by creating employment opportunities and earning of foreign exchange (Samanta, 2001) [18].
In West Bengal *Anabas* is considered as a lucrative fishery due to its high and regular demand (Roy, 1994) [17].
The testicular maturity can be judged by visual observation on the morphology and histological survey (Rath *et al.,* 2000) [16]. However, many have classified development stages of testes as Stage I as resting phases, Stage II as late immature phase, stage III as maturing phase, Stage IV as mature phase and Stage V as spent phase. Testes are composed of a large number of seminiferous tubules or lobules, which are closely bound together by a thin layer of connective tissues (Rath *et al.,* 1984) [15]. During breeding season seminferous tubules are filled with sperm and a few numbers of spermatogonia are seen. After breeding season, empty and collapsing seminiferous tubules are seen, some of which contain residual or unexpelled sperm (Sanwal and Khanna, 1972) [19]. Das (2002) [6] studied testicular maturity of *Anabas* and identified three phase of testes i.e. spawning, post spawning phase and preparatory phase.
Despite moderate size, market demand and high price, *Anabas testudineus* is regarded as a highly esteemed and eye catching fish that has been attracting attention of the fish growers since older times. *Anabas* is not only attracting the fish growers but also to the conservationists because this fish has become a threatened species due to over exploitation from their natural ecosystem to meet the market demand. Therefore, the population of *Anabas* species is threatened and is considered as vulnerable in the state of West Bengal (Roy, 1994;

Mukherjee and Das, 2001 [17, 10]. To save this fish population from threatened status, the juvenile of the fish should be recruited to the natural ecosystem as conservation measures. This is possible through artificial propagation and larval rearing. For this we need to know a good knowledge on reproductive biology of this fish.

Hence, a proper knowledge of reproductive biology of *Anabas testudineus* is necessary before going for artificial breeding. And it is required to study the gonadal development (histological changes). Present study entitled "To determine the seasonal cyclicity of gonad by studying its histology during pre-spawning and spawning period of *Anabas testudineus* (Bloch) in natural environment" was conducted in the laboratory.

2. Material and Methods

The study was confined to the laboratory investigation for this the gonad development from the natural environment was conducted during January to June 2005 in the Department of Fisheries Resource Management, West Bengal University of Animal & Fishery Sciences, West Bengal.

2.1. Collection and Sampling of the Fish Species: Live adults of *A. testudineus* were collected from Mohanpur, Nadia, West Bengal, for study. Sampling was done in the 3rd week of every month. In every sampling, four to five male and four to five female fishes were studied for histology of the gonads. After morphological examination, the gonads were cut into 3 pieces as anterior, middle and posterior portion. The middle portion is preferred for the further study. Both the middle parts were taken for histological study.

2.2. Histological study

For histological study, the microscopic slides were prepared by the following procedure as followed by of Agarwal (1996) [1]. The development stages of germ cells in the testes and the change of the oocytes in ovary were studied by following methods.

2.3. Collection and fixation of tissue: For histological study, the middle parts of the gonadal tissues (testes and ovary) of *Anabas testudineus* were collected as stated earlier. The tissues were trimmed into 5 to 6 mm size for better penetration of fixatives into it. The tissues were put into Formaldehyde Saline (Baker, 1944) [4] for 24 to 48 hours as per size of tissues.

2.4. Post fixation treatment

Washing: The tissue (testes and ovary) were removed from the fixatives and subjected to overnight washing with flowing clear tap water until the formaldehyde odour were vanished.

Dehydration: The tissues were dehydrated perfectly with graded alcohols, starting from 30%, 50%, 70%, 90% and absolute alcohol (100%) to avoid the brittleness of the tissues.

De-alcoholization: Two changes of xylene (1 hr each) were made to clean the tissues from alcohol. For better impregnation of wax into the tissue, the xylene penetrates into the tissue to become transparent and the material comes up to float on the top.

Infiltration: Paraffin wax (melting point 58-60 °C of B.D.H) was used for infiltration of tissue. Three changes of wax (45 min each) were made to make the tissue xylene free.

Embedding: For the preparation of blocks, pure paraffin wax was melted in water bath in between 58-60 °C. Metal 'L' moulds were adjusted according to the size of blocking materials. The melted paraffin was taken from water bath and the blocking disc was filled. After permitting a layer of wax to be solidifying on the bottom disc, the completely infiltrated tissues were carefully taken from the paraffin wax and put inside the different blocking disc according to their size. Care was taken so that the wax on the top of the disc did not solidify during keeping the material in the blocking disc. For this reason, a heated needle or forceps was put only the upper portion or inside the wax of the disc. After the proper positioning of the tissues, the wax inside the disc was allowed to solidify. After few minutes, the 'L' moulds were removed from the wax block. And the prepared blocks were kept separately inside the labelled polythene packets.

Trimming and sectioning: The paraffin blocks were trimmed carefully to 6 to 7 mm^2 by sharp blades. The trimmed blocks were fixed to the wooden holder (peg) with the material facing away from it. Molten wax was poured on the holder and the block was kept on it. The block was padded with more wax at the base to make it strong. After being confirm, the blocks were firmly fixed with holder, the sectioning was done by using microtome (SPENCER 820 TYPE). On the microtome, each section was cut into 5µ thickness. The ribbons containing tissues were collected on clear glass slide (already a smear of egg-albumin was kept on that slide) with the help of a fine brush.

Spreading and fixing: Glass slides were cleaned properly by Chromic-acid solution then soap and finally with tap water. After cleaning, the slides were air-dried and a thin layer of Glycerin Egg Albumin was rinsed over it. Then the ribbons with materials (about 10 to 15 sections depending on the size) were spread over the clean glass slides. Thin tissues were made wrinkle free and allowed to fix on slides by keeping them on hot plates (30 °C) for 2 to 5 minutes.

De-waxing and staining: Tissues fixed on slides were de-waxed with descending order of alcohols (100%, 90%, 70%, 50% and 30%) and stained by the double staining method with Haematoxylin and Eosin by using standard techniques as described by Agarwal (1996) [1].

Mounting: One or two drops of DPX (mountant) were put on the dried slide which one was ready for mounting. Then, a cover slip or slide was slowly lowered when the mountant will flow ahead of the descending glass without trapping air bubble between the cover slip and slide. The excess of mountant on the slides was removed with xylene soaked in cotton. After mounting, the slides were allowed for drying. The excess of mountant on the slides was removed with xylene soaked cotton.

Labeling and storing: Labeling was done on the slide by glass marking pen to avoid future confusion. The slides were stored in slide box to protect them from dust and dirt.

Microscopic observation: The histological sections on the prepared slides were thoroughly observed under Advanced Trinocular Microscope (Olympus, MODEL 8 x 51, Japan) microscope at different magnifications. The developmental stages of germ cells in the testes and changes of the oocytes of ovary were noticed carefully. Colour photomicrographs of selected histological sections were taken as and when required.

3. Results and Discussion

3.1. Histological Observation

Testicular cyclicity: On the basis of histological observations of testes for 6 months, the seasonal testicular cycle of *Anabas testudineus* was categorised as follows: During January to February, tunica albuginea was thick and seminiferous lobules were not dense and less spermatogenic activity was seen. During this period, primary and secondary spermatogonia are seen (Plate 1, Table 4). Histological observation revealed that testicular and lobular wall were thin. The lobules were filled with spermatozoa and sperm cells (Plate 2, Table 1).

In teleosts, the testes are either lobular or tubular in the lobular type of testes, large numbers of lobules are present and they are separated from each other by a thin fibrous connective tissue layer. This type of testes is found in most of the fishes. Histological structure of testes is variable from species to species. According to Gaurya *et at*. (1995) [7], in the testes of fish, when undergoing reproductive activity (spermatogenesis) in the lobules, about six spermatogenic elements are produced from sperm mothers cells of germinal epithelium and passes through different maturation stages as primary spermatogonia secondary spermatogonia Primary spermatocytes, secondary spermatocytes spermatids and spermatozoa (Sperms). The spermatozoa contains nucleus, cytoplasmic components and acquire flagellum for mobility (Nagahama, 1983; Agarwal, 1996) [11, 1].

In this study, the testicular cyclicity of *A. testudineus* is concerned with the preparatory phase and spawning phase. In different species, the spermatogenic activity starts at different times of the year (Rai, 1965; Nair, 1966; Shresthra and Khanna, 1976; Nautiyal, 1985) [14, 12, 22, and 13]. In the present study, it was observed that *A. testudineus* attained maturity only during March to June, which is considered as breeding season. This result is corroborated with the observation of Mookherjee and Mazumder (1946) [9] in the *Anabas* species.

Ovarian cyclicity: During January and February, more number of immature oocytes stages like stage I, stage II and stage III are seen. The stage was characterised by the formation of minute yolk vesicles (early yolk vesicles) in the peripheral ooplasm. In the oocytes, at late perinucleolus stage the oocytes were observed with increased ooplasm (Plate 3, Table 2).

During March to June, the ovaries were very much enlarged and reached their maximum size and occupied the whole body cavity (Plate 3). In this phase, the ovarian wall became thin and the oocytes of late yolk vessicle stage and early yolk stage were found more in number than the late perinucleolus stage and early yolk vessicle stage. During this period, the oocytes showed an increased in size and ovaries are filled with ripe eggs (Table 1, Plate 4).

The seasonal ovarian cycle has been differentiated by number of workers based on several external morphological features and macro and microscopic factors like GnSI, ova diameter and histological study (Sathyanesan, 1959; Shrestha and Khanna, 1979; Agarwal, 1996) [20, 21, 1].

Histological observation of ovaries showed the different stages of ova in different quantity, which in turn regulate the ovarian cycle. It is covered by an ovarian wall and encloses a cavity, the ovocoel. During the spawning period the tunica albuginea is a thin layer but during the rest period, it is a thick layer. The germinal epitheleum consists of a single layer of cuboidal cells which possess very little amount of cytoplasm.

The maturation cycle of *A testudineus* based on the histology of ovary and Gonado Somatic Index, was divided into viz Preparatory phase, Maturing phase and Ripe phase This observation can be correlated with the study of Agarwal (1996) [1] in *S. plagiostomus* and Azad (1990) [3] in *Anabas.* In the preparatory phase, immature oocytes stages like stage I stage II and stage III are found. So this study is resembled with Arocha (2002) [2] in sword fish. In the spawning phase, the ovarian wall became thin and the oocytes of late yolk vessicle stage and early yolk stage were found more in number than the late perinucleolus stage and early yolk vessicle stage. The oocytes showed an increased in size and ovaries are filled with ripe eggs. This study can be corroborated with the study of Belsare (1962) [5] in the ovary of *Ophiocephalus punctatus.* It can be concluded from the study that *Anabas testudineus* breeds mainly during monsoon season and its peak spawning season is May to June.

Table 1: Histological changes and testicular cycle of *Anabas testudineus.*

Month	Histological changes observed	Testicular Cycle
January and February	Primary and secondary spermatogonia are seen	Pre-spawning
March	Primary spermatogonia and primary spermatocytes were present	Spawning
April	Labulars were completely packed with spermatozoa	
May & June	Te sticular wall become thinner and thin lobular wall and full of spermatozoa were present	

Table 2: Histological changes and ovarian cycle of *Anabas testudineus.*

Month	Histological changes observed	Testicular Cycle
January and February	More Number of immature oocytes stage-I, stage II and Stage-III are seen	Pre-spawning
March	The oocytes of late yolk vesicle stage and early yolk stage are	Spawning
April	Increase in size of oocytes	
May & June	Ovary is filled with ripe eggs	

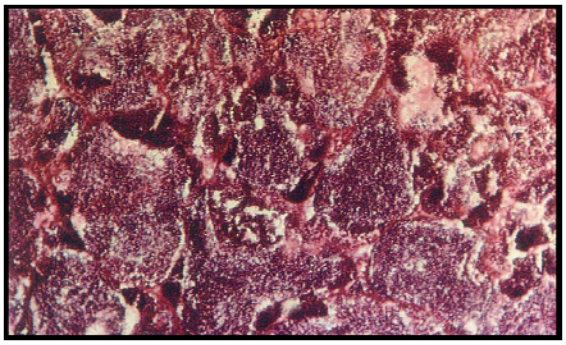

Plate 1: Photomicrograph of the testes (T.S) showing the seminiferous tubules filled with less numbers of primary spermatogonial (PSG) cells: E & H. X20.

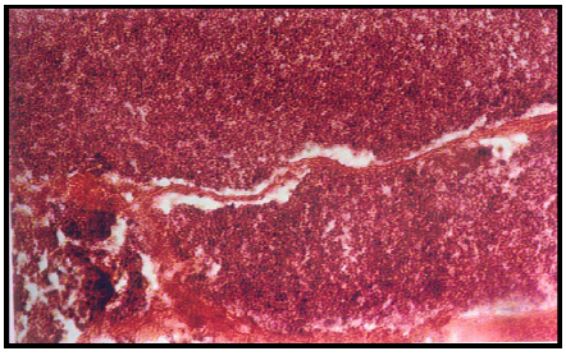

Plate 2: Photomicrograph of the testes (T.S) showing tunica albuginia (T. A.) and seminiferous tubules (S.T.) filled with sperm cells: E & H. X20.

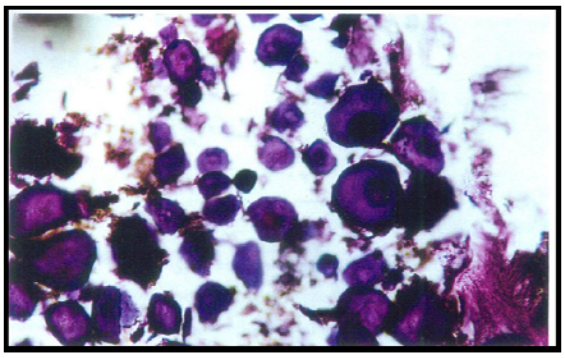

Plate 3: Photomicrograph of the ovary (T.S) showing the immature oocytes: E & H. X20.

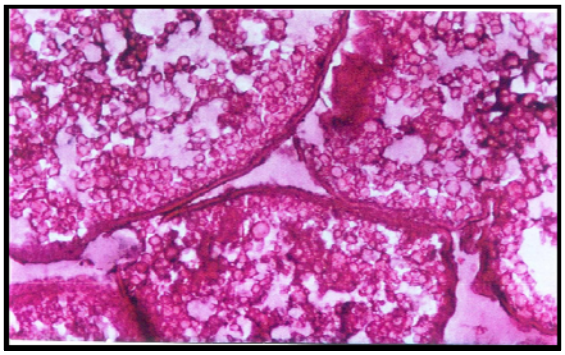

Plate 4: Photomicrograph of the ovary (T.S) showing the immature oocytes: E & H. X20.

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