Gonadal Developmental Stages of *Mystus gulio* in Twante channel at Yangon Region

Myint Myint Win[1], Thinzar Aung[2], Kyu Kyu Win[3]

Abstract

The present work was carried out gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (K) and the macroscopic and microscopic structure of gonad to predict the breeding season of the studied fish, *Mystus gulio*. The sample was monthly collected from the Twante canal, Yangon Division during the period of June 2017 to December 2018. According to the present finding, the highest GSI value of both female and male of *M gulio*, (22.93 ± 7.49, 2.57 ± 3.56,) was observed in June and the value of HSI was highest (3.31 ± 2.31, 1.77 ± 0.74) in August. From the histological study, all stages of oocytes were observed in June. Process of spermatogenesis was distinguished into four categories; spermatogonia, spermatocyte, spermatid, and spermatozoa while the process of oocyte development was identified into eight stages; Chromatin nucleolus stage, Prenucleolus stage, Primary yolk stage, Secondary yolk stage, Tertiary yolk stage, Germinalvesicle migration stage, Germinalvesicle breakdown stage and Spent stage. The spawning stage for males was found in June, July, and for females was May, June, and July. The size of the oocytes was ranged from 59.67 to 652µm. The highest cell percentages of germinalvesicles migration and germinalvesicles breakdown were observed in both months, June and May.

Key words: *Myistus gulio*, gonadosomatic index, hepatosomatic index, condition factor, macroscopic and macroscopic structure

Introduction

In Myanmar, the reproductive biology of marine fish and freshwater fish has been studied from different habitants in the natural water system and culture system. Fishes all over the world exhibit great diversity in reproductive strategies and associated traits (Helfman *et al*, 1997). A piece of complete knowledge on the reproductive system and biology of fishes is critically important to understand the reproductive strategy and annual reproductive cycle of many fish species and also the successful management of fisheries (Unver and Saraydin, 2004). Global importance of breeding of fish is for food, for recreation and aesthetic needs, or in the context of conservation of natural species against environmental harm. It is known that the ovarian cycle in the majority of freshwater teleosts which are seasonal breeders undergoes remarkable changes during various periods of the season. In fisheries study, the particular event of interest in the reproductive cycle of a species is the time of spawning, when fully developed gametes are released (King, 1995). The effectiveness of a histology classification system is for quantifying reproductive life-history traits and identifying the reproductive maturity stage (Cushion *et al*., 2008).

Mystus gulio (Hamilton Buchanan,1822) commonly known as long whiskered catfish is a euryhaline fish, occurring mostly in freshwater and has also been found to thrive in backwaters of low salinity (Pandian, 1966). *Talwar and Jhingran* (1991) have reported that this fish primarily inhabits brackish water and also enters and lives in freshwater. The market demand of this species as food fish is good as it's delicious taste (Haniffa, 2009; Begum *et al.*, 2010) and recently has been documented to be exported as indigenous ornamental fish India (Gupta and Banerjee, 2014). A piece of complete knowledge on the reproductive system and biology of fishes is critical to understand the reproductive strategy and annual reproductive cycle of many fish species. Information on reproductive biology is very much essential for the

[1] Lecturer, Dr., Department of Zoology, University of Mandalay
[2] Lecturer, Dr., Zoology Department, Bamaw University
[3] Professor(Retd:), Dr., Zoology Department, Lasho University

development of the aquaculture industry. Therefore the present study was carried out the reproductive strategy of *Mystus gulio.*

Materials and Methods

Study Area

The present work was carried out in Twante channel (between Twantewa village and Phayangoteto Village) 16˚ 41′ 14.92″ N and 95˚51 ′ 29.59″ E and 16 ˚ 43 ′ 28.79″ N and 96˚0 ′ 16.09″ E (Fig 1).

Study Period

The study period lasted from June 2017 to May 2018.

Sample Collection

A total of 351 of *Mystus gulio* were collected from the local fisherman at the study site during the study period (Plate 1).

Identification and Classification

The collected samples were identified according to Talwar and Jhingran (1991) and Jayaram (2010).

Morphometric Measurement

The monthly fish specimen was caught with the help of fishermen and total length (tip of the snout to the end of the caudal pentacle), Standard length (tip of the snout to the end of the caudal fork) was measured to the nearest millimeters (mm) by a measuring scale.

Total body weight was measured (g) by an electronic balance. Fish specimens were dissected out ventrally to remove gonads carefully and then surface moisture of gonads was removed using blotting paper. They were photographed and weighted before fixation in 10% natural buffer formalin for histology.

Gonadosomatic Index (GSI)

Gonadosomatic Index (GSI) for each fish was calculated using the formula, (Nikolsky,1963).

GSI = GW/BW x 100,

Where GW = gonad weight of fish in grams (g) and BW = Body weight of fish in grams.

Hepatosomatic Index (HSI)

Hepatosomatic Index (HSI) for each fish was calculated using the formula, (Biswas, 1993).

HSI = LW/BW x 100,

Where LW = liver weight of fish in grams (g) and BW = Body weight of fish in grams.

Condition Factor ‘K’

The condition factor ‘K’ for each fish was calculated using formula (Jones, 1970)

$K=W/L^3$ x100

K=condition factor, W=Body weight of fish in grams, L=Body length of fish in mm

Macroscopic and Microscopic observation of Gonad Maturation Stages

Macroscopic Examination

The gonads appearance, color, size, space occupied in the body cavity, size of the gonads were noted down. Macroscopic gonads maturity stages were classified according to Jacob (2005), Bucholtz (2008), and Selman (2003).

Microscopic Examination

For the histological study, gonads (testes and ovary) were collected by dissecting out the fish. The tissues were trimmed into 5 to 6 mm size for better penetration of fixatives into it. The tissues were put into 10% neutral buffer formalin for 24 to 48 hours as per the size of tissues.

Microscopic Observation

The histological sections on the prepared slides were thoroughly observed under light microscopes at different magnifications. The developmental stages of germ cells in the testes and changes of the oocytes of ovary were noticed carefully. Color photomicrographs of selected histological sections were taken as and when required. Developmental stages of gonads were classified according to Jacob, 2005, Lambert-JG, 1970, and Selman-K *et al*, 1993.

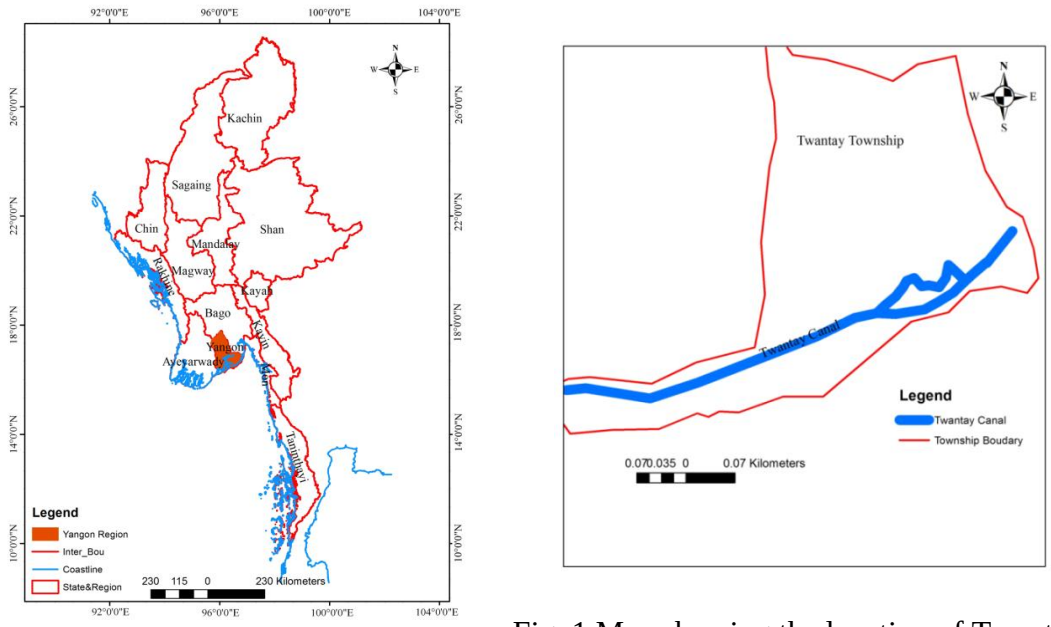


Fig. 1 Map showing the location of Twante canal

Source: Geography Department, Institute of Education

Results

Gonado-somatic (GSI) and Hepato-somatic (HSI) Index and Condition Factor (K)

The highest GSI value of females was 22.93 ± 7.49 in June and the lowest (0) in August. The highest HSI mean value (3.31 ± 2.31) was observed in August and the lowest HSI mean value (1.35 ± 0.36) was observed in October. Condition factor (K) value was highest (1.25 ± 0.31) in September and the lowest (1.01 ± 0.18) in July. In the male, the highest value of GSI (2.57 ± 3.56) was found in June followed by a steep decrease until August (0). The highest HSI mean value (1.77± 0.74) was observed in August and the lowest (1.15± 0.25) in December. The condition factor (k) was the highest 1.11± 0.41 in July and the lowest 0.82 ± 0.25 in May (Fig. 2, 3) (Table 1, 2).

Structure of Reproductive Organs

The ovary was elongated sacs, paired organs. Right, and left ovaries were fused forming a single, medium ovary. The right ovary was slightly longer than the left and observed various sizes according to their maturation stages. Testes were elongated paired organs, Y-shaped and consisting of numerous finger-like projections. Length and sizes were varied according to their maturation stages. Testes were located in the posterior half of the body cavity, just ventral to the trunk kidney (Plate 1 A, B).

Macroscopic Developmental Stages of Female Reproductive Organ

Immature Ovaries

The immature ovary was a small, elongated, transparent, and narrow tube-like structure. The right ovary was slightly longer than the left. Oocytes were not visible with the naked eye. Immature ovaries were found from September to November (Plate 2 A).

Developing Ovaries

Developing ovary was slightly larger than the above stages. The ovary was observed transparent in color and ova was visible through the thin ovarian wall. This stage was found from December to March (Plate 2 B).

Mature Ovaries

Ovaries size was became larger than the previous stages and filled with third-fourth of the body-cavity. The ovary was contained distinctly visible oocytes with obvious blood vessels. Ova were translucent and yellowish. This stage was found from April to May (Plate 2 C).

Ripe Ovaries

The ovary was yellow and filled the entire length of the body cavity. The ovarian wall was thin, ova were quite distinct and spherical.This ovary was found in June (Plate 2 D).

Spent Ovaries

The ovary was much shrunken, blood-shoot, and contained numerous small ova.This stage was found from July to August (Plate 2 E).

Macroscopic Developmental Stages of the Male Reproductive Organ of *M.gulio*

Immature Testes

Testes were tiny, thin, and transparent in color. This stage was found from September to November (Plate 3 A).

Developing Testes

Developing testes were found from December to March. In developing stage testes lobes were increased in size and length, slightly thicken whitishly and occupied one half of the body cavity length (Plate 3 B).

Mature Testes

Mature testes were observed from April to May. Testes were reached the largest, more than one half of the body cavity, and released milt on applying pressure on the abdomen (Plate 3 C).

Ripe Testes

In the ripe stage, testes were occupied two-thirds or more of the body cavity. The weight and volume were more increased than the mature stage. Testes were becoming opaque and milky white. Ripe testes were observed in June (Plate 3 D).

Spent Testes

Spent testes were found in August. Testis shape was found like fats in the body cavity.

Microscopic Developmental Stages of Oogenesis of *M. gulio*

Oogonia were a very small spherical cell. Cell diameter was ranged 35.28±10.26 μm, thin indistinct peripheral cytoplasm was observed. A large pale nucleus has appeared which ranges in diameter from 10.83 to 18.99 μm.

First Growth Phase

Chromatin-nucleolus Stage

In this stage, the cell was the polygonal or hexagonal shape, varied in diameter with 59.67 ± 15.01 μm. Nucleus (N) was a large spherical which varied in diameter with 50±16.96 μm. Nucleoli (Nu) were varied in number between 2 to 4 (Plate 4 A, Fig. 3).

Pre-nucleolus Stage

It was the final stage of the immaturities period. The oocyte was 75.25± 9.01μm in diameter. Nucleus (N) was increased in size and reached to 50.5± 9.3 μm in the average with several nucleoli (Nu) about 6 to 17. Nucleoli were arranged in the periphery of the nucleus, diameter ranged from 2.8 to 4.6 μm (Plate 4 B, Fig. 3).

Second growth phase

Primary Yolk Stage

In this stage, yolk vesicles have appeared in the periphery of the cytoplasm. Oocytes size was reached to 309.66 ± 61.62 μm. Nuclear diameters were ranged from 56.25±18.98μm. The outer membrane was surrounded by zona radiate (Zn) coated with a follicular epithelial layer (Plate 4 C, Fig. 3).

Secondary Yolk Stage

The yolk globules have appeared in the periphery of the cytoplasm. Oocytes were range in diameter 418 ± 90.65 μm. Nucleus appeared granulated with irregular boundary and its diameter range from 38 to134 μm. Nucleoli were stilled ranged on the periphery of the nucleus. Yolk vesicles were distributed at the outer border of the cytoplasm. The average thickness of the yolk granule was 204 μm, the thickness of the zona radiata was 2 μm and coated with follicular epithelium (Plate 4 D, Fig. 3).

Tertiary Yolk Stage

Yolk accumulation was preceded rapidly. In this stage, the oocyte was remarkably increased diameter of 593.33 ± 95.28 μm. Nucleus was appeared with a diameter of 98 μm on average. Yolk granules were densely packed, occupied mostly the total volume of the cytoplasm. Zona radiata was increased in thickness varying from 2 to 4 μm. The follicular epithelium was about 4 to 16 μm in thickness (Plate 4 E, Fig. 3).

Germinal Vesicle Migration Stage

Oocyte diameter was ranged from 500 to 780 μm. Nucleus (N) diameter was ranged from 16 to 112 μm. Nucleus (N) was migrated towards the periphery. Nucleus (N) became amoeboid in shape. Nucleoli (Nu) were scattered in the nucleus and reached 52 in number. Zona Radiata (Zn) was reached about 3.4 μm in diameter. The follicular epithelium was recorded at about 6.2 μm in an average (Plate 4 F, Fig. 3).

Germinal Vesicle Breakdown Stage

In this stage, the maturation process was complete. Oocyte diameter was 652 ± 68.35 μm. Zona radiate (ZR) became well-differentiated to reach about 4μm. Nucleus (N) could not be seen, lost in the cytoplasmic mass. The nuclear membrane was disappeared and lost its shape and became nuclear material (Plate 4 G, Fig. 3).

Postovulatory Follicle Stage or Spent Condition

In this stage, deplete ovary nest and loosely yolk granules were found. Primary oocytes were present in large numbers. Remnants of the atretic oocytes of the preovulatory ovary were seen (Plate 4 H, Fig. 3).

Microscopic Developmental Stages of Spermatogenesis of *M.gulio*

Spermatogonia Stage

Spermatogonia were the first appear cell and observed as the nest. Spermatogonia were large in size and spherical in shape. They distributed all along the germinal epithelium (Plate 5 A).

Spermatocytes Stage

In developing testis contained spermatogonia and spermatocytes. Spermatocytes were formed from the spermatogonia. Nucleolus was not visible in all cells (Plate 5 B).

Spermatids Stage

The Spermatids were produced from the spermatocytes. Spermatocytes were much smaller, compact dark dot-like structures. They appeared as deeply stained with hematoxylin and eosin (Plate 5 C).

Spermatozoa Stage

Spermatozoa were the smallest size in spermatogenesis with distinct tail and darkly stained nucleus and they derived from Spermatids. Accumulation of mature spermatozoa was found in the lumen of seminiferous lobules (Plate 5 D).

Monthly Percentage Composition of Gonad Maturity Stages

In *Mystus gulio*, only two stages of immature; chromatin nucleolus stage (47.55%) and prinucleolus stage (52.45%) were observed in September. Seven developing stages; chromatin nucleolus (16.18%), prinucleolus (3.12%), primary yolk (2.25%), secondary yolk (0.27%) and tertiary yolk (3.38%), germinal vesicles migration (9.52%) and germinal vesicles breakdown (61.68%) were found in June (Fig. 4).

Discussion

Developmental stages of gonads of four species; *Mystus gulios* were examined from Twante canal, Yangon region.

Among teleost fishes, the testes are generally a paired organ that develops longitudinally, elongating horizontally concerning the body axis, even though testes morphology varies among species, and they are located either dorsally or ventrally in the

abdominal cavity (Jobling, 1995; Helfman *et al*., 1997). In the present result, testes were examined on the dorsal side of the body cavity in *Mystus guli*.

In most teleosts, the ovary is a hollow paired organs, however, in some species paired structures become fused into one solid, single organ during their early development (Priyadharsini *et. al* 2013). The ovaries were observed elongated sacs, paired organs, and right and left ovaries fuse forming a single, medial ovary and two lobes were not the same size according to this result.

According to the results of gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (K), the peak value of GSI was found once a year, the highest value of GSI was found in June. The value of GSI and HIS were negatively correlated as the oocyte development in fish is intimately associated with hepatic synthesis of egg-yolk precursor protein vitellogenin which is secreted into the blood and ultimately transported to developing oocyte and deposited as yolk (Sudarshan and Kulkarni, 2013). However, in the recent study results coincide with Sudarshan and Kulkarni, 2013 in male of *M.gulio* (Fig. 4.2, Fig. 4.8).

Different authors have divided the gonad developmental stages according to their prominent features of changes during the process of gametogenesis (Jacob, 2005; Thida Aung, 2006; Tin Hnin Wai, 2010). Based on gross morpho-histological changes occurred in the ovary and testes, the occurrence of developing oocytes was divided into eight stages, and observation of developing spermatogonia was divided into four stages during the study period.

Oogonia give rise to immature oocytes with multiple peripheral nucleoli, in which the number of nucleoli was not more than 20 in the present study. Yolk vesicles were the first type of inclusions appearing in the cytoplasm of second-growth phase oocytes. These vesicles were likely to contain lipid, as they appeared as empty non-staining vacuoles on paraffin sections, suggesting that their contents were dissolved during routine histological procedures (Jacob, 2005). In the present study, during oocyte maturation, the lipid vesicles were found in the peripheral of the cytoplasm, which might help the eggs to keep buoyant but the cytoplasm was filled with yolk granules instead of lipid droplets. Zona radiate was also distinctly appeared in the mature stage of oocytes in this study. The distinction between germinalvesicle migration and germinalvesicle breakdown was histologically differentiated as the migration of the nucleus to the animal pole in the former stage and absent of the nucleus in the later stage.

As a result of histological stages of testes, accumulation of unstained spermatogonia and dot-like structure of stained spermatocytes were dominant over immature stages, all stages, spermatogonia, spermatocytes, spermatids, and spermatozoa were observed in developing. The abundance of a deep stain, basophilic, and oval-shaped head with distinct tail spermatozoa were observed in maturation stages during this study period.

During oogenesis, the size of the oocytes increased considerably due to a progressive accumulation of lipid and protein-yolk within the cytoplasm by vitellogenesis (Montchowui *et al*. 2012). The present study agrees with this statement as the sizes of oocytes were larger nearer to the germinalvesicle breakdown stage, ripe stage (59.67 µm to 652 µm).

The monthly GSI analysis and histological findings of the present study showed that ovarium types *were* group synchronous and this species spawn once in a breeding season. According to the finding results, the highest mean value of GSI corresponds to where the gonads were at ripe and while the lowest value indicates spent stage or starting developing stage. There are seven developmental stages; chromatin nucleolus stage, prinucleolus stage, primary yolk stage, secondary yolk stage, tertiary yolk stage, germinal vesicle migration stage, and germinal vesicles breakdown stage were found in the month where the values of GSI was highest. However, the percentage of germinalvesical migration and germinalvesicle breakdown

stages was dominant over the other stages. In the findings of this study, the highest percentage of germinalvesical migration and germinalvesical breakdown occurred in June.

Indeed, the present finding of the different developmental stages of gonads of the studied fish species from natural brackish water, Twante Canal will provide valuable information of fishery resources concerning with their breeding season and also evidence outcomes for the closing season to conservation and management on threatened fishes from natural water fishery resources.

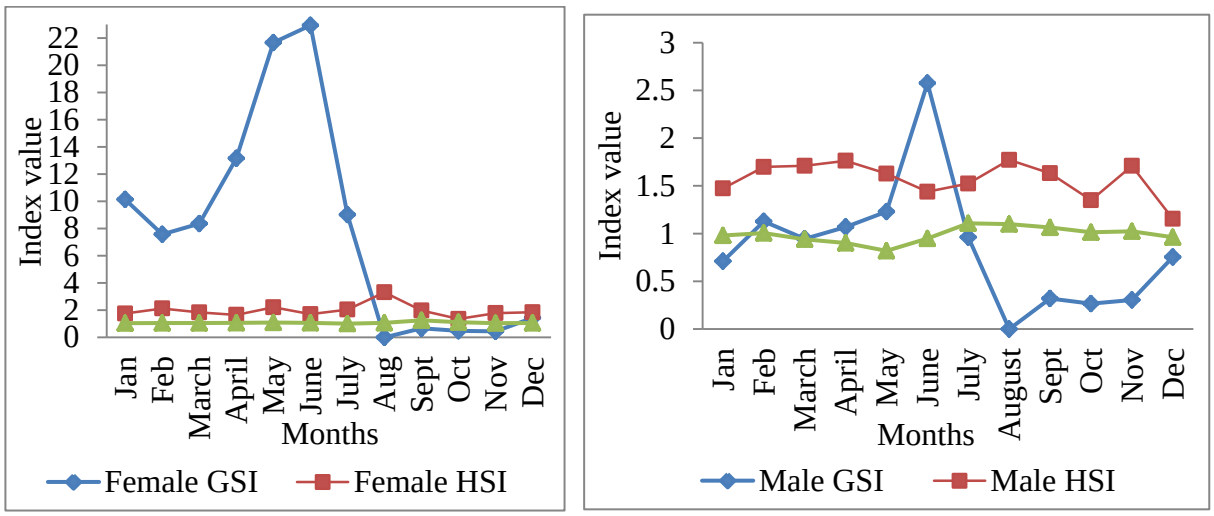


Fig. 2 Monthly changes in GSI, HSI and K of *Mystus gulio* (female and male)

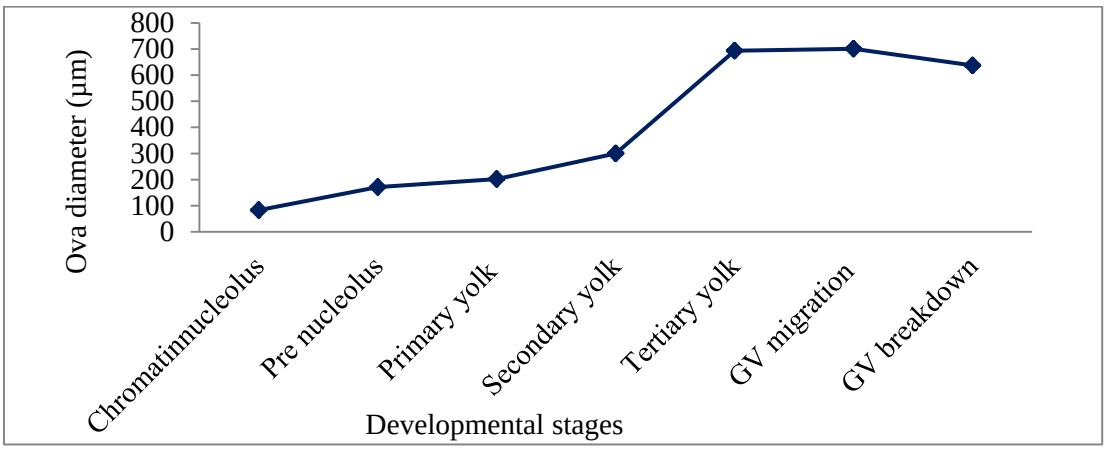


Fig. 3 Measurement oocyte diameter for *Mystus gulio*

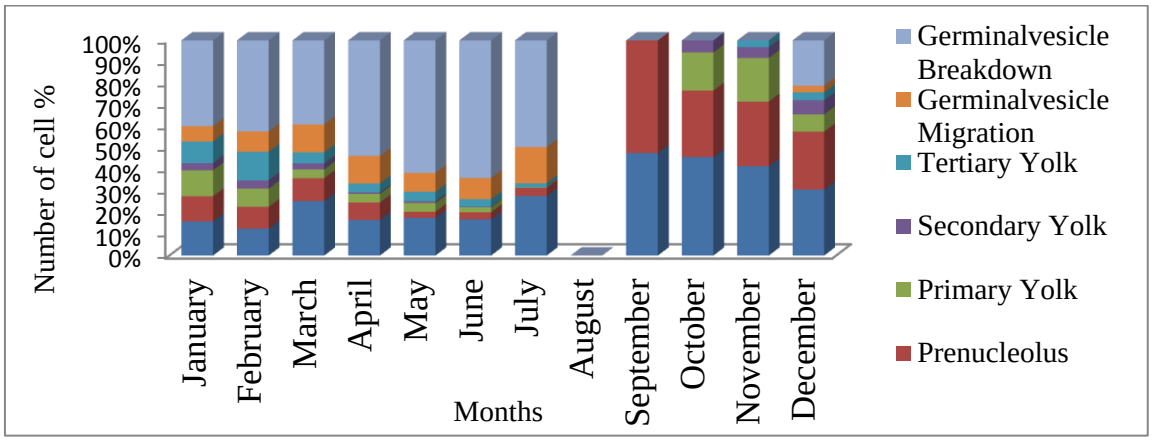
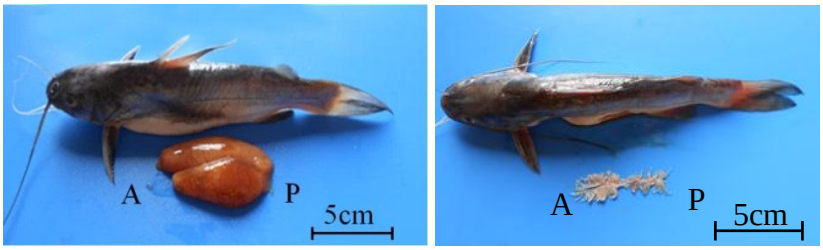
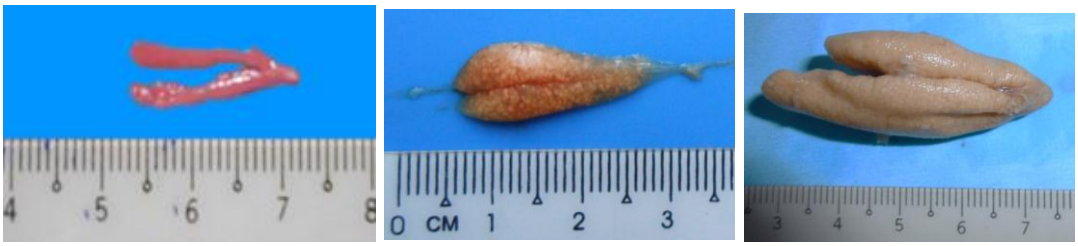


Fig. 4 Monthly cell composition (%) of *Mystus gulio*

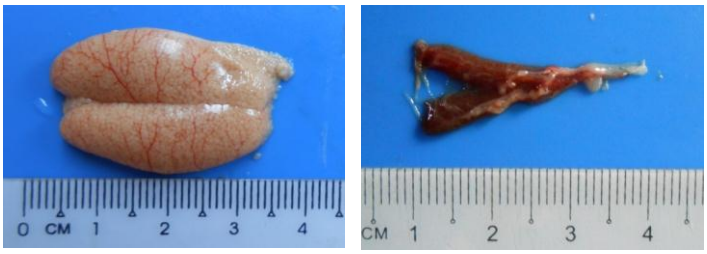


A. Ovary of *Mystus gulio* B. Testis of *Mystus gulio*

Plate 1 Reproductive organs of *Mystus gulio* (A = Anterior part , P = posterior part)

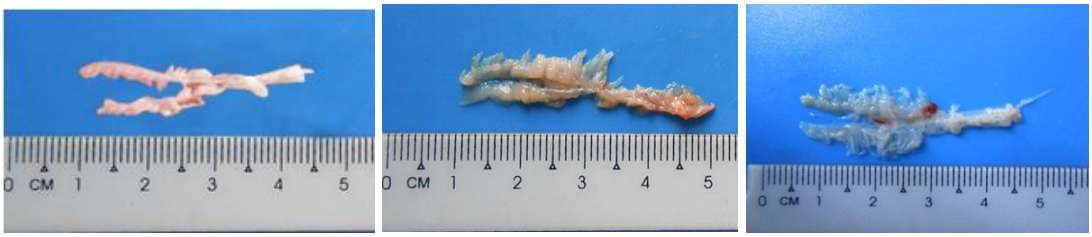


A. Immature stage B. Developing stage C. Mature stage



D. Ripe stage E. Spent stage

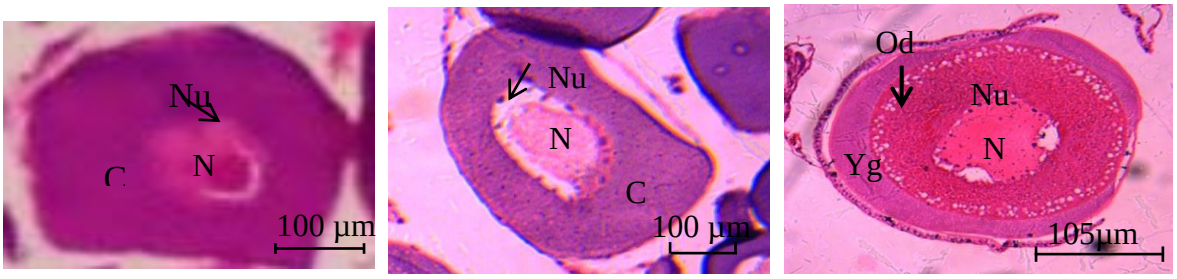
Plate 2. Macroscopic developmental stages of female reproductive organ of *M. gulio*



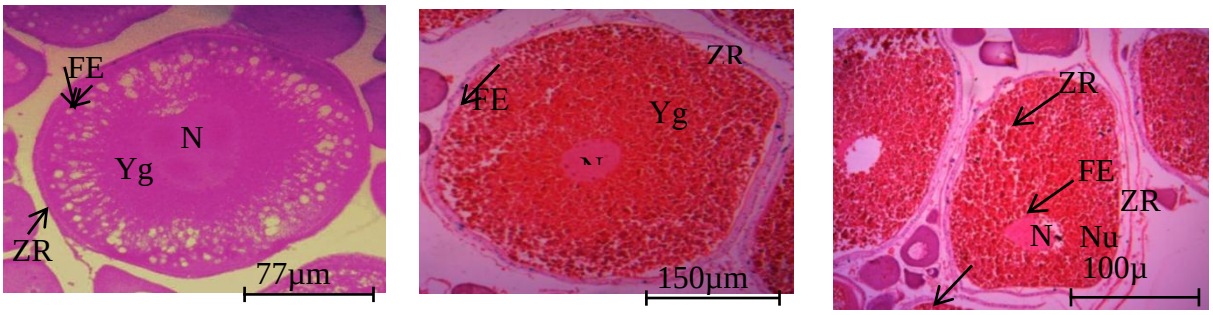
A. Immature stage B. Developing stage C. Mature stage

D. Ripe stage

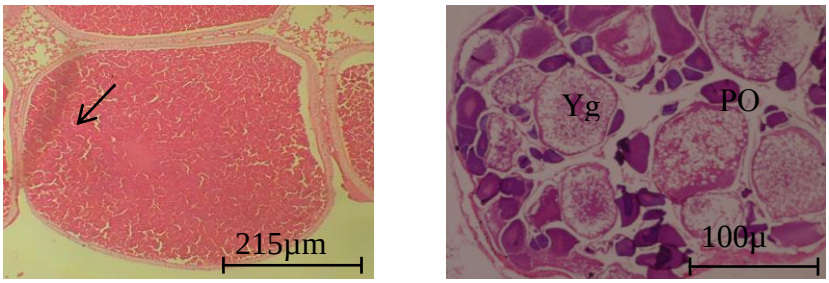
Plate 3 Macroscopic developmental stages of male reproductive organ of *M.gulio*



A. Chromatin nucleolus stage B. Prenucleolus stage C.Primary yolk stage

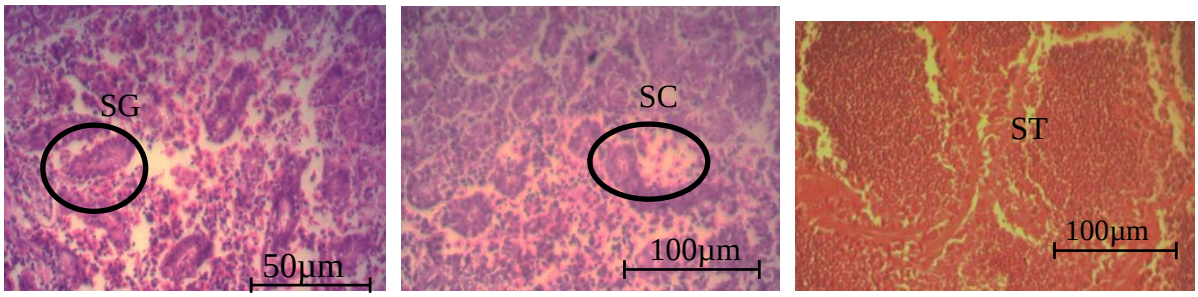


D. secondary yolk stage E. Tertiary yolk stage F. Germinal vesicle migration

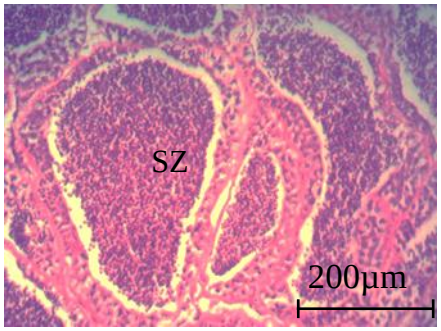


G. Germinal vesicle breakdown H. Spent stage

Plate 4 Continued Microscopic developmental stages of oogenesis in *M.gulio* H & E stain, transverse section, Cytoplasm (Cy), Nucleus (N), Nucleolus (No),Yolk granule (Yg), Oild droplet(Od),Follicular epithelium (FE), Zona radiate(ZR)



A. Spermatogonia (SG) B. Spermatocytes (SC) (c) Spermatid (ST)



D. Spermatozoa (SZ)

Plate 5. Microscopic developmental stages of spermatogenesis in *M.gulio*, H&E stain, transverse section, Spermatogonia (SG), Spermatocytes (SC), Spermatid (ST) and Spermatozoa (SZ).

Acknowledgements

I wish to express my gratitude to Dr Kay Thi Thin, Dr Myin Zu Minn, and Dr Mi Mi Gyi, Pro-Rectors, University of Mandalay. We are very grateful to Dr Thant Zin Professor and Head, Dr San San Myint, Professor, and Dr Moe Kyi Han, Professor, Department of Zoology, the University of Mandalay for their kind suggestions and encouragements.

References

Begum M., Pal H.K., Islam M. A., Alam M.J. 2010. Length weight relationship and growth condition of Mystus gulio (Ham.) in different months and sexes. University Journal of Zoology, Rajshahi University,28:73-75.

Biswas, S.P.1993. Manual of Methods in Fish Biology. Absecon Highlands, NJ, USA: International Book Co.

Bucholtz, R, H.,Tomkiewicz,J.& Dalskov,J. 2008.Manual to Determine gonadal maturity of herring (*Clupea harengus L.*). DTU Aqua-report 197-08, Chalottenlund; National Institude of Aquatic Resources. 45p.

Cushion,N., Cook,M., Schull, J., Sullivan-Sealey,K.M.2008. Reproductive classification and spawning seasonality of Epinephelus striatus (Nassau grouper), E.guttatus (red hind) and Mycteroperca venenosa (yellowfin grouper) from the Bahamas. Proceeding of the 11th International Coral Reef Symposium, Ft Lauderdale, Florida.

El-Halfawy M.M., Ramadan AM., Mahmoud WF. 2007. Reproductive Biology and Histological Studies of the grey mullet, *Liza ramada* (Risso,1826) in Lake Timsah, suez Canal.Egypt.J.Aquat.Res.33:434-454.

Gupta, S., and Banerjee,S.,2014,Indigenous ornamental fish trade of West Bengal. Narendra Publishing House, New Delhi.63.

Haniffa M.A.2009.Native catfish culture a technology package for fish farmers. Aquaculture Asia Magazine,14(3):22-24.

Helfman,G.S., Collette,B.B.&Facey, D.E. 1997. The diversity of fishes. Black wellscience,London,England.p.529.

Hoda, S.M.S., and Ajazuddin.S., 1992.Some aspects of Reproductive Biology of Two Sciaenids,*Otolithes cuvieri* and *Johnius elongates*: Maturation, Spawning, Sex ratio and Fecundity. *Pakistan Journal of Marine Sciences,Vol.1(2),95-110.*

Jacob,P.K.2005.Studies on Some Aspects of Reproduction of Female *Anabas testudineus* (Bloch). Doctor of Philosophy. Cochin University of Science and Technology India.

Jones,A.,1970. Some aspects of the biology of the turbot (*Scaphthalmus maximus* L) with special reference to feeding and growth in the juvenile stage. *Ph D Dissection*, University of East Anglia,kk.145p

King, M., 1995. *Fisheries Biology*, Assessment and Management,1st edn. Fishing News Books,

Mohamed, Abd.EL-G.AL-Absawy.2010. The reproductive biology and the histological and ultra structral characteristics in ovaries of the female gadidae fish *Merluccius merluccius* from the Egyptian Mediterranean water. *African Journal of Biotechnology Vol.9(17), pp 2544-2559.*

Pandian,T.J.1968.Feeding and reproductive cycles of the fish *Mystus gulio* in the Cooum backwaters, Madras. Indian Journal of Fisheries, 13(1&2) 320-333.

Priyadharsini S.,J Manoharan, D Varadharajan and A Subramaniyan. 2013.Reproductive Biology and Histological Study of Red Lionfish Pterois volitans from Cuddalore, South East Coast of India. *J Aquac Res Development 4: 201*

Selman K, Wallace RA (1989) Cellular aspects of oocyte growth in teleosts. Zoological Science 6: 211-231.

Sudarshan.S, R.S Kulkarni, 2013. Determination of Condition Factor (K) Somatic Condition Factor (Ks) Hepatic and Gonado Somatic Indices in The Fresh Water Fish Notopterus Notopterus. International Journal of Scientific Research. Volume : 2

Talwar, P.K., Jhingran, A.G. 1991.*Inland Fishes of India and Adjancent Countries*. Vol. I & II. Oxford & IBH Publishing Co.Pvt. Ltd. New Delhi.

Thida Aung.2006. Comparison of Age and Developmental stages of *Polynemus paradiseus* (Linnaeus, 1758) and *Glossogobius Giuris* (Hamilton,1822). *PhD Thesis.* Department of Zoology, University of Yangon.

Tin Hnin Wai. 2010. The Reproductive Biology of *Otolithoides pama* (Hamiltion and Buchanan, 1822) Pama Croaker in Pathein River, Ayeyarwady Division. *PhD Thesis.* Department of Zoology, University of Yangon.

Unver, B. and saraydin, S.U.2004. Histological examination of ovarian development of shemaya *Chalcalburnus chalcoides* living in Lake Todurge (Sivas/Turkey).Folia Zoologica, 53:99-106.