J. Appl. Biosci., 48(1): 1-30, June, 2022

ISSN (Print) 0975-685X
ISSN (Online) 0975-864X

HYPOTHALAMO-HYPOPHYSIAL REGULATION OF REPRODUCTION IN TELEOSTS WITH SPECIAL REFERENCE TO *LABEO ROHITA* (HAMILTON-BUCHANAN, 1822)

B.N. SHUKLA[1], N.K. CHADHA[1], KIRAN DUBE[1], P.B. SAWANT[1] and A.K. PANDEY[*]
[1]ICAR-Central Institute of Fisheries Education, Off Yari Road, Mumbai-400061, India
[*]ICAR-National Bureau of Fish Genretic Resources, Canal Ring Road, Lucknow-226002, India

ABSTRACT

Reproduction in most of the tropical, subtropical fish species is periodic and the peak reproductive event, spawning occurs in the most suitable time of the year to ensure maximum survival and growth of the offsprngs. Annual fluctuation in photoperiod and its dependent variable temperature are considered as the primary environmental factors regulating reproductive cycle of fishes. By and large, proper favorable synchronization of different environmental factors determines the reproductive success in teleosts mainly through the endocrine regulation of hypothalamo-hypophysial-gonadal axis. Proper understanding and knowledge of these endocrine secretions and mechanism of their regulations are of paramount importance not only because of difficulty in manipulating the environmental variable but also to exercise varying degree of control over the different stages of reproduction. The Indian major carp, *L. rohita,* commonly known as "Rohu" in many parts of India is a cultivable fish of high consumer preference. This fish attains maturity after second year of life and breeds once in a year during the monsoon season. The annual reproductive cycle of the Indian major carp (I.M.C.) have been divided into six periods - (i) Immature phase (December-January), (ii) Developing phase (February-March), (iii) Early maturing phase (April), (iv) Ripening phase (May-June), (v) Mature gravid (July-August) and (vi) Regressing stage (September-November). Ovarian growth is coincided with the enhancement of temperature and photoperiod from the month of March when oocytes are characterized with the inclusion of yolk vesicles and yolk granules. These oocytes are transformed in yolky mature follicles during maturation phase (April-May) and attain the maximum oocyte diameter during spawning phase (June-July). Ovary undergoes regression with decline of water temperature during September-October. Attempt has been made to correlate the changes occurring hypothalamus (NPO and NLT) and pituitary gonadotrophs (cynophil cells) with ovarian maturation in the commercially important Indian major carp, *Labeo rohita.*

KEY WORDS: Hypothalamus, Pituitary, Ovary, Reproduction, *Labeo rohita*

INTRODUCTION

Carps form the mainstay of the culture fishery sector of India, supported by a strong traditional knowledge base and scientific inputs. Carps and other cyprinids contribute the largest share in the total global aquaculture production (Cai *et al*., 2019). These fishes are cultivated extensively in Asian countries because of their consumer preference and suitable climate in these areas for their growth (Acosta & Gupta, 2005). Indian major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) are native to the Indo-Gangetic riverine ecosystems (Jena, 2009). Indian major carps and exotic carps together contribute more than 87% to the total fish produced from the freshwater aquaculture in India (Ayyappan *et al*., 2011). The last three decades have witnessed a phenomenal growth in carp farming in India, as a result, the market demand for fish fry also has increased. For carp seed production, the fish seed industry is exploring more and more possibilities to manipulate reproduction (Zohar & Mylonas, 2001; Singh & Pandey, 2009).

There has been growing interest in teleost reproduction because of a number of commercial reasons (Zohar & Mylonas, 2001; Melamed & Sherwood, 2005; Babin *et al*., 2007; Mylonas & Zohar, 2009; Zohar *et al.*,

*Corresponding author email: akpandey.ars@gmail.com

2010). Altering sexual cycles, induction of advanced, delayed maturation and multiple breeding, ovulation and artificial fertilization are to be practiced where reproductive physiology might help for faster progress in carp seed production (Singh & Pandey, 2009; Lubzens *et al.*, 2010; Phelps, 2010). Production of viable eggs is obviously essential for survival and propagation of the species (Babin *et al*., 2007; Bhattacharyya & Homechaudhuri, 2009; Singh & Pandey, 2009). Knowledge of oocyte development is a prerequisite for proper evaluation of the reproductive condition of the fish (Nagahama & Yamashita, 2008) which is often monitored either by representative sampling of individual fish or by a relatively simple biopsy through the genital pore in large fish (Singh & Pandey, 2009; Ryu *et al*., 2013).

Majority of teleostean fishes are seasonal breeders. The seasonal reproductive cycle, involving gonadal recrudescence, ovulation and spawning is precisely maintained by the endocrine cycle. Reproduction in fish is predominantly a periodic phenomenon. By and large, proper favorable synchronization of different environmental factors determines the reproductive success in teleosts mainly through the endocrine regulation of the hypothalamo-hypophysial-gonadal axis (Pandey & Mani, 2009). Though there is evidence of the involvement of other endocrine glands such as thyroid, interrenal etc., but their effect appears to be indirect (Singh & Lal, 2009). Proper understanding and knowledge of these endocrine secretions and mechanism of their regulations are of paramount importance not only because of difficulty in manipulating the environmental variables but also to exercise the varying degrees of control over the different stages of reproduction (Zohar & Mylonas, 2001; Melamed & Sherwood, 2005; Zohar *et al.*, 2010).

In higher vertebrates, the secretions of pituitary gland (hypophysis) control a wide variety of physiological processes directly or indirectly. Pituitary plays an important role in various metabolic activities of animals by elaborating trophic hormones. Studies involving hypophysectomy as well as hormone replacement therapies have revealed that hypophysis secretes at least nine hormones like somatotropic hormone (STH), adreno-corticotropic hormone (ACTH), thyrotropic hormone (TSH), gonadotropic hormone (GtH), prolactin or lactotropic hormone (LTH), oxytocin, vasopressin and anti-diuretic hormone (ADH) which regulate the various functions of the body (Tonsfeldt & Chappell, 2012; EI-Sayyad *et al*., 2019). Using specific stains, gonadotrophs have been identified in proximal pars distalis (PPD) of the hypophysis that shows cyclicity in relation to gonadal maturation (Grandi *et al.*, 2014; Balci & Ikiz, 2017). The gonads on being activated by the pituitary hormones secrete the important sex hormones like androgens, estrogens, and progesterone, which in combination with the pituitary hormones, regulate reproduction in fishes. Further, the external environmental factors like temperature, photoperiod, rainfall and pheromones influence the gonads, thyroids and other endocrine systems through the hypothalamus and hypophysis.

The Indian major carp, *Labeo rohita,* commonly known as "Rohu" is a cultivable fish of high consumer preference due to its delicious taste among the Indian major carps. It is distinguished by its relatively small or pointed head, almost terminal mouth with fringed cover lip. Body elongated with moderately convex abdomen, back brownish grey, dull reddish scales on the sides and pink reddish fins. *L. rohita,* attains maturity in the second year of life and breeds once in a year during the monsoon season (Thomas *et al*., 2003). Day *et al*. (2004), Bhattacharyya & Maitra (2006) and Lone & Hussain (2009) have divided the annual reproductive cycle of the Indian major carps into six stages- (i) Immature phase (December-January), (ii) Developing phase (February-March), (iii) Early maturing phase (April), (iv) Ripening phase (May-June), (v) Mature or gravid phase (July-August) and (vi) Regressing phase (September-November). Ovarian growth coincides with the enhancement of environmental temperature and photoperiod from the month of March when oocytes are characterized with the inclusion of yolk vesicles and yolk granules. These oocytes are transformed in yolky mature follicles during the maturation phase (April-May) and attain the maximum oocyte diameter during the spawning phase (June-July) after germinal vesicle breakdown (GVBD). Ovary undergoes regression with a decline of water temperature during September-October. Further, it has been possible to advance maturation of *L. rohita* to as early as March-April by favorable photo-thermal, hormonal and dietary manipulations (Pandey *et al*., 2007; Lone & Hussain, 2009).

The endocrine system acts as a regulatory link between the environmental events and maturation and release of gametes in fishes. The appearance of secondary sexual characteristics during the breeding season, breeding behavior including courtship and the timing of reproduction is some of the activities controlled by pituitary hormones. A reciprocal relationship exists between the hypophysis and other endocrine glands as well as between the hypophysis and the central nervous system. Hypothalamus in the vertebrate brain comprises groups of neurosecretory cells that mediate the organismic endocrine responses and adjustments to the environmental changes through the secretion of various tropic hormones of the pituitary and hypothalamus by elaborating releasing (-RH) and inhibiting hormones (-IH) (Amano, 2010; Chen & Frenald, 2010; Zohar *et al.*, 2010). Hypothalamus also contains receptors specifically

sensitive to the hormone which, in turn, regulates its activity through feedback mechanism (Zohar *et al.*, 2010). There is increasing evidence that in fishes too, the hypophysial functions are modulated by the hypothalamic neurohormones but the regulatory mechanisms have not yet been clearly understood (Yaron *et al*., 2003; Singh & Lal, 2009; Zohar *et al*., 2010; Kitahashi *et al*., 2013). Several workers have described the hypothalamus of various teleosts inhabiting freshwater (Bano, 2012; Rincon *et al*., 2017; Ray, 2019), brackishwater (Lal & Pandey, 1998; Bose & Chakrabarti, 2018) and marine ecosystems (Pandey & Mohamed, 1999). Attempts have been made in the past to understand the response of the neurosecretory cells of the hypothalamus to various exteroceptive stimuli and some workers also tried to correlate the activities of different neurosecretory centers with ovarian development of fish (Pandey & Mani, 2009; Khuroo, 2013).

However, secretory activity of the various neurosecretory centers in the brain has not yet been correlated with gonadal maturation of the teleosts. Therefore, attempt has been made in the present study to record the changes occurring in the nucleus preopticus (NPO) and nucleus lateralis tuberis (NLT) of the hypothalamus of the female *L. rohita* with ovarian maturation. Furthermore, effects of exogenous hormonal drugs (HCG and GnRH) administration on different neurosecretory centers of the female teleosts have been recorded to understand the role of these centers in the regulation of reproductive processes (ovarian maturation) and breeding of the fish. It was noticed that low doses of HCG and GnRH advance maturation in teleosts, either by directly influencing ovary or enhancing the activity of the gonadotrophs of the pituitary gland (Kanungo *et al*., 1999; Zohar & Mylonas, 2001).

An attempt has been made to document the different hypothalamic (neuroendocrine) centers in the brain and their rioles in ith ovarian maturation in teleosts and also evaluate the effects of HCG and GnRH-ovatide in egg maturation and breeding performance with special reference to the commercially important Indian major carp, *L. rohita.*

REVIEW OF LITERATURE

Knowledge of the reproductive physiology of fish is prerequisite for successful production of viable fish seed for commercial purposes. Most of the fishes breed in one season every year and are called seasonal breeders but some breed in all seasons (throughout the year) and are called year-round spawners. Generally, major carps are seasonal breeders and exhibit rhythmic changes in the structure and physiology of ovary in different seasons.

Ovarian Cycle

Production of viable eggs is obviously essential for species survival and propagation. There have been growing interests in teleost reproduction during the recent years because of a number of commercial reasons. It is desirable to know the reproductive condition of a population of fish that is often monitored either by representative sampling of individual fish or in large fish by a relatively simple biopsy through the genital pore. In either case, knowledge of oocyte development is a prerequisite for a proper evaluation of reproductive condition of the fish (Singh & Pandey, 2009; Ryu *et al*., 2013).

The development stages of ovarian maturity can be judged by visual observation on the morphology or through histological survey. Morphological staging of ovary is generally done on the basis of its appearance, color, size, weight and intensity of blood vascularization and maturity of ova (Ryu *et al*., 2013). The seasonal reproductive cycles of a number of teleosts inhabiting Indian waters have been studied (Jacob, 2005; Khanna, 2006; Pandey & Mani, 2009; Lone *et al.,* 2012; Roy &

Table 1: Pattern of the staging ovarian maturity in fishes (Wood, 1930)

Maturity stages	Female Fish
Immature	Ovaries very small, colorless and translucent; ova invisible to the naked eye.
Maturing-I	Ovary slightly less than half the length of the body cavity, eggs small, unyolked, visible to the naked eye.
Maturing-II	Length of ovary more than half of the body cavity and distinctly granular in appearance, ova opaque, colored, yolked and visible to the naked eye.
Maturing-III	Ovary occupying about 3/4th length of the body cavity, eggs opaque, yolked and larger in size.
Mature	Ovaries extending over the entire length of the body cavity, abdomen bulging; ovary colored, eggs large but not transparent.
Spawning	Eggs nearly transparent with a central yolk mass and are easily extruded by slight pressure on the abdomen.
Spent	Ovaries shrunken considerably, flaccid and blood shot in appearance.

Table 2: Macroscopic and histological description of the maturity stages of the ovary of *Labeo bata*

Developing stages of Ovary		Macroscopic description	Histological description
Resting phase (November-December)	Oogonia	Thread like transparent, no oocyte visible with naked eye.	Well-developed ovigerous fold, oogonia dominant in this stage, found in nests.
Preparatory phase (early) (January-February)	Stage-I	Whitish colour, thickness just higher than resting phase ovary.	Perinucleolar oocytes dominant. Yolk nucleus in cytoplasm, nucleolus was found in the periphery of nuclear membrane.
Preparatory phase (late) (March)	Stage-II	Ovaries were pale yellow color, blood Vessel prominent on the surface of ovary.	Cortical alveoli stage oocyte appeared containing cortical alveoli and yolk granules in cytoplasm.
Maturation phase (early) (April)	Stage-III	Longer, thicker, fully yolked ova were ivory yellow color. Eggs were visible witk naked eye.	Large vitellogenic oocyte dominant containing well developed zona radiata and yolk globule.
Maturation phase (late) (May)	Stage-IV	Ovary larger, deep yellowish color, round oocytes were present in gonad.	Mature follicles (MF) showing germinal vesicle migration (GVM) and yolk granules in cytoplasm were found.
Spawning phase (June- August)	Stage-V	Ovary larger, deep yellowish color, round oocytes were present in the gonad Ovary shrunken and transparent oocyte visible.	MF with fused yolk globules, hydrated and large. Nuclear Membrane disappear (GVBD) in most of the MF and some oocyte collapsed to atresia.
Post-spawning phase (September-October)	Stage- VI	Ovary long and transparent color.	Residual primary oocytes are dominant. Some atresia follicles found and empty space between ovigerous folds.

Mandal 2015). Wood (1930) recognized seven stages of maturity and the International Council of Exploration of Sea in the Herring Scheme adopted the following scale which is now accepted by most of the workers. These are as follows (Table 1).

According to the above classification on maturity stages, the female fish in stage 5 is considered ripe; stage 6 is reached actual spawning and stage 7 is that of spent fishes or stage of resorption of ovary.

The process of the ovarian development has been described in many fish and divided into seven to eight maturity stages (Ryu *et al*., 2013; Verma, 2013). However, in many of the Indian teleosts, annual changes in ovary are divided into four to six stages (Day *et al.,* 2004; Jacob, 2005*;* Pandey & Mani, 2009; Roy & Mandal, 2015). Commonly, these changes are demarcated into five phases such as- (1) Resting phase, (2) Preparatory phase, (3) Maturing phase, (4) Spawning phase and (5) Post-spawning phase (Rath, 2011).

Resting Phase

Resting phase extents from November to January in most of the teleosts of tropical region (Roy & Mandal, 2015. Mahmud *et al*., 2016). Lone *et al.* (2012) in *Catla catla* and Lone & Hussain (2009) in *L. rohita* have reported that the gonadosomatic index will be the lowest during this period and the ovary is in immature state containing nests of oogonial cells (first oocytes) at different phases of growth and a few second stage oocytes. Primary oocytes have larger germinal vesicles which are chromophobic, relatively small ooplasm which is chromophilic and undifferentiated oocyte envelopes.

During this stage, the first meiotic division of nucleus is initiated and the same is arrested at pachytene stage. These oocytes are also characterized by the presence of yolk nucleus (Balbiani's vitelline body) in the ooplasm. Initially, these bodies are found adjacent to the nucleus and as it grows, gets separated from the wall of nucleus and get scattered in the ooplasm after undergoing fragmentation. Later, they disappear. In spite of it universal occurrence and several histochemical and electron microscopic studies, the precise role of these bodies is not yet clearly understood. According to some workers like Sen *et al.* (2002), Roy & Mandal (2015) and Mahmud *et al*. (2016), the yolk nucleus provides the basic cytoplasmic machinery for future vitellogenesis that takes place inside the ooplasm. Stage II oocytes are larger in size, the oocyte envelope get differentiated into the outermost thecal layer, middle granulosa layer and the innermost chorion or zona pellucida or oolemma.

Preparatory Phase

This phase is noticed from February to March in most of the fishes. In places where winter is not severe, it may be observed from the latter part of January itself. According to Mahmud *et al.* (2016), oocytes in the preparatory phase (stage III oocytes) are characterized by further growth in size and development of cortical alveolii at periphery of ooplasm. This is beginning of viteilogenesis in the oocyte cytoplasm. Oocyte envelopes get differentiated into thecal and granulosa layers assuming the steroidogenic function. Cells of the granulosa layers start producing the female hormone, estradiol. Side by side, synthesis of vitellogenin takes place in the liver under the influence of estradiol. Vitellogenin is released into the blood from liver and the same gets incorporated into the ooplasm through the oocyte envelope. Vetellogenin is the precursor for yolk and the same is deposited in the oocyte cytoplasm. This process of formation of yolk in the ooplasm is called vitellogenesis (Lone & Hussain, 2009; Khanna, 2013).

Maturing Phase or Pre-spawning Phase

This phase extends from March to May and is characterized by the intensive process of vitellogenesis by which ooplasm of an oocyte is loaded with yolk granules leaving a small portion of ooplasm with germinal vesicle at the pole. The oocyte envelopes are highly differentiated and oocytes are large in size. The size of the ovary is maximum during this phase and GSI will be maximum at this stage. The similar observation has also been made by Day *et al.* (2004) in *Catla catla*. The ovary gets distended, highly vascularized to cater to the nutritional and metabolic requirements of oocytes. The ovary undergoes rapid growth in size due to the accumulation of yolky oocytes.

Spawning Phase

It is characterized by the gravid ovary containing ripe oocytes. During spawning, follicles of the fully ripe oocytes rupture as a result the oocytes are released into the ovocoel in case of fishes with cystovarian ovary. Mikolajczyk *et al*. (2004) reported that in *Cyprinus carpio* having gymnovarian condition of ovary, the eggs are released into the body cavity from where they pass out through the genital aperature into the water. Spawning phase extends from June to August in most of seasonal breeders of the tropics.

Post-spawning Phase

During this phase which is seen from early/late September to early November, the ovary exhibits a collapsed appearance as evacuated follicles are seen after the release of eggs. These observations were also recorded by in *Catla catla* (Lone *et al.*, 2012) and in *Clupisoma garua (*Pasha *et al*., 2019). If any yolky oocytes remain unovulated, it may undergo regressive changes/atresia, finally reabsorbing the unovulated oocyte. Oogonial cells and a few stage I, stage II oocytes also can be seen at this phase. The annual reproductive cycle of female *Labeo bata* has been described into six different phases (Roy & Mandal, 2015) (Table 2).

Pituitary Gonadotrophs

In Indian major carps, development of gonads and the process of reproduction is controlled by the pituitary gland which is known to mediate between external environment and the reproductive organs (Khanna, 2013). Pituitary gland or hypophysis plays important role in various metabolic activities of the animals through its hormones. Studies involving hypophysectomy as well as hormone replacement therapies revealed that this gland secretes about nine hormones which regulate the various activities of the body (Khanna, 2006; Kriegsfeld & Silver, 2006; Haus, 2007; Pandey & Shukla, 2007; Tonsfeldt & Chappell, 2012). This gland has been widely studied in a number of teleostean species (El-Sakhaawy *et al*., 2011; Ursani *et al*., 2012; Kharat & khillare, 2013; Gadekarp & Baile, 2015).

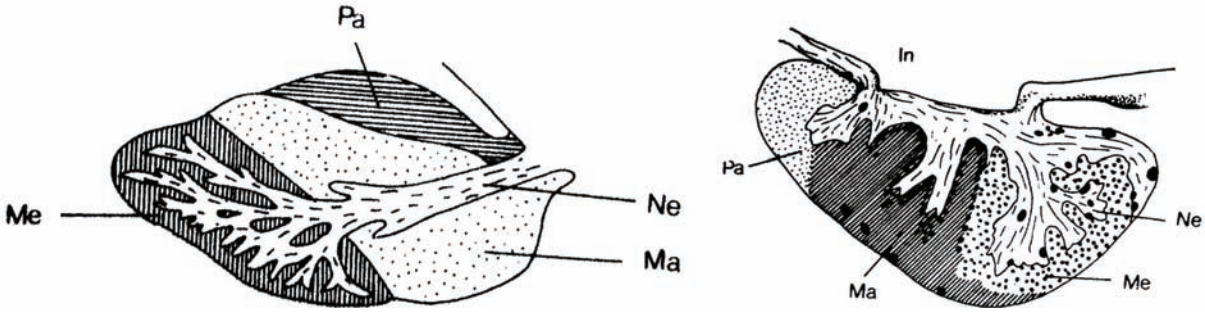

Fig. 1: Diagram showing component parts of the pituitary gland and distribution pattern of different cell types of *Tor tor (Barbus)* and *L.rohita,* respectively (modified from Rai, 1973; Jose & Sathyanesan, 1980). Pa, Proadenohypophysis; Ma, Mesoadenohypophysis; Me, Metaadenohypophysis; Ne, Neurohypophysis.

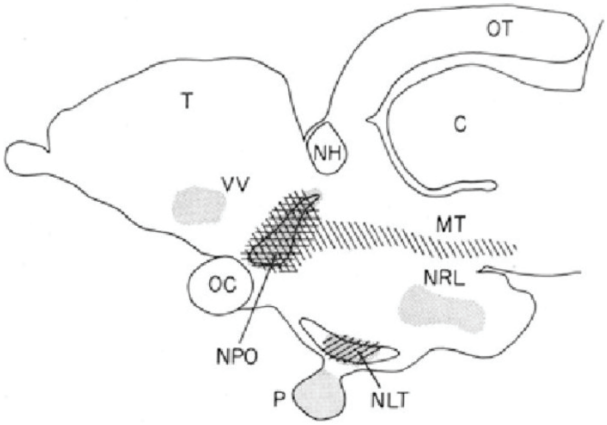

Fig. 2: Sagittal section of hypothalamus and pituitary. Note NPO and NLT and attachment of pituitary with brain in *teleost fish* (modified from Crews & Silver, 2015). NPO, Nucleus preopticus; NLT, Nucleus lateralis tuberis; P, Pituitary

Origin

Pituitary gland occupies the same central part in the endocrine signaling system of fish that it has in the mammals. This endocrine gland originates embryologically from the two sources. One as ventral down growth of a neural element from the diencephalon called the infundibulum to join with another, an ectodermal up growth from primitive buccal cavity. These two outgrowths are thus ectodermal in origin and enclose mesoderm in between them, which later on supply blood to pituitary gland, originating from the interrenal carotid artery.

Location

The pituitary gland of Indian major carp is located below the diencephalon (hypothalamus), behind the optic chiasma and anterior to saccus vasculosus and attached to diencephalon by a stalk or infundibulum (Pickford & Atz, 1957; Ekici & TImur, 2013). The size of infundibulum varies according to the species in cyclostomes usually it is smaller but increases in bony fishes, with prominence in groove or depression of parasphenoid bone receiving the gland (Khanna, 2006; Farrell, 2011). The short, thick walled, hollow infundibular stalk contains a lumen, which continues with the third ventricle.

Shape and colour

The pituitary gland is a small pear-shaped, whitish soft body and dorso-ventrally compressed. On ventral aspect, the gland gradually tapers caudally from rounded anterior end. The dorsal surface of pituitary gland is concave, ventrally it is slightly convex. The pituitary gland is completely enveloped by a delicate connective tissue capsule. Generally, male pituitary glands are smaller than those of female pituitary glands (Schreibman, 1986).

Anatomy of the Pituitary Gland

Microscopically, the pituitary gland of Indian major carp is divided into two parts- Adenohypophysis which is a grandular portion originated from the oral ectoderm. Neurohypophysis which is nervous portion originated from the infundibular region of the brain. Both parts are present in close association. Pickford & Atz (1957) divided adenohypophysis into three parts, proadenohypophysis, mesoadenohypophysis and metaadenohypophysis while Gorbman (1965) divided adenohypophysis into three parts and called them as the anterior grandular region- rostral pars distalis (RPD), middle grandular region- proximal pars distalis (PPD) and posterior grandular region- the pars intermedia (PI). However, nomenclature is synonyms as follow-proadenohypophysis- rostral pars distalis (RPD), mesoadenohypophysis- proximal pars distalis (PPD) and metaadenohypophysis- pars intermedia (PI)

Adenohypophysis

Earlier workers identified cells of adenohypophysis on the basis of staining procedures. The procedures used were Heidenhain's azon method, Masson's poncean acid fuschin anilin blue, the periodic acid Schiff's reaction (PAS), aldehyde fuchsin technique (AF) and then they made cell counts.The cells of pituitary secrete hormones which are stored in granules present in the cytoplasm (Khanna, 2006; Pandey & Shukla, 2007; Pogoda & Hammerschmidt, 2007). The cells are, therefore, classified on the basis of staining properties of granules of these cells. Cell types of adenohypophysis, on the basis of staining reaction, to the mixture of acidic and basic dyes with secretory granules are called as acidophilic and basophilic (Sakhaawy *et al*., 2011; Timur & Ekici, 2011; Kharat & khillare, 2013). The acidophilic cells are PAS- and AF- negative cells. The acidophilic cells are PAS- and AF- positive.

Recently on the basis of immunocytochemistry, the cells are classified according to hormones released by the proadenohypophysis. For example, the cells which take basophilic stain but produce adrenocorticotropic hormone, they are called ACTH cells but if secrete thyroid stimulating hormone, these cells are called thyrotrophs (TSH cells) and if they secrete FSH and LH hormones, they are called gonadotrops (GtH) although they are basophilic in nature (Rose & Pawlina, 2003). The cells of adenohypophysis when stained with periodic acid Schiff and aldehyde fuschin methods, if do not take stain, they are PAS- and AF- negative.

The topographical relationship is best seen in a mid-

sagittal section of the gland. The RPD occupies the antero-dorsal portion of the gland. The PPD lies in the middle region while the pars intermedia is located ventrally to the other two regions forming the apex of the gland (Bose & Chakrabarti, 2014). The RPD is the smallest portion of the adenohypophysis and the PPD, respectively. The PPD comprises the largest portion of the gland and is separated by the neurohypophysis (NH) into two pairs. It occupies the entire central port and is separated from the PPD by means of a notch.

The infundibulum branches out into the pituitary gland to form neurohypophysis. (NH) The main trunk is visible in a mid-sagittal section of the gland when it passes downwards through the middle of the PPD, dividing the latter into two halves. It extends to the pars intermedia (PI) where it branches out much more extensively than in the rostral and proximal pars distalis. The neurohypophysis passes through the middle of the PPD and consists of thick nerve fibres, the latter may be straight or wavy and extend length-wise along the main trunk (Bose & Chakrabarti, 2014).

Rostral pars distalis (RPD)

The rostral pars distalis consists mainly of acidophils. During the breeding season (June-July), however, a few cells take up periodic acid Schiffs (PAS), alcian blue (AB) and aldenyde fuchsin (AF) stains thus indicating that they contain glycoproteinaceous granules (Ekici & Timur, 2013). These cells are obviously cyanophils. The acidophils of RPD are mainly of two types of acidophils- acidophil I and acidophil || cells. The former take up either fuchsin, azocamine or the erythrosine color depending on the staining technique employed (Ursani *et al.*, 2012). These acidophils, however, do not stain with PAS reagent and hence may not be glycoproteinaceous in nature. The acidophil II cells take up orange color when Mallen or Romie"z Azan methods are employed. The cyanophils are generally elongated or angular and scattered in the rostral pars distalis. The latter can be distinguished from the proximal pars distalis during the breeding season by the difference in number and size of the eranophils.

Proximal pars distalis (PPD)

The proximal pars distalis is formed mainly of acidophils, cyanophils and chromophobes. The PPD is not clearly demarcated from the rostral part, but can be easily differentiated from the pars intermedia due to extensive ramification of the pars nervosa in the latter. This region increases or decreases in size during different periods of the year. The cyanophils are the chief constituents during maturation and spawning periods while the acidophils are more numerous during the rest of the year. The acidophils are stained with azocarmine or orange G while the cyanophils are stained with aniline blue, alcian blue, PAS, AF, aldehyde thionin stains (Balci *et al.*, 2006; Zohar *et al.,* 2010).

According to Kharat & Khillare (2013), during the breeding period, the cyanophils of PPD may be distinguished into two types- cyanophil I and cyanophil II cells. The cyanophil I cells are strongly positive to PAS, AF, aniline blue and alcian blue. The concentration of granules may vary from season to season. Cyanophil I cells are usually uniformly distributed in the PPD. The cyanophil I cells, certain globules of various sizes. They may be situated either at the centre or one side of the cells. Each cell may contain one or more globules. When the globules are smaller in size, they are numerically more abundant. They stain deep in cells when granulation has set in a few cells, however, the globules may be present inside the nucleus or on its periphery.

The cyanophil II cells are weakly positive to PAS, aniline blue and alcian blue. They can easily be distinguished when stained with AF. They are generally distributed along the branches of the neurophysis or present within the aggregation of cyanophil I cells and acidophils. The nucleus is centric, being proportionately larger in size to the cell body when compared to the other types of cells, except those of the chromophobes of the PPD. Two types of cyanophils have been distinguished in many species on the basis of their shape, size and staining affinity (Williams, 1995). The chromophobes do not take any stain.

Pars intermedia (PI)

Usually occupies the hinder region of the hypophysis. It partly or completely surrounds the distal end of the neurohypophysis and is abundantly supplied by the branches of the pars nervosa. It varies greatly in size in different species. Two distinct cell types are generally distinguished by differences in the staining reactions. These are cyanophils staining with aniline blue or acidophils that stain with azocarmine or orange G. Amphiphil cells taking both the aniline blue and orange G have also been described in some species. Besides, there may be few chromophobes (Shrestha & Khanna, 1978). The cyanophils of this region are negative to PAS, AF and aldehyde thionin stains. Pituitary gland of several species of fishes have shown two distinct cell types in pars intermedia, when stained with PAS followed by lead hematoxylin. PAS-positive cells are generally oval in shape and occur along the border of the neurohypophysis. The second types of cells are stained with lead hematoxylin. This region of the hypophysis is generally well vascularized and the existence of two distinct cell types suggests the production of two distinct hormones by this region.

Neurohypophysis

The neurohypophysis (NH) occupies considerable portion of the gland and possesses many interesting and distinctive features. The neurohypophysis comprises connective tissue, neuroglia cells and loosely tangled network of nerve fibres. These nerve fibres are scattered horizontally along the dorsal part of adenohypophysis and run vertically, which are generously interspersed with granular material, large irregularly shaped amorphous masses and large nuclei. They are located in the mid-dorsal region. The amorphous masses are called "Herring bodies", which are in intimate relation with the diencephalic neurosecretory cells called nucleus preopticus (NPO) by means of a fibre tract known as the preoptic neurohypophysial tract (Ekici & Timur, 2013). The diencephalon and other parts of the brain contain a group of neurons and each group is called nucleus. The nucleus preopticus (NPO) and nucleus lateralis tuberis (NLT) are important neurosecretory centres of the hypothalamus as their axons are in association with both adenohypophysis and neurohypophysis. These possess neurosecretory cells.

Seasonal Changes in Pituitary Gland

Proximal pars distalis (PPD) of pituitary gland undergoes a great deal of seasonal variations in relation to reproductive cycle. Using specific stains, gonadotrophs have been identified in PPD of the hypophysis and they show cyclicity in relation to gonadal maturation (Arukwe & Goksoyr, 2003; EI-Sakhaawy *et al*., 2011; Ursani *et al*., 2012). Grandi *et al*. (2014) reported that in *Ctenopharyngodon idella,* PPD undergoes seasonal variations and percentage composition of acidophilis and cyanophilis show a reciprocal relationship during the year. The acidophilis predominate during the resting phase of the gonads while the cyanophilis preponderate among the different cell types during the breeding season. The cyanophilis of the pituitary gland undergo changes with change in gonadal activity. Concentration of glycoproteinaceous material is extremely high during spawning period. The latter discharge their contents and become vacuolated during the post-spawning period. Therefore, it is assumed that as the cyanophils are undergone changes with the gonadal activity of fish, these are gonadal. Acidophils present in the rostral pars distalis and pars intermedia do not appear to be related to the gonadal activity of the fish.

Hypothalamus

Hypothalamus in the vertebrate brain comprises groups of neurosecretory cells that mediate the organismic endocrine responses and adjustments to the environmental changes through the secretion of various tropic hormones of the pituitary by elaborating releasing (-RH) and inhibiting hormones (-IH) (Goos *et al*., 1999; Bano, 2012). Hypothalamus also contains receptors specifically sensitive to the hormone which, in turn, regulates its activity through feedback mechanism (Zohar *et al*., 2010). The hypothalamus functions as an intermediary between nervous and endocrine effector systems and thus between nervous and endocrine effector systems and thus between the animal's external and internal environment. Besides, there exist data suggesting that in fishes also reproductive cycles are controlled by this interplay. The preoptic nucleus was first identified in bony fishes as long as 1891 by Herrick and its neurosecretory function and direct connection with neurohypophysis were established by Scharrer (Maksimovich, 1987). There are increasing evidences that in fishes too, the hypophysial functions are modulated by the hypothalamic neurohormones but the regulatory mechanisms have not yet been clearly defined (Evans, 1998; Bano, 2012). Several workers have described the hypothalamus of various teleosts inhabiting freshwater (Bano, 2012; Rincon *et al*., 2017; Ray, 2019), brackishwater (Lal & Pandey, 1998; Bose & Chakrabarti, 2018) as well as marine environment (Pandey & Mohamed, 1999). However, secretory activity of the various neurosecretory centres in the brain has not yet been correlated with gonadal maturation of the teleosts. Histomorphology of the hypothalamus and its relation with the pituitary gland has been studied in a number of species such as *Tor putitora* (Pandey *et al*., 2000); *Decapterus russelli* (Pandey, 2008); *Xenentodon cancila* (Bano, 2012); *Liza parsia* (Bose & Chakrabarti, 2018) and *Mastacembelus armatus* (Ray, 2019).

The hypothalamic neurosecretory system is made up of two nuclei rich areas viz., nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and their axonal pathways (Bose & Chakrabarti, 2018). Bano (2012) reported that the NPO is vertically organized and the cells are arranged in an oblique plane on either side of the third ventricle. The larger cells viz., pars magnocellularis (PMC) are dorsally placed whereas smaller cells, pars parvocellularis (PPC) are placed ventrally to the third ventricle in *Xenentodon cancila*. Bose & Chakrabarti (2018) suggested that in *Liza parsia* the cells of NLT are paired and situated above the pituitary gland. The cells of NPO and NLT exhibited both quantitative and qualitative variations during different periods of the testicular maturation. During growth and maturation periods, cells of PMC and NLT were characterized by intense staining and dense homogeneous granules along with deposition of neurosecretory materials. During spawning period, slight decrease in staining affinity of the cells of PMC and NLT were recorded.

Fig. 3: Measurement of length and weight *of Labeo rohita*

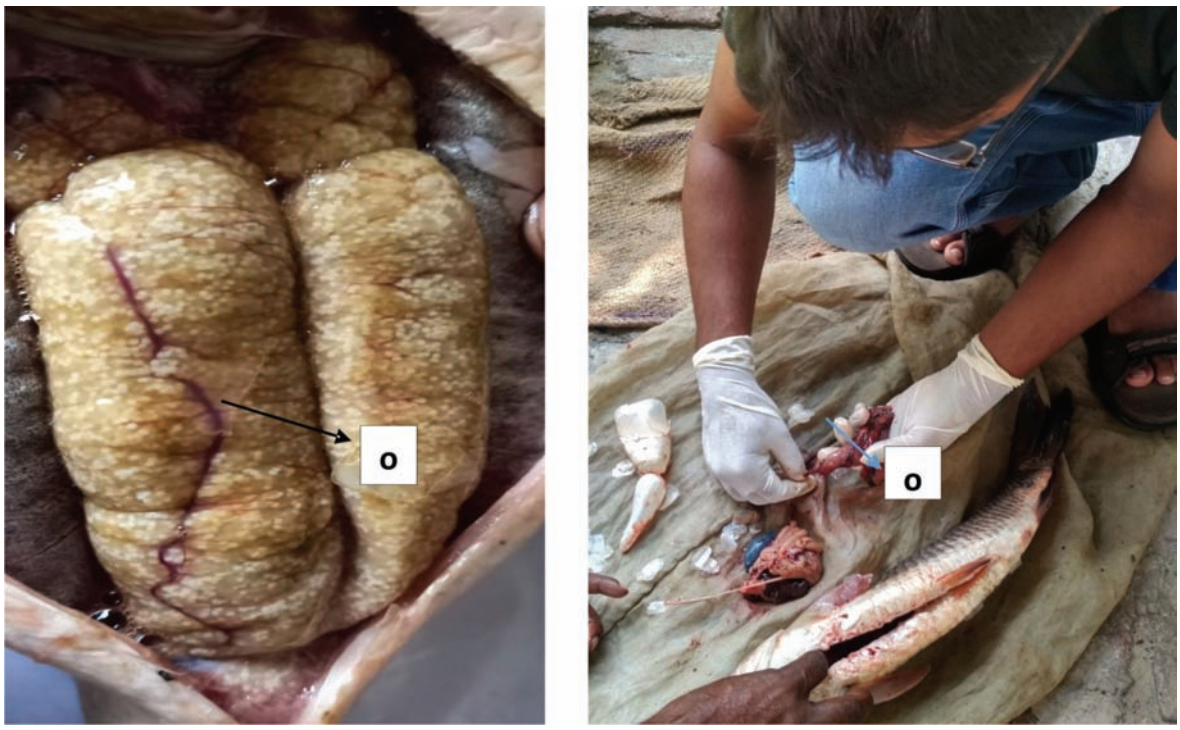

Fig. 4: Collection of ovary (O).of *Labeo rohita*

Effects of HCG and GnRH Administration on Ovarian Maturity and Spawning

Hormones

Hormones are chemical substances released by specific endocrine gland that can serve as a messenger possessing specific or diverse actions on some target organs (Biran & Sivan, 2018). Hormones actions are particularly related to metabolic activities that are anabolic and catabolic in the cytoplasm. The nervous and endocrine mechanism use basic properties of living cells, such as secretion and the propagation of impulses (Fsgbenro *et al.,* 1991). The hypothalamus in vertebrate brain contains certain neurosecretary cells specialized for the production of hormones. In many cases, neurons secrete a hormone for synaptic transmission (Biran & Sivan, 2018). Endogenous controls are mediated through actions of various hormones along the brain-hypothalamus-hypophysial-gonad axis (Biran & Sivan, 2006; Zohar *et al*., 2010).

Effects of HCG (human chorionic gonadotropin) on ovarian maturity and spawning

Human chorionic gonadotropin (HCG), a protein hormone is produced by human placenta and excreted through the urine during pregnancy. HCG is also known as sialoprotein or glycoprotein because of the carbohydrates molecules being attached to the protein molecules. HCG is an important factor in the maintenance of prudency during the initial stages. Compared to pituitary gland, HCG is inexpensive. It has a standard potency, long shelf-life and easy to store and transport (Chaudhury, 1976). HCG is prepared by an Indian Pharmaceutical Company named "Infar India Ltd.", Calcutta. It is extracted from the urine of pregnant women and is in the form of white or cream color powder. The light yellowish liquid extract containing HCG was used for injecting the carp fishes (Menon, 1982). High doses of HCG has been successfully used for fish breeding on larger scale in China and limited scale in India and other countries. Dosage of HCG for breeding different fishes varied greatly depending on the maturity stage of the recipients (Zohar & Mylonas, 2001; Haniffa & Sridhar, 2002). Pituitary extract was also prepared according to the standard procedure.

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) of the pituitary play an important role in the normal reproduction of fish in promoting the development of gonads, growth, maturity and spawning (Zhang *et al.,* 2014). HCG is almost similar in character and function to FSH and LH. As pituitary gland is used for induced fish breeding, HCG can also be used for early ripening of gonads (Piska & Naik, 2007). HCG has been used as low doses in different fishes by various researchers to study their impact on reproductive functions and breeding performance (Rath, 2000). Selvaraj & Francis (2007) reported that gonadal development, gonadosomatic index, histology and level of serum steroid hormones in captive striped murrel (*Channa striatus*) implanted with low doses of HCG for a period of five months after implantation. HCG implants induced a significant increase in the GSI of male and female fish. The GSI values were highest in the fourth month and lowest in first month.

Mani & Pandey (2007) studied the effect of HCG administration on ovarian maturation and spawning of *H. fossilis*. The broodstock (2+ years) of *H. fossilis* were given intramuscular injection of HCG (two doses: 25 IU and 50 IU/kg body weight) at weekly intervals for 28 days during June in order to record the effects on maturity in the catfish. The response of 50 IU HCG was more pronounced as most of the oocytes exhibited germinal vesicle migration (GVM) towards the periphery. The eggs of drug administered catfish were greenish-brown, well-developed, separated and released after a gentle pressure on the abdomen whereas the eggs of control catfish were released in lumps only after a heavy pressure on the abdomen and were bluish-brown in color. Fertilization percentage and hatching success were better in HCG treated catfish as compared to the control.

There exist reports that low doses of HCG and GnRH advance maturation in teleosts, probably by acting directly on the gonad or enhancing the activity of the gonadotrophs of the pituitary gland (Kanungo *et al*., 1999; Zohar & Mylonas, 2001). Even oral administration of HCG advanced maturation by 1.5-2.0 months in *H. fossilis* (Kanungo *et al*., 1999). Das & Singh (1990) recorded enhanced activity in gonadotrophs and ACTH cells of *L. rohita* concurrently with the ovaian development (maturation) in response to 50 IU HCG administration (4 doses, weekly intervals).

Effects of GnRH on ovarian maturity and spawning

Ovatide, a commercial induce-spawning agent was evaluated by (Reddy & Thakur, 1998; Marimuthu *et al*., 2000, 2009). The drug having a base of synthetic peptide structurally related to the naturally occurring gonadotropin-releasing hormone (GnRH) is successfully used for fish breeding which is a mixture of a synthetic GnRH analog and a dopamine analog and a dopamine antagonist pimozide. The advantage of ovatide over other commercially available spawning agent is that it is a low viscosity injectable solution, highly active and cost effective (Pandey *et al*., 2001, 2002a,b,c; Pandey & Koteeswaran, 2004; Mishra *et al*., 2011; Yadav *et a*l., 2011). Mylonas *et al.* (2004) reported that the reproductive biology of the shi drum (*Umbrina cirrosa*) in captivity

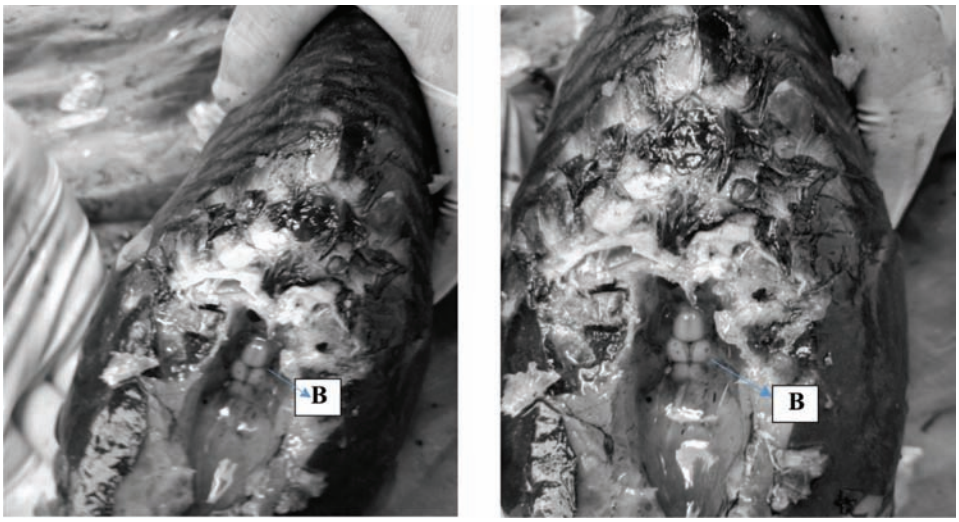

Fig. 5: Dorsal view of brain (B) of *Labeo rohita*

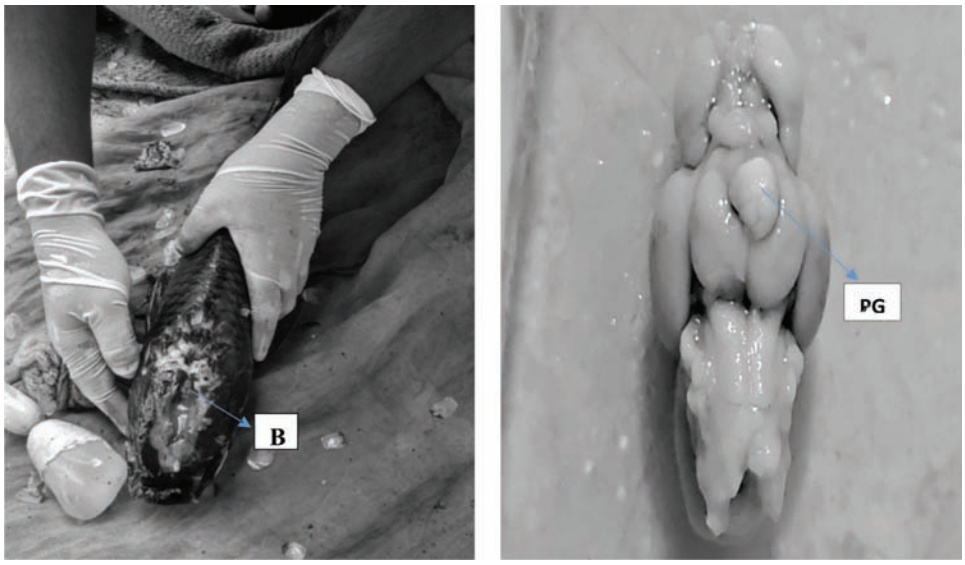

Fig. 6: Collection of brain (B) and pituitary gland (PG) of *Labeo rohita*

Resting phase (December-January) | Early maturing phase (February-March) | Advanced maturing phase (April) | Mature phase (May-June) | Spawning phase (July-August) | Spent phase (September-November)

Fig. 7: Different maturity stages in ovary of *L. rohita.*

and induced for spawning using GnRH-a. They used GnRH-a (10 µg/kg) and a normal saline injection as control. For spawning induction and gonadal maturation, the fishes were injected with GnRH injections @ 10 µg/kg before 10 days and 20 days of the final inducing dose. The results underlined the success of female Shi Drum in culture to undergo final oocyte maturation where GnRH-a injection was effective in inducing spawning of viable eggs (fertilization 65% and hatching 42 to 76%). However, multiple treatments did induce multiple spawning. Khan (1972) reported that low doses (0.1-0.2 ml/kg body weight) of pituitary extract given before 15 days of induced spawning improves the quality and quantity of gametes in the Indian major carps, silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*). Yadav *et al*. (2011) remarked that a low preparatory dose of the drug administered 45 days prior to the spawning gave better results in terms of fertilization and hatching in the catfish, *Clarias batrachus*.

Studies on induced breeding of fishes were carried out in our country by various workers (Marimuthu *et al.,* 2000; Singh *et al*., 2002; Das, 2004; Pandey & Singh, 2003; Pandey & Koteeswaran, 2004; Haniffa *et al.,* 2007). The study was conducted to optimize the dose of synthetic hormone for induced breeding of catfish to determine the fertilization rate and hatching success. The ovaprim and ovatide treated *H. fossilis* yielded better results compared to HCG, Wova-FH treated fish in terms of fertilization and hatching success.

Effects of HCG and GnRH on different neurosecretory centres

Recent research in field of fish endocrinology have led to a better understanding of hormonal factors involved in the control of gamete production, mode of their action and regulation of their secretion during different stages of reproductive cycle (Zohar & Mylonas, 2001; Dufour *et al.*, 2005; Singh & Pandey, 2009; Taranger *et al.*, 2010; Zohar *et al.*, 2010; Kim *et al.*, 2012). The majority of fishes breed at a particular time of the year and the seasonal reproductive cycle is precisely maintained by the endocrine rhythm. Environmental stimuli like photoperiod, temperature and rainfall are perceived by the brain which releases a decapeptide hormone, gonadotropin-releasing hormone (GnRH) (Alok *et al.*, 2000; Okubo *et al.*, 2002; Yashuvi *et al.*, 2006; Kah *et al.*, 2007; Crossin *et al.*, 2010; Kim *et al.*, 2012).

Attempts have been made to understand the neuroendocrine regulation of ovarian maturation by correlating the changes occurring in the two important hypothalamic nuclei- nucleus preopticus (NPO) and nucleus lateralistuberis (NLT) with the egg maturation in the Indian fishes (Pandey *et al.,* 2000,2007b,2011; Pandey & Mani, 2009). Generally, the neurosecretory cells of NLT exhibited enhanced cytological activity with the advancing maturity while neurosecretory cells of NPO displayed accumulation of secretory granules in matured specimens and excessive vacuolization during peak spawning period.

EXPERIMENTAL PROTOCOLS

Collection of Samples

Adult specimens of *L. rohita* (body weight range 582-810, g) were collected from the fields adjoining Lucknow (Uttar Pradesh) at monthly intervals during the study period (September 2015-August 2016). The maturity stages were observed by external morphological examinations and confirmed by the histological studies. The specimens for the experimental studies were maintained in the large glass fibre tanks of 5000 litres capacity at the Carp Hatchery, ICAR-NBFGR, Lucknow. They were fed on the formulated feed (crude protein content 30-32%; lipid content 11.0%; gross energy 3.90 kcal/g (Singh *et al*., 2000; Gupta *et al*., 2013). Temperature of water ranged between 26-30^{0}C and normal photoperiod were maintained during the experimental period.

Determination of Gonadosomatic Index

Gonadosomatic index (GSI) of ovary of 8 specimens at each maturity stage was determined based on the following formula and the mean value was calculated.

$$\text{Gonadosomatic index} = \frac{\text{Weight of the ovary (g)}}{\text{Weight of the fish (g)}} \times 100$$

At each stage, 28 oocytes were measured with the help of ocular micrometer along its long and short axes and average value calculated.

Histological and Histochemical Procedures

Staining of ovary

Based on the gross anatomical appearance, ovary of *L. rohita* was divided into six maturity stages which was later confirmed by the histological studies. The maturity stages of the carp were- resting phase, maturing phase, advance maturing phase, mature or pre-spawning phase, spawning phase and spent phase. For general histology, portions of ovarian tissues from anterior, middle and posterior regions were fixed in freshly prepared Bouin's as well as calcium formal solutions. After routine processing, they were dehydrated in the ascending series of alcohol, cleared in xylene and embedded in praffin wax at 60^{0}C. 6-8 µm sections were cut on the rotary microtome and stained in hematoxylin-eosin (H&E), toludine blue (1%), bromophenol blue and Mallory triple (for differentiation of various tissues) Composition of the stain is given in Appendix.

Table 3: Macroscopic and histological description of the different maturity stages of the ovary of *L. rohita*

Maturity stage	Macroscopic description	Histological description
Resting phase (December - January)	The ovaries at this stage were translucent, pale or dirty white in colur with inconspicuous vascularization. The ovaries occupy only a small part of the body cavity and ova were not visible to naked eye. This stage the ovaries do not show any remarkable changes in their morphology.	The small ovaries at this stage had oogonia and primary oocytes with some oocytes in peri-nucleolus stage. The nucleoli may be one or several, depending upon the size and maturity (Pate 1).
Early maturing phase (February-March)	Ovaries become slightly larger, thicker and opaque were brown to light yellowish in color. There was an increase in the weight of the ovary and they occupy about ½ of the body cavity.	Oogenesis has started in earnest. Two types of oocytes can be seen first maturing and second immature in the ovigerpus lamellae. At this stage, many oocytes possess a yolk nucleus, lying close to the nuclear membrane in the cytoplasm. The nucleoli was arranged along the periphery of the nucleus in the chromatin reticulum and few nucleoli pass out of the nuclear membrane and are seen in the cytoplasm of oocyte (Pate 2, 3).
Advanced maturing phase (April)	In this period, ovaries increase in the size and weight. They were occupy two third to three fourth of the body cavity.	At this stage in the oocyte the number and size of yolk vesicles further increases so that the ooplasm was completely filled with yolk vesicles. The ovarian wall becomes thin, vascular supply increases and the blood capillaries become conspicuous (Pate 4).
Mature phase (May-June)	Ovaries were enlarged in volume and occupying almost the entire body cavity. They were turgid, deep yellow in color and a large number of spherical ova were visible to the naked eye through the thin ovarian wall. Both translucent and opaque ova were present and the ovaries attain their maximum weight.	In this stage the yolk globules were present throughout the cytoplasm. There was heavy deposition of yolk globules which are fairly large in size. The yolk vesicles also fuse and become large. The nucleus gradually migrates towards the periphery and around the nucleus, the yolk globules were in the form of liquid. The nucleoli almost disappear in the nucleus. Some yolk vesicles pushed towards the periphery of the egg and form cortical alveoli (Pate 5, 6).
Spawning phase (July- August)	In this phase, ovaries were very much enlarged occupying the entire body cavity. They were turgid and yellow in color with large number of translucent eggs. Ovaries were full in mature yolky follicles and ovarian wall was very thin, almost transparent. Eggs were present in the oviduct also and the fish spawn during this period. At the beginning of this phase, ova were extruded by applying a gentle pressure on the abdomen.	The oocytes were greatly increased in diameter at this stage. Fusion of yolk granules. Atretic follicles or oocytes sparsely seen(Pate 7, 8)
Spent phase (September-November)	The ovaries were flaccid, shrunken and sac-like, reduced in volume and color became yellowish-red. Some unspawned large ova and a large number of small ova were present.	The ovary shows atretic and discharged follicles. Some oocytes showed coalescing of the yolk. This decrease in oocyte size caused a decrease in weight and size of the ovary.

Hematoxylin-eosin

It has polychrome properties which may be brought out with proper differentiation. For staining purpose, paraffin sections were first cleared in xylene and then hydrated through descending series of alcohol to water, kept for 10 minutes in haematoxylin, rinsed in tap water, brought upto 70% alcohol, stained with eosin for 5 minutes, dehydrated further upto absolute alcohol, cleared in xylene and mounted in DPX.All the stained slides were examined under light microscope and different stages of oocytes, their activities and substances were studied.

Staining of pituitary gland and hypothalamus neurosecretory cells

Mallory's triple stain

It is commonly used stain for neurosecretory cells (NSCs). Sections of brain along with pituitary of the female *L. rohita* were de-paraffinized in xylene and dehydrated in descending alcohol series upto water, kept in mordant (see Appendix) for 10 minutes, rinse in distilled water and stained in acid fuchsin for 10 seconds. Sections were again moderated in phosphomolybdic acid for 1 minute thereafter stained in Mallory's triple for 2 minutes after differentiated in water for 3 minutes. Then in 90% alcohol for 10 seconds, dehydrated and mounted in DPX.

HCG and GnRH (ovatide) Administration on Ovarian Maturity and Spawning

The present work was carried out on *L. rohita* (body weight range 1840-2470 g) in order to record the effects of HCG (two doses) and GnRH (ovatide) administration on ovarian maturity, spawning success. The fishes for the experimental studies were maintained in the rectangular tanks of 5000 litres capacity at the Carp Hatchery, ICAR-NBFGR, Lucknow. The fish were fed with commercial pelleted feed (crude protein content 32%; lipid 4 %) at the rate of 4% of body weight. Temperature of water ranged between 28-36^{0}C. Broodstock of *L. rohita* were randomly divided into four groups of 12 specimens each.Group 1: Fish were given physiological saline (0.20 ml/kg body weight) and served as control. Group 2: Fish were given intramuscular (im) injection of human chorionic gonadotropin (HCG) @ 25 IU/kg body weight (UNI-SANKYO, Hyderabad). Group 3: Fish were given intramuscular (im) injection of HCG @ 50 IU/kg body weight (UNI-SANKYO, Hyderabad). Group 4: Fish were given intramuscular (im) injection of GnRH (ovatide; Hemmo Pharma, Mumbai) (0.10 ml/kg body weight).

All the injections were given at weekly intervals during the month of June. After 28 days of injection, fish were checked for ovarian maturity by catheter method. Success of the maturity of all the groups of fish were evaluated through induced breeding performance by using ovatide (Hemmo Pharma, Mumbai). The recommended dose of GnRH-based drug for female *L. rohita* was 0.20-0.40 ml/kg body weight (single dose). The eggs were released to the water after 6-8 hours of the drug administration. The fertilization was external, sperm penetrated into the oocyte at a specific point known as micropyle. The embryos were hatched out within 14-18 hours after fertilization. The rate of fertilization and hatching success were recorded.

Gonadosomatic index

After 28 days of injection, from each groups three females were collected and weighted during experiment. Ovary form the fish was removed by dissecting the abdomen. The gonadosomatic index of the female was calculated by using the following formula:

$$\text{Gonadosomatic index} = \frac{\text{Weight of the ovary (g)}}{\text{Weight of the fish (g)}} \times 100$$

Determination of fertilization rate

After 8-10 hours, the eggs were placed on a Petridis containing acetone and observed under a magnifying glass. The fertilized eggs were counted with the help of soft thin brush. The fertilized eggs were separated from the unfertilized eggs in terms of color of the egg shell. Transparent eggs were identified as fertilized ones and opaque white ones were identified as unfertilized eggs. The fertilization rate was determined by the following formula:

$$\text{Fertilization rate} = \frac{\text{Number of fertilized egg}}{\text{Total number of egg}} \times 100$$

Determination of hatching rate

When hatching was completed, the hatchlings were collected in a pot (dish) and counted by visual observation using magnifying glass and recorded. The hatching rate was determined by the following formula:

$$\text{Hatching rate} = \frac{\text{Number of Hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) in the first and second experiments using statistical software SPSS (version 22.0). The significant differences between the treatments were determined by Duncan's multiple range test (DMRT) and the statistical significance level was assumed at $p<0.05$ in both the experiments.

Table 4: Changes in gonadosomatic index (GSI) and oocyte diameters (Mean ± SE) of *L. rohita* during various stages of ovarian maturation

Maturity stage	Number of fish	Body length (cm)	Body weight (g)	Ovary weight (g)	Ovary length (cm)	GSI (%)	Average ova diameter (µm)
Resting phase (December- January)	8	44.25^{e}±0.41	857.43^{e}±9.16	13.07^{e}±0.50	12.00^{f}±0.15	1.52^{e}± 0.06	108.43^{f}± 2.43
Early maturing phase (February-March)	8	46.61^{d}±0.34	964.62^{d}±14.46	18.41^{e}±0.35	12.9^{e}±0.22	1.90^{e}± 0.04	203.37^{e}±6.27
Advanced maturing phase(April)	8	47.90^{c}±0.34	1269.06^{c}±11.70	106.9^{c}±3.60	15.75^{c}±0.14	8.42^{c}± 0.24	576.87^{c}±12.63
Mature phase(May-June)	8	49.12^{b}±0.51	1567.87^{b}±14.85	207.62^{b}±16.21	18.90^{b}±0.16	13.24^{b}±1.08	683.06^{b}±23.57
Spawning phase (July-August)	8	50.93^{a}±0.40	1753.37^{a}±9.90	375.93^{a}±8.63	21.69^{a}±0.30	21.44^{a}±0.50	785.18^{a}±7.61
Spent phase (September-November)	8	51.25^{a}±0.44	1306.06^{c}±17.99	55.87^{d}±1.09	13.89^{d}±0.19	4.28^{d}± 0.11	378^{d} ±21.74

Data are expressed as mean ± S.E. (n=8). Different superscript in the same column differ significantly ($p<0.05$).

RESULTS

Female Reproductive System

In *L. rohita,* ovaries were paired structures extending along the whole length of the body cavity situated on the dorsolateral side of the air bladder. Both ovaries were elongated sac-like structure. They were lying in the posterior half of the body cavity and present on both sides of the stomach and intestine ventral to the kidneys. They remain suspended in position from the mid-dorsal side of the body cavity by mesenteries (mesovarium). Both the ovaries were enclosed in an ovisac. Generally, both ovaries were equal in size but occasionally they were unequal also. The ovaries show many variations in the shape and size depending upon the stage of their maturity. The anterior ends of the two ovaries were free but their caudal ends become united into one. The hinder end of each ovary was continued posteriorly into a short oviduct. The two oviducts fuse and open to the exterior by a separate genital aperture. They were thin, flaccid and translucent when immature but on maturity stage, they become greatly enlarged, lobulated and the ripe ova were seen bulging out.

Ovarian maturity stages

On the basis of gross size, shape, coloration and histo-morphological features of the ovaries of female *L. rohita*, development of ovary were categorized into six maturation phases. The details have been summarized in Table 3, 4.

Gonadosomatic index (GSI)

The value of GSI is indicator of the status of gonadal development and maturity of individuals. The phase wise distribution of gonadosomatic index of female *L. rohita* is given in Table 4 and Fig. 10. The minimum value of GSI was detected during resting phase (1.52±0.06). The maximum value of GSI appeared during spawning phase (21.44±0.50). The GSI was significantly different ($p<0.05$) during the sampling period.

Oocyte diameter

Oocyte diameter is a measurement to determine oocyte growth. The phase wise distribution of average ova diameter of female *L. rohita* is given in Table 4 and Figure 11.The lowest value of oocyte diameter was detected during resting phase (108.43±2.43 µm). The highest value of oocyte diameter appeared during spawning phase (785.18±7.61µm). The oocyte diameter was significantly different ($p<0.05$) during the sampling period.

Pituitary Gonadotrophs

Pituitary gland of *L. rohita* was situated behind the optic chiasma and protected within a bony chamber of the para-sphenoid bone. In general, the pituitary gland of *L. rohita* was an oval structure, compressed dorso-ventrally and attached to the ventral region of the hypothalamus. The colour of fresh pituitary gland was creamy white. Pituitary gland of *L. rohita* was on a typical teleostean pattern. Histologically, it was divided into a glandular component (adenohypophysis) and a neural component (neurohypophysis). The glandular region was distinguished into three parts- rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) placed serially one after the other along the anterio-posterior axis. The rostral pars distalis (RPD) was the smallest region of the glandular component and pars intermedia (PI) occupies

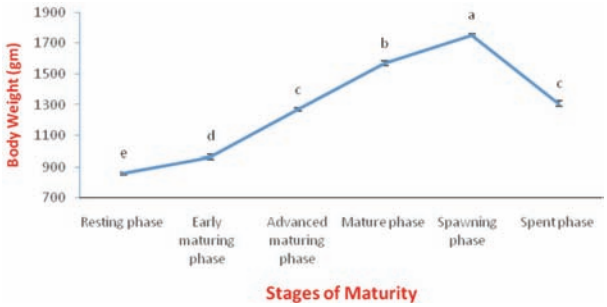

Fig. 8: Graph showing the body weight (mean ± SE) in different stages of ovarian maturation in *L. rohita.* Data expressed as mean ± SE, n=8; the bar bears different superscript differ significantly (p<0.05).

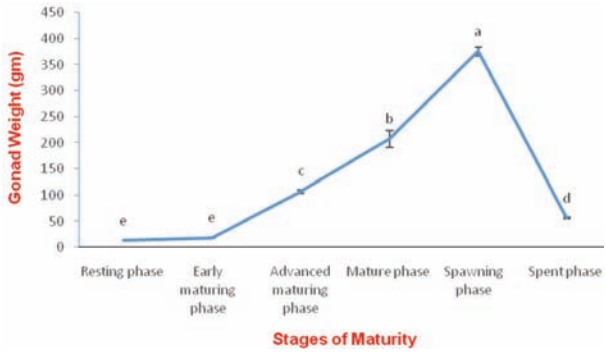

Fig. 9: Graph showing the gonad weight (mean ± SE) in different stages of ovarian maturation in *L. rohita.* Data expressed as mean ± SE, n=8; the bar bears different superscript differ significantly (p<0.05).

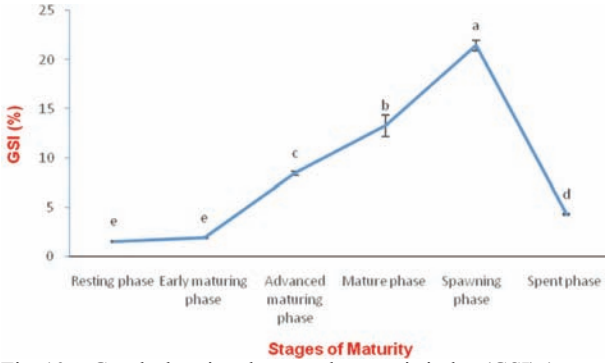

Fig. 10: Graph showing the gonadosomatic index (GSI) (mean ± SE) in different stages of ovarian maturation in *L. rohita.* Data expressed as mean ± SE, n=8; the bar bears different superscript differ significantly (p<0.05).

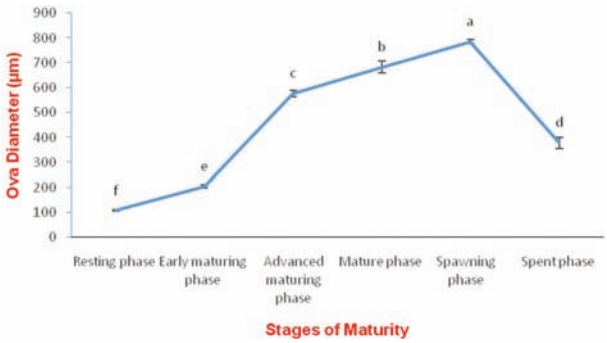

Fig. 11: Graph showing ova diameter (mean ± SE) in different stages of maturation in *L. rohita.* Data expressed as mean ± SE, the bars with different superscript differ significantly (p<0.05)

Table 5: Maturity stages and levels of granulation in gonadotrophs of *Labeo rohita*

Maturity stage	Body weight (g)*	Ovary weight (g)*	GSI*	Levels of granulation (%)
Resting phase (December-January)	857.43^{e}±9.16	13.07^{e}±0.50	1.52^{e}±0.06	10.0±1.5
Early maturing Phase (February-March.)	964.62^{d}±14.46	18.41^{e}±0.35	1.90^{e}±0.04	20.0±1.0
Advanced maturing phase (April)	1269.06^{c}±11.70	106.9^{c}±3.60	8.42^{c}±0.24	35±2.5
Mature phase (May-June)	1567.87^{b}±14.85	207.62^{b}±16.21	13.24^{b}±1.08	65±4.5
Spawning phase (July-August)	1753.37^{a}±9.90	375.93^{a}±8.63	21.44^{a}±0.50	40±3.0
Spent phase (September- November)	1306.06^{c}±17.99	55.87^{d}±1.09	4.28^{d}±0.11	25±1.5

*Data are expressed as mean ± S.E. (n=8). Different superscript in the same column differ significantly (p<0.05).

Table 6: Maturity stages and levels of granulation in NPO neurosecretaory cells (NSCs) of *L. rohita*

Maturity stage	Body weight (g)*	Ovary weight (g)*	GSI	Levels of granulation (%)
Resting phase (December- January)	857.43^{e}±9.16	13.07^{e}±0.50	1.52^{e}±0.06	10.0±1.0
Early maturing Phase (February- March.)	964.62^{d}±14.46	18.41^{e}±0.35	1.90^{e}±0.04	25±1.0
Advanced maturing phase (April)	1269.06^{c}±11.70	106.9^{c}±3.60	8.42^{c}±0.24	45±2.5
Mature phase (May-June)	1567.87^{b}±14.85	207.62^{b}±16.21	13.24^{b}±1.08	85±4.5
Spawning phase (July-August)	1753.37^{a}±9.90	375.93^{a}±8.63	21.44^{a}±0.50	30±3.0
Spent phase (September to November)	1306.06^{c}±17.99	55.87^{d}±1.09	4.28^{d}±0.11	20±1.5

*Data are expressed as Mean ± S.E. (n=8). Different superscript in the same column differ significantly (p<0.05).

almost one third posterior part of the pituitary gland whereas rest of the region was occupied by the proximal pars distalis (PPD). Thus the PPD was the largest part of the glandular component of the pituitary gland. PPD of *L. rohita* mainly comprised somatotrophs, gonadotrophs and thyrotrophs. Somatotrophs were found distributed among the gonadotrophs. They were acidophilic, stained positive with erythrosine and somatotrophs and predominated during non-breeding phases (Plate 7). Gonadotrophs were cynophilic and stained positive with alcian blue (AB) and aniline blue. Ovarian status and levels of granulation of gonadotrophs (cynophils) in *L. rohita* at various stages of maturity have been summarized in Table 4. Number of gonadotrophs increased considerably and became the major cell type of PPD as the advanced maturing phase. During maturing phase, granulation of these cells initiated and was heavily granulated during mature phase (Plate 8, 9). During or early spawning and spawning peak, there was a progressive and excessive degranulation of the cynophil cells (Plate 10, 11). In post-spawning period, these cells depicted scanty granulation and appeared exhausted (Plate 12). In resting, preparatory and immature phases, the gonadotrophs exhibited inactivity or low profile of activity with scanty accumulation of secretory granules. Some cynophils which do not exhibit hypertrophy or degranulation in relation to maturity were identified as thyrotrophs. In anterior neurohypophysis (ANH), 3-5 acid fuchsin+ve Herring bodies (HB) of varying sizes were also encountered in the matured *L. rohita*.

Hypothalamus

Hypothalamo-neurosecretory complex of *L. rohita* consisted mainly of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and their axonal tracts. NPO was a paired structure situated topographically on either side of the third ventricle dorsal to the optic chiasma and looks inverted L-shape in sagittal section (Plate 13). The horizontal limb of NPO comprises sparsely distributed neurons whereas the neurosecretory cells were closely packed in the vertical limb. Based on the size of the neurosecretory cells, NPO was further divided into dorsal pars magnocellularis (PMC) formed of larger neuronal cells and a ventral pars parvocellularis (PPC) comprising smaller cells (Plate 15, 16). Thus, a progressive reduction in the size of neurons from dorsal to ventral aspect of NPO was seen in the *L. rohita*. Generally, neurons of PMC and PPC were bipolar and contributed beaded axons to form left and right neurohypophyseal main tracts. The neurosecretory cells of NPO were laden with the secretory material in the matured specimens (Plate 16) while they exhibited excessive vacuolation during spawning phase of the ovarian cycle.

NLT cells were distributed uniformly in the infundibular floor adjacent to the pituitary stalk. Based on the distribution and size, NLT was divided into pars anterior, pars posterior and pars inferior. This structure was highly vascular and several neurons were seen in close association with the blood vessels. The neurosecretory cells of NLT of *L. rohita* exhibited heightened activity during mature/pre-spawning phase of the reproductive cycle (Plate 18, 19). Neuro-hypophyseal tract enters the pituitary gland of *L. rohita* (Plate 20).

Effects of HCG and GnRH (ovatide) administration on the ovarian maturity and spawning of *Labeo rohita*

Effects of human chorionic gonaditropin (HCG) (25 IU/Kg and 50 IU/Kg) and ganadotropin-releasing hormone (GnRH) (ovatide) on the ovarian maturity and breeding performance of *L. rohita* have been summarized in Table 7. Though all the three hormonal drugs did enhance gonadosomatic index (GSI) and ovarian maturity in *L. rohita*, but the response of GnRH (ovatide) (Group 4) was more pronounced as the oocytes exhibited germinal vesicle migration (GVM) towards the periphery in most of the cases. Interestingly, all the fishes of Groups 2-4 bred successfully after 6-8 hours of intramuscular administration of ovatide (@ 0.10-0.20 ml/kg body weight for males and 0.20-0.40 ml /kg body weight for females). The eggs of Group 2-4 fishes were reddish-white in colour, round shape, well developed and separated after spawning whereas the eggs of control fishes (Group 1) released in lumps only after spawning and were whitish in colour.

DISCUSSION

Ovarian Cycle and Maturation in *L. rohita*

Seven stages of ovarian maturation was suggested for fish by the International Council of Exploration of Sea (Wood, 1930- cited by Qasim, 1973; Prakash & Paliwal, 1974) and subsequently adopted by various workers. However, Khanna & Pant (1967) and Srivastava & Saxena (1996) recorded 5 maturity stages while Smith & Walker (2004) and Khanna (2013) have assigned the maturity stage on 6 point scale. Even Qasim, 1973 also felt for reducing the number of maturity stages to 5 for marine species for ease in collecting data at the landing centres. The process of ovarian development has been described in many fish and divided into seven to eight maturity stages (Shirali *et al*., 2011; Verma, 2013). However, in many of Indian teleosts, annual changes in ovary divided into four to six stages (Day *et al.,* 2004; Roy & Mandal, 2015).

Following the standard methods for judging the maturity stages described by Lone & Hussain (2009), Khanna (2013) and Verma (2013) based on shape, size, colour of the ovary and other histomorphological features,

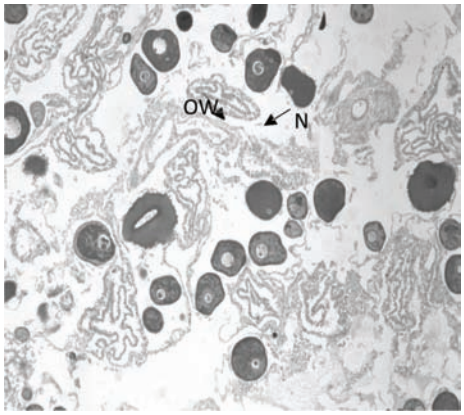

Plate 1: Ovary of *L. rohita* showing immature oocytes in ovigerous lamellae. H&E x 400. N; Nucleus, OW; Oocyte wall.

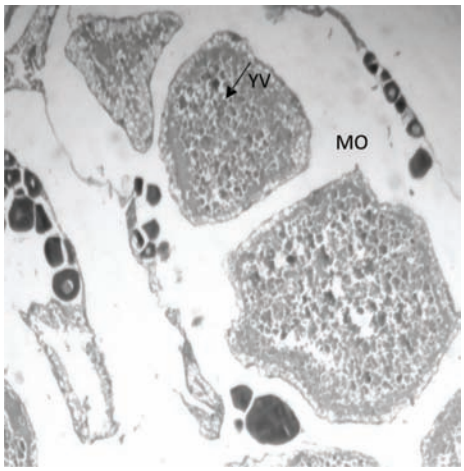

Plate 2: Ovary of *L. rohita* showing maturing oocytes. Also mark the presence yolk vesicles towards the periphery. H&E x 10. MO; Mature oocyte, YV; yolk vesicles.

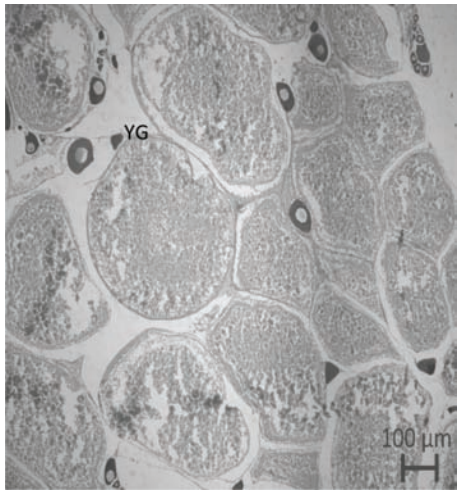

Plate 3: Ovary of *L. rohita* showing maturing oocytes containing yolk globules. H&E x 10. YG; Yolk globules.

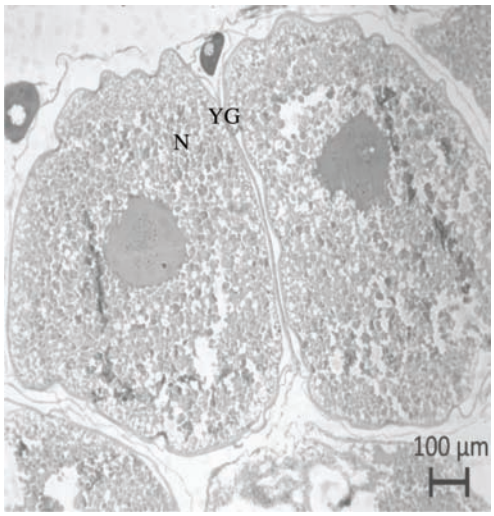

Plate 4: Ovary of *L. rohita* exhibiting advanced maturing oocytes showing large number of yolk globules near the nucleus. H&E x 10. N; Nucleus, YG; yolk globules.

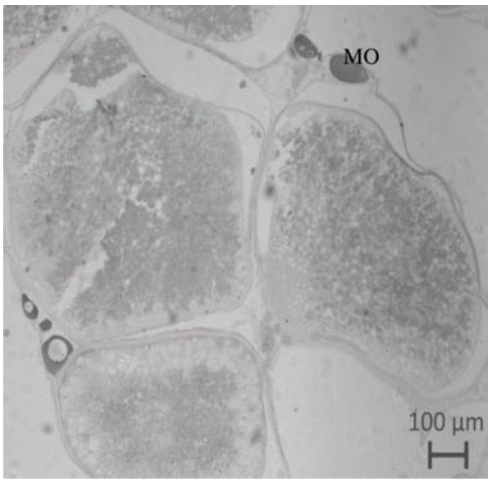

Plate 5: Ovary of *L. rohita* exhibiting matured oocytes with deposition of yolk. H&E x 20. MO; Matured oocyte.

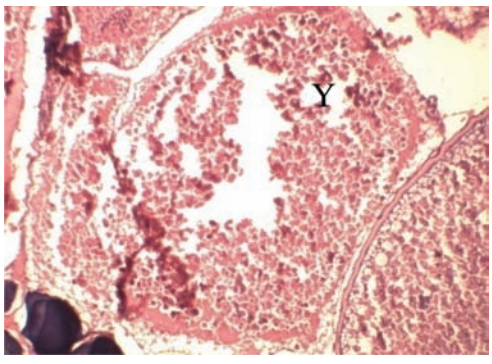

Plate 6: Ovary of *L. rohita* exhibiting ripe eggs with deposition of yolk. H&E x 20. Y; Yolk.

six maturity stages such as (i) Resting phase (December-January), (ii) Early maturing phase (February-March), (iii) Advanced maturing phase (April), (iv) Mature or pre-spawning phase (May-June), (v) Spawning phase (July-August) and (vi) Spent phase (September to October) in *L. rohita* were recognized.

The reproduction of all Indian freshwater fish exhibit annual periodicity and maturation of their gonads and spawning occurs in a particular period of the year. Photoperiod, temperature and rainfall are the important external factors influencing breeding periodicity (Davis *et al*., 1999; Bhattacharyya & Maitra, 2006). The reproductive cycle in female fish is determined by the most suitable method to observe seasonal development changes in the gonad (Sivakumaran *et al*., 2003). The mechanism of oogenesis and oocyte maturation among teleosts seems to be similar except variations in the timing of recruitment and maturation of oocytes (Lone & Hussain, 2009; Roy & Mandal, 2015).

In the present study, *L. rohita* exhibits seasonal cyclic changes in the ovarian histology with peak activity during matured and spawning phases of reproductive cycle. Similar observations were also reported for *L. rohita* (Day *et al*., 2004; Lone & Hussain, 2009), *Common carp* (Mikolajczyk *et al*., 2004) and *Labeo bata* (Roy & Mandal, 2015). The function of the follicular epithelium in fish oocyte development remained controversial. The granulosa cells were believed to be responsible for the deposition of yolk in the developing ovum and also for its removal in ova which degenerates and become atretic. Besides these, the granulosa cells may also be responsible for the secretion of ovarian hormone estradiol, which is responsible for the production of vitellogen (Zohar *et al.,* 2010). Though atretic follicles were more predominant during post-spawning phase, they also encountered even during early maturation, matured and spawning phases of the ovarian cycle. Day *et al*. (2004) in *Catla catla*, Mikolajczyk *et al*. (2004) in *Cyprinus carpio*, Mani & Pandey (2006) in *Heteropneustes fossilis*, Lone & Hussain (2009) in *Labeo rohita* and Roy & Mandal (2015) in *Labeo bata* have encountered such follicles during the phases of the ovarian cycle. In the present study in *L. rohita*, all the four stages of atretic follicles, described by Lone & Hussain (2009) were noticed. Though endocrinological functions was assigned to the atretic follicles, the main function appears to limit the number of oocytes that could undergo vitellogenesis and become mature for ovulation (Mani & Pandey, 2006; Farrell, 2011; Khanna, 2013; Roy & Mandal, 2015).

The GSI is one of the main parameters used to evaluate gonadal development in fishes and this method is easier and cheaper to utilize. The high correlation of GSI with number of matured female could be utilized to extrapolate peak spawning season. In the present study, GSI which indicates growth and maturation of gonad was higher during spawning stage and lower in resting stage. Like

Table 7: Maturity stages and levels of granulation in NLT neurosecretaory cells (NSCs) of *L. rohita*

Maturity stage	Body weight (g)*	Ovary weight (g)*	GSI*	Levels of granulation (%)
Resting phase (December- January)	$857.43^{e}\pm9.16$	$13.07^{e}\pm0.50$	$1.52^{e}\pm0.06$	10.0±1.0
Early maturing Phase (February- March.)	$964.62^{d}\pm14.46$	$18.41^{e}\pm0.35$	$1.90^{e}\pm0.04$	35±1.0
Advanced maturing phase (April)	$1269.06^{c}\pm11.70$	$106.9^{e}\pm3.60$	$8.42^{c}\pm0.24$	65±2.5
Mature phase (May-June)	$1567.87^{b}\pm14.85$	$207.62^{b}\pm16.21$	$13.24^{b}\pm1.08$	80±4.5
Spawning phase (July-August)	$1753.37^{a}\pm9.90$	$375.93^{a}\pm8.63$	$21.44^{a}\pm0.50$	45±3.0
Spent phase (September to November)	$1306.06^{c}\pm17.99$	$55.87^{d}\pm1.09$	$4.28^{d}\pm0.11$	25±1.5

*Data are expressed as Mean ± S.E. (n=8). Different superscript in the same column differ significantly ($p<0.05$).

Table 8: Breeding responses of *L. rohita* in response to various hormonal treatments.

Treatment	Sets taken	Gonadosomatic Index (GSI)	Fertilization (%)	Hatching success (%)
Control	8	$15.39^{d}\pm0.94$	$22.71^{c}\pm1.18$	$12.27^{d}\pm0.87$
HCG 25 IU/Kg	8	$19.26^{c}\pm0.62$	$81.83^{b}\pm1.42$	$71.53^{c}\pm1.03$
HCG 50 IU/Kg	8	$21.26^{b}\pm1.06$	$86.57^{a}\pm0.81$	$75.07^{b}\pm1.09$
GnRH (ovatide)	8	$23.91^{a}\pm1.62$	$88.35^{a}\pm0.72$	$81.13^{a}\pm1.13$

Data are expressed as mean ± S.E. (n=8). Different superscript in the same column differ significantly ($p<0.05$).

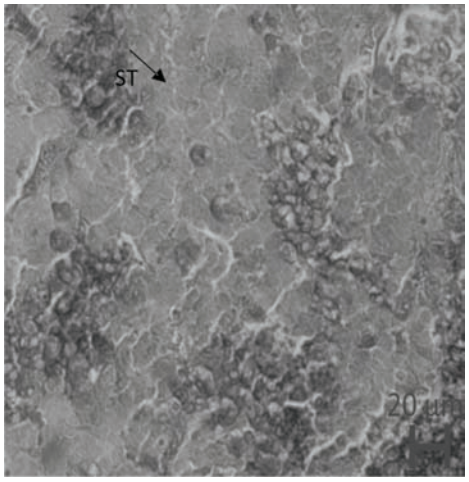

Plate 7: Pituitary of female *L. rohita* in resting phase showing predominance of somatotrophs. Mallory's triple stain x 40. ST; Somatotrophs.

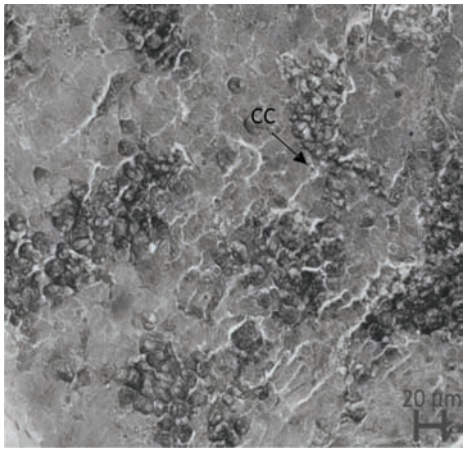

Plate 8: Pituitary gland of maturing female *L. rohita* showing granulation in cynophil cells. Mallory's triple stain x 40. CC; Cynophil cells.

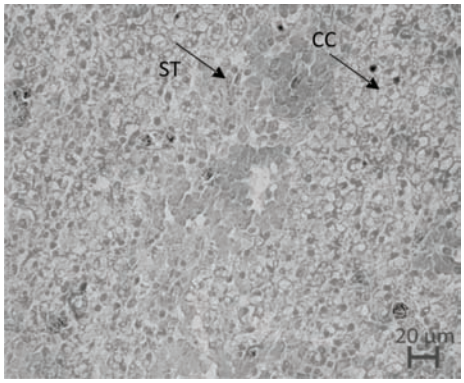

Plate 9: Pituitary of mature female *L. rohita* showing partial degranulation in cynophil cells. Mallory's triple stain x 20. CC; Cynophil cells.

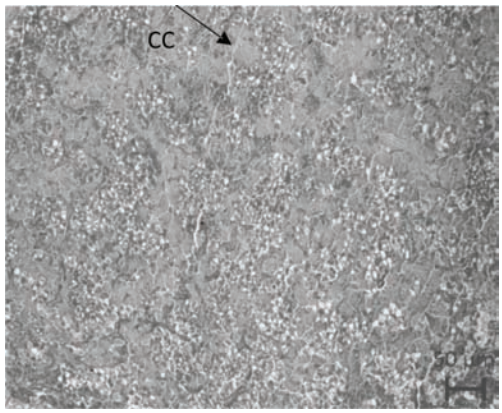

Plate 10: Pituitary of mature female *L. rohita* showing partial de-granulation in cynophil and somatotrophs cells. Mallory's triple stain x 40. CC; Cynophil cells, ST; somatotrophs

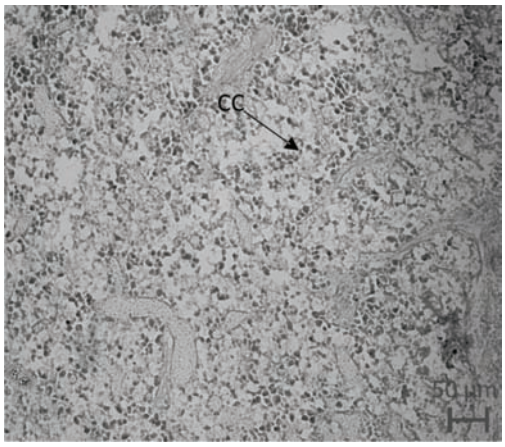

Plate 11: Pituitary of female *L. rohita* during spawning period showing excessive degranulation in cynophils cells. Mallory's triple stain x 20. CC; Cynophil.

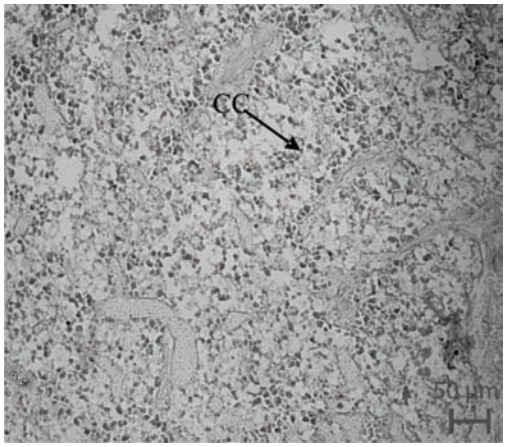

Plate 12: Pituitary of female *L. rohita* during post-spawning period depicting degranulated and exhausted cynophils cells. Mallory's triple stain x 20. CC; Cynophil.

the present findings, Lone *et al.* (2012) also reported that GSI of *Catla catla* was highest during spawning stage and lowest in resting phase. The highest GSI and lowest GSI were correlated with ovarian development of *Labeo bata* too (Roy & Mandal, 2015).

As the highest oocyte diameter recorded in spawning phase, it implied that the oocytes reached maturity in this period and ovulation is likely to occur and lowest oocyte diameter was seen in resting phase. Therefore, the fish would spawn around this month under suitable conditions. Lone *et al.* (2012) also noted a highest oocyte diameter in spawning phase and lowest in resting phase which coincided with the findings of the present study.

Pituitary Gonadotrophs

The pituitary gland in *L. rohita,* like in all vertebrates, is a master internal secretion organ controlling growth and reproduction, enabling body electrolyte balance and controlling all other endocrine glands (Farrell, 2011). In the anatomical examination made of *L. rohita,* it was seen that the pituitary gland is pear/ oval shape, creamy white coloured, ompressed dorso-ventrally and attached to the ventral region of the hypothalamus. Similar anatomical observation has also been reported for the pituitary of *Oreochromis niloticus* (EI-Sakhawy *et al.,* 2011) and *Mystus vittatus* (Chatterjee & Chakrabarti, 2014).

Based on the tinctorial properties and distribution, adenohypophysis has been subdivided in rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) innervated by the neurohypophysis process. In the present observation in *L. rohita,* various cell types located in the constituent parts of the pituitary gland on the basis of staining reaction in the cytoplasmic content with different methods employed. Ursani *et al*. (2012) and Grandi *et al*. (2014) also identified various cell types and their distribution in the pituitary of *Oreochromis mossambicus* and *Ctenopharyngodon idella*, respectively. In the present investigation PPD of *L. rohita* mainly comprised somatotrophs (STH), gonadotrophs (GTh) and thyrotrophs (TSH). Somatotrophs were found distributed among the gonadotrophs. Similar pattern of distribution of somatotrophs are also ireported by Bose & Chakrabarti (2014) in *Liza parsia*, Ekici & TImur (2013) in *Cyprinus carpio* and Kharat & Khillare (2013) in *Nemacheilus moreh*. In the present study, somatotrophs were acidophilic, stained positive with erythrosine and orange G and predominated during non-breeding phases. This observation was similar to the findings of Bose & Chakrabarti (2014) in *Liza parsia* and Grandi *et al*. (2014) in *Ctenopharyngodon idella*.

In present study, using specific stains like PAS, alcian blue and aniline blue, gonadotrophs have been identified in proximal pars distalis (PPD) of the hypophysis of *L. rohita*. Similar tinctorial location of gonadotrophs was recorded in various teleostean species (Pandey & Mani, 2009; Ekici & TImur, 2013). Histological changes of gonadotrophs during ovarian maturation have been studied in several species of teleosts. During the advanced maturing phase the gonadotrophs increased in number, size and occupied a considerable area in the PPD (AIi, 2003; AI-Absawy, 2004; Fahmy, 2006; Balci and Ikiz, 2017). In the present study, gonadotrophs reached to the maximum degree of granulation during maturing stage. These observations are supported by Ekici and TImur (2013) in *Cyprinus carpio*. In the present investigation in *L. rohita* during or early spawning and spawning peak, there was a progressive and excessive degranulation of the cynophil cells suggesting the release of the hormone in blood circulation (Pandey and Mani, 2009). In resting, preparatory and immature phases, the gonadotrophs exhibited inactivity or low profile of activity with scanty accumulation of secretory granules. Grandi *et al.* (2014) reported similar result that the gonadotrophs exhibited cyclicity in relation to ovarian maturation stages in *Ctenopharyngodon idella.*

In the present study, the gonadotrophs in immature and spent phases were observed more than through spawning time, while secretion granules of cynophil cells spread over a great area of the cell during spawning period. These variations in the granulation amount was reported for other species studied (Assem, 2004; Vongvatcharanon *et al.,* 2005; Chatterjee & Chakrabarti, 2014; Balci & kiz, 2017). In anterior neurohypophysis (ANH) of *L. rohita*,, 3-5 acid fuchsin+ve Herring bodies of varying sizes were also encountered. Herring bodies (HB) of varying shapes and sizes have also been reported in *Decapterus tabl* (Pandey & Mohamed, 1993), *Megalaspis cordyla* (Pandey, 1997), *Sphyraena obtusata* (Pandey & Mohamed, 1997), *Lates carcarifer* (Lal & Pandey, 1998) and *Decapterus russelli* (Pandey, 2008). Ekici & TImur (2013) reported that the secretory granules containing a specific type Herring bodies of these fibers exist in great numbers and various sizes in the anterior neurohypophysis of *Cyprinus carpio*. There are reports that Herring bodies are stored neurosecretory secretaty materials (Pandey, 1997; 2008).

Hypothalamus

Hypothalamo-neurosecretory system of the teleosts was comprised mainly of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and their axonal tracts (Subhedar *et al*., 1999; Pandey, 2008; Bose & Chakrabarti, 2018). The NPO of *L. rohita* was observed as paired structure located on either side of the third ventricle slightly above and anterior to the optic chiasma and having inverted L-shape, which is similar in line with the observations of Moitra & Medya (1980) in *Cirrhinus*

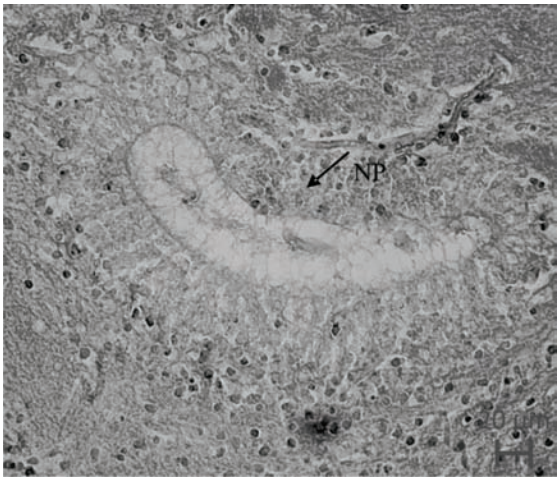

Plate 13: Nucleus preopticus (NPO/POA) of immature female *L. rohita*. Mallory's triple stain x 63.

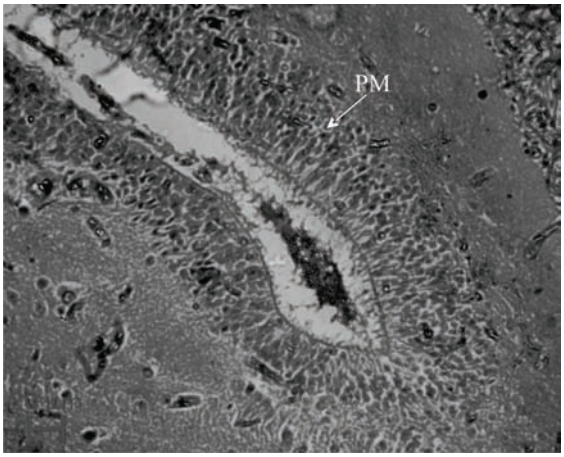

Plate 14: Pars magnocellularis (PMC) of maturing female *L. rohita.* Mallory's triple stain x 63.

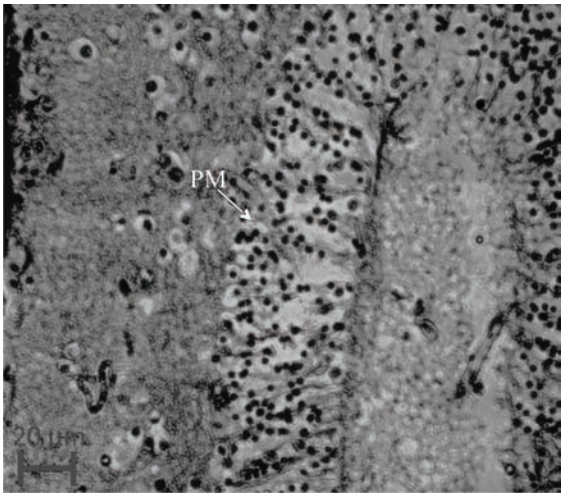

Plate 15: Pars magnocellularis (PMC) of matured female *L. rohita.* Mallory's triple stain x 63.

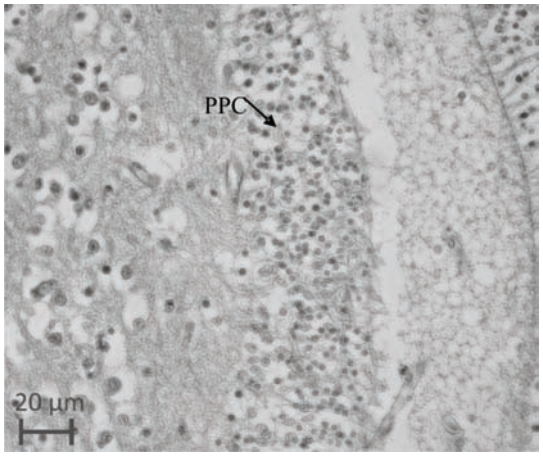

Plate 16: Pars parvocellularis (PPC) of matured female *L. rohita.* Mallory's triple stain x 63.

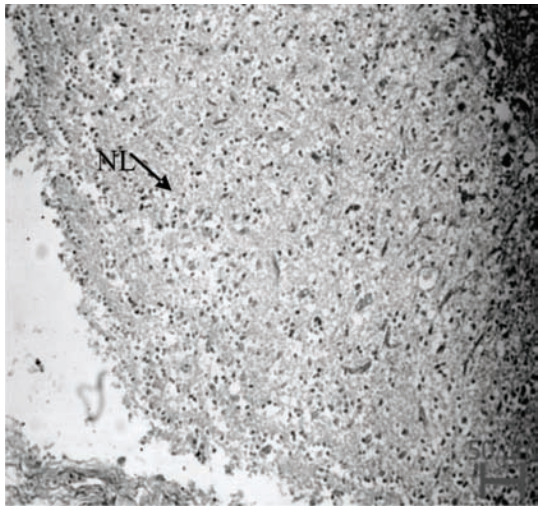

Plate 17: NLT of immature female *L. rohita* with enhanced staining affinity. Mallory's triple stain x 20.

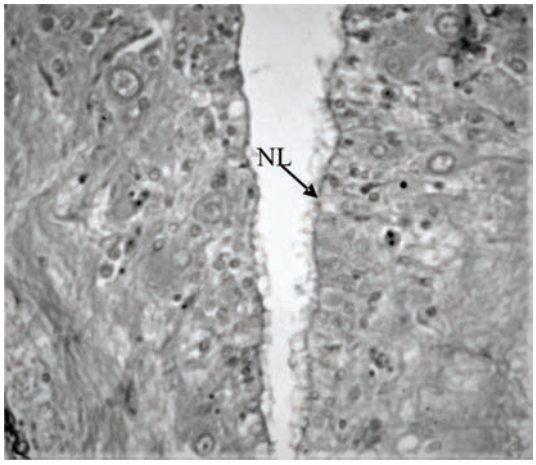

Plate 18: NLT of maturing female *L. rohita* with enhanced staining affinity. Mallory's triple stain x 20.

mrigala, Gur *et al*. (2000) in *Mylopharyngodon piceus,* Khuroo (2013) in *Xenentodon cancila* and Bose and Chakrabarti (2018) in *Liza parsia*. The NPO was divisible into a dorsal pars magnocellularis (PMC) consisting of large neurosecretory cells and a ventral pars parvocellularis (PPC) with small neuronal cells (Pandey, 2008; Bano, 2012; Bose & Chakrabarti, 2018; Ray, 2019). Thus, a progressive reduction in the size of neurosecretory cells was seen from the dorsal to the ventral aspect of NPO. NPO was a highly vascularized structure. A similar observation has also been identified in the other species like *Tor putitora,* (Pandey *et al*., 2000)*, Xenentodon cancila* (Bano, 2012) and *Mastacembelus armatus* (Ray, 2019). Generally, the neurosecretory cells of NPO stain with AF and CAHP but they were also stainable with acid fuchsin in a number of teleosts such as *Notopterus chitala* (Prakash *et al*., 1984a, b), *Rastrelliger kanagurta* (Pandey, 1993), *Megalaspis cordyla* (Pandey, 1997), *Decapturus tabl* (Pandey & Mohamed, 1993), *Lates calcarifer* (Lal & Pandey, 1998), *Sphyrena obtusata* (Pandey & Mohamad, 1997), *Arioma indica* (Pandey & Mohamed, 1999) and *Liza parsia* (Bose & Chakrabarti, 2018).

The cells of PMC and PPC of *L. rohita* were laden with the neurosecretory secretory material in maturing and matured phases whereas they are partially vacuolated in during spawning phase. Most of the PMC and PPC neurosecretory cells of the carp were bipolar and contribute beaded axons to form the neurohypophysial tract. Similar observations have also been recorded by Pandey & Mohamed (1993, 1997, 1999); Lal & Pandey (1998), Pandey (2008) and Bano (2012). There were reports that NPO are involved in spawning activities and its secretions influences gonadal maturation and spawning among teleosts (Das & Sinha, 1988; Pandey & Mohamed, 1993; Lal & Pandey, 1998). Vacuolation was observed in the neurosecretory cells of PMC and PPC of matured *L. rohita* during spawning phase. Moitra & Medya (1980) in *Cirrhinus mrigala*, Zolotnitskiy (1980) in *Scopthalamus maeoticus*, Rai & Pandey (1986) in *Colisa fasciata*, Bano (2012) in *Xenentodon cancila* and Bose & Chakrabarti (2018) in *Liza parsia* also noticed depletion of neurosecretory material during breeding season.

Nucleus lateralis tuberis (NLT) was the second important neurosecretory centre in the teleostean hypothalamus (Pandey & Mohamed, 1993,1997,1999; Lal & Pandey, 1998; Pandey *et al*., 2000; Pandey, 2008; Mohan, 2014). In contrary to this, there are various reports regarding the complete absence of NLT in some fishes (Saksena, 1979; Prakash *et al*., 1984a) although it has great implication on gonadal maturation. Kobayashi *et al*. (1959) had remarked that season or age factors might be responsible for the absence of stainable neurosecretory material. In NLT, the neurosecretory cells are distributed unevenly in the infundibular floor adjacent to the pituitary stalk. This feature of NLT was also observed by various authors (Moitra & Medya, 1980; Maksimovich, 1987; Bose & Chakrabarti, 2018). These cells are generally negative to aldehyde fuchsin (AF) and chrome-alum-haematoxylin-phloxine (CAHP) but stain readily with acid fuchsin in Mallory's triple stain. The neuroseccretory cells of NLT stained readily with acid fuchsin. NLT cells of other teleosts like *Catla catla* (Sathyanesan, 1973), *Rastelliger kanagurta* (Pandey, 1993), *Decapterus tabl* (Pandey & Mohamed, 1993), *Sphyrena obtusata* (Pandey & Mohamad, 1997), *Ariomma indica* (Pandey & Mohamad, 1999) and *Tor putitora* (Pandey *et al*., 2000) also exhibited almost similar staining response.

Based on distribution and size of the neurosecretory cells, the nucleus lateralis tuberis (NLT) may be divisible into pars anterior, pars posterior and pars inferior. The neurosecretory cells of NLT are variously shaped and their sizes range from very small to the larger ones with polymorphic nuclei. These neurons are generally bipolar but a few multipolar cells were also observed in NLT of *L. rohita.* NLT was a highly vascularized structure in *L. rohita* and a few neurosecretory cells are seen in close association of blood vessels. A similar observation has been recorded in the other species like *Lates calcarifer* (Lal & Pandey, 1998), *Liza parsia* (Bose & Chakrabarti, 2018) and *Mastacembelus armatus* (Ray, 2019). Neurohypophysial tract (NHT) enters the pituitary gland of *L. rohita* through infundibulum. NLT cells of maturing as well as matured *L. rohita* appeared active in the present study. Viswanathan & Sundararaj (1974) in *Heteropneustes fossilis* and Rai & Pandey, (1986) in *Colisa fasciata* also recorded enhanced activity in the NLT cells after estrogen administration and during breeding season, respectively.

Effects of HCG and GnRH (ovatide) Administration on Ovarian Maturity and Spawning

HCG (25 IU and 50 IU) and GnRH (ovatide) induced maturity and enhanced breeding performance in *L. rohita*. These hormonal drugs increased gonadosomatic index (GSI) and ovarian maturity in the carps resulting in the better breeding responses. There exist reports that lower doses of HCG and GnRH advance maturation in teleosts (Lam, 1982; Donaldson & Hunter, 1983; Das & Singh, 1990; Kanungo *et al*., 1999; Bandyopadhyay *et al.*, 2009; Zohar and Mylonas, 2001). Though Zohar & Mylonas (2001) remarked that HCG acts directly on gonads, Das & Singh (1990) recorded enhanced activity in gonadotrophs and ACTH cells of *L. rohita* concurrently with the ovarian development (maturation) in response to 50 IU HCG administration (4 doses, weekly intervals).

Proper favourable synchronization of different environmental factors determines the reproductive

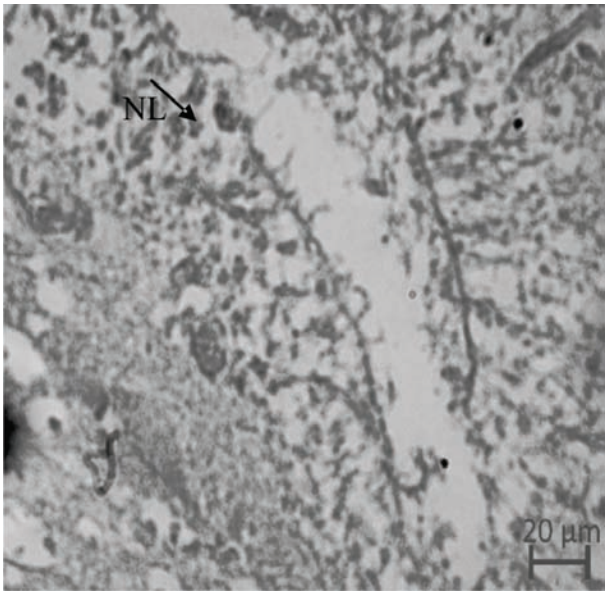

Plate 19: NLT of matured female *L. rohita* with enhanced staining affinity. Mallory's triple stain x 63.

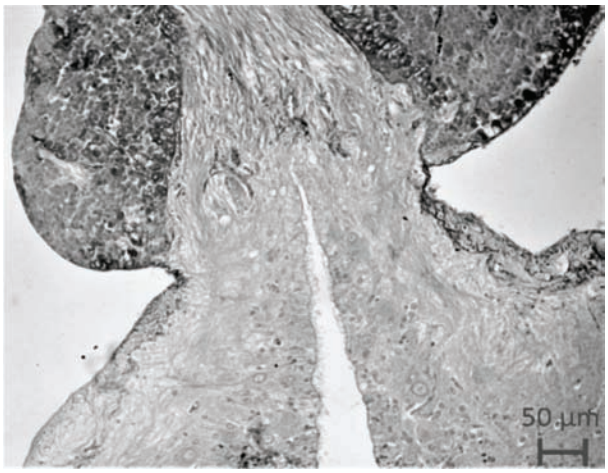

Plate 20: Hypothalamo-hypophyseal tracts entering pituitary (PI). Mallory's triple stain x 20.

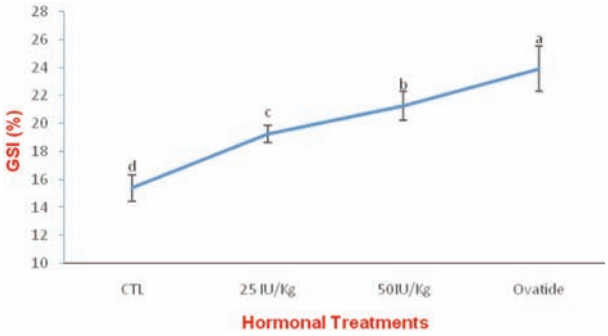

Fig. 12: Gonadosomatic index (GSI) (mean ± SE) of *L. rohita* in different hormonal treatments. Data expressed as mean ± SE (n=8). The bar bears different superscript differ significantly ($p<0.05$).

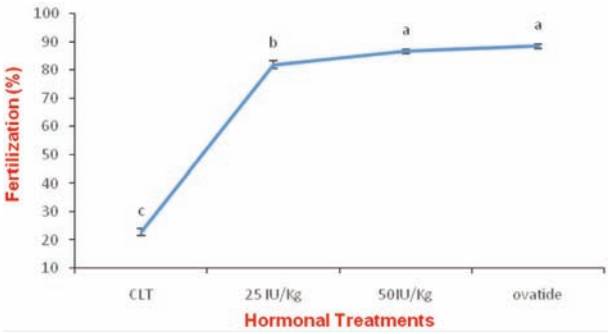

Fig. 13: Fertilization (%) (mean ± SE) of *L. rohita* in different hormonal treatments. Data expressed as mean ± SE (n=8). The bar bears different superscript differ significantly ($p<0.05$).

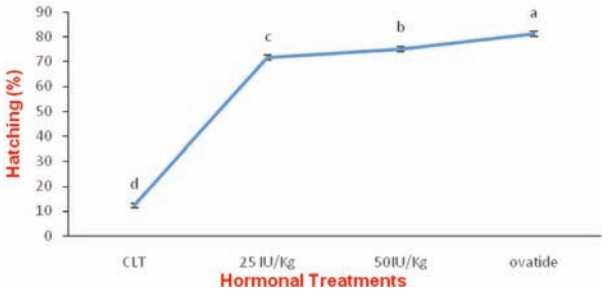

Fig. 14: Hatching (%) (mean ± SE) of *L. rohita* in different hormonal treatments. Data expressed as mean ± SE (n=8). The bar bearing different superscript differ significantly ($p<0.05$).

success in teleosts mainly through the endocrine regulation of hypothalamo-hypophysial-gonadal axis. Proper understanding and knowledge of these endocrine secretions and mechanism of their regulations are of paramount importance not only because of difficulty in manipulating environmental variables but also for exercising varying degree of control over the different stages of reproduction. This study has attempted to bring all the above into light.

Summary and Conclusion

The steadily growing importance of culture fisheries during the recent years owing to stagnation of marine fish production worldwide has stimulated to improve the techniques necessary for securing the basic requirement, production of young ones (fry and fingerlings) for stocking. Therefore, the artificial propagation technique needs constant refinement for obtaining quality fish seed at the desired times of year by closing the life cycles (Pandey. 2013; Zohar, 2021). Recent advances in fish endocrinology have led to a better understanding of the hormonal factors involved in the control of gamete production, mode of their action and regulation of secretions during different phases of reproductive cycle. Environmental stimuli like photoperiod, temperature and rainfall are perceived by the brain which releases gonadotropin-releasing hormone (GnRH). Though GnRH appeared first in cnidarians (coelenterates), it has also been recorded from molluscs, echinoderms and protochordates. This neuropeptide (10 amino acids) has also been reported from non-hypothalamus tissues where it appears to performs autocrine/paracrine functions.

With evolution of hyothalam-hypophysial-ganad (HPG) axis, GnRH plays pivotal role in neuroendocrine regulation of reproduction in chordates. It binds specifically to receptors in the pituitary gonadotrops and stimulates secretion of gonadotropic hormones (GTh-I, II). The circulating GTh-I functions at the target site in two ways- it induces synthesis and secretion of estradiol-17 β during pre-vitellogenic phase which, in turn, induces vitellogenesis or yolk production during post-vitellogenic phase, GTh-II triggers synthesis and secretion of maturation-inducing hormone, 17α,20β-dihydroxy-progesterone (17,20-P) which is responsible for the final maturation of gametes leading to ovulation and spermiatioin. The recent identification of three GnRH isoforms (GnRH-1, GnRH-2 and GnRH-3), kiss proteins and two kiss genes (kiss-1. kiss-2) and two kiss receptors (GPR-54)-kiss-1r and kiss-2r as well as cytochrome P450 aromatase gene (CYP19) in brain and gonads (ovary and testis) have given better insight into the mechanisms of hormonal interactions in fish reproduction (Zohr *et al*, 2010, Pandey. 2013; Kitahashi *et al*., 2013; Zohar 2021). Further, role of pheromones are also gaining importance during advanced phases of reproduction involving the synchronization of maturity among both the sexes, attraction of prospective mates, triggering spawning behaviour and release of gametes (Pandey, 2012).

ACKNOWLEDGEMENTS

Senior author (BNS) received Senior Resesrch Fellowship (SRF) from ICAR-CIFE (Deemed University), Mumbai. He is grateful to the Director of ICAR-CIFE, Mumbai, ICAR-NBFGR, Lucknow and ICAR-CIFRI. Barracpore for providing the laboratory facilities to carry out this work.

REFERENCES

Acosta, B.D. & Gupta, M.V. (2005). The status of introduced carp species of Asia. In: Carp Genetic Resources for Aquaculture in Asia (eds. Penman, D.J., Gupta, M.V. & Dey, M.M). World Fish Center, Penang, Malaysia, 121-128.

Ali, H.H. (2003). Reproductive Biology and Physiology of *Solea impar* in Abu-Kir Bay, Ph.D. Thesis. Faculty of Science Alexandria University, 327 p.

Al-Absawy, M.A. (2004). Reproductive biology and fine structure for gonads of *Trachinotus ovatus* (Family Carangidae) in the Egyptian Mediterranean Sea Ph.D. Thesis. Faculty of Science: Zagazig University, 423 p.

Alok, D., Hassin, S., Kumar, S.R., Trant, J.M., Yu, K.L. & Zohar, Y. (2000). Characterization of pituitary GnRH-receptor from a perciform fish, *Morone saxatilis*: functional expression in a fish cell line. *Mole. Cell. Endocrinol*., 168: 65-75.

Amano, M. (2010). Reproductive biology of salmoniform and perciform fishes with special reference to gonadotropin-releasing hormone (GnRH). *Aqua BioSci. Monogr*., 3(2): 39-72.

Arukwe, A. & Goksoyr, A. (2003). Egg shell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenetic, population, and evolutionary implications of endocrine disruption. *Comp. Hepatol.,* 2(1): 4.

Assem, S.S. (2004). Histochemical studies, cell type distribution and seasonal variation of gonadotropin cells in pituitary gland of female *Dicentrarchus labrax* in relation to maturation of gonads. *Egypt. J. Agua. Res.,* 30: 374-389.

Ayyappan, S., Moza, U., Gopalakrishnan, A., Meenakumari, B., Jena, J.K. & Pandey, A.K. (2011). Handbook of Fisheries and Aquaculture. Directorate of Knowledge Management in Agriculture (DKMA), Indian Council of Agricultural Research, New Delhi, 1116 p.

Babin, P.J., Cerda, J. & Lubzens, E. (2007). The Fish Oocyte: From Basic Studies to Biotechnological Applications. Springer, Dordrecht, The Netherlands.

Balci, B.A. & Ýkiz, R. (2017). Light and electron microscopic examination of the pituitary gland of common Pandora

(*Pagellus erythrinus L.,* 1758). *Intern. J. Fish. Aqua. Stud.,* 5 (6): 97-102.

Balcý, B., Ýkiz, B.R. & Mutaf, B.F. (2006). Histomorphological comparison of pituitary gland of dusky grouper (*Epinephelus guaza* L., 1758) and blacktip grouper (*Epinephelus alexandrinus V.,* 1828). JFAS., 23: 183-186.

Bancroft, J.D. & Stevens, A. (1977). Theory and Practice of Histochemical Techniques. Churchill Livingston, New York., p. 436-445.

Bandyopadhyay, M.K. & Singh, S.K. (2009). Pheromone- a probable biostimulant for sympathetic spawning of the Indian major carps. In: Recent Advances in Hormonal Physiology of Fish and Shellfish Reproduction (eds. Singh, B.N. & Pandey, A.K.). Narendra Publishing House. Delhi, p. 261-264.

Bano, Z. (2012). Correlative cyclical chances in the hypothalamo-neurohypophysial system and gonads of a Gar fish, *Xenentodon cancila* (Ham.). *Bull. Env. Pharmacol. Life Sci.*, 1(12): 60-68.

Bhattacharyya, M. & Homechaudhuri, S. (2009). Assessment of captive breeding of *Anabas testudineus* with synthetic hormone, ovaprim. *Proc. Zool. Soc.* (Calcutta), 62: 23-27.

Bhattacharyya, S. & Maitra, S.K. (2006). Environmental correlate of the testicular events in a major carp, *Catla catla* in an annual reproductive cycle. *Biol. Rhythm Res.,* 37: 87-110.

Biran, J. & Sivan, B.L. (2018). Endocrine control of reproduction, Fish. In: Encyclopedia of Reproduction., 2nd Edn (Skinner, M.K.). Academic Press, San Diego, p. 362-368.

Bose, S. & Chakrabarti, P. (2014). Distribution of different cells and seasonal variations of gonadotropin cells in the pituitary gland of *Liza parsia* (Hamilton) in relation to maturation of ovary. *J. Zool. Stud*., 1: 33-49.

Bose S. & Chakrabarti, P. (2018). Changes in the hypothalamus in relation to testicular cells during growth, maturation and spawning phases in the brackishwater teleost, *Liza parsia* (Ham.). *Indian J. Fish.,* 65: 40-46.

Cai, J., Zhou, X.,Yan, X., Lucente, D. & Lagana, C. (2019). Top ten species groups in global aquaculture. FAO Fisheries and Aquaculture Department. Rome, p. 11-12.

Chatterjee, N. & Chakrabarti, P. (2014). Distributional pattern of different cells with special emphasis on the seasonal variations of gonadotrophs in the pituitary gland of *Mystus vittatus* (Bloch) in relation to testicular activities. *Intern. J. Fish. Aqua. Stud.,* 2: 79-87.

Chaudhury, H., (1976). The use of hormones in induced spawning of carps. *J. Fish. Res. Bd., Can.,* 33: 940-947.

Chen, C. & Frenald, R.D. (2010). GnRH and GnRH receptors: distribution, function and evolution. *J. Fish Biol*., 73: 1099-1120.

Crossin, G.T., Hinch, S.G., Cooke, S.J., Patterson, D.A., Lotto, A.G., van der Kraak, G., Zohar, Y., Klenke, U. & Farrell, A.P. (2010). Testing the synergistic effects of GnRH and testosterone on the reproductive physiology of pre-adult pink salmon, *Orcorhynchus gorbuscha*. *J. Fish Biol.,* 76: 112-128.

Das, R.C. & Sinha, Y.K.P. (1988). Histomorphology of the neurosecretory regulation of ovarian maturity in the teleost, *Labeo bata* (Hamilton). *Veterin. Arhiv*., 58: 11-21.

Das, R.C. & Singh, B.N. (1990). Histophysiological studies of pituitary-ovarian relationship under the influence of HCG in a carp, *Labeo rohita* (Ham.). *J. Freshwater Biol.,* 2: 107-110.

Das, S.K. (2004). Evaluation of a new spawning agent, Ovopel in induced breeding. *Asian Fish*. *Sci*., 17: 313-322.

David, C. & Rae, S. (2015). Reproductive physiology and behaviour interactions in nonmammalian vertebrates. https://www.researchgate.net/publication/274080565.

Davis, B., Bromage, N. & Swanson, P. (1999). The brain-pituitary-gonadal axis of female rainbow trout, *Oncorhynchus mykiss*: effects of photoperiod manipulation. *Gen. Comp. Endocrinol*., 115: 155-166.

Day, R., Bhattacharya, S. & Maitra, S.K. (2004). Temporal pattern of ovarian activity in a major carp, *Labeo rohita* and its possible environmental correlate in an annual cycle. *Biol. Rhythm Res.*, 35: 329-353.

Donaldson, E.M. & Hunter, G.A. (1983). Induced final maturation, ovulation and spermiation in cultured fishes. In: Fish Physiology. IXB :Reproduction (eds. Hoar, W.S., Randall, D.J. & Donaldson, E.M.). Academic Press, Orlando, Florida, p. 351-403.

Dufour. S., Weltzien, F.A., Sebert, M.E., Le, Belle N, Vidal B, Vernier, P. & Pasqualini, C. (2005). Dopaminergic inhibition of reproduction in teleost fishes: ecophysiological and evolutionary implications. *Ann. N.Y. Acad. Sci.,* 1040: 9-21.

EI-Sakhaawy, M.A., Shamma, M.A., Abd Rabou, M.I., Seba, A.A. & Hussein, S.H. (2011). Seasonal histology and histochemistry of the adenohypophysis of Nile tilapia (*Oreochromis niloticus)*. *J. Vet. Anat*., 4: 39-60.

EI-Sayyad, H., EI-Gamal, A.E.E, Darwih, S.T. & Sheha, M.A. (2019). The correlation between the hypophysial gonadal axis with special reference to the role of fatty acids and isoenzyme during the ovarian maturation in female grass carp, *Ctenopharyngodon idella*. *Egyptian J. Aquat. Biol. Fish*., 24(1): 311-328.

Ekici, A. & Timur, M. (2013). An anatomical and histochemical examination of the pituitary gland of carp (*Cyprinus carpio*). *Turkish J. Vet. Anim. Sci.,* 37: 399-403.

Evans, D.H. (1998). The Physiology of Fishes. 2nd Edn. CRC Press, Boca Raton & New York.

Fahmy, A.F.A. (2006). Reproductive Biology and Induced Spawning of One Species of Family Bagridae. Ph.D Thesis. Faculty of Science. Zagazig University.

Farrell, A.P. (2011). Encyclopedia of Fish Physiology: From Genome to Environment. Academic Press, San Diego.

Gadekarp, G. & Baile, V.V. (2015). Localization of acid phosphatase in the different tissues of developmental stages of indian major carp, *Labeo rohita* (Hamilton). *IOSR-J. Pharm. Biol. Sci.,* 11(6): 5-12.

Goos, H.J.Th., Senthilkumaran, B. & Joy, K.P. (1999). Neuroendocrine integrative mechanisms in the control of

gonadotropin secretion in teleosts. In: Comparative Endocrinology and Reproduction (eds. Joy, K.P., Krishna, A. & Haldar, C.). Narosa Pub. House, New Delhi,113-135.

Gorbman, A. (1965). Vascular relationship between the neurohypophysuis and adenohypophysis of cyclostomes and the problem of evolution of hypothalamic endocrine control. *Arch. Anat. Microscop.. Morrphol. Exp*., 54: 163-194.

Grandi, G., Marchetti, M.G., Lanzoni, M. & Chicca, M. (2014). Immunocytochemical and ultrastructural identification of adenohypophyseal cells in *Ctenopharyngodon idella* (Cypriniformes: Cyprinidae) during gonadal differentiation. *Fish Physiol. Biochem.,* 40: 1115-1139.

Gur, G., Melamed, P., Gissis, A. & Yaron, Z. (2000). Changes along the pituitary-gonadal axis during maturation of the black carp, *Mylopharyngodon piceus*. *J. Exp. Zool*., 28: 13-40.

Gupta, A.K., Kumar, R. & Rai, B.P. (2013). Development of high-nutrient-dense, low-pollution diets and prediction of aquaculture wastes using biological approaches. *Aquacul*., 124: 293-305.

Hamilton, F. (1822). Account of the Fishes Found in the River Ganges and its Branches. Edinburgh, London. 405 p.

Haniffa, M.A. & Sridhar, S. (2002). Induced spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using human chorionic gonadotropin and synthetic hormone (ovaprim). *Vet. Arch.,* 72: 51-56.

Haniffa, M.A., Sethuramalingam, T.A., Dhanaraj, M., Muthuramkrishnan, C., Kumar, Y.A. & Singh, S.V.A. (2007). Breeding and larval rearing of the native Indian catfish, *Heteropneustes fossilis*. 17WOFISH–International, Centre for Aquaculture Research and Extension (CARE), St Xavier's College (Autonomus), Pallayamkottai, Tamil Nadu (India). 6: 16-19.

Haus, E. (2007). Chronobiology in the endocrine system. *Adv. Drug Deliv. Rev.,* 59: 985-1014.

Herrick, C.L. (1891). Studies on the brains of some American freshwater fishes A topography. *Comp. Neurol*., 1: 228-252.

Jacob, P.K. (2005). Studies on Some Aspects of Reproduction in Female *Anabas testudineus* (Bloch). Ph.D. Thesis. Cochin University of Science & Technology, Cochin.

Jena, J.K. (2009). *Labeo rohita* (Hamilton, 1882) (Cyprinidae). In: Cultured Species Fact Sheets (eds. Crespi, V. & New, M.). FAO, Rome. 1-12.

Jose, T.M. & Sathyanesan, A.G. (1980). Pituitary cytology of freshwater teleost, *Anabas testudineus* (Bloch). *Z. mikroscop.- anat. Forsch*., 94: 123-132.

Kah, G., Lethimonier, C., Somoza, G., Guilgur, L.G., Vaillant C. & Lareyre, J.J. (2007). GnRH and GnRH receptors in metazoan: a historical, comparative and evolutionary perspective. *Gen. Comp. Endocrinol*., 153: 146-164.

Kanungo, G., Sarkar, M., Singh, B.N., Das, R.C. & Pandey, A.K. (1999). Advanced maturation of *Heteropneustes fossilis* (Bloch) by oral administration of human chorionic gonadotropin. *J. Adv. Zool*., 20: 1-5.

Khan, R.A. (1972). Studies on the Biology of Some Important Major Carps. M.Sc. Thesis. Department of Zoology, Aligarh Muslim University, Aligarh 185 p.

Khanna, S.S. (2006). An Introduction to Fishes. Silverline Publications, Ghaziabad, 226-229

Khanna, S.S. (2013). An Introduction to Fishes. Silverline Publications, Ghaziabad, 221-232

Khanna, S.S. & Pant, M.C. (1967). Seasonal changes in the ovary of a sisorid catfish, *Glyptosternum pectinopterum*. *Copeia,* 1: 83-88.

Kharat, S.S. & Khillare, Y.K. (2013). Seasonal changes in the cytology of adenohypophysis, testes and ovaries of the hili-stream loach, N*emacheilus moreh.* (Sykes). *J. Bionano Front*., 6(2): 226-231.

Khuroo, Z.B. (2013). Cyclic changes in the hypothalamo neurohypophysial system of *Xenentodon cancila* (Ham.). *Indian J. Pharm. Biol. Res.,* 1(1): 12-19.

Kim, D.K., Cho, E.B., Moon, M.J., Park, S., Hwang, J. I., Do Rego J.L. & Vidal, H. (2012). Molecular co-evolution of neuropeptides gonadotropin-releasing hormone and kissprotein-coupled receptors. *Front. Neurosci.,* 6: 288-298.

Kitahashi, T., Shahjahan, M. & Parhar, I.S. (2013). Hypothalamic regulation of pituitary gonadotropins. In: Sexulal Plasticity and Gametogenesis in Fish (ed. Senthilkumar, B.). Nova Science Publications, New York.

Kobayashi, H., Ishii, S. & Gorbman, A. (1959). The hypothalamic neurosecretory apparatus and the pituitary gland of a teleost *Lepidogobius lepidus*. *Gunma J. Med. Sci*., 8: 3-321.

Kriegsfeld, L.J. & Silver, R. (2006). The regulation of neuroendocrine function: timing is everything. *Horm Behav*., 49: 557-574.

Lal, K.K. (1991). Studies on the Reproductive Physiology of *Lates calcarifer* (Bloch). Ph. D. Thesis. Cochin University of Science & Technology, Cochin.

Lal, K.K. & Pandey, A.K. (1998). Hypothalamo-neurosecretory system of the female seabass, *Lates calcarifer* (Bloch), with special reference to gonadal maturation. *Indian J. Fish*., 45: 51-60.

Lam, T.J. (1982). Applications of endocrinology in fish culture. *Can. J. Fish. Aquat. Sci*., 39: 111-137.

Lone, K.P. & Hussain, A. (2009). Seasonal and age-related variations in the ovaries of *Labeo rohita* (Hamilton, 1822): a detailed gross and histological study of gametogenesis, maturation and fecundity. *Pakistan J. Zool.,* 41: 217-234.

Lone, K.P., Sahar, S. & Fatima, S. (2012). Age-related changes in ovarian gross and Histological characteristics during pubertal development in captive *Catla catla* (Hamilton, 1822) of age 18-29 months. *Pakistan J. Zool.,* 44(1): 159-172.

Lubzens, E., Young, G., Bobe, J. & Cerda, J. (2010). Ooogenesis in teleosts: how fish eggs are formed? *Gen. Comp. Endocrino*l., 165: 367-389.

Mahmud, N., Rahman H.M.H., Mostakim G.M., Khan M.G.Q.,

Shahjahan M., Nahid, S.N.S. & Islam, M.S. (2016). Cyclic variations of gonad development of an air-breathing fish, *Channa striata* in the lentic and lotic environments. *Fish. Aquat.Sci.,* 19: 5-11.

Maksimovich, A.A. (1987). Neurosecrectory hypothalamo-hypophysial system of teleostean fish. *J. Ichthyol*., 27(4): 92-106.

Mani, C.V. & Pandey, A.K. (2006). Occurrence of follicular atresia in ovary of freshwater catfish, *Heteropneustes fossilis* (Bloch). *J. Aqua.*, 14: 63-69.

Mani, C.V. & Pandey, A.K. (2007). Effects of HCG and WOVA-FH administration on ovarian maturation and spawning of *Heteropneustes fossilis* (Bloch). *J. Exp. Zool. India,* 10: 317-319.

Marimuthu, K., Muruganandham, M., Arokiaraj, J. & Haniffa, M.A. (2000). Induced spawning of the Indian catfish, *Heteropneustes fossilis* (Singhi) using a synthetic hormone, ovatide. *Fishing Chimes*, 19(10&11): 105-106.

Marimuthu, K., Haniffa, M.A. & Rahman, M.A. (2009). Spawning performance of native threatened spotted snakehead fish, *Channa punctatus* (Actinopterigii: Channidae: Perciformes) induced with ovatide. *Acta Ichthyol. Piscat*., 19: 1-5.

Melamed, P. & Sherwood, N. (2005). Hormones and Their Receptors in Fish Reproduction. World Scientific, New Jersey & London.

Melamed, P. & Zohar, Y. (2009). Controlling fish reproduction in aquaculture. In: New Technologies in Aquaculture. Improving Production Efficiency, Quality and Environmental Management. Woodhead Pub. Series, Sawston, Cambridge, p. 109-142.

Menon, K.B. (1982). Inland Fisheries in Australia. *Fishing Chimes,* 2(1): 60-65.

Mikolajczyk, T., Chyb, J., Szczerbik, P., Sokolowska-Mikolajczyk, M., Epler, P., Enright, W.J., Filipiak, M. & Breton, B. (2004). Evaluation of the potency of azaglynafarelin (GnRH analogue), administered in combination with different formulations of pimozide, on LH secretion, ovulation and egg quality in common carp (*Cyprinus carpio* L.) under laboratory, commercial and natural conditions. *Aquaculture,* 234: 447-460.

Mishra, R.K., Yadav, A.K., Varshney, P.K., Pandey, A.K. & Lakra, W.S. (2011). Comparative effects of different hormonal preparations in induced spawning of the freshwater catfish, *Heteropneustes fossilis*. *J. Appl. Biosci.,* 37(1): 27-30.

Mohan, R.M. (2014). Sea Water Fish, *Glossogobius giuris* at Last Lives in Fresh Water Now. *J. Fish. Livest. Prod.,* 2: 123.

Moitra, S.K. & Medya, B.C. (1980). Histomorphology of the hypothalamo-neurohypophysial system in relation to gonadal maturation in *Cirrhinus mrigala*, a freshwater Indian carp. *Anat Anz*., 148: 409-421.

Mylonas, C.C., Kyriakou, Y., Sigelaki, I., Georgiou, G., Stephanou, D. & Divanach, P. (2004). Reproductive biology of the shidrum (*Umbrina cirrosa*) in captivity and induction of spawning using GnRHa. *Israeli J. Aquacult. Bamidgeh.,* 56: 77-94.

Nagabhushanam, R., Sarojini, R. & Mirajkar, M.S. (1983). Manual of Research Methods for Marine Invertebrate Reproduction. Special Publication No. 14. CMFRI, Cochin.

Nagahama, Y. & Yamashita, M. (2008). Regulation of oocyte maturation in fish. *Curr. Topic Dev. Biol*., 30: 103-45.

Okubo, K., Mitani, H., Naruse, K., Kondo, M., Shima, A., Tanaka, M., Asakawa, S, Shimizu, N., Yashiura, Y. & Aida, K. (2002). Structural characterization of GnRH Loci in the medaka gene. *Gene.*, 293:181-189.

Pandey, A.K. (1993). Hypothalmo-neurosecretory system of the Indian mackerel, *Rastrelliger kanagurta* Cuvier. *Nat. Acad. Sci. Letters.*, 16: 265-268.

Pandey, A.K. (1997). Studies of the hypothalamo-neurosecretory system of *Megalospis cordyla* Linnaeus. *J. Mar. Biol. Assoc. India,* 39: 132-135.

Pandey, A.K. (2008). Hypothalamo-neurosecretory system of the marine teleost, *Decapterus russelli* with particular reference to gonadal maturation. In: Perspectives in Animal Ecology and Reproduction. Vol. 5. (eds. Gupta, V.K. & Verma, A.K.). Daya Pub. House, New Delhi, p. 369-381.

Pandey, A.K**.** (2012). Recent advances in fish pheromone research with emphasis on their potential applications in aquaculture and fishery management. *J. Exp. Zool. India*, 15: 1-22.

Pandey, A.K. (2013). Dietary and hormonal manipulations for gonadal maturation and seed production of Indian major carps and catfishes. *J. Exp. Zool., India*, 16: 19-37.

Pandey, A.K. & Mohamed, M.P. (1993). Histomorphology of the hypothalamo-neurosecretory system of the Indian scad, Decapterus tabl (Berry, 1968). In: Proceedings of the Third Indian Fisheries Forum (eds. Joseph, M.M. & Mohan, C.V.). College of Fisheries, Mangalore, 131-134.

Pandey, A.K. & Mohamed, M.P. (1997). Hypothalmo-neurosecretory system of the marine teleost, *Sphyraena obtusata* Cuvier. *Indian J. Fish*., 44: 191-200.

Pandey, A.K. & Mohamed, M.P. (1999). Hypothalmo-neurosecretory system of the marine teleost, *Ariomma indica* (Day, 1870). In: Proceedings of the Fourth Indian Fisheries Forum (eds. Joseph, M.M. & Mohan, C.V.). College of Fisheries, Mangalore, p. 27-29.

Pandey, A.K. & Singh, B.N. (2003). Recent advances in brood stock management, induced breeding and larval rearing of the Indian catfish, *Heteropneustes fossilis* (Bloch). *J. Exp. Zool. India.*, 6: 163-168.

Pandey, A.K. & Koteeswaran, R. (2004). Ovatide induced breeding of the Indian catfish, *Heteropneustes fossilis* (Bloch). *Proc. Zool. Soc.* (*Calcutta*)., 57: 35-38.

Pandey, A.K. & Mani, C.V. (2009). Histo-morphological changes in the hypothalamo-neurosecretory cells and gonadotrophs of *Heteropneustes fossilis* (Bloch) in relation to ovarian maturation. *J. Appl. Biosci.,* 35(1): 43-50.

Pandey, A.K., Lal, K.K. & Mahanta, P.C. (2000). Histomorphology of the hypothalmo-neurosecretory system of the endangered golden mahseer, *Tor putitora*

(Hamilton-Buchanan). *Indian J. Fish*., 47: 65-69.

Pandey, A.K., Mahapatra, C.T., Sarkar, M., Kanungo, G., Sahoo, G.C. & Singh, B.N. (2001). Ovatide induced spawning in Indian major carp, *Catla catla* (Hamilton-Buchanan), for mass scale seed production. *J. Adv. Zool.,* 22: 70-73.

Pandey, A.K., Mahapatra, C.T., Sarkar, M., Kanungo, G. & Singh, B.N. (2002a). Ovatide induced spawning in Indian major carp, *Cirrhinus mrigala*, for mass scale seed production. *J. Exp. Zool. India.,* 5: 81-85.

Pandey, A.K., Mahapatra, C.T., Sarkar, M., Kanungo, G. & B.N. (2002b). Ovatide induced spawning in Indian major carp, *Labeo rohita* (Hamilton-Buchanan). *Aquacul.,* 3: 1-5.

Pandey, A.K., Koteeswaran, R. & Singh, B.N. (2002c). Breeding of fishes with synthetic hormone drug ovatide for mass scale seed production. *Aquacult.,* 3: 137-142.

Pandey, A.K., Singh, B.N., Sarkar, M., Mahapatra, C.T., Kanungo, G. & Arvindakshan, P.K. (2007). Advanced maturation in the broodstock of Indian major carp, *Labeo rohita* (Hamilton-Buchanan), maintained on lysine and methionine supplemented diet. *J. Appl. Biosci.,*33: 150-152.

Pandey, A.K., Rani, M., Mahapatra, CT., Kumari, A. & Shrivastava, P. (2011). Hypothalamo-neurosecretory system of five spot herring, Hilsa kelee Cuvier. *J. Exp. Zool. India.,*14: 583-586.

Pandey, K. & Shukla, J.P. (2007). Fish & Fisheries (2nd ed.). Rastogi Publications, Meerut, 208-221.

Pasha, R.H., Anjum, M.Z., Ullah, I., Khan, M.A., Ali, A., Rehman, S.U., Batool, S. & Emmanuel, A. (2019). Seasonal variation in the microscopic anatomy of gonads and gonadosomatic index of *Clupisoma garua*. *Pak. J. Agric. Res.,* 32: 670-674.

Pearse, A.G.E. (1968). Histochemistry: Theoretical and Applied. Vol. 1. Churchill Livingston, Edinburgh.

Phelps, R.P. (2010). Recent advances in fish hatchery management. *Rev. Bras. Zootec*., 39: 95-101.

Pickford, G.E. & Atz, J.W. (1957). The Physiology of the Pituitary Gland of Fishes. New York Zoological Society, New York.

Piska, R.S. & Naik, J.K. (2007). Freshwater Aquaculture Fisheries. II Year Paper I (ed. Piska, R.S.) Intermediate Vocational Course, State Institute of Vocational Education and Board of Intermediate Education, Hyderabad.

Pogoda, H.M. & Hammerschmidt, M. (2007). Molecular genetics of pituitary development in Zebra fish. *Cell Dev. Biol.,* 18(4): 543-558.

Prakash, M.M., Shrivastava, S.S. & Belsare, D.K. (1984a). Preoptico-hypophysial neurosecretory system in tropical air-breathing fish, *Notopterus chitala* (Ham.). *Z. mikrosk.-anat. Forsch*., 98: 205-212.

Prakash, M.M., Shrivastava, S.S. & Belsare, D.K. (1984b). Correlative cyclical changes in the hypothalamus-hypophysial-gonad system in *Notopterus chitala* (Ham.). *Z. mikrosk.-anat. Forsch*., 98: 225-240.

Prakash, R. & Paliwal, J.C. (1974). Histo-morphological studies on the preoptico-hypophysial system in a teleost, *Eutropiichthys vacha* (Bleeker). *Proc. Nat. Acad. Sci. India*, 44B: 209-214.

Rsai, B.P. (1973). On the neurosecretory centres in the hypothalamus and their relationship in reproduction in *Tor* (*Barbus*) *tor*. *Acta Anat*., 85: 429-433.

Rai, S.C. & Pandey, K. (1986). Correlative seasonal changes in the hypothalamic nuclei, adenohypophysial cells and gonads of the tropical perch, *Colisa fasciata* (Bl. & Sch.). *Bull. Inst. Zool., Acad Sinica*, 25: 57-66.

Rath, R.K. (2000). Freshwater Aquaculture. 2nd Edn. Scientific Publishers (India), Jodhpur, 103-172.

Rath, R.K. (2011). Freshwater Aquaculture. 3nd Edn. Scientific Publishers (India), Jodhpur, 120-159.

Ray, S. (2019). Characterization of hypothalamic nuclei in indian freshwater spiny eel, *Mastacembelus armatus* (Lacepede*). Biol.Biotech. Res. Asia*., 17(4): 685-689.

Reddy, A.K. & Thakur, N.K. (1998). Ovatide- a new hormonal preparation for induced fish breeding. *Fishing Chimes,* 18(4): 28-30.

Rincón, L., Obando-Bulla, M.J., Tovar-Bohorquez, M.O., Pandolfi, M., Hurtado, H., Rincón, L. & Hurtado, H. (2017). Topological and histological description of preoptic area and hypothalamus in cardinal tetra, *Paracheirodon axelrodi* (Characiformes: Characidae). *Neotrop. Ichthyol*., 15(1): e160145.

Ross, M.H. & Pawlina, W. (2003). Histology: A Text and Atlas. 5th edn., Lippincott Williams & Wilkins, Philadelphia., 716-719.

Roy, K. & Mandal, D.K. (2015). Maturity stages of ovary of a minor carp, *Labeo bata* (Hamilton-Buchanan, 1822). *Intern. J. Fish. Aquat. Stud.,* 2(6): 19-24.

Ryu, Y.W., Hur, S.P., Lee, C.H., Lim, B.S. & Lee, Y.D. (2013). Characterization of pubertal development phases in femaile longtooth grouper, *Epinephelus bruneusvia* classification of body weight. *Dev. Reprod*., 17: 55-62.

Saksena, D.N. (1979). The hypothalamo-neurohypophysial system in Indian freshwater goby, *Glossogobius giuris*. *Z. mikrosk.-anat. Forsch*., 93: 1137-1158.

Sathyanesan, A.G. (1973). Hypothalamo-hypophysial neurosecretory system of the freshwater teleost, *Catla catla* (Ham.). *Zool. Beitr*., 19: 163-178.

Schreibman, M.P. (1986). Pituitary gland. In: Vertebrate Endocrinology: Fundamentals and Biomedical Implications. Vol. 1. Morphological Considerations (eds. Pang, P.K.T. & Schreibman, M.P.). Academic Press, San Diego, p. 11-56.

Selvaraj, S. & Francis, T. (2007). Influence of human chorionic gonadotropin on maturation in striped murrel, *Channa striatus. Asian Fish. Sci.,* 20: 23-39.

Sen, U., Mukherjee, D., Bhattacharyya, S.P. & Mukrerjee, D. (2002). Seasonal changes in plasma steroid levels in Indian major carp, *Labeo rohita*: Influence of homologous pituitary extract and steroid production and development of oocyte maturational competence. *Gen. Comp. Endocrinol*., 128: 123-134.

Shrestha, T.K. & Khanna, S.S. (1978). Seasonal changes in the

testes of hillstream teleost, *Garra gotyla. Acta. Anat.,*100: 210-220.

Shirali, S., Majd, E., Mesbah, M. & Shapoori, M.R. (2011). Histological and histometrical study of common carp ovarian development during breeding season in Khouzestan Province in Iran. *Intern. Jour. Vet. Res.,* 5(4): 260-268.

Singh, B.N. & Pandey, A.K. (2009). Recent Advances in Hormonal Physiology of Fish and Shellfish Reproduction. Narendra Pub. House, New Delhi, 415 p.

Singh, B.N., Das, R.C., Sahu, A.K., Kanungo, G., Sarkar, M., Sahoo, Nayak, P.K. & Pandey, A.K. (2000). Balanced diet for broodstocks of *Catla catla* and *Labeo rohita* and induced breeding using ovaprim. *J. Adv. Zool*., 21: 92-97.

Singh, B.N., Das, R.C., Sarkar, M., Kanungo, G., Sahoo, G.C. & Pandey, A.K. (2002). Balanced diet for broodstock of Indian catfish, *Heteropneustes fossilis* (Bloch) and induced breeding using ovaprim. *J. Ecophysiol. Occup. Hlth*., 2: 57-64.

Singh, T.P. & Lal, B. (2009). Current status of gonadotropin and growth hormone in control of reproduction in teleosts. In: Recent Advances in Hormonal Physiology of Fish and Shellfish Reproduction (eds. Singh, B.N. & Pandey, A.K.). Narendra Pub. House, New Delhi. p. 1-19.

Sivakumaran, K.P., Brown, P., Stoessel, D. & Giles, A. (2003). Maturation and reproductive biology of female wild carp, *Cyprinus carpio*, in Victoria, Australia. *Environ. Biol. Fish.,* 68: 321-332.

Smith, B.B. & Walker, K.F. (2004). Spawning dynamics of common carp in the river Murray, South Australia, shown by macroscopic and histological staging of gonads. *J. Fish Biol.,* 64: 336-354.

Srivastava, S.J. & Saxena, A.K. (1996). Morphological changes in hepatocytes in relation to reproductive cycle of a freshwater female catfish, *Heteropneustes fossilis* (Bloch). *J. Adv. Zool*., 17: 98-104.

Subhedar, N., Khan, F.A., Saha, S.G., Burade, V.S. & Sarkar, S. (1999). The hypothalamus of teleosts. In: Comparative Endocrinology and Reproduction (eds. Joy, K.P., Krishnan, A. & Halder, C.). Narosa Publishing House, New Delhi, p. 54-68.

Subramoniam, T. (1982). Manual of Research Methods for Marine Invertebrate Reproduction. Special Publication No. 9. CMFRI, Cochin.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Welzien, F.A., Dufour, S., Karlsen, O., Norberg, B., Andersson, E. & Hansen, T. (2010). Control of puberty in farmed fish. *Gen. Comp. Endocrinol.,* 165: 483-515.

Thomas, P.C., Rath, S.C. & Mohapatra, K.D. (2003). Breeding and Seed Production of Finfish and Shellfish. Daya Publishing House, New Delhi, 402 p.

Timur, M. & Ekici, A. (2011). Microscopic examination of adenohypophysis of common carp (*Cyprinus carpio*). *Istanbul Univ. J. Fish. Aquat. Sci*., 26: 1-12.

Tonsfeldt, K.J. & Chappell, P.E. (2012). Clocks on top: The role of the circadian clock in the hypothalamic and pituitary regulation of endocrine physiology. *Mol. Cell Endocrinol.*, 349: 3-12.

Ursani, T.J., Shaikh, S.A., Soomro, S.M. & Malik, S. (2012). Histological changes in pituitary gonadotrophic hormone cells in relation to seasonal changes in ovaries of the fish, *Orieeochromis mossambicus. Sindh Univ. Res. J.(Sci. Ser.).,* 44: 107-14.

Verma, R. (2013). Seasonal changes in the histological profile of the testicular and ovarian cycle of *Labeo dyocheilus*. *Intern. J. Fish. Aquacult. Sci*..3: 143-149.

Viswanathan, N. & Sundararaj, B.I. (1974). Seasonal changes in the hypothalamo-hypophysial-ovarian system in the catfish, *Heteropneustes fossilis* (Bloch). *J. Fish Biol.,* 6: 331-340.

Vongvatcharanon, U., Kirirat, P., Suwanjarat, J. & Boonyoung, P. (2005). Alteration of gonadotrophs in the pituitary gland during the annual reproductive cycle of the adult female sand goby, *Oxyeleotris marmoratus*. *J Sci Technol*., 27: 437-445.

Williams, R.H. (1995). Textbook of Endocrinology. W.B. Saunders, Philadelphia & London.

Wood, H. (1930). Scotish herring shoals: pre-spawning and spawning movements. *Scotland Fish. Bd. Sci. Invest*., 1: 1-71.

Yadav, A.K., Mishra, R.K., Singh, S.K., Varshney, P.K., Pandey, A.K. & Lakra, W.S. (2011). Induced spawning of Asian catfish, *Clarias batrachus*, with different doses of GnRH-based drugs. *J. Exp. Zool. India.,* 14: 199-202.

Yaron, Z., Gur, G., Melamed, P., Rosenfeld, H., Elizur, A. & Levavi-Sivan, B. (2003). Regulation of fish gonadotropins. *Int. Rev. Cytol*., 225: 1310-185.

Yaron, Z. & Sivan, B. (2006). Fish reproduction. In: Physiology of Fishes. 3rd Edn (eds. Evans, D.H. & Claiborne, J.B. Taylor and Francis, Boca Raton, 345-388.

Yashuvi, Y., Klenke, U., Palevitch, O., Abraham, E., Zohar, Y. & Gothilf, Y. (2006). Ontogeny of gonadotropin-releasing hormone (GnRH) neurons in hybrid striped bass, *Morone* spp. whole-mount *in situ* hybridization analysis. *J. Fish Biol.,* 69(Suppl. A): 20-30.

Zhang, Z., Zhu, B. & Ge, W. (2014). Genetic analysis of zebra fish gonadotropin (FSH and LH) functions by TALEN-mediated gene disruption. *Mol. Endocrinol.,* 29(1): 76-98.

Zohar, Y. (2021). Fish reproductive biology: reflecting on five decades of fundamental and transalational research. *Gen Comp Endocrinol.* 2021 Jan 1;300:113544. doi: 10.1016/j.ygcen.2020.113544.

Zohar, Y. & Mylonas, C.C. (2001). Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture.,*197: 99-136.

Zohar, Y., Munoz-Cueto, J.A., Elzur, A. & Kah, O. (2010). Neuroendocrinology of reproduction in teleost fish. *Gen. Comp. Endocrinol*., 165: 438-455.

Zolotnitskiy, A.P. (1980). The morphofunctional characteristics of the hypothalmo-hypophysial neurosecrectory system of the Black sea turbot, *Scophthalamus maeoticus*, in connection with reproductive cycle. *J. Ichthyol.*, 20: 104-111.