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Seasonal Germ Cell Proliferation and Maturation Patterns in Indian Major Carp, *Labeo Rohita* (Hamilton, 1822) and Tilapia, *Oreochromis Niloticus* (Linnaeus, 1758)

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Abstract

The present work assessed the seasonal changes in germ cells (GC) and the maturation of rohu, *Labeo rohita* and tilapia, *Oreochromis niloticus*. Here, we showed two species representing two diverse types of spawning behavior, having two distinct GC proliferation and spawning patterns. During July, a single peak spawning season for rohu was recorded with the highest GSI (female: 19.5±1.29 and male 4.2±0.24), which is the peak monsoon time in Eastern India. However, two peak spawning seasons were marked in tilapia during the pre-monsoon (April) and post-monsoon (October) period with GSI values of male: 0.9±0.21, female: 3.9±1.32 and male: 1.1±0.3, female: 4.4±1.1 respectively. Gonadal histology in synchrony with GSI provides insights into the effects of various seasonal climate changes on the reproductive status of fish. Further, GC ultra-structure revealed various development stages in rohu and tilapia in different seasons using a scanning electron microscope. Here, comparative models were proposed for GC proliferation and maturation during different parts of the year in rohu and tilapia that may be used to plan breeding programs and hatchery management of these two species.

Introduction

A successful reproduction depends upon the function of two end products viz. sperm and egg, that develop in the testis and ovary respectively (Nishimura & Tanaka, 2014). However, the puberty of the testis and ovary must be attained through the activation of a sufficient number of germ cells (Migaud et al., 2010). The initiation of germ cell (GC) proliferation and subsequent maturation and reproductive cycle of fish depends on the environmental stimuli, which are seasonal in many species. The reproductive cycle is one of the most striking features providing clues to the biological processes, synchronized with seasonal changes in climate, day length and food availability (Migaud et al., 2010; Routray et al., 2007; Verma et al., 2009a, Gupta & Rath, 2006). This synchronization during fish spawning is adjusted in such a way that spawn and fry are produced in an environment where the probability of survival is maximum (Migaud et al., 2010; Akhtar et al., 2018). This precision is believed to be due to the biological clock genes conserved in fish having reproductive significance (Jaiswal et al., 2021).

During the maturation process in the teleost, early-stage germ cells develop through a series of basic biological processes (Harvey & Carolsfeld, 1993; Yang et al., 2018). These highly specialized cells play an important role in gonadal development and gametogenesis (Yang et al., 2018). GC proliferation is one such biological process responsible for giving rise to

the next generation of species (Van Doren, 2010). Germ cells could develop into either spermatogonia or oogonia and their proliferation and terminal maturation varies under different environmental conditions (Verma et al., 2009a; Chilke, 2012; Patra et al., 2015). Earlier, the GC maturation in teleost has been reported in some species that are limited to spawning season only (Verma et al., 2009a, Patra et al., 2015, Ribeiro et al., 2017; Wildner et al., 2013; Schulz et al., 2005; Lahnsteiner et al., 1997). Similarly, the ultra-structure of terminally differentiated GCs (matured gametes) has been studied in some cyprinids (Psenicka et al., 2006; Verma et al., 2009b; Fürböck et al., 2010; Neznanova, 2012; Kim, 2006; Guan & Afzelius, 1991; Shamna et al., 2021) and cichlids (Quagio-Grassiotto et al., 2003; Thünken et al., 2007; Siqueira-silva et al., 2012). The morphological features of sperm and egg are highly adapted to environmental conditions and they are responsible for reproductive success and seed production (Brooks et al., 1997). While emphasizing these studies, it is pertinent to mention that the seasonal variations in GCs cannot be generalized as they are species-specific in nature. However, it would be fascinating to know the GC proliferation pattern in two fish having diverse spawning behavior such as rohu (carp) and tilapia (cichlid). The knowledge about their seasonal proliferation in GCs and the final products (terminally differentiated gametes) will immensely help in the management of aquaculture in general and seed production in particular.

It is pertinent to note that the carps and tilapia are the most extensive and widely cultivable fish species around the world (De silva, 2003). A majority of teleost fishes are seasonal spawners while a few are prolific spawners. Though prolific spawners like tilapia breed independent of season, the regulatory role of environmental cues are not understood properly (Negassa & Getahun, 2003). The genus *Sarotherodon* displays seasonal reproductive activity even though temperature and day-length are almost constant throughout the year (Billard & Breton, 1978), but the vast majority of the freshwater fishes like Indian major carps (IMCs) including rohu breed seasonally during the monsoon season (June-August) when rainfall is heaviest (Jhingran, 1975; Guerrero et al., 2009). Among the Indian major carps, rohu is an important freshwater species having group synchronous breeding behavior (Pandey, 2013). Similarly, Nile tilapia is a predominant species worldwide with asynchronous breeding behavior (Tave, 1988). These two types of spawners could be the best models to study how seasonal manifestations of reproductive products such as GCs are controlled by seasonal cues. The GC proliferation under different seasons like pre-monsoon (Feb-May), monsoon (June-September) and post-monsoon (October-Jan) in these two different types of spawners (rohu and tilapia) have not been studied widely. The changes in seasonal parameters cause gonadal regression in fish, including incomplete or complete loss of germinal elements that might alter GC generation and proliferation (Routray et al., 2011; Patra et al., 2015). The impairment of GC proliferation may ultimately affect the GSI, fertility and reproductive performance in fish.

Here, a comparative study was conducted to assess the seasonal germ cell proliferation and maturation patterns in two types of spawners *Labeo rohita* and *Oreochromis niloticus* that belong to two different orders.

Materials and Methods

Collection and Rearing of Spawners

For this study 400 2-year old rohu, *L. rohita* (male: 1349 ± 75.23g; female: 1464 ± 85.23g) and 400 6-month old tilapia, *O. niloticus* (male: 191 ± 12.4g; female: 175 ± 21.6 g) were obtained from the Carp and Tilapia Breeding Unit of ICAR-CIFA, Bhubaneswar, India. All the brood fish were transported under a stress free condition with fish hammock (Varghese et al., 2021). After the initial acclimation for 7 days, the fish were stocked at the rate of 1500 kg/ha i.e. 200 rohu and 200 tilapias in each replicate. All the fish were communally reared in two experimental earthen ponds (0.2 ha area, size: 50 m x 20 m, 2 m depth) in duplicate for 12 months in the farm facility of ICAR-CIFA (Lat. 20.2961° N, long. 85.8245° E) following standard procedures (Nandi et al., 2007). The fishes were reared for 12- months consisting of three major seasons such as pre-monsoon (Feb-May), monsoon (June-September) and post-monsoon (October-January).

The physico-chemical parameters of pond water such as pH (digital pH meter), dissolved oxygen (mg/l) (winkler's method), free CO2 (mg/l) (titration method), total alkalinity (mg/l) (alkalinity test kit), P2O5 (mg/l) (calorimetric method), NH4-N and NO3-N (mg/l) (spectrophometry) and conductivity (mho/cm) (digital conductivity meter) were measured every week following (APHA,1998). Fish were fed with floating pellet feed (30% crude protein) ad lib twice a day (09:00 hr and 16:00 hr).

Assessment of Maturation and GSI

For the assessment of maturation and GSI, each time 10 males and 10 females of rohu and tilapia were collected randomly from both the replicate ponds by drag netting at the end of each month. Moreover, the sample size (n) is 40 males and 40 females of each fish in each season (pre-monsoon, monsoon and post-monsoon). The sampled fish were anesthetized using 2-phenoxyethanol (MP Biomedicals, Inc. Ohio 44139) at a rate of 100 ppm and kept in a 200-litre capacity tank with aeration for further study. All the fish were sacrificed humanely to remove the gonads. A ventral incision was made to expose gonads for determination of gonadal maturation rate by physical observation of gonads (i.e., the presence of eggs or milt) and the final

maturation% was estimated following the formula:

$$\text{Maturation rate (\%)} = \frac{\text{No.of fish matured}}{\text{Sample size (n)}} \times 100$$

Similarly, GSI (Gonado Somatic Index) was calculated as per Alam & Pathak (2010).

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

Gonadal Histology for Light Microscopy

To check the GC status, every time, the middle part of gonads of either male or female was excised, sliced and immediately fixed in 10% neutral buffered formalin. After dehydrating through an ascended sequence of ethanol, tissues were cleared in xylene and embedded in paraffin wax. Tissue sections of 5-6µm thickness was cut using an ultramicrotome (Leica, Germany) and stained using hematoxylin and counterstained with eosin (Merck, India Ltd) following the methods of Luna (1968). The histological changes were marked following Blazer (2002) and photographed using a photomicroscope (Nikon, Japan). The changes in exposed sections of gonads were compared between the two spawners during different seasons.

Assessment of Germ Cell Morphology Through Electron Microscopy

The germ cells from gonads were collected as described earlier to study of ultrastructural morphology by scanning electron microscope (SEM). The SEM fixation was done as described by Marquez & Ogasawara, 1975. GC smears were fixed for 3h at 4 °C temperature in modified Karnovsky's fixative with 0.1 M sodium phosphate buffer at pH 7.4. After washing the GC smear with fresh buffer and repeatedly washed three times in double distilled water for 15 min each. The samples were air dried and coated with 20 nm gold (Hitachi E-1010) in a sputter coater and observed under a Hitachi S3400N scanning electron microscope at 15 KV.

Statistical Analysis

All the data represent mean ± standard error of mean (SEM). The significance of the differences in the maturation and GSI of rohu and tilapia in different seasons were analyzed by the Student's t-test and ANOVA- analysis of variance. A probability value of $P<0.05$ was taken as statistically significant. All the statistical analysis was performed using SPSS-16.

Results

Physico-chemical Parameters

The month wise variations in the physico-chemical water parameters of the rearing pond was assessed for a period of 12-months that are shown in Table 1. The monthly minimum average temperature of pond water was recorded in January as 24.51 °C, whereas, a maximum temperature 34.8 °C was recorded in May. Similarly, the minimum and maximum monthly average atmospheric temperatures at the site were 15.2 °C in the month of January and 37.2 °C in May respectively and the mean values are represented in Figure 1.

Maturation Rate

The seasonal GC proliferation and subsequent maturation of rohu and tilapia were studied here. This was divided into three phases such as pre-monsoon, monsoon and post-monsoon months. The maturation rate in rohu and tilapia varied during different months of the season is depicted in Figure 2. The maturation pattern was dissimilar in these two species and the highest maturation rate (100%) among the males and females of rohu was seen in July that falls in the monsoon season. However, two maturation rates (%) peaks were observed in April and October for both sex of tilapia. It was also seen that few tilapia (Nearly 20-30%) also matured in June and September. In the pre-monsoon season, rohu males had an average maturation rate of 34% and the highest value of 60% recorded in May. None of the matured males were

Table 1. Physico-chemical parameters of rearing ponds during different months of the year (Mean ± SEM)

Months	Mean water Temperature(°C)	Min Atmospheric Temperature (°C)	Max Atmospheric Temperature (°C)	pH	Dissolve oxygen(mg/l)	Ammonia (mg/l)	Nitrate (mg/l)	Hardness (ppm)
Feb-20	26.64±2.5	18.7±1	31.4±2.5	7.4±0.02	5.4±0.02	0.006±0.002	0.02±0.001	112±0.14
Mar-20	29.81±3.4	22.2±1.5	34.9±2.1	7.7±0.11	4.6±0.05	0.007±0.004	0.01±0.002	110±0.07
Apr-20	32.84±2.7	25±1.8	36.9±2.3	7.2±0.23	4.7±0.03	0.005±0.002	0.02±0.001	120±0.16
May-20	34.80±2.5	26.2±1.5	37.2±3.1	6.8±0.12	5.2±0.02	0.004±0.002	0.03±0.001	122±0.04
Jun-20	32.87±2.1	26.1±2.1	35.3±2.4	6.7±0.02	5.5±0.03	0.008±0.001	0.02±0.003	110±0.09
Jul-20	27.32±2.35	25.2±1.5	32.2±3.4	7.2±0.23	6.1±0.02	0.007±0.001	0.03±0.01	112±0.07
Aug-20	27.40±2.4	25.1±1.4	31.6±3.1	7.1±0.01	6.2±0.01	0.006±0.002	0.02±0.003	130±0.09
Sep-20	28.4±2.4	24.8±2.3	32.1±2.2	6.8±0.56	6.5±0.04	0.006±0.001	0.02±0.001	122±0.05
Oct-20	27.14±2.1	23±2.51	32.32±1.5	7.1±0.47	5.2±0.03	0.005±0.001	0.03±0.01	122±0.08
Nov-20	27.32±2.5	19.4±1.6	30.4±1.8	7.4±0.62	5.4±0.02	0.005±0.001	0.03±0.01	130±0.07
Dec-20	26.05±2	15.8±1.5	29±2.4	7.2±0.14	5.0±0.05	0.006±0.001	0.02±0.003	128±0.06
Jan-21	24.51±2.2	15.6±1.2	28.7±1.8	7.8±0.04	5.7±0.04	0.008±0.001	0.03±0.002	108±0.09

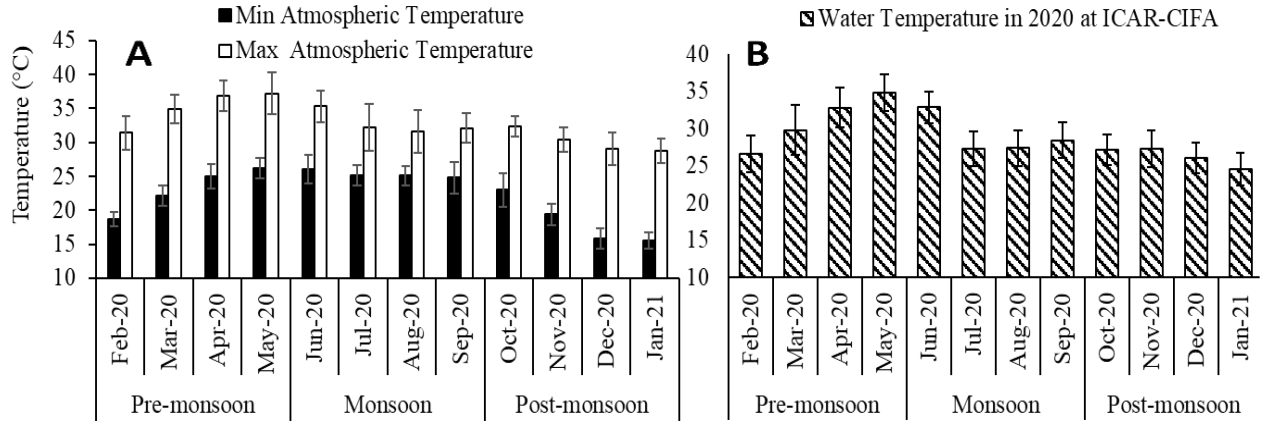

Figure 1. Seasonal variations in the atmospheric and water temperatures in the experimental ponds during the 12-month study period (Feb-2020 to Jan-2021). **A:** minimum and maximum atmospheric temperature; **B:** average water temperature in the rearing ponds at ICAR-CIFA. Data shown as mean ± S.E.M (vertical bars).

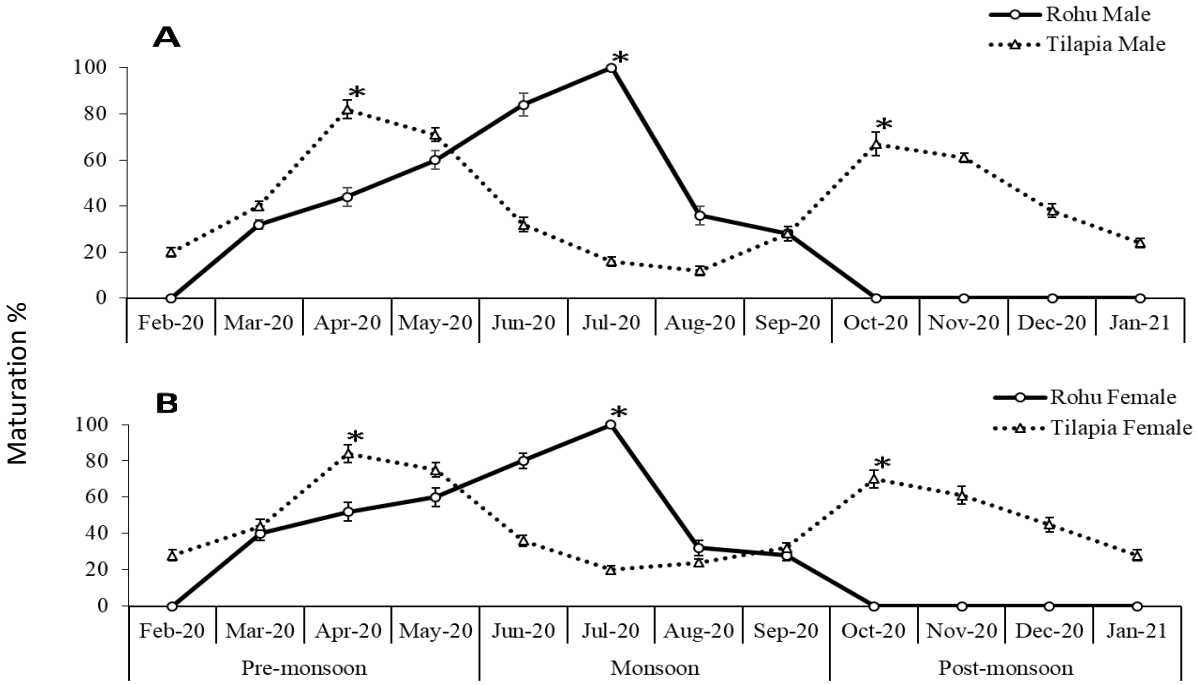

Figure 2. Changes in the maturation pattern of *L. rohita* and *O. niloticus in* different seasons. **A:** male of rohu and tilapia; **B:** female of rohu and tilapia. Data shown as mean ± S.E.M. Asterisks indicate the significant values.

found in post-monsoon months. On the contrary, Nile tilapia male showed a different trend and the highest maturation was observed in pre-monsoon (80%) and post monsoon months (63%). The average maturation rates for male tilapia in pre-monsoon and post-monsoon months were 38% and 42% respectively. A similar trend was also observed in female rohu and tilapia. The rohu females had an average maturation rate of 38% and 60% during pre-monsoon and monsoon season months respectively and none found matured in post-monsoon. Two peak maturation rates were observed in tilapia during pre-monsoon and post-monsoon months. The average maturation rates in these two seasons were 38% and 42.5% respectively. The lowest maturation was recorded in monsoon (22%).

Gonad-somatic Index (GSI)

GSI is the indicator of maturity status and the same were assessed monthly for rohu and tilapia (male and female) are represented in Figure 3. In male rohu, the maximum mean GSI value was 2.21 in monsoon whereas two peak values were observed in tilapia viz., 0.56 and 0.63 in pre-monsoon and post-monsoon respectively. The female rohu recorded a highest GSI value in monsoon (9.84), whereas two highest values were recorded in tilapia such as 2.77 and 2.76 during pre-monsoon and post monsoon months respectively. This variation in these two species corresponds to their reproductive activities including gonad development during that period. The morphology of excised intact

gonads of rohu and tilapia collected during three seasons is shown in Figure 4.

Gonad Histology

Further evidence on the maturity status and germ cells was assessed by gonad histology during three seasons in rohu and tilapia (Figure 5 & 6). The gonad histological analysis of rohu and Nile tilapia showed active gametogenesis. Gonadal development in rohu revealed its group synchronous type that releases gametes once in a year or once in the spawning season, whereas gonadal development in Nile tilapia is asynchronous type dominated by matured gametes at different parts of the year/seasons. The gonad histology revealed active gametogenesis in rohu during monsoon. However, it was recorded during pre and post monsoon periods in Nile tilapia. The testis development and maturation in rohu can be seen from pre-monsoon as depicted in figure 5 with the developing primary, secondary spermatocytes, spermatids and lobules consist of few spermatozoa. Lobules were filled with spermatozoa and thin tunica characterized in monsoon. In post-monsoon few lobules are filled with spermatids and few residual spermatozoa could be observed. However, in tilapia, the gonad development and maturity started from pre-monsoon as they showed an asynchronous type of breeding. It could be seen that there were all stages of male germ cells (primary spermatocyte, secondary spermatocyte, spermatids and spermatozoa) in the histological section indicating active spermatogenesis and spermiogenesis round the year and more prominently during pre and post monsoon periods. Pre-monsoon was characterized by the presence of primary germ cells, spermatogonia and secondary spermatocytes that were converted to spermatids. In monsoon, the activity of spermatogenesis was reduced as indicated by low GSI whereas in post-monsoon the lobules were filled with developed spermatozoa.

By the onset of pre-monsoon, the ovary was filled with two stages of oocytes (primary and secondary). In the monsoon season, the ovary reached its peak ripening stage with densely packed yolk granules and clearly visible group maturation of oocytes. This peak breeding is exclusively visible in the monsoon season which indicates a synchronous spawning behavior. After the spawning, some tertiary oocytes undergoing atresia were noticed in the post-monsoon season (Figure 6). The primary oocytes were identifiable in both species by a few peripherally located nucleoli and small localized areas of intense basophilia in the cytoplasm. The secondary oocytes were noticeable by the appearance of yolk vesicles, that was emergence of vitelline envelope. During tertiary oocyte stage, the oocytes increased in size due to deposition of the yolk vesicles. In the mature oocytes, the nucleus was dissolved and the ooplasm consisted of yolk bodies. The ovary development of Nile tilapia showed peaks in pre-monsoon and post-monsoon but all the oocyte stages were observed in almost all seasons that represents the batch spawning nature of tilapia (Figure 6). The female Nile tilapia had two maturity peaks that can be seen from the GSI in pre-monsoon and post-monsoon months. Increase in the oocyte size with small yolk granules in the cytoplasm and later cytoplasmic area is completely packed with yolk granules in the pre-monsoon. All stages of oocytes were present but

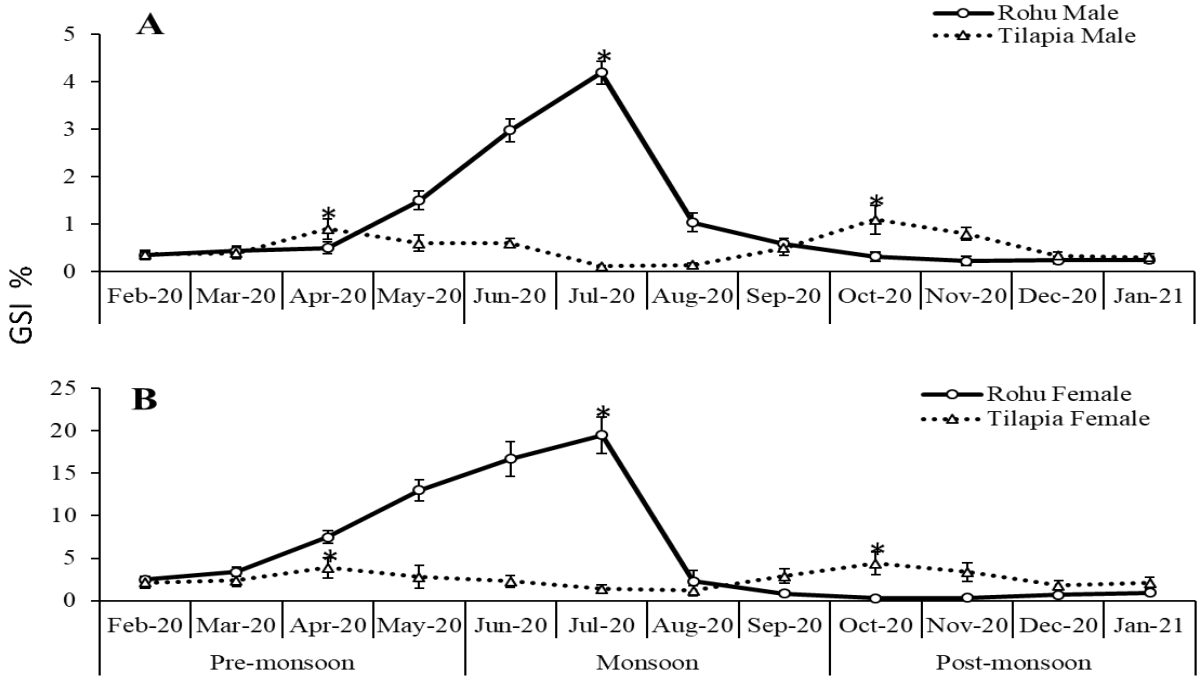

Figure 3. Changes in the GSI of *L. rohita* and *O. niloticus* during different seasons. A: male of rohu and tilapia; B: female of rohu and tilapia. Data shown as mean ± S.E.M. Asterisks indicate the significant values.

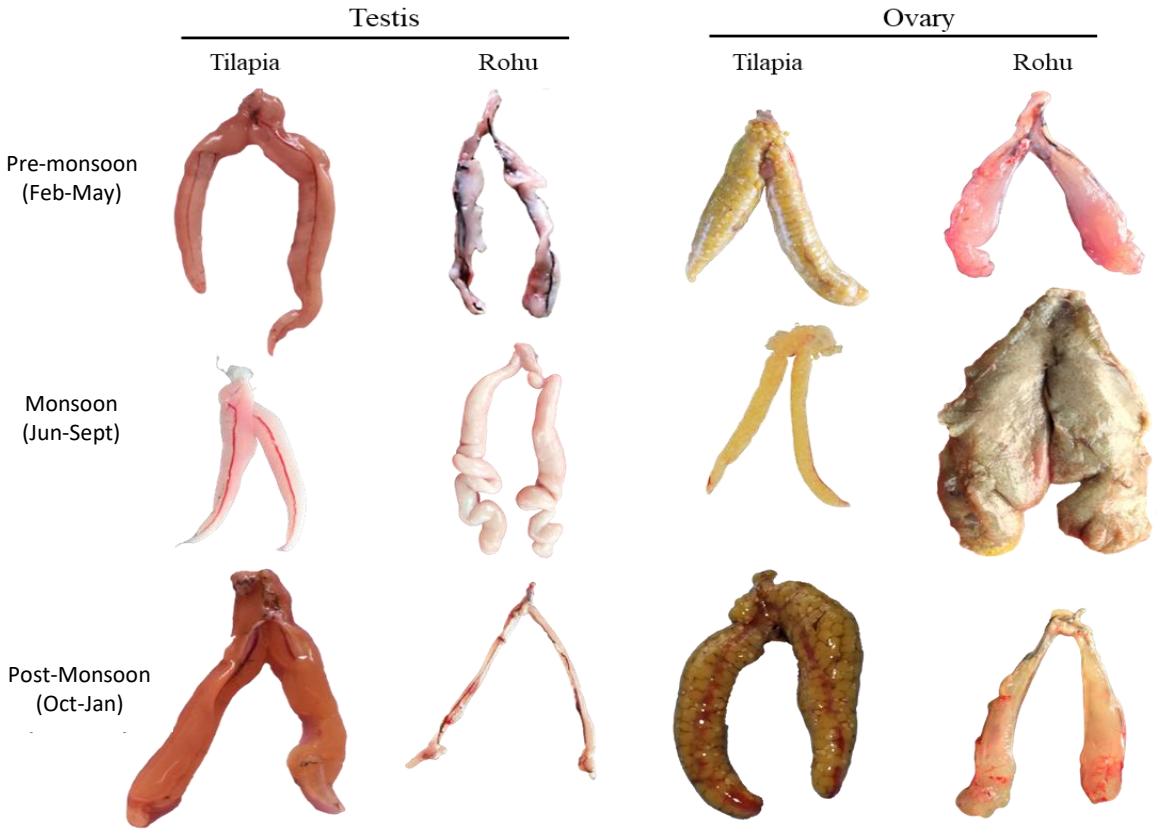

Figure 4. Typical morphology of gonads of *L. rohita* and *O. niloticus* observed in pre-monsoon, monsoon and post-monsoon months.

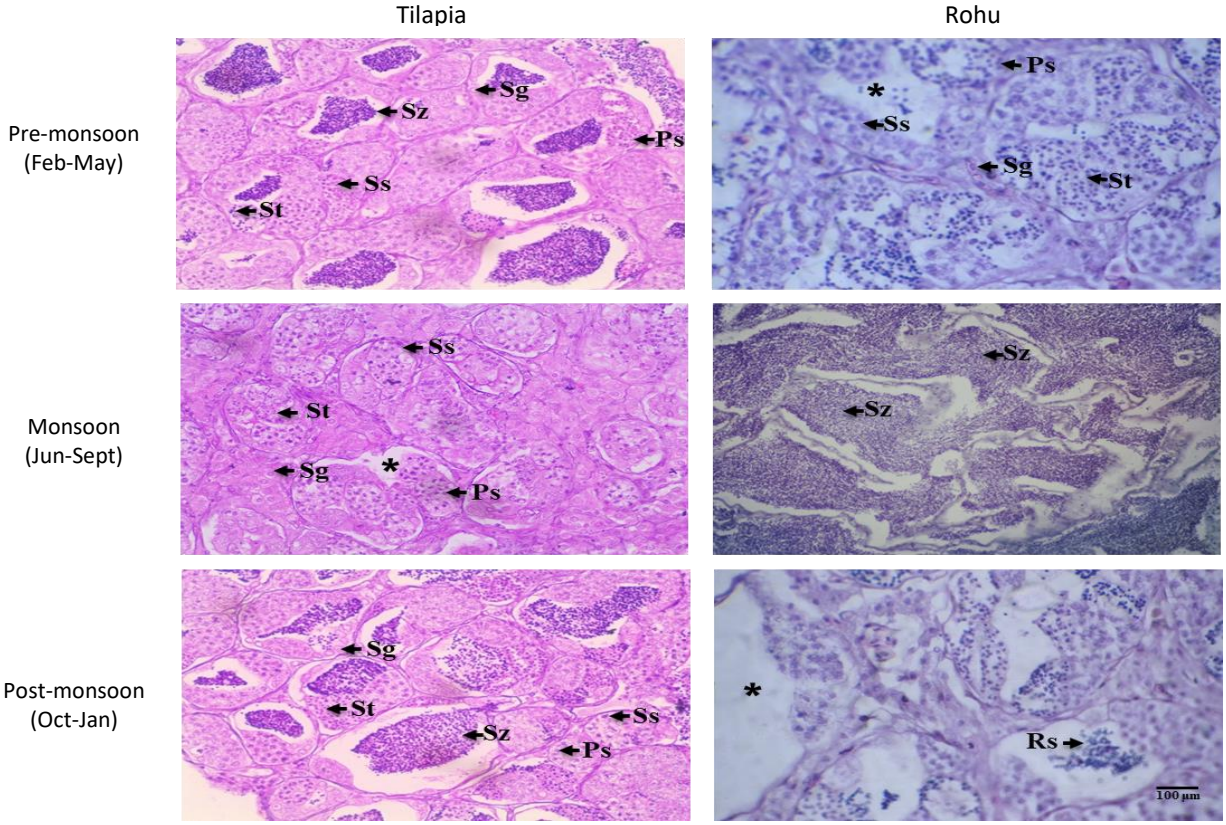

Figure 5. Changes in the testis histology of *L. rohita* and *O. niloticus* during different seasons, all sections were stained with hematoxyline-eosin. (Sg-spermatogonia, Ps-primary spermatocytes, Ss-secondary spermatocytes, St-spermatids, sz-spermatozoa, Rs-residual spermatozoa, * indicates empty seminiferous lobules). Bar = 100 µm.

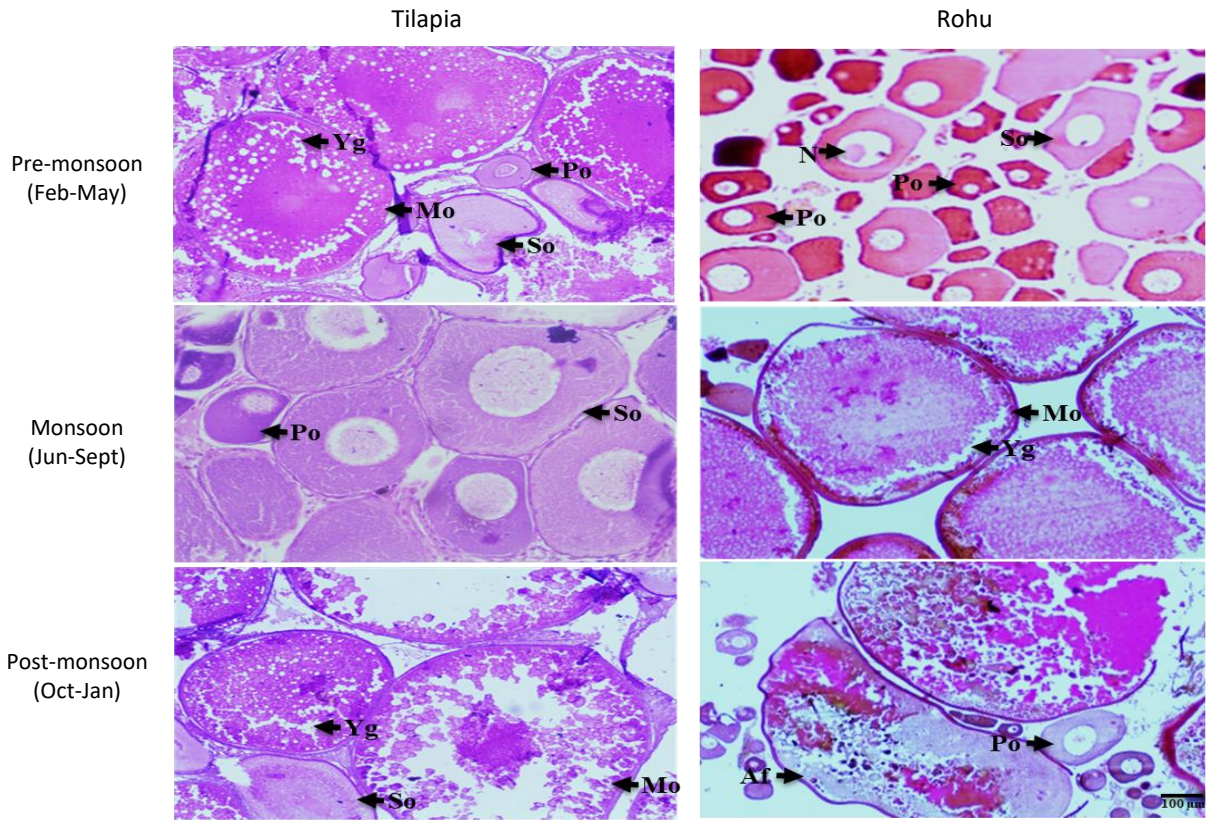

Figure 6. Changes in the ovary histology of *L. rohita* and *O. niloticus* during different seasons, all sections were stained with hematoxyline-eosin. *(*Po-primary oocyte, So-secondary oocyte, Mo- matured oocyte, Yg- Yolk granules, Af-atretic follicles). Bar=100 µm.

dominated by tertiary oocytes with increased size in the post-monsoon. During the monsoon the GSI was considerably low characterized by the presence of few tertiary oocytes and secondary oocytes with a central nucleus, the nucleus contained one to four nucleoli.

Ultra Structure of Germ Cells

The electron micrograph of GC suspension displayed active gametogenesis in both species during different months of the three seasons (Figure 7 & 8). In the pre-monsoon season, the GC suspension of rohu testis showed the presence of spermatogonia, primary spermatocytes and secondary spermatocytes, but in case of Nile tilapia the presence of all the stages of GCs were noticed indicating active spermatogenesis. In the monsoon months matured GCs (spermatozoa) of rohu was visible while in Nile tilapia more primary and secondary spermatocytes and less spermatozoa were present. During post-monsoon, a smaller number of remnant spermatozoa could be seen in rohu but a greater number of spermatozoa were observed in case of tilapia. The GC suspension of the ovary in both the species showed oocytes at different stages of development (Figure 8). The dimensions of GCs (oocytes) varied during different seasons. Here, the oocytes of rohu showed maximum growth in monsoon with length and width as 758.5 µm and 784.4 µm respectively. In tilapia, the length and width of oocytes had no significant differences between the pre-monsoon and post-monsoon periods. The oocytes (egg) of teleost's consist of a funnel-shaped depression called micropyle through which spermatozoa enters. The diameter of the micropyle canal (4.1 µm ± 0.2) of rohu was larger than the width of the sperm head (1.6 µm). Similarly, the tilapia micropyle canal diameter was 5.5 ± 0.3 µm larger than the sperm head (1.8 µm).

The electron micrograph of spermatozoa showed a primitive acrosome less sperm differentiated into a nucleus (head), a small midpiece and a flagellum in Figure 7. In tilapia, spermatozoa had an ovoid head with 2.03 µm length and 1.8 µm width. The middle piece has a cylindrical length and width of 0.81 ± 0.02 µm and 0.94 ± 0.03 µm respectively. The flagellum length was 16.5 ± 0.25 µm and the total length of spermatozoa was 19.34 ± 0.31 µm. In case of rohu sperm, head length and width were 1.9 ± 0.2 µm and 1.6 ± 0.2 µm respectively. The middle piece has a length and width of 0.6 ± 0.03 µm and 0.5 ± 0.07 µm, respectively. The flagellum and total length of spermatozoa were 21.5 ± 1.2 µm and 24.0 ± 0.4 µm respectively.

Discussion

Germ cells are inseparable part of sexual reproduction in animals under optimal climatic conditions. They are the first cells in gametogenesis and these cells differentiate to produce end products like

male and female gametes, sperm and unfertilized eggs (oocytes or ova). The production of GCs regulates maturation and reproduction in fish that varies from species to species. Some mature and spawn once in a season like Indian major carps while others like tilapia does this act multiple times. For better management of aquaculture of these two species, it is important to study their GC proliferation and maturation patterns. The study of seasonal variation in germ cell proliferation of carps and tilapia is limited. Here, an attempt has been made for the first time to assess the GC status and correlate seasonal variations in two types of spawners rohu and Nile tilapia having two diverse germ cell proliferation, maturation and spawning patterns. Patra et al. (2015) reported that increase and decrease in water temperatures arising from seasonal climate change, may advance or retard spermatogenesis and oogenesis through endocrine signalling pathway. Billard et al. (1995) and verma et al. (2009b) reported seasonal variation in quality of male gametes of some carps. Here, the GC proliferation and maturation pattern of rohu and tilapia showed that the maturation pattern is regulated by season. The peak activity of GC proliferation and maturation in rohu was observed in monsoon months only whereas in tilapia two peak activities recorded in pre and post monsoon months. These activities in rohu and tilapia were confirmed through assessment of gross gonad anatomy, GSI, histology, dye uptake and electron microscopy. A comparative cyclic GC proliferation and maturation model is shown in Figure 9.

GSI is used to determine the degree of ripeness of the gonads. In rohu seasonal variations in GSI and the percentage of matured fish were quite evident and the pattern was more or less similar in both sexes. Indeed, fish with well-ripened gonads and matured eggs were noted exclusively in monsoon season. In our study, gonads of rohu started developing in March when the water temperatures started rising and ultimately showed a peak in July. This means that in pre-monsoon when the water temperature was 30 °C, the ovary started increasing in weight and showed histological advancement. Later on, when the temperature in monsoon rise to nearly 32 ºC, a fully developed ovary was observed. Differences in the mean GSI values and percentage of matured male and female of rohu may be summarized as monsoon>pre-monsoon>post-monsoon. GSI values and percentage of matured fish indicated that breeding of rohu was seasonal and its activity was highest during monsoon season. Hora (1945) reported that in Indian rivers, rohu spawns once a year, during the monsoon season. Similarly, Parmeswaran et al. (1974) stated in their reports that GSI was higher in the rainy season (monsoon) than in the dry season (pre-monsson) in *L. gonius*, conforming that monsoon is most suitable for success of reproduction in carps. Kaur et al. (2018) reported that the low GSI of carp fish may be due to dormancy of gonads in post-monsoon

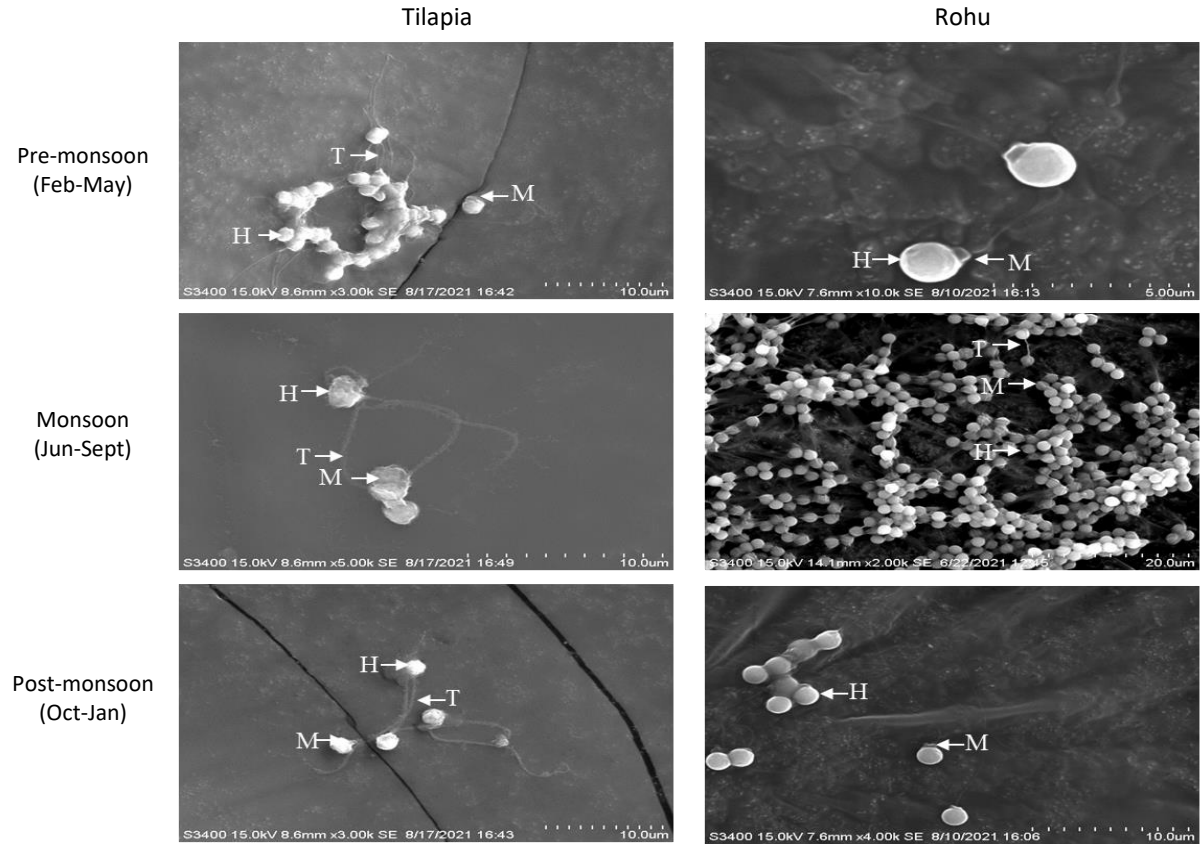

Figure 7. Electron micrographs (SEM) of tilapia, *O. niloticus* and rohu, *L. rohita* males showing changes in GC morphology during different seasons of the year. (H-Head; M-Middle piece; T-tail/flagellum).

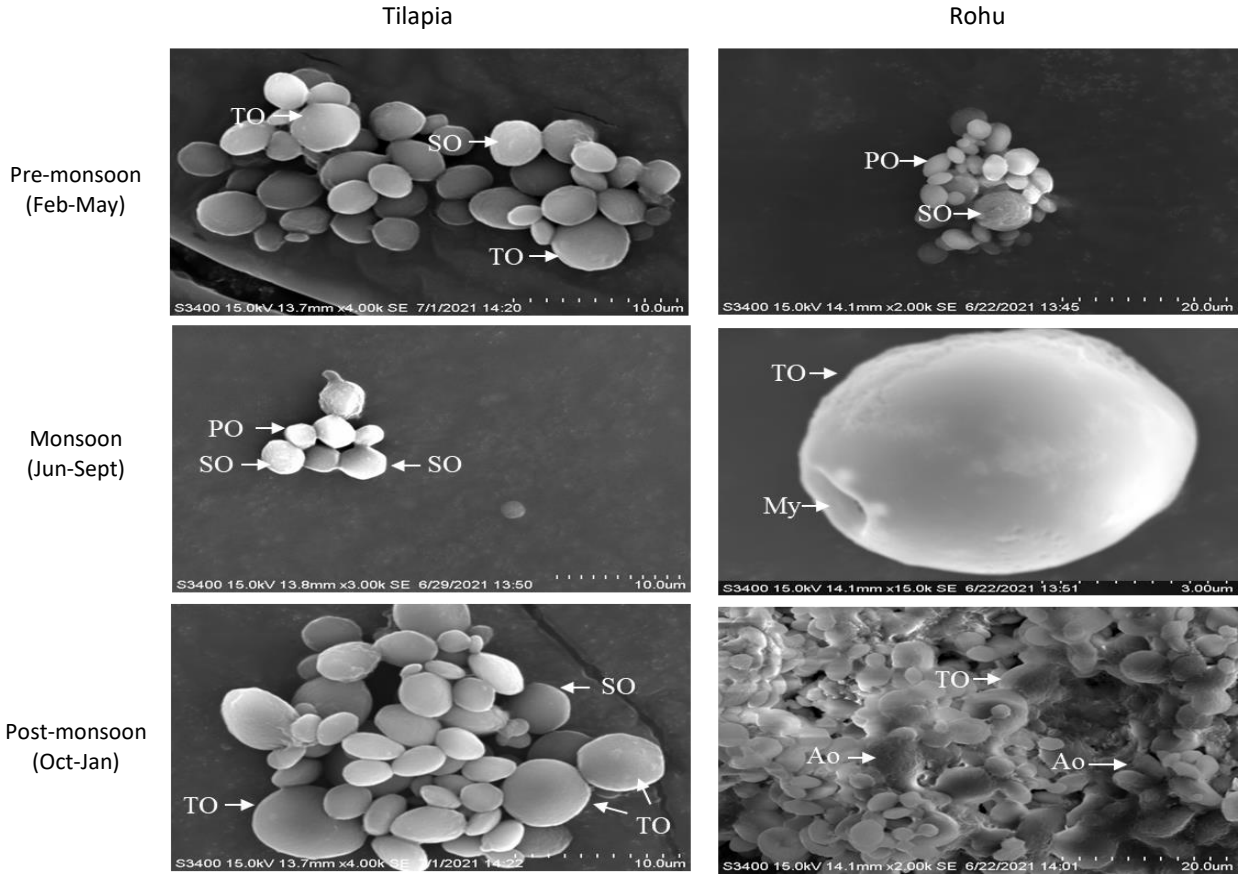

Figure 8. Electron micrographs (SEM) of tilapia *O. niloticus* and rohu, *L. rohita* females showing changes in GC (oocyte) morphology during different seasons of the year. (PO-Primary oocyte; SO-secondary oocyte, TO- tertiary oocyte, My-Micropyle, Ao-Atretic follicle).

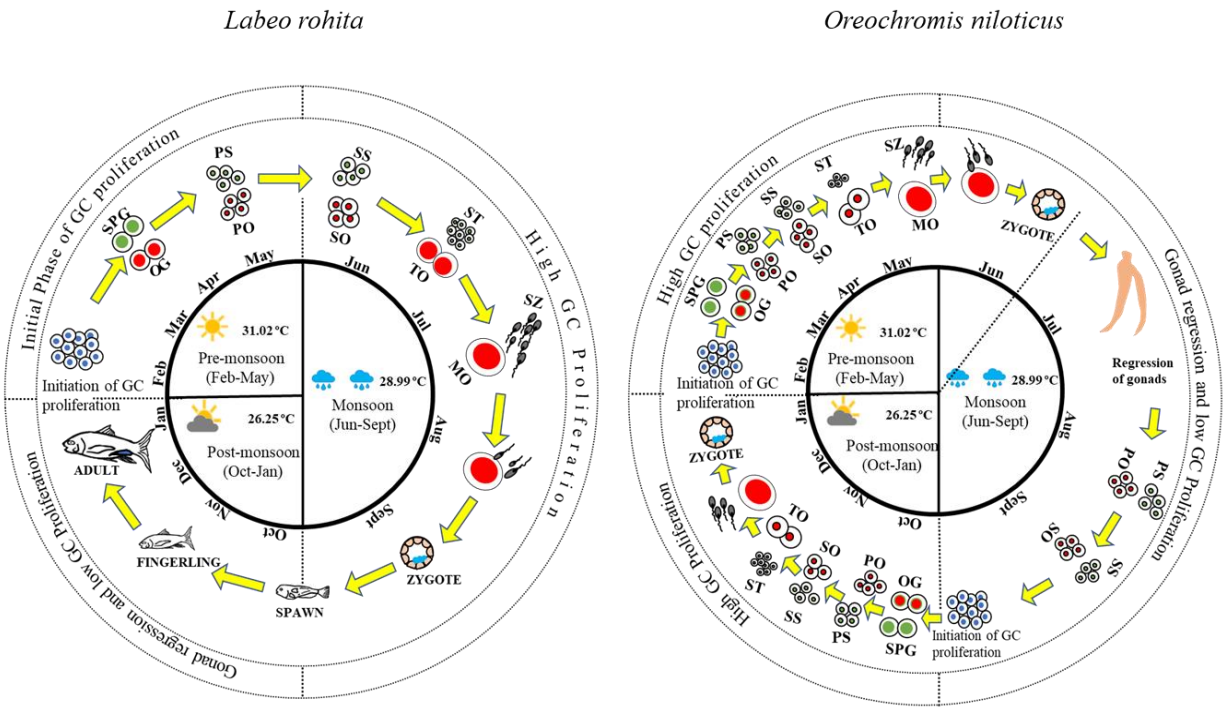

Figure 9. A cyclic depiction of seasonal GC proliferation and maturation in *Labeo rohita* and *Oreochromis niloticus* based on our studies in the state of Odisha, India. PGC- primordial germ cells, SPG- spermatogonia, PS- primary spermatocyte, SS- secondary spermatocyte, ST- spermatids, SZ-spermatozoa, OG- oogonia, PO- primary oocyte, SO- secondary spermatocyte, TO- tertiary oocyte, MO-mature oocyte.

season. The GSI in asynchronous batch spawners is usually lower than that in synchronous total-spawning species (Tyler & Sumpter, 1996; Rinchard & Kestemont, 2003). Here, GC patterns and maturation in rohu and tilapia indicated that the former is a total and synchronous spawner whereas tilapia is a multiple spawner and breeds at least twice in a year in natural systems.

In tilapia, the GSI and percentage of matured fish did not differ significantly during pre-monsoon and post monsoon seasons (Pre-monsoon = Post-monsoon > Monsoon). GSI values and percentage of matured fish indicated that breeding in tilapia was year-round and highest recorded in pre-monsoon and post-monsoon months. Admassu (1996) reported gonadosomatic index (GSI) values showed two peaks in a year, a major peak observed during January–March and a less pronounced one during July–September. Jubb (1952) reported in *T. mossambica*, as the temperature of water gets warmer breeding activity get started. Akel & Moharram, (2007) reported the higher values GSI during the period from June to September with a peak in July, while the lower ones occurred during the period from October to February in *T. zillii*.

Histological observation confirmed the gonadal maturation and spawning season in these two species. The use of histology in maturity studies has become more and more widespread as it is more consistent and reliable (Tomkiewicz et al., 2003). The histological analysis of female rohu revealed that the size range of oocytes was similar to that observed in other cyprinids like bream (Domagała et al., 2015) or roach (Geraudie et al., 2010). In post-monsoon, the spent ovary displayed atretic oocytes which is a common phenomenon in the teleost ovary (Saidapur, 1978; Witthames et al., 1995; McDermott et al., 2007). In the testis of rohu, the changes in seasonal rhythms were determined according to the presence of spermatogenic elements in the testes. In pre-monsoon, the active spermatogenesis with primary spermatocytes, secondary spermatocytes and fewer spermatids were observed. Lone et al. (2009) reported a similar histological observation in carps during the pre-monsoon. In the ovary of tilapia, different stages of oocytes were observed from pre-monsoon to post-monsoon but in the peak spawning season the number of matured oocytes increased. Our study was similar to Babiker & Ibrahim (1979) in which the size frequency distribution of intra-ovarian oocytes in ripe ovaries were commonly found in pre-monsoon and post-monsoon. This is indicative of the presence of two generations of oocytes in ripe ovaries. Many studies have also suggested that tilapia's ovary has all the stages of oocyte development throughout the year (Admassu, 1996; Tave, 1988; Negassa & Getahun, 2003). The tilapia testis also showed similar peak breeding in pre-monsoon and post-monsoon with more spermatozoa in seminiferous lobules. El-Sakhawy et al. (2011) reported the testicular tubules showed different activity where some tubules were filled with spermatozoa in spring, summer and autumn seasons. Craig et al. (2000) reported evidence for testicular recrudescence (proliferation of spermatogonia) in March, with the first spermiating male in July. In tilapia different workers reported different spawning periods which were overlapping (Admassu, 1996; Tave, 1988; Negassa & Getahun, 2003; El-Sakhawy et al., 2011).

In the present study, the predominance of mature and ripe ovaries from pre-monsoon to monsoon indicated that rohu has a precise spawning season with individuals spawning during peak monsoon, which is also confirmed by the maximum values of GSI. The estimation of GSI revealed that the fish spawned once in a year mainly from June to August. This was in accordance with the observations of Lashari et al. (2007) in case of *Cirrhinus reba*. It is reported that temperature plays a vital role in gonadal reappearance and timing of ovulation. If the optimum temperature cannot be maintained the maturation may delay or the eggs may release spasmodically and total amount of eggs may also be reduced (Hanumantharao, 1971).

Ultrastructure studies of spermatozoa of rohu and Nile tilapia were reported here. The morphological structure displayed by spermatozoa of rohu in this study showed structural similarities to those described in most of the teleostean fishes in which lack of acrosome is a common character (Mattei, 1970). A variety of acrosomal-like structures are found in fish spermatozoa (Mattei, 1970; Stanley, 1971). Further, sperm length differs across fishes between buccal and substrate spawners (Balshine et al., 2001) and between internal and external fertilizers (Stockley et al. 1996). The acrosome reaction occurs inside or on the surface of the egg envelope to allow sperm penetration in mammals where as in case of teleostean fish such as carps, spermatozoa reach the egg plasma membrane through a narrow micropyle because the sperm lacks an acrosome (Morisawa, 1995). The sperm has a slightly ovoid nucleus in head and mid-piece oriented with centrioles and mitochondrion followed by flagellum having axonemal structure for movement (Jamieson, 1991).

The ultrastructure of spermatozoa may be varying more or less broadly within the families (Verma et al., 2009b). For instance, the spermatozoa of two diverse species revealed that each was characterized by a specific organization of sperm organelles within a broad range representing the whole family (Baccetti et al., 1984). Egg diameter of fish also varies from species to species. It depends onfactors like fecundity, which is influenced by environmental factors such as temperature (Idowu, 2017).

In summary, we may say with certainty that the rohu is a group synchronous spawner and ovulates in the monsoon months once in a year due to activation of the hypothalamo-hypophyseal-gonadal axis after getting environmental stimuli. However, tilapia is a multiple spawner and their gonads are mainly dominated by matured gametes in the peak season,

leading to intermittent fry production. Further, this study implies that the GCs can be dyed with florescent dye without compromising cell viability.

Conclusion

In conclusion, the seasonal changes including changes in water temperature is shown to affect the maturation pattern of rohu and tilapia due to variation in germ cell proliferation. The GC content and electron micrographs in these two species showed a clear and varied pattern of maturation and spawning behavior.

Ethical Statement

Prior to the experimental design and initiation, the ethical clearance of the Institute Animal Ethical Committee was also obtained.

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Author Contribution

B. Panda planned and carried out the work and ms preparation, O. Gopi Krishna, S. Manna, D. K. Verma, participated in the experimental set up, analysis of data, L. Samanta and P. Routray: Overall planning, experimental design, data analysis and ms preparation. All co-authors read the final manuscript, corrected appropriately, and approved it for publication.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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