TFR
Trends in Fisheries Research
An International Peer-Reviewed Journal
www.sciencejournal.in

SEASONAL CHANGES IN THE CYTOLOYG OF ADENOHYPOPHYSIS, TESTES AND OVARIES OF THE HILL STREAM LOACH NEMACHEILUS MOREH

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ABSTRACT
An attempt was made to study the correlation of seasonal changes in the gonadotrops (GTH) from the adenohypophysis with the simultaneous changes in the ovaries and testes according to spawning season throughout study period. In *N. moreh*, most of the PAS+ve basophils assumed to be gonadotrophs on the basis of staining properties and reveal one kind of gonadotrophs, but appearance of granulation in cytoplasm is of two types and hence may be considered as two types of gonadotrophs. During the spawning period, the pituitary has an overwhelming number of granulated basophils. During post spawning period the granulation of basophils gradually decrease, may due to releasing large amounts of FSH and trace of LH which are responsible for oogenesis and spermatogenesis.

KEY WORDS: Pituitary gland, Gonadotrophs, Adenohypophysis, Testes, ovary

INTRODUCTION

Present study has been designed to study the edible fish *Nemacheilus moreh* from Mula - Mutha river system in northern Western Ghats, Pune in Maharashtra, situated between 17°54" and 19°21" N Latitude and 73°24" to 75°14" E Longitude in western India. Most of the studies on reproductive biology of teleost have been described through development of gonad in terms of stage of maturity of ovums, (Kesteven,1960), (Nikolsky, 1963) and of gonadosomatic index, (LeCren, 951), (Craig, 1974). It is well known fact that the pituitary gland play important role in the maturation of gonads .Spawning in teleost during a particular phase of the reproductive cycle represents a spawning behavior. Some species are annual breeder, some breeds at regular intervals throughout the year. Most of the teleostean species manifest annual rhythm of breeding, (De vlaming,1975). The pituitary-gonad interrelationship has been variously assayed by hypophysectomy, (Mathews, 1939), injection of pituitary extracts ,(Houssay, 1930. 1931), (Cardeso, 1934). Experiments on the induction of spawning by pituitary extracts in Indian species have been attempted by (Khan,1938) with appreciable degree of success, (Alikunhi and Choudhary, 1957), and (Ramaswamy, 1958). Reports on the correlative changes of the pituitary and gonads are available only in isolated instances, (Brestchneider and deWit, 1947); (Scruggs 1951); (Stolk 1950), (Ursani et al., 2012). Present study is an attempt to correlate the functional mechanism of the pituitary and gonads in fish, *Nemacheilus moreh.*

MATERIALS AND METHODS

The fish *Nemacheilus moreh* were collected from Mutha river, in each month during January 1998 to 2001. Live fishes were brought to the laboratory. Length, weight, sex, gonad weight were recorded. Monthly samples of the ovaries, testes and pituitary glands were fixed in Bouin's fluid, for histological preparations. Tissues were sectioned at 5 to 6 µ on rotary microtome. H/E staining was used to study general histological examination of ovaries and testes. Sections of pituitary gland were stained with Aldehyde fuschin –Orange G – Light green and Periodic acid Schiff technique for demonstration of gonadotrophs.

RESULTS

Seasonal changes in the structure of the pituitary, especially gonadotrophic cell and the gonads in every month were recorded simultaneously.

Prespawnig phase (January and August)

During these months, ovaries become vascularised, and the ova were richly ladden with yolk granules and the abdominal cavity was occupied by the ovary. Developing oogonia appeared, fig. 7, in the wall of ovigerous lamellae.

The testes fully matured, number of spermatogonia and sypermatocytes decreased relatively. The spermatids also show considerable decrease in relative abundance, whereas the spermatozoa increased to the maximum level, fig 5 and 6. The interstitial cells show maximum size and granulation. Pituitary shows many basophils of various stages of granulation, fig. 1 and 2. The nuclei of the basophils were large, rounded and vesicular and situated almost in the centre of cell cytoplasm and uniformly packed with blue staining granules,fig.2 and 3. Chromophobes were very few with indistinct cell boundary.

Fig.1. L.S. pituitary of of *N. Moreh*. (100 X), RPD- Rostral Pars distalis, PPD- Proximal pars distalis, PI- Pars Intermedia, NHP- Neurohypophysis.

II. Spawning phase (February-March and September-October):

Nemacheilus moreh has two spawning cycles once in September and other in February in the year. During this period, the eggs were surrounded by three membranes yolk nucleus disappears and contains continuous mass of yolk, fig.8 . A new crop of oogonia was a distinguishing character of the ovary during phase. The ovary shows decrease in weight and volume due to intermittent discharge of ripe ova. A large number of ruptured follicles were detected indicating spawning reached its peak. Seminiferous lobules shows increase in number of spermatogonia and spermatocytes, but spermatids and spermatozoa were greately decreased. Empty seminferous lobules with traces of mature sperms were observed, fig. 1. The sertoli cells were prominent. The intestitial cells show degranulation.

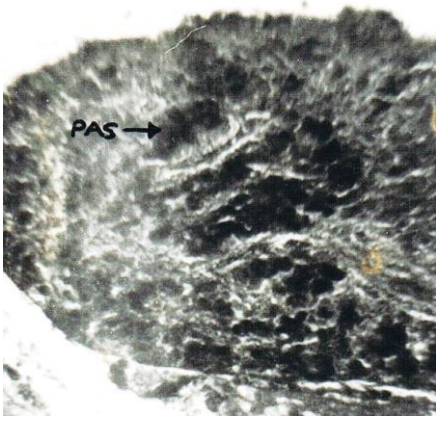

Fig.2. Proximal pars distalis (PAS +ve), GTH cells .

Pituitary gland in this phase, reached to its maximum activity with increase in number of granulated basophils and acidophils. The basophils were (PAs+ve) noticed in major part of RPD. The PAS+ve cells in RPD were more indicating migration and increase in number of GTH cells, fig.1 and2. Some of the basophils were showing degraulation. Acidophils were not indicate any sign of degranulation, fig. 4.

1II. Post-spawing or resting ppase (October-November and April-May).

The ovaries were flaccid and dull coloured, and reduced in size. Tunica abuginea layer appears in these months. Discharged follicles and atretic follicles show various phases of resorptions. Testes were very small having predominant spermatogonia and stress of spermatocytes. No marked changes occurred in interstitial cells.

PPD of pituitary gland shows polygonal or rounded chromphobes with definite cell boundaries. Acidophils were few. Due to the degeneration and disappearance of the cytoplasmic content, large number of basophils looks like chromophobes, fig.4. Therefore, chromophobe predominates this phase. Empty basophils coincide with resting phase of gonads. However, some basophils show granulation in their cytoplasm.

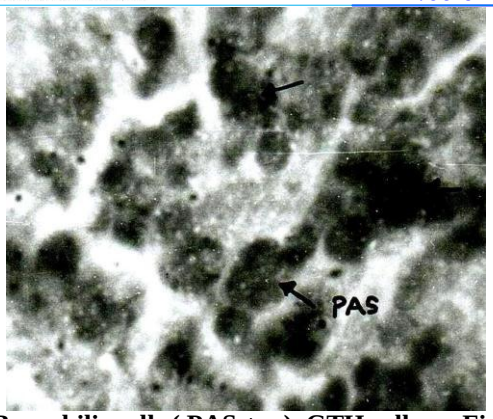

Fig.3. Basophilic cells (PAS +ve), GTH cells .

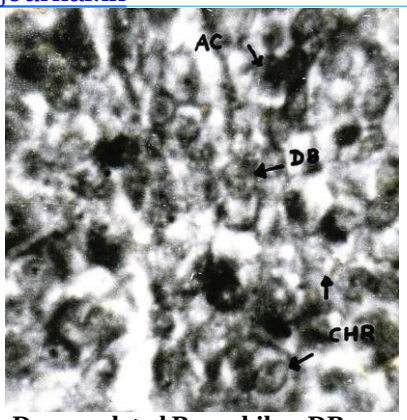

Fig.4. Degranuleted Basophils – DB, CHR- Chromophobes, AC- Acidophils.

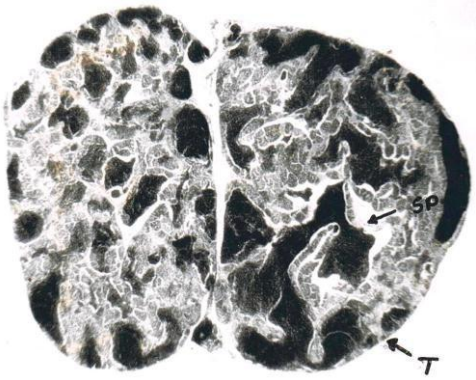

Fig.5. T.S. of testis (40 X)
SP- Sperms, T- Tunica albuginea.

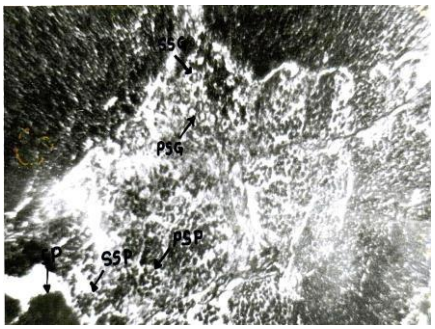

Fig.6. T.S. of Testes (200 X).
PSG - Primary spermatogonia.
SSG – Secondary spermatogonia.
PSP – Primary spermatocytes.
SSP – Secondary spermatocyte, SP – Sperms.

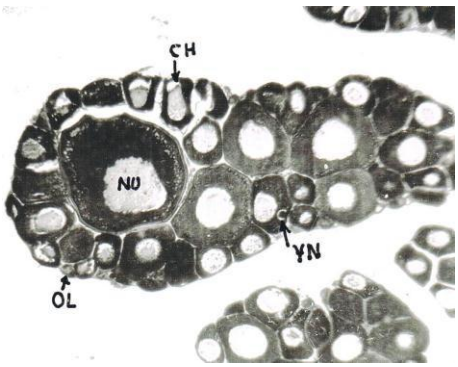

Fig.7. T.S ovary (100 X).
(Single ovigerous lamellae)
CH – Chromatin, YN- Yolk nucleus,
NL- Nucleoil, NU - Nucleus, OL- Ovigerous lamellae.

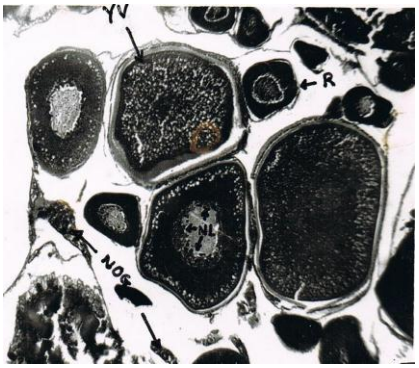

Fig.8. T.S. ovary (100 X)
NOG- New crop of oogonia, R- Resting oogonia,
YV- Yolk vesicle, VM- Vitelline membrane,

IV. Preparatory phase (June and December)

The ovary became opaque, vascular and pale yellow in colour. Ovigerous damage starts swollen and ova start laden with yolk and increased in size. The testes gradually increased in size and weight. The spermatogonia were reduced greatly. Primary and secondary spermatocytes were firstly increased in size and number, but later on decreased. The spermatids were predominant.

The GTH cells of the pituitary gland, basophils were degranulated and still predominant. Increased granulation of basophils indicates the accumulation of hormone requires further in oogenesis and spermatogenesis.

DISCUSSION

The endocrine system provides a link between the external environment and the internal physiological state, as a result reproductive behavior is nicely synchronized with the gonads maturity and the environmental conditions. Reproductive behaviour and the annual cycle are described by (Van Lesrel 1953). Very few authors have described the seasonal changes in the epithelial component in the transitional lobe in teleost fishes, (Mathews, 1936); (Kerr, 1948); (Scruggs, 1951); (Sundarraj, 1959), (Sathynesan, 1960), (El-Sakhawy et al.,2011). These investigators have noticed an increase in the basophilic content and its correlation with spawning. The pituitary gland and gonads of *Nemacheilus moreh*, exhibits marked seasonal changes. The pituitary, like the mammalian counterpart, secretes gonadotrophic hormone, the follicle stimulating (FSH) and the luteinizing (LH) hormones and the action of FSH and LH is said to be synergistic. In mammals, former is responsible for the growth of the ova, latter brings about ovulation, (Burrows, 1949), (Turner, 1952), (Williams, 1955). The FSH is responsible for the development, growth and ripening of the oocyte, while the LH promotes ovulation and spawning.

Histological changes in the PAS(+) basophils, during sexual maturation have been described in numerous species of teleost *Petromyzon marinus, (*Percy et al.,1975), *Sleropages formosus,* (Scott and Faller, 1976) *Carassius auratus, Clarius batrachus, Clarius macrocephalus, Cyrprinus carpio, Fundulus heteroclitus, Lebistes reticulatus, Oncho orhynchusketa,* O. *nerka, O.nerka kenneryli, Xiphores maculates,* (Saurez, 1976), *Heteropneustus fossilis, H*aider, (1978), *Salvelinus leucomaenis,* (Ueda, 1980) and (Tan, 1985). *Nemacheilus moreh* resembles with *Carassius* species, (Scruggs, 1951) and *Mystus* species, (Sathyanesan, 1960) with the occurance of basophils and the incidence secretion during the prespawning and spawning period. The PAS+ basophils showed an increase in cytoplasmic staining intensity, degree of granulation and hypertrophy during sexual maturation in the milk fish, (Tan,1985). Some workers claim to demonstrate the presence of two types of gonadotrophs in some teleost, while others are able to demonstrate single type, (Sage and Bern 1971); (Nagahama, 1973); (Scheribman, *et al.,* 1973). On the basis of staining properties and histological changes, (Tan, 1985) described only one type of gonadotroph in milk fish, *Clarias chones.* During present study in *Nemacheilus moreh,* most of the PAS+ve basophils assumed to be gonadotrophs on the basis of staining properties and reveal one kind of gonadotrophs, but appearance of granulation in cytoplasm is of two types and hence may be considered as two types of gonadotrophs.

During the spawning period, the pituitary has an overwhelming number of granulated basophils. In some pituitaries the basophils are observed in RPD also. During post spawning period the granulation of basophils gradually decrease it may probably due to releasing large amounts of FSH and trace of LH. These are probably responsible for oogenesis and spermatogenesis. Similar changes in gonadotrophic activity of cyprinids have been reported, (Beach, 1959), (Bhargava and Raizada, 1973); (Nagahama, 1973) and (Chiba *et al.,* 1978). In *Triturus,* Copeland, (1943) recorded that, in the annual cycle of the male, "the globular basophills" increase with the testis growth and discharge with testis maturation. Aplington, (1942), observed correlation between the testes and the pituitary during the growth period with increase in the number of granular basophils. According to him, during the discharge of sperm, degranulation of the granular acidophils takes place which furnishes evidence of correlation. In *Heteropneustus fossilis* ovary, Sundararaj (1959) reported that, during the prespawning period, basophils undergo process of degranulation and granulation. The granulated basophils start degranulation during postspawning period and this process is correlated with preparatory phase of ovary indicating direct correlation of GTH cells with ovary. In *Nemacheilus moreh,* during the reproductive cycle, both the gonads, testis and ovary, undergoes the similar fashion of activities and the results obtained indicating direct correlation of pituitary with the development of gonads.

ACKNOWLEDGEMENT

Authors are thankful to UGC, Delhi, Pune University and Abasaheb Garware College, Pune for awarding the Teacher Fellowship to first author. Thanks are also due to Head, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for providing laboratory facilities.

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