GONADAL DEVELOPMENTAL STAGES OF *JOHNIUS COITOR* (HAMILTON, 1822) IN GYAING RIVER SEGMENT BETWEEN GADOE AND KAWBEIN AREA

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Abstract

Amphidromous croaker *Johnius coitor* (Hamilton, 1822) is one of the commercially important demersal fish of the Gyaing River segment between Gadoe and Kawbein Area. The irregular pattern of GSI and HSI of studied species were now an evidence of potential indicator which directly reflect the hazard conditions of aquatic medium. Macroscopic and microscopic studies were made on the gonadal development of this species, *J.coitor*. Microscopic study for male spermatogenesis were identified into four stages; spermatogonia phase, spermatocyte phase, spermatid stage and spermatozoa stage. For oogenesis, oocytes were identified as chromatin nucleolus phase, prenucleolus phase, primary yolk phase, secondary yolk phase, tertiary yolk phase, germinal vesicle migration phase, germinal vesicle breakdown phase and spent phase. The female gonadal developmental stages will be compared with that of "oocytes ranged from 13.10µm to 520.40µm in *Johnius coitor*. Thus, this study suggested to take necessary steps are needed to monitor the aquatic medium to protect the fish reproductive physiology, thus fish population as a whole. These findings are very important to fishery management to fix closed season and closed areas for conservation of fish. Suggestions for future works outlined.

Keywords: GSI, HSI, Condition factor, macroscopic and microscopic structure, J. *coitor*,

Introduction

Johnius coitor, commonly known as coitor croaker, is an amphidromous fish species belonging to family Sciaenidae under order Perciformes, popularly known as Jew fish, croakers or drums. Besides India, coitor croaker is widely distributed in Indo-West Pacific region with reports of occurrence from Australia, Bangladesh, Brunei, Indonesia, Malaysia, Myanmar, Nepal and Singapore (Froses and Pauly 2017).The family Sciaenidae consists of approximately 300 species from genera throughout Atlantic, Indian and Pacific Oceans, in coastal marine, brackish and freshwater; many associate with muddy or sanday bottons (Chao *et al.*, 2015; Lin *et al.*, 2019). Some sciaenids form large aggregations for spawning, feeding and over-wintering, which makes them vulnerable to overfishing (Liu and Sadovy de Mitcheson 2008). In recent years, small- and medium-sized croakers enter fisheries and become predominate in Chinese and adjacent water, e.g., the genera *Collichthys, Johnius* and *Pennahia* (Yamaguchi *et al.*, 2006; Wang *et al*., 2011; Xie *et al.*, 2012).*Johnius coitor* is an amphidromous fish species and popularly known as Jew fish, croakers or drums are commercial fish species of the family Sciaenidae. This species can be caught by Gyaing River almost year-round and is important local food fish.

Gonadosomatic index (GSI) is a suitable indicator of the gonad development that can be used for determining the reproductive period (Le Cren, 1951). Hepatosomatic Index (HSI) provides an indication on status of energy reserve in an animal. Condition factor (K) is widely

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used in fisheries and fish biology studies. 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 development of the aquaculture industry. Therefore the present study was carried out the reproductive strategy of *Johnious coitor*.

Therefore, the objectives of this paper are

- To determine GSI, HSI, and K values of the studied fish species
- To investigate the macroscopic and microscopic gonad developmental stages of *Johnious coitor*

Materials and Methods

Study area and period

The present work was carried out in Gyaing River segment between Gadoe and Kawbein Area. It is located at North latitude 16º34´29.98"N and East longitude 97º39´20.07"E (Fig .1). The study period lasted from January, 2023 to December, 2023

Figure. 1 Topographic map of the study area (Source: Office of Land Record, Hpa-an Township, Kayin State, 2022)

Specimen collection

A total of 220 specimens of *Johnius coitor* were collected and recorded from the local fisherman at the study site during the study period (January 2023 to December 2023). In this research work, 20 fresh fish samples of the species were randomly collected monthly from the local fishermen in the Gyaing River segment between Gadoe and Kawbein Area (Plate 1).

Identification and classification

The collected samples were identified referring to Talwar and Jhingran 1991 and Talwar 1995.

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 a digital 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.

Gonado-somatic index (GSI)

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

GSI= GW/BWx100

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).

HIS= 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)

$K= W/L^3 \times 100$

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

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 a few day as per the size of tissues.

Results

Correlation between GSI, HSI and K

In male *Johnius coitor*, the GSI values fluctuated throughout the study period and then increased in December as the peak GSI value (6.8%). The lowest GSI value was found in January (0.03%). But for females, the peak GSI values were observed in December (11.29%). The lowest GSI values were found in January (1.45%) A gradual increase in GSI value was found during the spawning period and gradually decreased during the post-spawning and resting periods(Fig. 2&3) In male *J.coitor*, the peak HSI values were found to be 2.71% in May, and the lowest values were found to be 0.44% in August and 0.71% in December. But *J.coitor* for female the highest HSI values were observed in May (2.76%), and the lowest HSI values were found in December (0.44%) (Fig. 2&3). The monthly values of condition factor (K) for males and females were determined from the number of fish studied per month. The K values ranged from 0.41 to 1.0 in

males and 0.40 to 1.05 in females. The K value is close to 1, therefore males and females were in good condition in the study area (Fig.2&3).

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 January to December (Plate 3 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 stages was found from January, May, August to December (Plate 3,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 in January –April and July – August (Plate 3, C)

Ripe Ovaries

The ovary was yellow and filled the entire length of the body cavity. The ovarian wall thin, Ova were translucent and yellowish. This stage was found in April – May and July – November (Plate 3, D)

Spent Ovaries

The ovary was much shrunken, blood-shoot, and contained numerous small ova. This stages was found in August and October (Plate 3, E)

Macroscopic Developmental Stages of the Male Reproductive Organ of *Johnius coitor*

Immature Testes

Testes were tiny, thin, and transparent in color. This stage was found from January to November (Plate 4, A)

Developing Testes

Developing testes were found from May and August to November. In developing stage testes lobes were increased in size and length, slightly thicken whitish and occupied one half of the body cavity length (Plate 4, B)

Mature Testes

Mature testes were observed in January – April and July to December. Testes were reached the largest, more than one half of the body cavity, and released milt on applying pressure on the abdomen (Plate 4, 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 April and July - November (Plate 4, D)

Spent Testes

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

Microscopic Developmental Stages of Oogenesis of *J.coitor*

Oogonia

Germ cells or oogonia were embedded in the ovigerous tissue. Oogonia cells were observed in cluster and originated from the germinal epithelium, 13.10±0.87µm (Plate 5, A)

Chromatin nucleous stage

These cells were youngest cells, strongly basophilic cytoplasm with large nucleus and one to two darkly stain nucleoi were found. The shape of the cells were irregular and sizes were small, 90.50±5.17µm (Plate 5, B)

Pre-nucleolus stage

Oocytes were rounded or oval or polygonal shape. In this stage, cytoplasm was less basophilic and different size of nucleoi was situated in the periphery of the nucleus. This stage was found throughout the year. The sizes of the cells were 141.03±12.09µm (Plate 5, C)

Primary yolk stage

Oil droplets (Od) and yolk granules (Yg) were initially appeared in the cytoplasm. Cytoplasm was weak basophilic and nucleus was still presented in central position. The size of the cells were 234.63±27.74µm (Plate 5, D)

Second yolk stage

Cells were rapid growth and yolk granules (Yg) and oil droplets (Od) were increased in number and coalescence. Nucleus was irregular shaped. The size of the cells were 244.63±10.10µm (Plate 5, E)

Tertiary yolk stage

Size and number of yolk granules (Yg) and oil droplets were increased. The size of the nucleus was small and it seen nearly disappeared. The sizes of the cells were 590.50± 48.64µm (Plate 5, F)

Germinal vesicle migration stage

In this stage, nucleus (N) was moved toward the pole and oil droplets (Od) were accumulated in the cyctoplasm (Cy). The nucleolus (Nu) were scattered in the nucleus. The size of the cells were 433.17±46.14µm (Plate 5, G)

Germinal vesicle breakdown stage

In this stage, nucleus substance were released into the cytoplasm (Cy) and nucleoli (Nu) were not clearly seen in the nucleus. Yolk globules (Yg) were fused to each other in the cytoplasm. The size of the cells were 520.40±35.62µm (Plate 5, H)

Postovulatory follicle stage

In this stage, various types of postovulatory follicle (Pof) was found, Hydrolyse yolk granules, Primary growth oocyte and oogonia were observed found in this stage (Plate 5, I)

Microscopic developmental stages of spermatogenesis of *Johnius coitor*

Spermatogonia stage

The largest size of spermatogonia cells were observed and localized at the inner layer of seminiferous tubule wall. This cell was contained basophilic nucleus and unstained cytoplasm (Plate 6, A)

Spermatocytes stage

Spermatocytes cell became smaller in size than the spermatogonia. Spermatocytes were found the most abundant stage during the process of spermatogenesis (Plate 6,B)

Spermatids stage

Spermatids was strongly basophilic spherical cell and observed dense as clusters. They were found in the inner layer of seminiferous tubules (Plate 6, C)

Spermatozoa stage

Mature spermatozoa were contained spherical nucleus at the head region and a faint long tail. They were found in cluster in the central position of the lumen of the lobules (Plate 6, D)

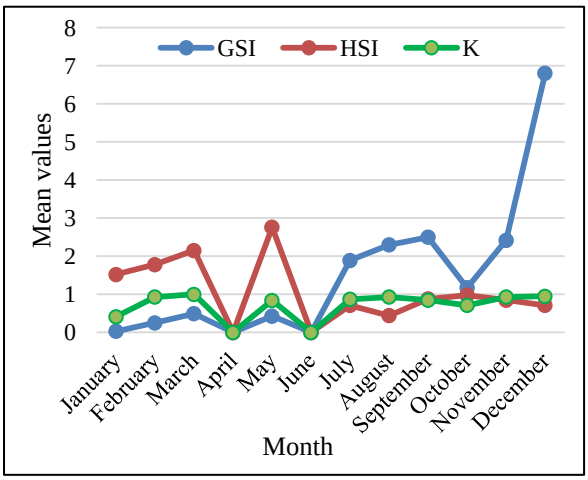


Figure.2 Monthly values of GSI, HSI, and K of male *Johnius coitor*

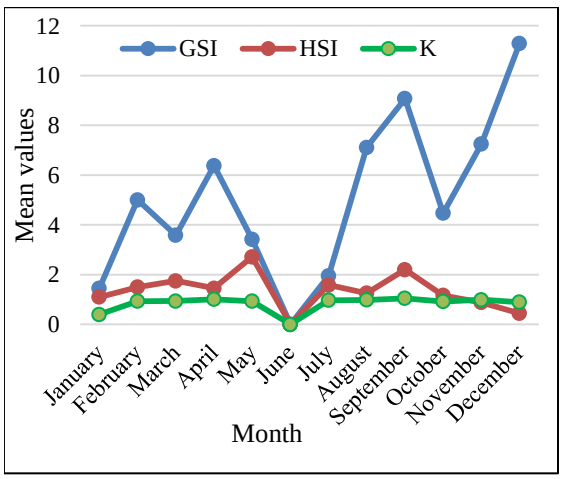


Figure.3 Monthly values of GSI, HSI, and K of female *Johnius coitor*

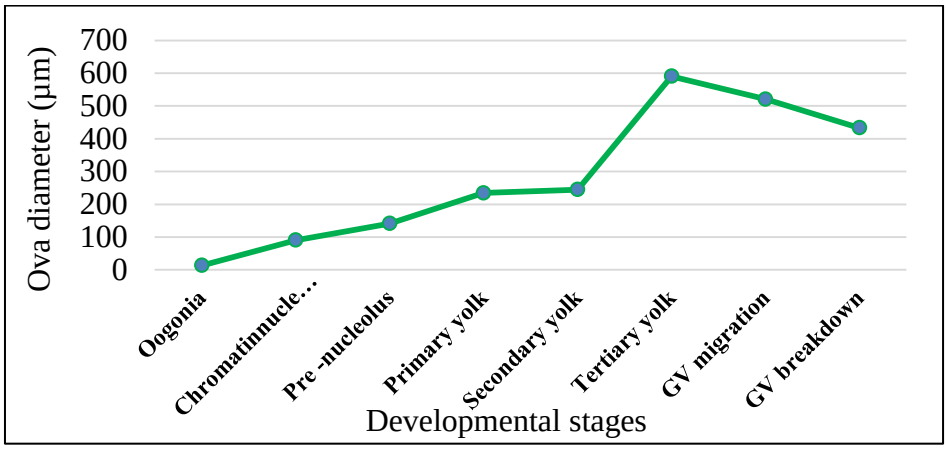
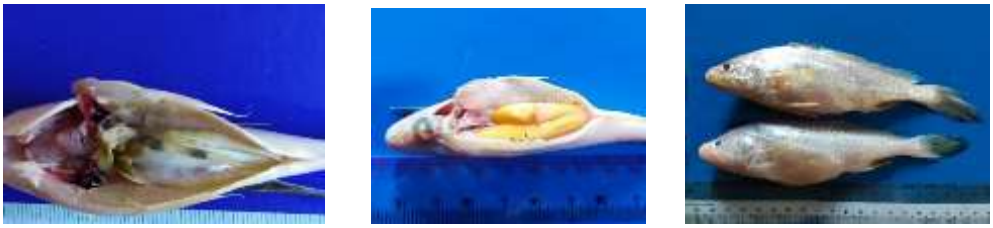


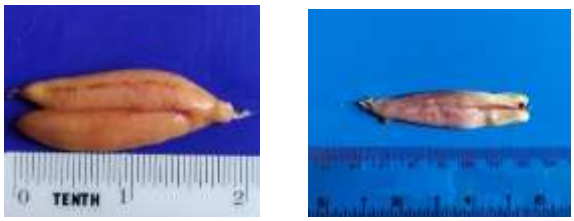
Figure. 4 Measurement oocyte diameter for *Johnious coitor*



(A) Dissected male (B) Dissected female C. male& female of

Plate 1 Reproductive organ of *Johnius coitor*

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

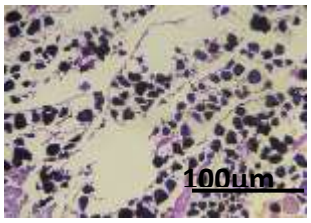


D. Ripe stage E. Spent stage

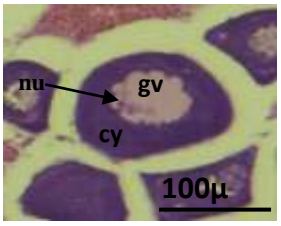
Plate 3. Macroscopic developmental stages of female reproductive organ of *J.coitor*

A.Immature stage B. Developing stage C. Mature stage D. Ripe stage E. Spent stage

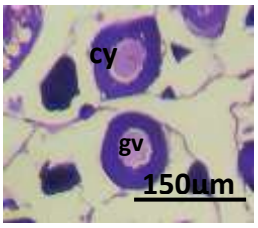
Plate 4. Macroscopic developmental stages of male reproductive organ of *J.coitor*



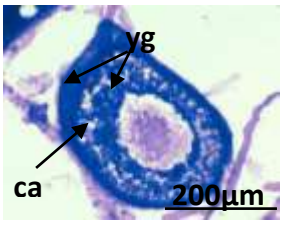
A. Oogonia



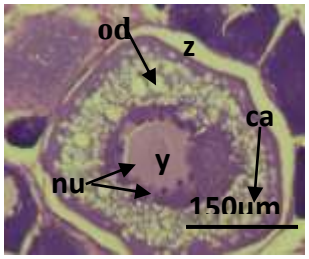
B. Chromatin nucleolus stage



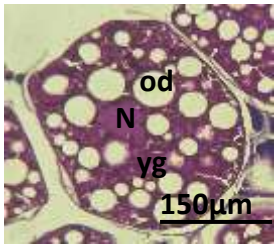
C. Prenucleolus stage



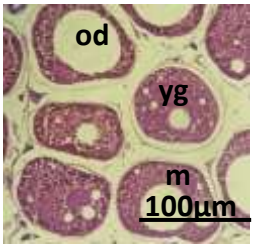
D. Primary yolk stage



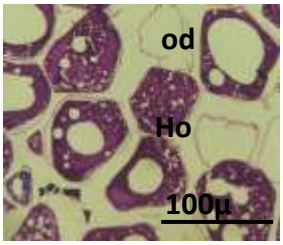
E.Second yolk stage



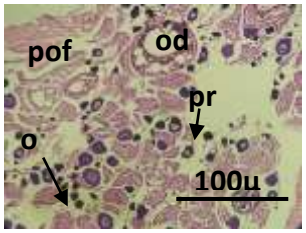
F.Tertiary yolk stage



G. Germinal vesicle

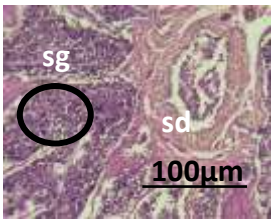


H. Germinal vesicle Breakdown stage

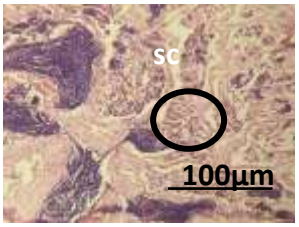


I.Spent stage

Plate 5. Continued Microscopic developmental stages of oogenesis in *J.coitor* H& E stain, transverse section, Oogonia (Og),Cytoplasm (Cy), Nucleus (N), Primary growth oocytes (Pr),Nucleolus (Nu), Yolk granule (Yg), Oil droplet (Od), Zona radiate (Zr), Migration nucleus (Mn), Hydrated oocyte (Ho), and Post ovulatory follicle (Pof)



A.Spermatogonia(Sg)



B. Spermatocytes (Sc)

C. Spermatid (St)

D. Spermatozoa (Sz)

Plate 6. Microscopic developmental stages of spermatogenesis in J.coitor, H &E stain, transverse section, Spermatogonia (Sg), Spermatocytes (Sc), Spermatid (St) and Spermatozoa (Sz).

Discussion

Developmental stages of gonads of *Johnius coitor* were examined from Gyaing River segment between Gadoe and Kawbein Area.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 (Helfman *et al.,* 1997). In the present result, testes were examined on the dorsal side of the body cavity in *Johnious coitor*.

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.

Lagler (1962) staged that the several fish species spawn more than once a year and more or less continually. The GSI values are higher in spawning period and declining in the post spawning period (Le Cren, 1951). According to the monthly GSI, *Johnius coitor,* peak GSI values were found in December for both males and females. The lowest GSI value was found in January for both males and females. So that peak GSI value indicates the peak spawning season of *J.coitor* is during post-monsoon periods. Similar results are found in Kumar *et al.,* (2013), who described that during peak breeding season (maximum July–September > January–May >October–December minimum) throughout the year for *J.coitor.* Quang Minh Dinh *et at.,* (2022) also concluded that the fish (*J.coitor*) was a multi-spawner releasing eggs all year-round with a peak in the wet season.

In the present finding, the highest HSI value of *J.coitor* was found in May for both males and females. The lowest HSI value occurred in August for males and in November to December for females. The GSI and HSI were negative 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).Wootton (1990) also stated that the liver weight (HSI) decreased as the ovary weight increased during vitellogenesis. This showed that HSI has reversed action on GSI.

Sarkar *et al.,* (2018) recorded that condition factor (K) of *J.coito*r was lower in December and higher in January. In the present study, condition factor (K) values of male was higher in March and December and lower in January and then of female was higher in April and September and lower in January. The present finding was disagreement with the previous author. It may be suggested that different in food resources, environmental temperature or evolutionary adaptation of different population to the specific ecological properties of specific ecosystem.

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; Thinzar Aung 2018;).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 the 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 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 agreed with this statement as the size of oocytes were larger nearer to the germinalvesicle breakdown stage, ripe stage (13.10µm to 520.40µm).

The monthly GSI analysis and histological finding 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 eight developmental phase or stages; Oogonia, chromatin nucleolus phase, prenucleolus phase, primary yolk phase, secondary yolk phase, tertiary yolk phase, germinal vesicle migration phase, and germinal vesicle breakdown phase were found in the month where the values of GSI was highest. However, the number of germinalvesicle migration and germinal vesicle breakdown phases was dominant over the other stages. In the findings of this study, the highest number of germinalvesical migration and germinal vesical breakdown occurred in October to December.

Indeed, the present finding of the different developmental stages of gonads of the studied fish species from natural brackish water, Gyaing River 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.

Conclusion

The irregular pattern of GSI and HSI of the studied species, *J.coitor* were now evidence of potential indicators that directly reflect the hazardous condition of aquatic medium. The study on maturity stages of oocytes in the ovaries of these species was firmly supported by ovarian developmental stages of histological slides with photographs. Indeed, the present finding of the different developmental stages of gonads of the studied fish species from natural brackish water,The data provides valuable baseline information for the fishery management of this species, specifically for implementing closed fishing seasons or areas during the peak spawning months (October–December) to ensure the sustainability of the population.

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