INT. J. BIOL. BIOTECH., 15 (3): 541-548, 2018.

STUDY ON SPAWNING FREQUENCY OF *CYPRINUS CARPIO* WITH RESPECT TO GONADO SOMATIC INDEX

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ABSTRACT

A correlated study between gonadal cycle and pituitary cells has been carried out in terms of GSIs on *Cyprinus carpio*. The main purpose of study was to understand the effect of various seasons on breeding cycles of the experimental fish. The fish was found as a multiple times breeding species. Its breeding cycle has been divided into four stages naming pre, 1st, 2nd and post breeding season. Also the pituitary cells were found small sized granulated during preliminary season, large sized granulated during pre-breeding, fully granulated/chromophilic during first breeding and degranulated / chromophobic at the onset of second breeding season. Highest peak of GSI **(12.1 ± 1.2)** was first found in the month of March while second peak was observed in the month of June **(12.06 ± 0.72).** The study results will benefit the fish farmers in terms of economic benefits as this is a multiple breeding fish, thus farmers can get the yields twice as compared to other teleost fishes.

Key-words: GSIs, granulated, degranulated , economic benefit, breeding season.

INTRODUCTION

Cyprinus carpio, commonly known as Common Carp or Gulfham is a commercial carp of Central Asia. It is most extensively cultivated in tropical, sub-tropical and temperate regions of the world because it is the most adaptable species in terms of variable climatic and environmental conditions as well as different feed varieties. It tolerates cold as well as hot temperatures and the salinity ranges upto 7ppt.

Many scientists and researchers have worked on annual reproductive cycle and seasonal changes in the gonadosomatic index and/or histological changes in the ovaries or testes, (De Vlaming, 1972, 1974, 1975; Egami 1954; Sundararaj and Vasal, 1976; Jalali and Haider 1985, Saikh and Jalal 1991; Coetzee, 2015; Gadekar *et al.*, 2014.; Kirti *et al*., 2014).

A lot of researchers agreed that all fresh water temperate zone fishes that spawn in spring or early summer have gonadal reactivation in the winter or spring in response to long photoperiods and warm temperatures (de Vlaming 1972, 1974; Egami, 1954; Billard 1977; Breton 1980; Harrington 1957; Vasal,1976; Jalali and Haider, 1985; Shaikh and Jalali, 1991; Akio Shimizu, 2002; Sivakumar *et al.*, 2003).

In India, it has been observed that *C. carpio* presumably known as a group spawner, possess two peak breeding seasons;

1. Jan- March
2. June-August

while mature males and females can be found throughout the year. During the breeding season females are found with soft, bulging and prominently protruded vent while male also have bulky and bulging bellies but are differentiated because of the presence of pectoral fins and tubercles on the scales. The egg lying ability of the female depends upon its age and size. Oocyte may vary in size from 1.0 to 1.4 mm in diameter. Sivakumran *et al.* (2003) and others scientists observed mature males and females of Common carp throughout the year, the highest numbers are seen during January to March and July to August.

For the sake of convenience, breeding cycle of Common Carp has been divided into three seasons,

1. Pre-monsoon; As early as , March – May
2. Monsoon; After 45 days , June-July (Gupta *et al.*, 1975)
3. Late monsoon; August-September

MATERIAL AND METHODS

Experimental Design: Conditioned males and females were procured from Maqbool Aijaz Hatchery located at Ver road Thatta. The mean body weight of 250 individuals of *Cyprinus carpio* was 557 ± 26.78 g. They were fed with the test diets prepared from non-conventional food stuffs. After carried out preliminary steps, Ten males and Ten females were randomly selected to study the patterns of maturational changes in gonadal cycles in relation to

the pituitary gonadotrophs. Morphometric data were taken before the administration of chloroform for further proceeding. Extraction of pituitary gland and gonads were taken followed by fixation in Bouins solution for histological examination. Prior to making histological slides, pituitary gland and gonads were weighed separately. Gonado somatic index (GSI) was calculated with the help of obtained information by the formula:

GSI= Gonadal weight/ total body weight x 100

Histological Examination:

The tissues separated from pituitary gland, testes and ovaries were washed in tap water and forwarded for dehydration and hydration. Treated tissues were embedded in paraffin wax for sectioning as thin as 6 micron meter by using rotary microtome. These sections were stretched on the albumenized glass slides by using slide warmer.

Staining of the Sections:

Staining of gonads and pituitary gland were done separately. Gonadal sections were stained in haematoxylin and eosine whereas specialized techniques were used to stain the sections of pituitary gland. These techniques include;

1. PAS Technique (Ahmed *et al*., 2011)
2. AB_PAS_OG Technique (Alcian Blue_PAS_Orange)
3. Combined Stained Technique (Ahmed *et al.*, 2011)
4. PbH Stain Technique

All processed sections of pituitary and gonads were studied histologically under the light microscope.

RESULTS

Reproductive process in fishes is correlated with the seasonal changes. Among Carps particularly *Cyprinus carpio* respond in accordance with the onset of monsoon season. Different stages of maturation are noted during the whole reproductive cycle that include preparatory phase, pre-spawning phase, spawning phase and post spawning phase. These phases are demonstrated by the values of gonado somatic indices and histological studies.

Gonado somatic index in male fish;

The calculated value of GSI was remain less in October **(6.21 ± 0.36)** and increased progressively with respect to proceeding months as much as **(12.1 ± 1.2)** in the month of March and **(12.06 ± 0.72)** in June. The subsequent increase in GSI values from October- February governed the active proliferation of spermatogenic cells where as a sudden drop is noted during April and May indicating a second proliferating phase (Table 1).

Gonado somatic Index in female fish;

Data pertaining to GSI calculations for female fish is given in table 2. Lowest GSI value **(3.10** ± **0.5)** was noted in October and creeping rapidly as much as **(23.61** ± **2.62)** in the month of March showing the fully grown ovaries as first phase of spawning. These values were found to be least in the month of April **(8.5** ± **0.87)** and again approach to its second increase up to (**22.7** ± **2.32)** in the month of June followed by subsequently decreasing factor at the end of experimental duration.

Table 1. Mean values for growth and reproduction ± SE in male fish *cyprinus carpio.*

Months	Total Length (cm)	Total body Weight (g)	Total Gonadal Weight (g)	GSI
October	34.4 ± 0.24	557.7 ± 26.78	35.0 ± 3.95	6.21 ± 0.36
November	31.91 ± 0.31	419.0 ± 12.04	44.16 ± 3.44	9.0 ± 0.95
December	36.38 ± 0.90	644.0 ± 44.95	60.0 ± 5.09	9.55 ± 0.23
January	30.03 ± 0.81	468.3 ± 25.18	44.16 ± 3.8	10.09 ± 0.78
February	37.7 ± 0.46	713.0 ± 52.60	80.0 ± 4.0	11.61 ± 1.0
March	37.5 ± 1.22	758 ± 42.78	95.0 ± 6.2	12.10 ± 1.2
April	34.0 ± 0.48	490 ± 26.07	47 ± 3.89	9.54 ± 1.0
May	41.8 ± 1.29	893.3 ± 31.32	68.33 ± 3.22	7.45 ± 0.91
June	41.16 ± 1.16	1020 ± 69.84	123.33 ± 8.94	12.06 ± 0.72
July	38.1 ± 0.89	725 ± 70.47	55 ± 6.01	7.55 ± 1.0
August	36.9 ± 0.35	658 ± 35.38	52 ± 3.04	7.95 ± 0.87
September	37.9 ± 0.35	848 ± 58.76	69.8 ± 18.03	7.98 ± 0.23

Table 2. Mean values for growth and reproduction ± SE in female fish *cyprinus carpio.*

Month	Total Length (cm)	Total Body weight (gm)	Gonadal WT (gm)	G.S.I
October	35.2 ± 3.51	472 ± 30.19	14.4 ± 1.51	3.10 ± 0.5
November	31.3 ± 0.60	418 ± 22.16	42.6 ± 3.76	10.07 ± 1.0
December	39.37 ± 0.40	955 ± 40.38	120 ± 7.90	12.74 ± 1.02
January	31.46 ± 1.16	534 ± 36.16	90 ± 7.54	18.00 ± 1.78
February	34.1 ± 0.79	543 ± 24.31	113 ± 12.45	20.68 ± 1.90
March	38.9 ± 0.87	908 ± 62.12	214 ± 22.9	23.61 ± 267
April	37.25 ± 1.15	710 ± 47.56	60 ± 7.07	8.5 ± 0.87
May	35.5 ± 1.21	630 ± 46.77	99.5 ± 9.36	15.9 ± 91.20
June	39.0 ± 3.97	953.3 ± 160.76	221.66 ± 93.39	22.7 ± 2.32
July	39.1 ± 1.31	791 ± 41.25	115 ± 18.26	14.58 ± 1.23
August	36.7 ± 1.36	701 ± 46.65	101 ± 9.63	14.93 ± 1.26
September	37.75 ± 1.13	873.75 ± 86.01	83.75 ± 7.78	9.81 ± 0.96

Histological Examination of Testis

Histological examination of tests revealed that reappearance of spermatocytes was noted twice during experimental duration.The timespan from Nov. to Jan. was specific for proliferation of primary spermatocytes **(Fig.1b)** whereas secondary spermatocytes and spermatids were noticed in Feb. and may be concluded as pre breeding period **(Fig.2b)**. Later on histological sections exhibit that lobules were loaded with spermatozoa **(Fig.3b)** along with some spermatogonial cells **(Fig.4b)** of testicular section show the presence of spermatozoa in lobules and concluded as the second spawning period in the month of June. Later stages of experimental duration i.e. from August to October, testes were comprised of only spermatogenic material and picnotic cells **(Fig.5b)**.

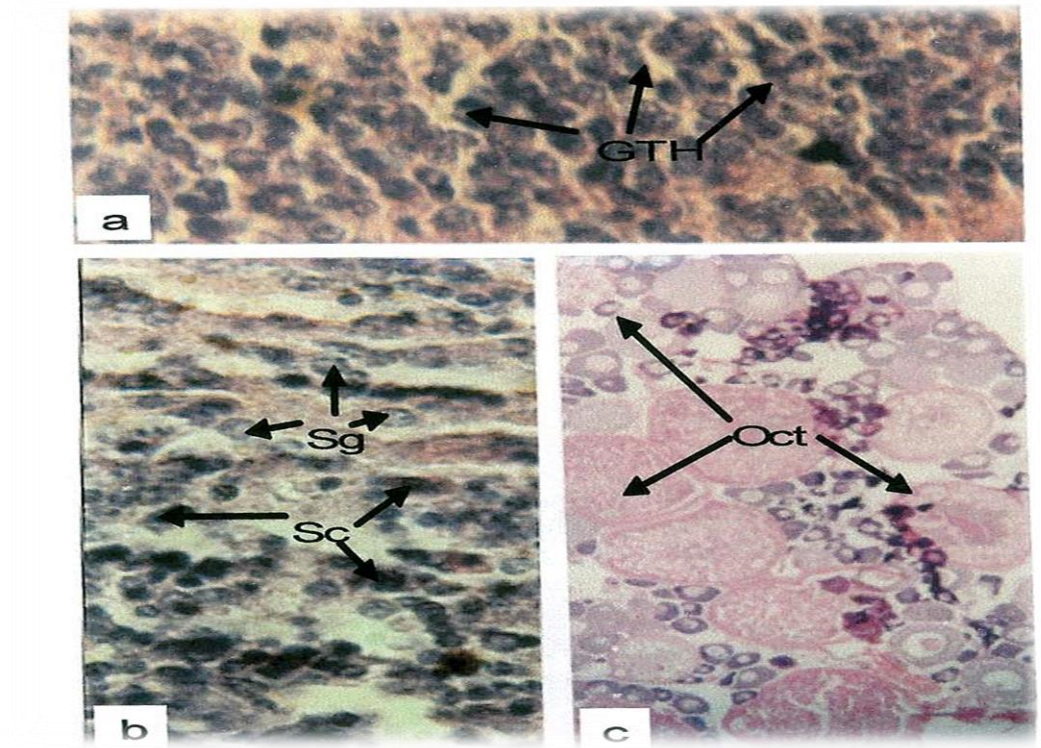

Fig.1. Preliminary Season (Nov - Jan), (a) Small-sized granulated GTH cells (X100).
(b) Some spermatognia (sg) and specifically spermatocytes (sc) (X100) (c) Ovary shows nearly every type of oocyte (oct) (X40)

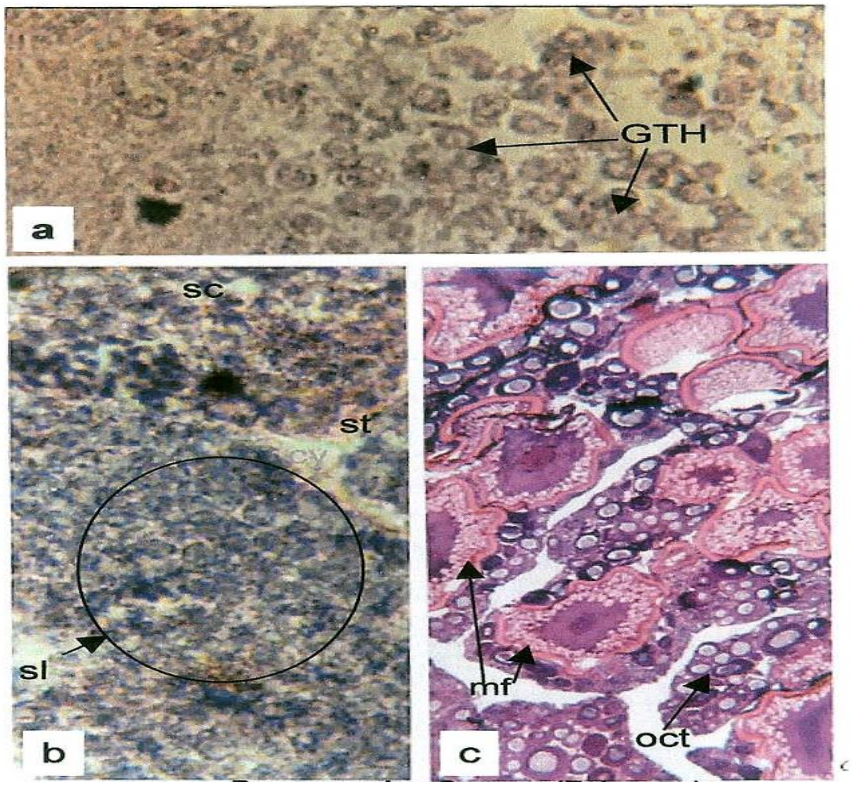

Fig.2:. Pre-Breeding Season (February).
(a) Large sized, granulated GTH cells (X100) (b) Testis showing seminiferous lobules (sl) loaded with bulky cyst (cy) filled with spermatocytes (sc) and spermatids (st) (X100)
(c) Ovarian follicles with oocytes

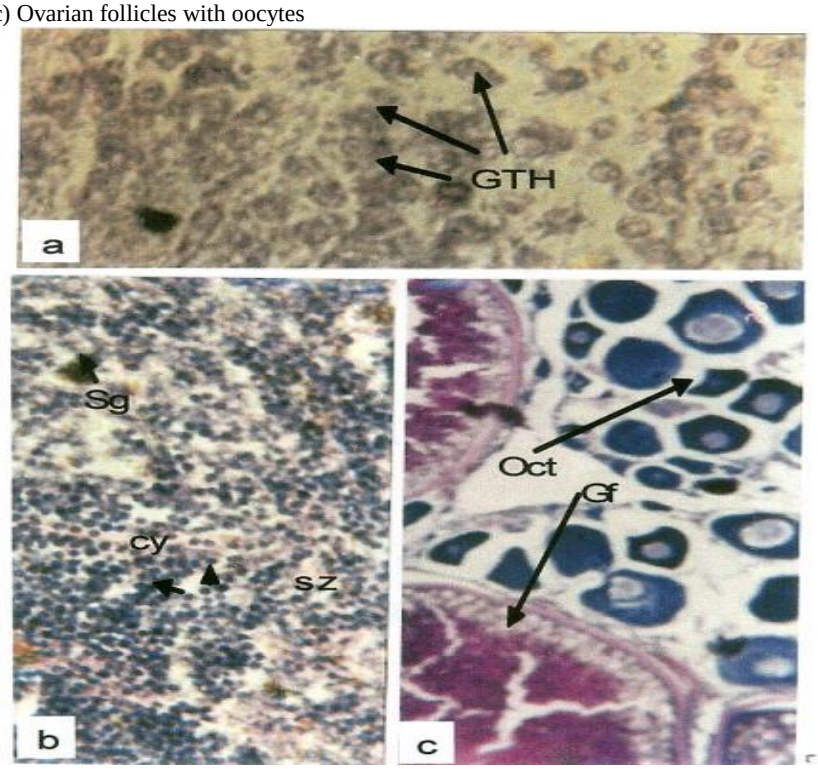

Fig.3. First Breeding Season (March).
(a) Completely granulated GTH cells (X100) (b) Testicular cysts (cy) loaded with spermotozoa (sz) and some spermotognia (sg) (X100) (c) Riped Ovarian Graffian follicles (Gf) and multiple maturing oocytes (oct) (X40)

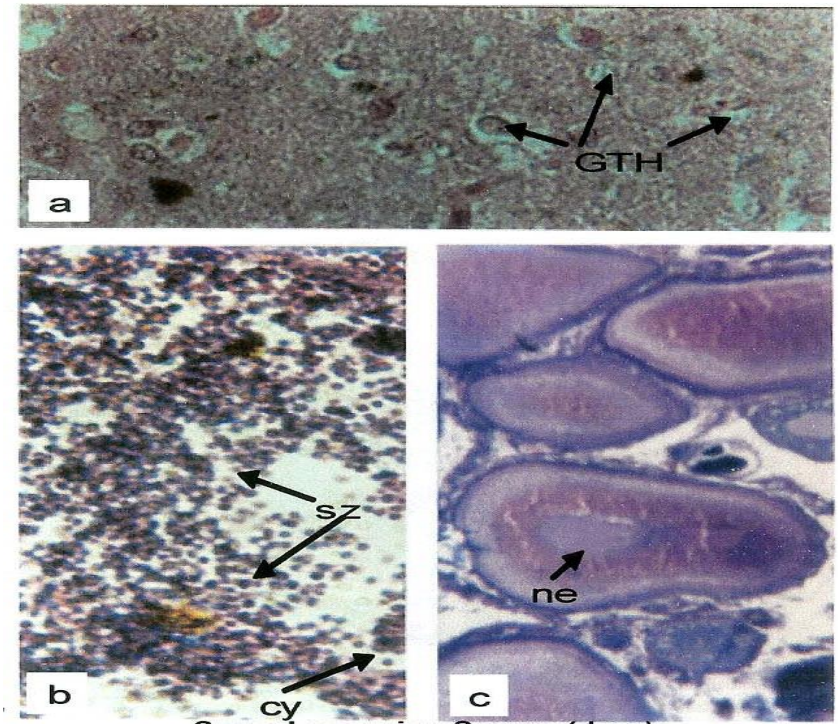

Fig.4. Second Breeding Season (June).
(a) Large degranulated GTH cells(X100), (b) Large cysts (cy) with mature spermatozoa (Sz) (X100) , (c) Large Ovarian follicles with collapsed nuclear envelope(ne)

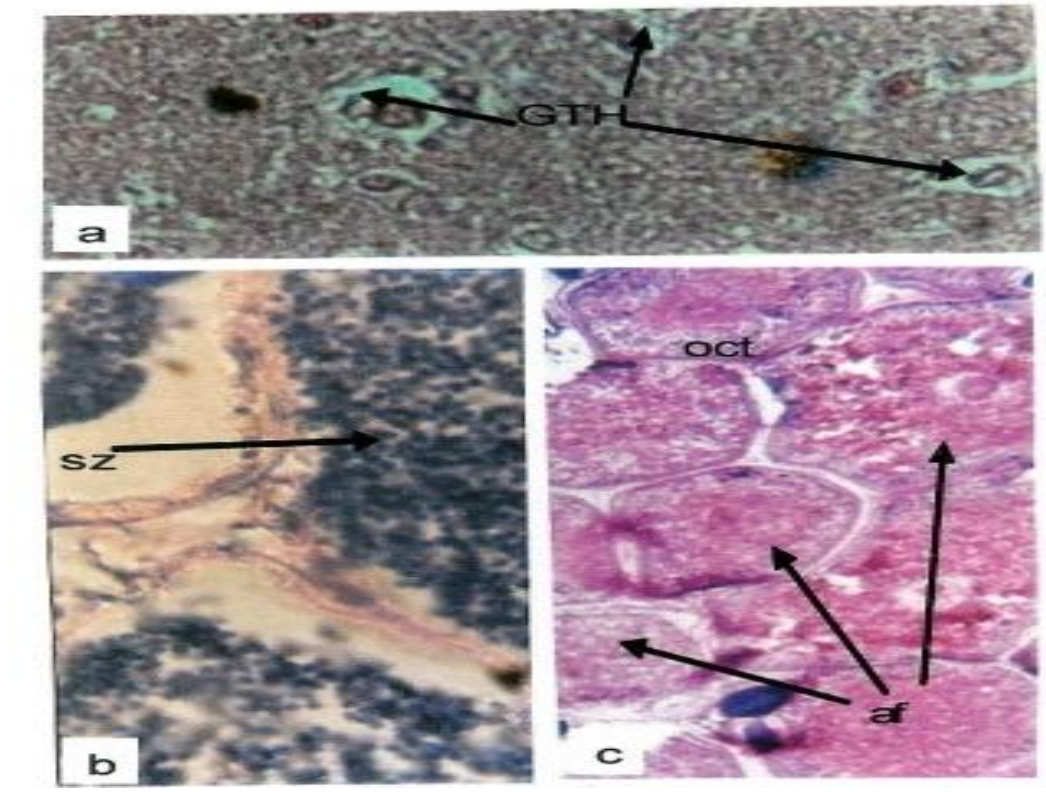

Fig.5. Post Breeding Season (August-September).
(a) Pour out GTH cells (X100) of Pituitary gland, (b) Remaining leftover spermatozoa (sz) (X100) in testis, (c) atresic follicles (af) and some oocytes (oct) (X40) in Ovary.

Histological Examination of Ovary

Numerous immature follicles were noted contained all types of oocytes from Nov. to Jan **(Fig.1c)**. In accordance with the advancement in ovarian cycle matured follicles were clearly visible by means of appearance of nuclear membrane **(Fig.2c)**. Ovulated follicles were occupied in major part of ovaries and ready to ovulate in the month of March **(Fig. 3c).** The left over immature oocytes were now transformed into matured follicles after an interval of two months **(Fig. 4c)** in June. The tissue samples from August to October exhibit the post breeding season with the presence of residual atretic follicles **(Fig. 5c)**.

Histological Examination of Gonadotrophs

It is visible from **(Fig. 1a)** that the Gonadotrophs (GTH) cells were seen as small granules in the anterior part of piyuitary gland and become significant in numbers by the increasing dimensions during pre breeding season **(Fig. 2a)**. The conversion of small sized GTH cells into larger ones were noticed before the beginning of first breeding season **(Fig. 3a).** The appearance of large sized nuclei was observed in the month of June **(Fig. 4a)**. By the completion of spawning season, the GTH cells were chromophobic **(Fig. 5a)** and this process prolonged up to the next reappearance at the time of start of breeding cycle.

DISCUSSION

It was expected by the experiments on *Cyprinus carpio* that some fruitful results will become the eminent source of our success in case of relationship between Spawning frequencies and GSIs. Also the current study helps us to understand the effects of various hormones on different reproductive phases of Cyprinus carpio.

In our study, *Cyprinus carpio*, a Chinese fish, is found to spawn twice in a year that shows its nature of spawning at multiple times. Its reproductive cycle has been divided into two phases, Ist spawning (in the month of March) and 2nd spawning (in the month of June). Also the months show that there are three stages of its reproductive cycle as Pre Spawning phase, Spawning phase and Post Spawning phase. Thus our studies revealed that *Cyprinus carpio* is a fish having strong effects of weather on its reproductive cycle and growth as well. Also it is a group spawnner fish.

Different studies have been put forward to explain the reproductive biology as well as reproductive behavior of various teleost fishes. Crossland (1977) described *Chrysophrys auratus* , Manooch,1976 presented *Pagrus pagrus* (Red Progy). Hussain and Abdullah (1977) worked on *Acathopagrus latus* and *Acathopagrus cuvieri*, Jalali and Haider (1985) explained *Barilius vagra* (Ham) and Shaikh and Jalali (1991) proved *Cyprinion watsoni* as group spawnner fishes. A lot of studies are already done on the reproductive behavior of Teleosts (Hour, 1969; Polder, 1971; Cyrus and Blaber, 1984; Grier *et al*., 1980; Grier , 1981). Short work has been done to explain the reproductive psychology, spawning frequency and seasonal effects on reproduction and growth of Cyprinids. (Leatherland and Sonstegard, 1978; Jalali and Haider, 1985; Shaikh and Jalali, 1989-91; Ursani *et al*., 2011; Zarina *et al.*, 2011).

Our studies disclosed Gonado-Somatic Indices (GSIs) in male and female fishes are at its peak in the months of March and showing the full breeding season which suddenly falls in the month of April. While a second rise appear in the month of June showing its second breeding season. Different investigators have found different readings in the GSIs. Abu Hakima in 1984 found maximum GSI in the month of Feb-March for Acanthopagrus species. Marcus and Kusumiji (1980) showed maximum readings for males of *Ilishiya africana* in the months of May- July and Oct-Dec. while its females show maximum maturation in the months of May to Oct. and Dec. Shaikh and Jalali (1991) presented highest peak in the months of March–April in Cyprinion watsoni. Ursani *et al*. (2011) declared highest level in the months of May and August in *C.carpio*. Zarina *et al*. (2011) found two top readings during reproductive cycle of same female fish in the months of Feb. and July; Ashwani and Ghanbahadur in 2012 has proved two peak values from July to August and from Jan- March; Emam in 2014 described March-May and June-August as two peaks for Catfish, *Clarias lazera while Kiran* in 2015 presented the evidence of three spawning seasons for Salmostoma untrahi.

The maturation of GTH cells is characterized by the appearance and disappearance of granules within the basophil cells. Large number of granulated and a few degranulated basophil cells show the onset of reproductive period. After period is over, the basophil cells start degranulating until they become reactivate again. The similar condition of GTH cells during male and female breeding cycle has been reported (Sundararaj, 1959; Peter and Crim, 1979; Stacey, 1984; Kobayashi *et al.*, 1988; Ursani *et al.*, 2011).

The histological studies of pituitary and gonadal sections show various seasons during reproductive cycle of *Cyprinus carpio.* Many spermatocytes, a few spermatogonia , some ripped follicles and differential oocyte stages are visible along with the cytoplasmic granulated GTH cells. The presence of cytoplasmic granules stimulate the GTH

cells for the production of gonadotrophin which in turn prepare leydigs cells in the testes and granulosa and theca cells of the ovarian follicles to discharge androgen and estrogen respectively. Androgens are ready to develop spermatocytes whereas estrogen gets the maturation of oocytes. Also increased propagation of GTH cells and cytoplasmic granule are indicators of next preparatory reproductive period. In the month of Feb. the testes and ovaries show the enhanced stages of development. Our findings mostly match Sundararaj as He is also emphasizing on granulation and degranulation of GTH cells as a sign of large quantity of FSH and a little bit of LH for development of oocytes.

Cyprinus carpio is a serial spawner fish, can spawn twice a year. First spawning occurs in the month of March while second spawning occurs in the month of June. Testes show very large sized cysts with many spermatozoa and ovarian sections show ripped follicles with mature ova. Maturation of both the gonads occurs by the GTH cells.

During the month of March, the GTH cells show the large number of granulated and a few degranulated basophil cells that indicate the onset of first breeding cycle. Degranulated cells are indicating the maturation of gonadal cells and release of LH for ovulation of mature ova (Sundararaj, 1959). Also the GTH cells are similar in the post breeding season as no LH or FSH is needed. These degranulated cells are visible in the months of July-October to express the pre and post breeding periods.

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(*Accepted for publication June 2018*)