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# Seasonal Histological Changes of Testis in *Channa orientalis* Bloch & Schneider, 1801

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## Abstract

A total of 164 male *Channa orientalis* Bloch & Schneider, 1801 sampled from Thanmataw Village, Patheingyi Township were used to evaluate the reproductive condition during June 2007 to May 2008. Seasonal histological changes of testis were analyzed. Based on seasonal histological changes of testis, the reproductive cycle of the above species is designated into prespawning season (January – February), spawning season (March – October) and postspawning season (November-December). The testis development was microscopically characterized into five stages (proliferation, early developing, late developing, ripe and spent). The highest % of spermatozoa were found during March-October.

Keywords: Seasonal, histological changes, testis, *Channa orientalis*

## Introduction

The reproductive biology of fish has long been widely investigated. The study of the reproductive biology of fish is very important for proper planning of fishery management (Abdel and El-Greisy, 2005). Reproduction in fishes is the process by which species are perpetuated and by which in combination with genetic change, characteristics for new species first appear. The study and observation of gonadal development of fish at different seasons is one of the part of contributions to fish breeding, hoping more investigations concerned with the fish breeding is essential to be done (Lagler *et al*., 1962).

One of the most important studies of fishery biology is to determine the annual breeding cycle of a fish species. There are a few methods, such as gonad indexes, staging based on the external appearance of the gonad and measurements of gonad size and histology to assess the stage of gonad development of individual fish. Despite expensive and time-consuming procedures being used to determine gonad development, the most accurate technique is histological analysis (West, 1990).

Most utilized macroscopic staging of gonads and histological interpretation of gonads development may lead to rehabilitation measures.

Histological examination of gonads is a useful tool for assessing the maturity stage of fish (Crook and Robertson, 1999).

The histological description of gonad structure is fundamental to understanding reproduction. The spawning period in teleosts is determined from changes occurring within the gonad throughout the year. The macroscopic or histological observations of the gonad (qualitative method) and evaluation of oocyte diameter (quantitative method) are commonly applied (Karlou-Riga and Economidis, 1997) (cited in Garcia-Diaz *et al*., 2006).

The basic function of the teleost gonads like those of the higher vertebrates, is to produce gametes. During past few decades, several comprehensive studies pertaining to the gonadal morphology, gamete production by gonads have been made with the help of classical histological techniques (Sathyanesan, 1959; Rai, 1965; Nair, 1966; Lehri, 1967).

Gonadal staging is common in fisheries science. Numerous authors have usually limited their descriptions of the fish reproductive cycle to listing the maturity stages of gonads they examined. The dynamic of gonadal maturation is also a good tool to indicate the time of fish reproduction.

Thus, the reproductive process is an essential part of the biology of species. In addition, an understanding of the gonadal deveopment and reproductive cycle is of fundamental importance for culture purpose and fishery management.

The objectives of the present study are:

- to observe the seasonal changes in the testis
- to assess the reproductive season by determining the microscopic structure

## Materials and Methods

### Collection of the Samples

Specimens of *Channa orientalis* were collected from Thanmadaw Village in Patheingyi Township. Collection was made from June 2007 to May 2008.

### Histological Preparation of Testis

The testes from collected fish were removed and fixed in 10% formalin till the time of preparation of slides. Just before the preparation of slides, the tissues were transferred in Bouin's fluid prior to dehydration. The tissues were

then washed with 70% ethanol and embedded in paraffin. The tissues were sectioned at 5-7 μ and stained with haemotoxylin and eosin.

**Analysis of Histological Preparation**

Testis development was determined histologically by light microscope. Stages of spermatogenic cells were determined based on the histological criteria adapted from Htun Han, 1978b; Fujita *et al*., 1997; Calvo *et al*., 1999; Carrasson and Bau, 2003; Dahle *et al*., 2003; Arockiaraj *et al*., 2004; Sanchez-Cardenas *et al*., 2007. Histological appearance of the testis was taken as photomicrographs aided by Olympus microscope digital camera DP 12 (Bx 41 TF, Japan).

**Calculation of Mean Percent Frequency of Different Spermatogenesis Stages**

Among the prepared slides for each month, five well-prepared slides that represented the monthly maturity stages were selected. Three sections from each one of five slides were chosen. Again from selected each section, five areas were selected and counted the different maturity stages of testis.

The number of different maturity stages from each one of five areas were pooled and taken the mean value to represent each section. Again, the mean number of each one of three sections were pooled and taken the mean value to represent each slide. Further, the mean number of each one of five slides were pooled and taken the mean value to represent each month. Finally, the mean percent frequency of different spermatogenesis stages were calculated.

## Results

**Seasonal Changes in the Testis**

Spermatogenesis of *Channa orientalis* was divied into five stages, namely, proliferation (S I), early developing (S II), late developing (S III), ripe (S IV) and spent (S V).

Stages of maturity, macroscopic appearance and histological differentiation of testis at different reproductive periods are given in Table 1.Monthly changes in frequency (%) of spermatogenesis stages are given in Table 2 and Fig. 1.

Proliferation stage (S I) were found all year round. Early developing stage (S II) and late developing stage (S III) occurred during the months of January-February, when the frequency(%) of spermatozoa reached lowest level (Table2, Fig.1 and Plate 1A,B,C ).

Ripe stage (S IV) was observed from March-October, when the frequency (%) of spermatozoa reached the highest level (Table 2, Fig.1 and Plate 1 D). Spent stage (SV) was seen during the months of November-December (Table 2, Fig.1 and Plate 1 E ).

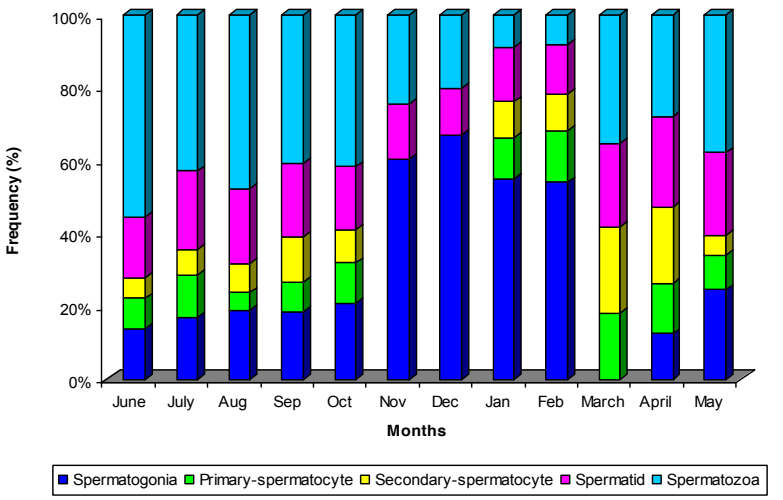


Fig. 1 Monthly changes in frequency (%) of spermatogenesis stages in *Channa orientlis*

Table.1 Stages of maturity, macroscopic appearance and histological differentiation of testis at different reproductive periods in male *Channa orientalis*

| Spawning periods | Maturity Stage | Stage | Macroscopic appearance | Histological differentiation | Period |
|---|---|---|---|---|---|
| Prespawning | Proliferation (Immature) | S I | small and compact, with a reddish-white colour | - Only spermatogonia are present either singly or in small groups. Somatic cells around the spermatogonia cells were clearly visible. | January - February |

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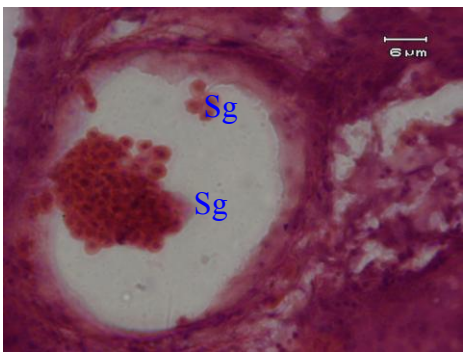
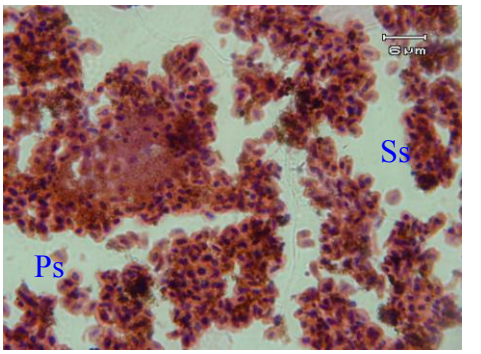
| | Early developing (Immature) | S II | Reddish-white or creamy-white | - The bulk of testis made up of primary spermatocyte, secondary spermatocyte and a few spermatids. | |
| | Late developing (Maturing) | S III | slightly larger, with a pale cream colour | - Secondary spermatocytes and spermatids predominate; a few ripe spermatozoa present but are attached to lobular wall. | |
| Spawning | Ripe (Fully mature) | S IV | Robust, with yellowish or creamy white | - Some spermatids present, but mainly consistof ripe spermatozoa unattached in the lumen. | March - October |
| Postspawning | Spent | S V | Flaccid, with a cream brownish colour | - Lumen of testis looks unfilled; a few spermatozoa left; many spermatogonia nests present. | November - December |

Table 2 Monthly changes in frequency (%) of spermatogenesis stages in *Channa orientalis*

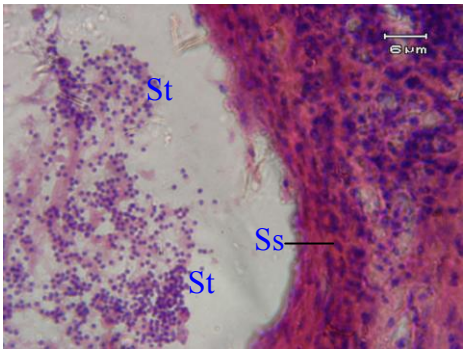
| Month | Spermatogonia | Primary-spermatocyte | Secondary-spermatocyte | Spermatid | Spermatozoa |
|---|---|---|---|---|---|
| June, 07 | 13-89 | 8.33 | 5.56 | 16.67 | 55.56 |
| July, 07 | 17-14 | 11.43 | 7.14 | 21.43 | 42.86 |
| Aug, 07 | 19-05 | 4.76 | 7.94 | 20.63 | 47.62 |
| Sep, 07 | 18-69 | 8.13 | 12.19 | 20.33 | 40.65 |
| Oct, 07 | 20-83 | 11.11 | 9.03 | 17.36 | 41.67 |
| Nov, 07 | 60-61 | - | - | 15.15 | 24.24 |
| Dec, 07 | 67-00 | - | - | 13.00 | 20.00 |
| Jan, 08 | 55-00 | 11.25 | 10.00 | 15.00 | 8.75 |
| Feb, 08 | 54-05 | 14.32 | 10.00 | 13.51 | 8.11 |
| March, 08 | 11-00 | 16.12 | 21.19 | 20.42 | 31.26 |
| April, 08 | 12.5 | 13.89 | 20.83 | 25.00 | 27.78 |
| May,08 | 24.53 | 9.43 | 5.66 | 22.64 | 37.74 |



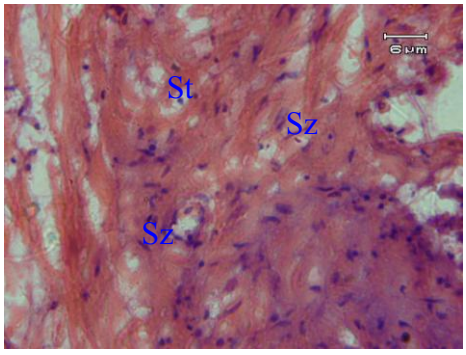

(A) Proliferation stage



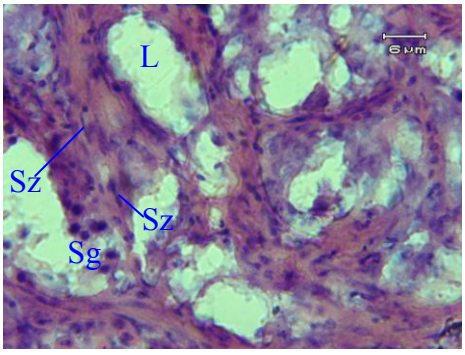

(B) Early developing stage




(C) Late developing stage




(D) Ripe stage




(E) Spent stage

Sg= spermatogonia, Ps = primary spermatocyte, Ss = secondary spermatocyte, St= spermatid, Sz = spermatozoa, L = lumen

Plate 1 Different stages of spermatogenesis in section of *Channa orientalis* testis

## Discussion

The histological description of gonad structure is fundamental to understand reproduction. The onset of maturity varies considerably among different populations of the same species, different species and also within the limits of a single population (Nikolsky, 1963).

Spermatogenesis was divided into five stages according to the most advanced type of germ cells present. They are proliferation (S I), early developing (S II), late developing (S III), ripe (S IV) and spent (S V).

Vazzoler (1996) gonadal maturation stages categorized the based on the variation of frequencies in gonad (cited in Lampert *et al*., 2004). In the present work, proliferation (S I), early developing (S II) and late developing (S III) stages were commonly observed during January-February. The lowest frequencies (%) of spermatozoa were 8.11 in February and 8.75 in January. Thus it is assumed that this period is prespawning time.

The highest frequencies (%) of spermatozoa were 55.56 in June and 47.62 in August. All stages of germ cells especially the most of ripe stages (S IV) were seen in March – October, suggesting the spawning time.

The frequency (%) of spermatozoa decreased to 24.24 in November and 20.00 in December and spermatids and many spermatogonia are found during the period of November-December. Thus these months are designated as postspawning time.

S I, S II and S III were observed during the prespawning period and the testes contained all germ cell types with the most spermatogonia and few spermatozoa.

S IV was observed during the spawning period. The thin testicular and lobular wall and the presence of spermatozoa were the characteristic features of this phase. However, some empty lobules were also present.

As spawning progressed, remaining early germ cell types became less frequent in the testes. This was characterized as spent stage (S V). The testes contained large empty lobular space except for some residual spermatozoa and spermatogonia.

Histological observation of gonad revealed that *C. orientalis* has a prolonged period of reproductive activity that extends from March to October.

## Conclusion

Studies of fish reproduction are biologically and ecologically important and constitute a valuable tool in assessing data for fisheries management purpose. The data included in the work provide the most accurate information on the reproductive biology of *C. orientalis*, much of which is needed for the current fishery management plan.

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