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DIETARY AND HORMONAL MANIPULATIONS FOR GONADAL MATURATION AND SEED PRODUCTION OF INDIAN MAJOR CARPS AND CATFISHES

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ABSTRACT - Recent advances in fish endocrinology have led to a better understanding of the hormones involved in control of gamete production, mode of action and regulation of their secretion during different stages of reproductive cycle. Environmental stimuli like photoperiod and temperature are perceived by the brain which releases gonadotropin releasing hormone (GnRH) that binds specifically to receptors in the pituitary gonadotrops and stimulates secretion of gonadotropic hormones (GtH - I & II). The circulating gonadotropins stimulate gonadal (ovarian and testicular) development and final maturation. GtH-I induces synthesis and secretion of estradiol-17β in previtellogenic phase of ovarian growth leading to vitellogenesis or yolk production while during post-vitellogenic phase, GtH-II triggers synthesis of 17α,20β-dihydroxy-progesterone (17,20-P) which is responsible for the final maturation leading to ovulation and spermiation. Role of nutrition in broostock management for quality seed production in fishes has been appreciated during the recent years. The artificial propagation technique being employed presently needs constant refinement for obtaining quality fish seed at the desired times of the year. Altering sexual cycles, induction of advanced, delayed maturation and multiple breeding, ovulation, spermiation and artificial fertilization of the commercially important species need to be refined where nutritive and reproductive physiology might help for faster progress in aquaculture. Role of reproductive pheromones in gonadal maturation, synchronization of reproductive processes and spawning as well as reproductive containment of invasive species may not be overlooked. In this communication, the importance of nutrition in broodstock management for better quality gamete output and recent advances in hormonal biotechnology in aquaculture with particular reference to the cultured Indian major carps and catfishes have been discussed.

Key words: Dietary, hormonal manipulation, gonadal maturation, seed production, Indian fishes.

INTRODUCTION

The steadily growing importance of culture fisheries has made it imperative that the fish culturists should improve the technique necessary for securing the basic requirement of fish culture, namely the production of young ones (fry and fingerlings) for stocking (Yaron, 1995; Zohar and Mylonas, 2001; Jakobsen *et al*, 2009; Taranger *et al*, 2010; Zohar *et al*, 2010; Amano, 2010; Lubzens *et al*, 2010; Phelps, 2010; Ayyappan *et al*, 2011; Kim *et al*, 2012). The artificial propagation technique, presently used, needs constant refinement for obtaining quality fish seed at the desired times of the year (Lin and Peter, 1996; Zohar and Mylonas, 2001; Taranger *et al*, 2010; Amano, 2010). Modern fish industry is highly specialized exploring more and more possibilities to manipulate reproduction (Patino, 1997; Zohar and Mylonas, 2001). Altering sexual cycles, induction of advanced, delayed maturation and multiple breeding, ovulation and spermiation and artificial fertilization are to be practiced where nutritive and reproductive physiology might help for faster progress in aquaculture (Amano, 2010; Lubzens *et al*, 2010; Phelps, 2010). In this communication, importance of nutrition in broodstock management for better quality gamete output and recent advances in hormonal biotechnology in aquaculture with particular reference to the Indian major carps and catfishes have been discussed.

Dietary Manipulations for Advancing Gonadal Maturation

Success of induced breeding depends on proper gonadal maturation of the broodstocks because fishes reared without adequate food supply do not show full maturity (Matty, 1985; Watanabe, 1985; Luquet and Watanabe, 1986; Bromage and Roberts, 1995; Izquierdo *et al*, 2001; Watanabe and Vassallo-Agius, 2003). Also, the breeding of females and males do not synchronize under improper rearing conditions (Singh *et al*, 2000a, 2002). Though the use of fish meal as animal protein source has been the natural choice of the feed manufacturers but its acceptance is severely limited due to high cost involved and dubious role in contributing to nitrogen load in the pond ecosystem (Viola and Lahav, 1991; Chakraborty and Chakraborty, 1998; Stibranyiova and Paraova, 2000). This compels the feed industry to resort to plant ingredients as a protein source (Webester et al, 1992; Gatlin, 2003; Forster, 2003; Pandey and Singh, 2003). The importance of broodstock nutrition in aquaculture has been realized during the recent years (Matty, 1985; Watanabe, 1985; Cumaranatunga et al, 1991; Bromage and Roberts, 1995; Singh et al, 2000a, 2002, 2009; Nandi et al, 2001; Pandey et al, 2003; Memis and Gun, 2004). Micronutrients such as polyunsaturated fatty acids (PUFA), vitamins C and E, carotenoids and various trace elements have been implicated in gonadal maturity and egg quality of fish. Since proteins and lipids are the major components of egg yolk, their role in reproduction is expected (Matty, 1985; Watanabe, 1985; Bromage and Roberts, 1995; Singh et al, 2000a, 2002, 2009; Nandi et al, 2001; Pandey et al, 2003). Unfortunately, very few studies have been devoted to understand the role of dietary protein on gonadal maturation and egg quality of fish (Matty, 1985; Watanabe, 1985; Shim et al, 1989; DeSilva and Radampola, 1991; Bromage and Roberts, 1995; Gunasekara et al, 1995; Degani and Yehuda, 1996; Singh et al, 2000a, 2002). The quality of protein refers to its amino acids contents with particular reference to essential amino acids (EAAs) (Wilson, 1986; Ravi and Devaraj, 1990; Lall, 1990; NRC, 1993). It has been demonstrated that deficiency of any of the ten essential amino acids result in reduced growth rate, poor feed conversion and in some cases poor appetite (Cowey and Sargent, 1979; Wilson and Robinson, 1982; Ketola, 1983; Halver, 1989; Lovell, 1989; Cowey et al, 1992; Li and Robinson, 1998). Methionine deficiency has been shown to cause bilateral cataracts in lake- and rainbow trouts (Poston et al, 1977), lysine deficiency results in reduced weight gain, fin erosion and mortality. Since oil-cakes and rice bran are poor in lysine and methionine contents and also in certain other essential amino acids (Robinson et al, 1980; Fauconneau, 1988; Lim and Dominy, 1989; Robinson, 1991; Webester et al, 1992; Bai and Gatlin, 1994), we tried to balance the diet rich in plant ingredients with lysine and methionine through commercially available lysomix and methiomix. Recently, we achieve success in advancing gonadal maturity among the Indian major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) and the freshwater catfish, *Heteropneustes fossilis,* maintained on the semi-balanced diet supplemented with lysine and methionine under field conditions (Pandey et al, 2003, 2004a, 2006a, b, 2007a).

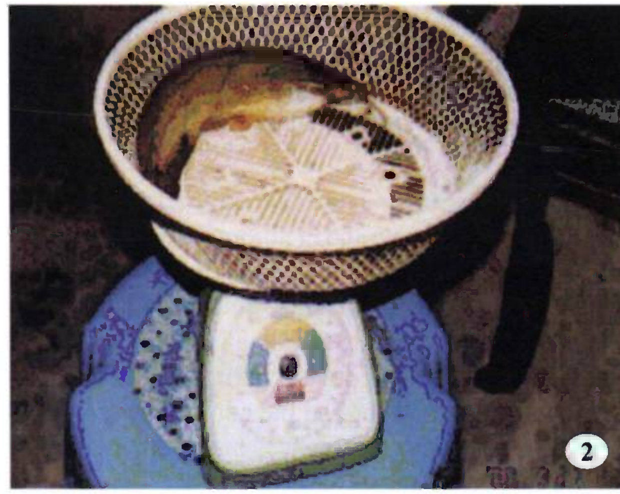
Dietary manipulation in Indian major carps

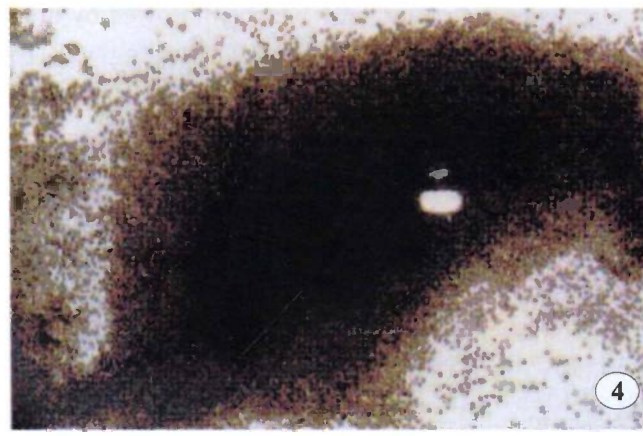
Broodstocks (2+years) of the Indian major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) were reared (from January onwards) on 5 mm pelleted semi-balanced diet composed of fish meal, groundnut oil-cake (GOC), soyabean oil-cake (SOC), rice bran, wheat flour, trace mineral mix, vitamin mix (crude protein content 30.65%; crude lipid 12.7%; gross energy 3,800 kcal/kg) in 0.04 ha ponds with stocking density @ 1,500 kg/ha (control group) whereas the experimental group broodstocks were maintained on the same diet lacking fish meal (as source of animal protein) but supplemented with lysine (0.5%) and methionine (0.5%) (Table 1). Protein requirement of the major carps was kept at the optimal level (Renukaradhya and Verghese, 1986; Ravi and Devraj, 1990; Singh, 1991). Fishes from both the groups were fed @ 2–3 % of the body weight daily. The carps maintained on the control diet matured during the late May/early June but with the dietary supplementation of lysine and methionine, gonadal maturity (both the sexes) was observed during the month of April. They were bred successfully with ovaprim @ 0.5 ml/kg body weight for females and 0.2 ml/kg for males (single injection). Natural spawning took place in the cemented tank after 6-8 hours of the drug administration. About 78-90% fertilization and 74-82% hatching success were recorded in these carps (Table 2).

The fertilization rate of the eggs of the Indian major carps kept on experimental diet ranged from 50-65%, 55-70%, 78-90% during April, May and June and hatching success was 18-32%, 30-52%, 74-82%, respectively during the corresponding months. Interestingly, eggs of the carps maintained on the control diet, the fertilization rate was 10-30%, 35-50%, 50-75 during April, May, June and hatching success was 0%, 20-31% and 35-46% during the corresponding months, respectively.

Dietary manipulation in *Heteropneustes fossilis*

Heteropneustes fossilis is a freshwater air-breathing fish distributed throughout India, Pakistan, Sri Lanka, Myanmar, Thailand and China (Tripathi, 1990). It is omnivorous in habit and can withstand hardy conditions of culture (Thakur, 1991). *H. fossilis* commands good consumer preference because it contains high amount of protein, iron and low fat and is recommended in convalescence. The catfish possesses accessory respiratory organs and reaches market in live condition fetching good price as compared to carps. The catfish can survive even in swampy and derelict water bodies with low oxygen content and can be cultured in high stocking density, hence, recommended for paddy-cum-fish as well as cage culture (Dehadrai et al, 1985; Thakur and Das, 1988; Dehadrai and Kamal, 1993). An attempt was made to record the dietary manipulation through supplementation of lysine and methionine on advancement of maturity and breeding responses of the catfish.





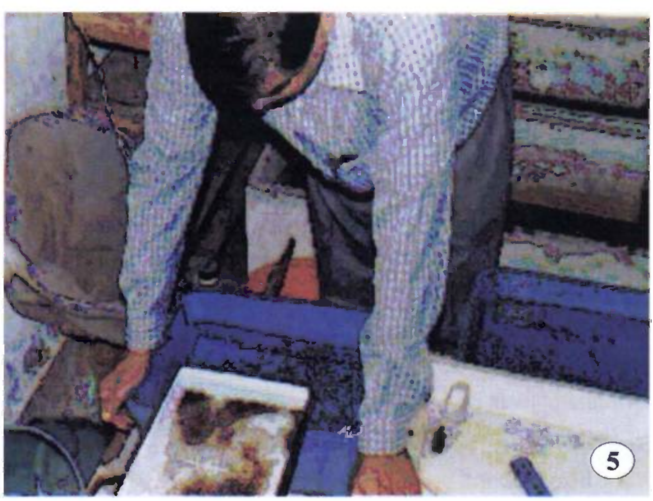


Fig. 1: Broodstock of *H. fossilis*. **Fig. 2:** Broodstock of female *H. fossilis*. **Fig. 3:** Eggs released from *Heteropneustes fossilis* after striping. **Fig. 4:** Fertilized eggs of *H. fossilis*. **Fig. 5:** Fertilized eggs of *H. fossilis*. **Fig. 6:** Juveniles of *H. fossilis* after 6 months of rearing under hatchery conditions.

Two 0.02 ha ponds were selected and prepared as described for the Indian major carps. *H. fossilis* (weight range 52-55 gm) were stocked during January at the density of 30,000/ha. Since the protein requirement of catfishes are slightly higher than carps (Singh, 1990, 1991; Pandey and Singh, 2003), the crude protein content of both the groups was kept at uniform level of about 35.28%. The basic ingredients of the control and experimental diets remained the same but feed (pelleted; size 2 mm) of the experimental group was supplemented with lysine and methionine @ 0.5% each (Table 3). Feeding was done twice daily @ 4% of biomass. Water quality parameters ranged as temperature 25-30°C, pH 7.2-8.1, dissolved oxygen 5.5-6.0 mg/l, total ammonia 0.12-0.60 mg/l and total alkalinity 45-70 mg/l during the experimental period of 150 days. Sampling was carried out at every 15 days interval and the feed was adjusted accordingly.

Catfish maintained on the experimental diet supplemented with lysine and methionine recorded better growth (increase in weight and weight gain %) and feed utilization as compared to those fed on control diet. Advancement in the maturity was also observed in *H. fossilis* (both sexes) kept on the experimental diet by end of April whereas those kept on the control diet matured by end of May. Breeding was carried out by intramuscular administration of ovaprim (0.6 ml/kg body weight of female; males 0.4 ml/kg body weight) followed by the stripping after 10-12 hours of the drug administration (van der Waal, 1985) (Fig. 1-6). The details of breeding responses of the catfish maintained on both the diets have been summarized in Table 4. The fertilization rate of the catfish kept on experimental diet ranged from 80-85% and 90-96% during May and June and hatching success was 70-72% and 80-85%, respectively during the corresponding months. Interestingly, fertilization rate of the eggs of the catfish maintained on the control diet was 65-68, 70-75% during May, June while the hatching success was 50-52% and 55-60% during the corresponding months, respectively.

Dietary protein supplementation has been reported to advance maturity and improve gametes quality in teleosts (Watanabe, 1985; Matty, 1985; Gupta *et al*, 1990; Somshekarappa *et al*, 1990; Bromage and Roberts, 1995; Singh *et al*, 2000a, 2002). We observed advancement in maturity (both sexes) of the Indian major carps, *Catla catla, Labeo rohita* and *Cirrhinus mrigala* as well as in the freshwater catfish, *H. fossilis,* with the dietary supplementation of lysine and methionine (Pandey *et al*, 2003, 2004a, 2006a, b, 2007a), most probably by improving feed utilization of the cultivable species under field condition (Singh *et al*, 2000b; Pandey *et al*, 2000a, 2001a, 2012; Muruganandam *et al*, 2001; El-Dahhar and El-Schazly, 2008). This is the first report demonstrating the role of two essential amino acids in advancing gonadal maturation of fish.

Hormonal Control of Reproduction

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 (Yaron, 1995; Zohar and Mylonas, 2001; Dufour *et al*, 2005; Singh and 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 and temperature are perceived by the brain which releases a decapeptide hormone, gonadotropin-releasing hormone (GnRH) that binds specifically to receptors in the pituitary gonadotrophs and stimulates secretion of gonadotropic hormones (GtH-I & II) (Amano *et al*, 1997; Alok *et al*, 2000; Okubo *et al*, 2002; Yashuvi *et al*, 2006; Kah *et al*, 2007; Crossin *et al*, 2010; Kim *et al*, 2012). The circulating GtHs (GtH-I & II) enhance gonadal development and final maturation (Yaron, 1995; Patino, 1997; Zohar and Mylonas, 2001; Amano, 2010; Lubzens *et al*, 2010; Taranger *et al*, 2010; Zohar *et al*, 2010). GtH-II regulates final maturation of the gametes by producing maturation-inducing steroids, 17α,20β-dihydroxyprogesterone (17,20-P) and 17α,20β,21-trihydroxy-4-pregnen-3-one (Nagahama, 1997; Delvin and Nagahama, 2002; Podhorec and Kouril, 2009). The GtH-I functions at the target site by stimulating synthesis and secretion of estradiol-17β during previtellogenic phase which, in turn, induces vitellogenesis or yolk production. During post-vitellogenic phase, GtH-II triggers the synthesis of 17α,20β-dihydroxyprogesterone (17,20-P) which is responsible for the final maturation leading to ovulation and spermiation (Nagahama, 1997; Patino, 1997). The hormonal cascade of events is perfectly coordinated with seasonal reproductive cycle of the fish to ensure spawning at specific time of the year (Singh and Lal, 2009; Zohar *et al*, 2010).

Brain peptides

Gonadotropin-releasing hormone (GnRH), is a prime mediator for neural control of reproduction in fish (Zohar *et al*, 2010; Crossin *et al*, 2010; Kim *et al*, 2012). The structure and function of GnRH are more or less conserved during the evolution (Amano *et al*, 1997; Kah *et al*, 2007). Till today, primary structures of twenty four species of variant GnRH have been determined. The chemical structures of the GnRH of a few chordates have been summarized in Table 5.

As in chicken, both in salmon and lamprey, two to three forms of GnRH molecule are detected. Soon after GnRH identified in different fishes, synthetic molecules were prepared and tested. Some GnRH analogues were found to be super-active in fish compared to the native GnRH. Attempts have also 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 lateralis tuberis (NLT) with the egg maturation in

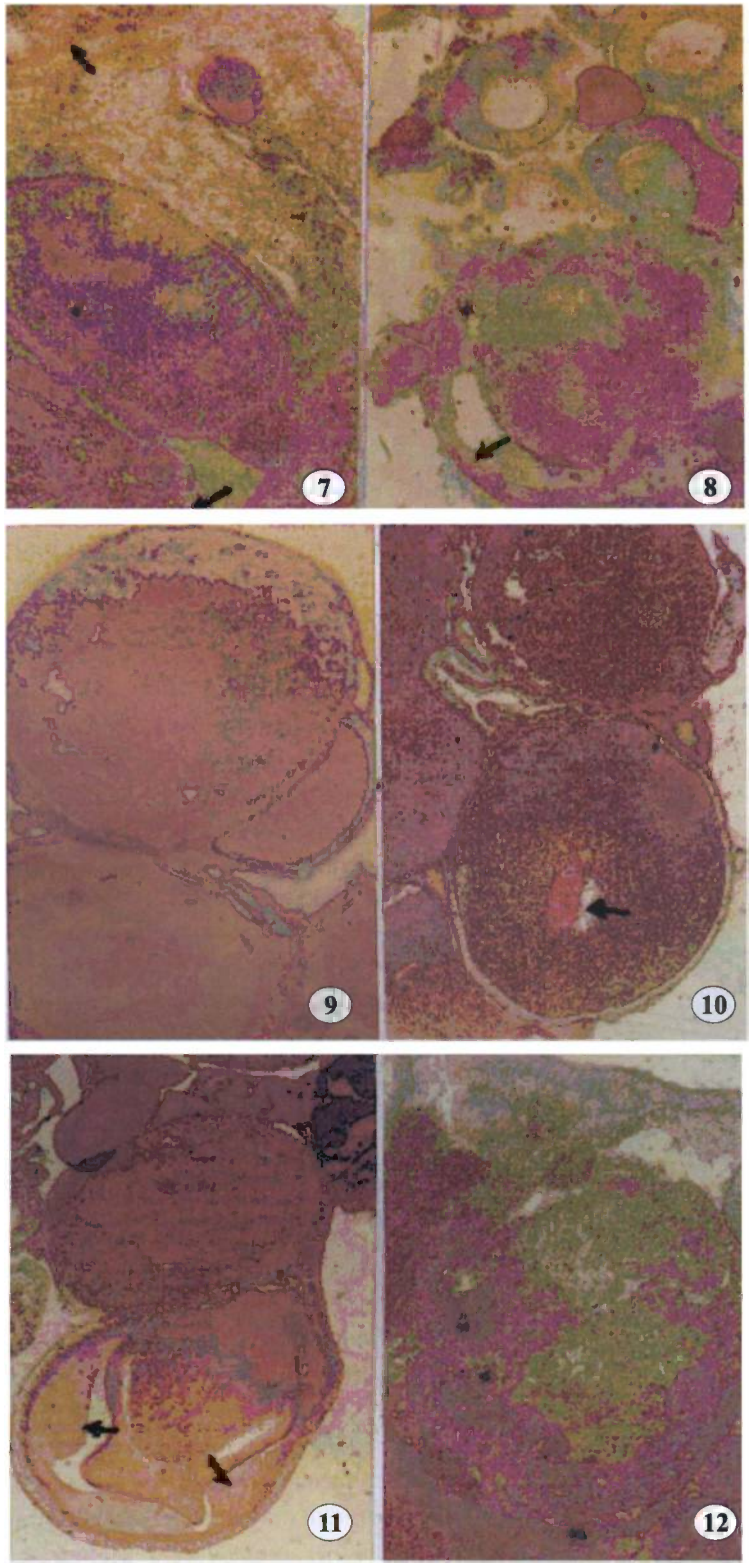


Fig. 7 : Previtellogenic atretic follicles *H. fossilis* depicting vacuolated/flocculent ooplasm and hypertrophied granulosa cells (arrow).
Fig. 8 : Previtellogenic atretic follicle *H. fossilis* showing prominent granulosa cells and separation of ooplasm from zona pellucida (arrow).
Fig. 9 : Vitellogenic follicles *H. fossilis* at early stage of atresia with prominent granulosa cells and vacuolization of the ooplasm at periphery.
Fig. 10 : Vitellogenic follicles ***H. fossilis*** at the early stage of atresia showing vacuolated germinal vesicle (arrow) and vacuolization of ooplasm at periphery.
Fig. 11 : Atretic vitellogenic follicle *H. fossilis* with vacuolated cytoplasm, thickened zona pellucida (arrow) and hypertrophied granulosa cells.
Fig. 12 : Vitellogenic follicle *H. fossilis* with advanced stage of atresia depicting disorganized ooplasm, obscured germinal vesicle and hypertrophied phagocytic granulosa cells.

the Indian fishes. Generally, the neurosecretory cells of NLT exhibited enhanced cytological activity with the advancing maturity while neurosecretory cells of NPO displayed excessive vacuolization during peak spawning period (Pandey, 1993, 1997, 2010; Pandey and Mohamed, 1993, 1997, 1999; Lal and Pandey, 1998; Pandey *et al*, 2000b, 2007b, 2011; Mani and Pandey, 2009).

Pituitary gonadotropin

As in other vertebrates, GtHs in fishes are the major hormone, secreted from the gonadotrophs located in the proximal pars distalis (PPD), regulating gonadal functions (Pandey and Mani, 2006; Pandey *et al*, 2007b). Two types of pituitary GtHs have been isolated and purified one is carbohydrate-rich and other is carbohydrate-poor subunit. Irrespective of its chemical structure, the function of pituitary GtHs in fishes is to control oocyte growth including vitellogensis, maturation, ovulation/spermiation through stimulating gonadal steroidogenesis (Patino, 1997; Delvin and Nagahama, 2002; Singh and Lal, 2009).

Gonadal steroid hormones

In fishes, the GtH levels in circulation begin to rise at the initial stages of annual reproductive cycle and GtH surge triggers a cascade of biochemical events which ultimately leads to final gonadal maturation. In male, the GtH stimulates the secretion of the fish androgen (11-ketotestostrone) from Leydig cells, which in turn, activate Sertoli cells to stimulate pre-mitotic spermatogonia formation. Spermatozoa, within the testicular lobules, are immotile and lack fertilization capacity. During spermiation, Leydig cells continue their steriodogenic activity under gonadotropic stimulation to convert 17α-hydroxrprogesterone to 17α,20β-dihydroxy-progestrone (17,20-P) which ultimately raises the spermduct pH and c-AMP level leading to sperm mortality. Mixing of saline in homogenized testis keeps the sperm quiescent until released into the water.

In the female, the two main steroids secreted are 17β-estradiol and 17α,20β- dihydroxyprogestrone (17,20-P). Estradiol is directly involved in the vitellogenesis by stimulating hepatic vitellogenin biosynthesis. While 17α,20β-P is considered as maturational-inducing hormone (MIH) in majority of the fishes, in some teleosts, 17α,20β,21-trihydroxy-4-pregnen-3-one function as MIS suggesting that a variety of steroids may act as MIH in different species (Patino, 1997; Delvin and Nagahama, 2002). The surge in synthesis and secretion of 17α, 20β-P during oocyte maturation is associated with the drop of estradiol-17β level.

Estradiol-17β has been shown to express the vitgellogenic gene. When vitellogensis is completed, the oocytes are termed as post-vitellogenic but they are still physiologically immature as they cannot be fertilized. To make them suitable for fertilization, oocyte should undergo the process of final maturation consisting of germinal vesicle breakdown (GVBD), chromosome condensation and extrusion of the first polar body for which three factors- GtH (particularly GtH-II), maturation inducing hormones (MIH) and maturation - promoting factor (MIF) have been found to be responsible (Nagahama, 1997; Patino, 1997; Delvin and Nagahama, 2002).

Maturation-promoting factor

Binding of the MIH to its membrane receptors is followed by the formation of a maturation- promoting factor (MPF) in the ooplasm which mediates its action on the meiotic process. MPF, isolated from carp unfertilized eggs, is a complex consisting of the cell cycle regulator, cdc-2-kinase and cycline B. When active, MPF contains a phosphorylated form of cdc-2-kinase. Using monoclonal antibodies against a genetically-engineered specific sequence of P34 cdc2 and against cycline B, it was possible to follow the changes in MPF components during maturation of oocytes induced *in vitro* by 17α,20β-P. Protein P34cdc2 was found in the oocyte prior to maturation and its concentration did not changes significantly during GVBD while cyclin B appeared only in oocytes undergoing maturation. The addition of recombinant cyclin B to immature oocyte extract activated P34fcdc2 and was associated with phosphorylation in both the components, identical to 17α,20 β-P induced oocyte maturation. This suggests that 17α,20β-P induces oocyte to produce cyclin B, which in turn, phosphorylates and activates pre-existing P34cdc2, called as maturation-promoting factor and involved in meiotic as well as mitotic processes (Delvin and Nagahama, 2002; Singh and Lal, 2009; Lubzens *et al*, 2010).

Induced Spawning

Fishes reproduce in their natural environment to produce offspring for continuation of their progeny. However, under controlled conditions and static pond water, the fishes do attain maturity but may or may not breed on their own. Thus, on the basis of breeding responses, fishes may be categorized into (i) free spawners-which breed freely in confined condition, and ii) non-spawners which attain maturity in static waters such as ponds but do not breed unless induced to spawn by application of hormones (Chaudhuri and Singh, 1984; Jhingran, 1991). For the first time, Houssay (1930) of Argentina used pituitary injections for successful spawning in fish. He showed that intra-peritoneal injections of pituitary extracts from *Prochelodus platensis* induced egg laying in *Cresterodon decemmaculatus*.

Development of hypophysation technique for induced spawning

Following the work of Houssay (1930), Brazil was the first country ever to develop a technique of hypophysation in 1934 by conducting experiments with various pituitary hormone injections to fish. The Brazilians first injected suspensions of fresh pituitary of the fish but soon Cardoso (1934) developed a technique of preserving the pituitary in acetone and calcium chloride (the latter was found unnecessary in subsequent experiments) which was then adopted by other workers. de Azeredo and de Oliveira (1939) used the pituitary preserved in alcohol following the example of Rugh (1937) and since then, this became a standard practice for fish breeding in Brazil.

In India, the first success in induced breeding of fish by fish pituitary extract was achieved by Chaudhuri (1955) who injected intraperitoneally *Catla catla* pituitary gland to induce breed *Esomus danricus*. Ramaswamy and Sundararaj (1956, 1957) reported successful breeding of catfishes, *Heteropneustees fossilis* and *Clarias batrachus* by hormone injections. Chaudhuri and Alikunhi (1957) successfully induce bred *Labeo rohita, Cirrhinus mrigala, C. reba, Labeo bata* and *Puntius sarana* by injecting carp pituitary extracts. The Chinese carps were also successfully bred in 1962 by hypophysation techniques (Alikunhi *et al*, 1963; Chaudhuri *et al*, 1966). Since then, this technique has spread widely as part of seed production of commercially important and threatened fishes in many parts of the country including *Tor putitora* (Tripathi, 1977; Pathani and Das, 1979; Joshi, 1981, 1986; Sehgal, 1991; Sehgal and Malik, 1991), *Tor khudree* (Kulkarni and Ogale, 1986) and *Tenualosa ilisha* (Sen *et al*, 1990).

In hypophysation technique for induced breeding of Indian major carps, females are given 2 split doses of pituitary extracts- (i) 1st dose 2-3 mg/kg and (ii) 2nd dose 5-10 mg/kg at 4-6 hours interval. The male brood fish is injected single dose of 2-5 mg/kg at the time of 2nd injection to female (Chaudhuri, 1955. 1960; Chaudhuri and Alikunhi, 1957; Alikunhi *et al*, 1963; Bhowmick *et al*, 1977; Chaudhuri and Singh, 1984; Mahanta *et al*, 1998). Catfishes, such as *Clarias batrachus* (magur) and *Heteropneustes fossilis* (singhi) are given a higher dose of more than 30 mg/kg of carp pituitary for successful spawning (Khan, 1972; Khan and Mukhopadhyay, 1975; Zonneveld *et al.* 1988; Rao and Janakiram, 1991). Even marine catfish pituitary has been employed for induced breeding of carps (Verghese and Rao, 1975; Verghese *et al*, 1975).

Substitutes of pituitary extracts

The large-scale collection and preservation of pituitary gland was realized to be cumbersome as well as expensive. Further, pituitary should be collected from mature specimens for greater efficiency. During late 1970's, it was felt that the need for pituitary gland for fish breeding was ever-increasing on national and global becoming it difficult to meet such high demands. Therefore, substitutes of pituitary glands were sought for and soon a number of hormonal/chemical preparations were tested and used for fish breeding. Some of these are summarized below:

(i) Human chronic gonadotropin (HCG): 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 (Zarin *et al*, 1992; Zohar and Mylonas, 2001; Haniffa and Sridhar (2002).

(a) In *Labeo rohita*, HCG @ 50 IU/kg in 4 weekly injections advanced maturation by one month but HCG at even 1500 IU failed to induce spawning in rohu. Low dose of HCG administered 3 months earlier to breeding in female Indian major carps resulted in higher rate of fertilization and hatching (Varshney *et al*, 1990).

(b) HCG in doses of 25 and 50 IU/weekly (oral/intramuscular) for 28 days increased gonadosomatic index (GSI) and advanced maturation in *H. fossilis* by one and half month (Kanungo *et al*, 1999; Singh and Pandey, 2009).

(c) HCG in dose of 25 and 50 IU/week for 28 days enhanced GSI and breeding success in *H. fossilis* (Mani and Pandey, 2007) (Table 6).

(d) Chinese carps respond well to HCG for breeding either with HCG alone or in combination with the carp pituitary extracts (CPE).

(e) The catfish, *C. batrachus,* breeds with single injection of high dose 4000 IU/kg of HCG. However, effective minimal dose of HCG for *C. macrocephalus* is 2000-5000 IU/ kg. HCG in combination with carp pituitary extracts (CPE) is more effective than HCG alone for breeding major carps. However, if HCG is not injected in proper dose, it affects hatching as in golden perch, *M. ambigua*, a dose of 200 IU/kg caused significant reduction of hatching rate compared to the optimal dose of 500 IU/kg.

(ii) Partially-purified fish gonadotropin: Partially-purified salmon gonadotropin or even purified gonadotropin (salmon GtH) is available from some research laboratories which are effective in induced breeding in fishes. Nayak *et al.* (2000a, b) observed encouraging results with low doses of SG-G100 in combination with steroid 17α,20β-dihyroxyprogesterone (17α,20β-P) in induced ovulation of *H. fossilis*. However, it has not found commercial use because of cost effectiveness.

(iii) Luteinzing hormone releasing hormone (LHRH) and analogues: LHRH is effective in inducing gonadotropin release and ovulation in fish but its superactive analogues (LHRH-a) are more effective in a variety of fishes including Indian major carps. However, LHRH-a in combination with pimozide or domperidone (a dopamine antagonist-dopamine, one of the GnRIF-gonadotropin release-inhibiting factor) is very effective in induced breeding in Indian major carps, Chinese carps, catfishes, salmon, trout *etc*, however, the level of dopaminergic inhibition of GnRH release from pituitary gonadotroph varies greatly in various groups of teleosts (Patino, 1997; Delvin and Nagahama, 2002; Podhorec and Kouril, 2009). For the Indian major carps, the effective dose is LHRHa (10-20 μg/kg fish)+PIM (10 mg/kg). This method is also referred to as Linpe-technique based on name of the two scientists, H.R. Lin and R.E. Peter (Peter *et al*, 1988).

(iv) Gonadotropin-releasing hormone (GnRH): GnRH, secreted in the hypothalamus, accelerates release of gonadotropin (GtHs) from the pituitary gland of fishes. GnRH and its analogue (GnRH-a) in combination with domperidone or pimozide is effective in induced breeding of fishes tested so far. About 10-20 μg GnRH+5 mg domperidone/kg fish injected to mature fish elicited successful breeding (Peter *et al*, 1988; Alok *et al*, 1993; Tharaknan and Joy, 1996; Podhorec and Kouril, 2009). Though three variants (forms) of GnRH have been identified in the same species but their precise role in reproduction has not yet been delineated (Yashuvi *et al.*, 2006).

A formulation of ^{s}GnRH [D-Arg^{6},Pro^{9}NET] and domperidone has been marketed as a spawning kit under the trade name of "Ovaprim" by Syndell Laboratories Inc., Vancouver (Canada). Ovaprim is being widely used in India for breeding of cultivable fishes on large scale (Nandeesha *et al*, 1990, 1993; Lakra *et al*, 1996; Pandey *et al*, 1998, 1999; Kanungo *et al*, 1999; Ogale, 1999; Ponniah *et al*, 2000; Nayak *et al*, 2001; Dash *et al*, 2000; Singh *et al*, 2000a, 2002; Rath *et al*, 2007; Srivastava *et al*, 2010; Mishra *et al*, 2011; Yadav *et al*, 2011; Chaturvedi and Pandey, 2012; Chaturvedi *et al*, 2012a, b, 2013a,b). Even threatened fishes have also been bred successfully through ovaprim administration under captive conditions (Sridhar *et al*, 1998; Bhowmik *et al*, 2000; Reddy, 2000; Radheyshyam and Sarangi, 2005; Sarkar *et al*, 2005, 2006; Hussain, 2006; Chakraborty *et al*, 2009; Chaturvedi *et al*, 2012c). Although dose of the drug for Indian major carps and catfishes varies among the species and between males and females depending upon the reproductive status of the individuals (Table 7), it is highly effective for mass scale seed production and also cost is bearable by farmers (Singh *et al*, 2000a, 2002; Chaturvedi *et al*, 2012a, b; Taslima and Ahmed, 2012)). Recently, similar formulations in the trade name of "Ovatide" (M/S Hemmo Pharma, Mumbai) and "WOVA-FH" (M/S Wockhardt Life Sciences Ltd., Mumbai) have been marketed which are equally effective in induced breeding of carps as well as catfishes (Mukherjee and Das, 2001; Mukherjee *et al*, 2002; Pandey *et al*, 2001b, 2002a, b, c, 2009; Pandey and Koteeswaran, 2004; Sahoo *et al*, 2005; Rath *et al*, 2007; Yadav *et al*, 2011; Mishra *et al*, 2011; Purkayastha *et al*, 2012). It is pertinent to remark 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, *C. batrachus* (Yadav *et al*, 2011). However, it is high time that GnRH from Indian fishes must be isolated, characterized and synthesized so that an indigenous and low-cost product becomes available for fish breeding in India. For breeding carps, it is better to observe nuclear migration in eggs from brood fish. For this, a few eggs are drawn from posterior region of the ovary using catheter and immersed in a solution containing 70% acetic acid and 30% alcohol for about 5 minutes. When the eggs are clear, the nucleus position is observed under microscope. Migration of nucleus from centre to the surface (periphery) of egg (GVM) indicates the readiness of fish for spawning (Singh and Pandey, 2009).

Teleosts have complex reproductive physiology and behaviour. Their reproductive responses depend on genetic, nutritional and environmental factors (Pandey and Mani, 2006; Babin *et al*, 2007; Jakobsen *et al*, 2009; Mani and Pandey, 2009). Among the above, nutritional factors can be easily controlled and manipulated (Pandey *et al*, 2003; 2006a, b, 2007a; Amano, 2010; Lubzens *et al*, 2010; Phelps, 2010). The water quality can also be managed to provide good environment but factors such as light and temperature are not easily manipulated in field/pond on large scale rearing of brood fishes. However, under severe cold conditions, progressive farmers/fish breeders can afford to reset "polythene enclosures" over the broodfish ponds providing the "Green House" effect that will maintain temperature at much higher level than outside surroundings. Photoperiod can also be easily maintained in such enclosures providing conducive environments for maturation. Such arrangements can be provided at an

Table 1: Composition of feeds used for rearing of broodstock of the Indian major carps.

Ingredients	Control feed (T-1)	Experimental feed (T-2)
Fish meal (%)	10	Nil
Groundnut oil-cake (%)	35	35
Soyabean oil-cake (%)	20	30
Rice bran (%)	24.27	23.27
Calcium di-phosphate (%)	0.2	0.2
Vitamin mix (%)	0.5	0.5
Vitamin C (%)	0.03	0.03
Wheat flour (%)	10	10
Lysomix (%)	Nil	0.5
Methiomix (%)	Nil	0.5
Composition of feed		
Crude protein (%)	30.65	30.58
Crude lipid (%)	12.70	12.80
Gross energy(kcal/kg)	3,800	3,820

Table 3 : Proximate composition of feeds used for *Heteropneustes fossilis* rearing.

Ingredients (%)	Control feed (T-1)	Experimental feed (T-2)
Fish meal	25	15
Groundnut oil-cake (GOC)	30	30
Soyabean oil-cake (SOC)	20	30
Rice bran	14.5	13.5
Wheat flour	10	10
Supplivite- M	0.5	0.5
Lysine	—	0.5
Methionine	—	0.5
Crude protein	35.28	35.21

Table 4 : Breeding responses of *Heteropneustes fossilis* to ovaprim administration.

	Sets taken	May June			
		T-1	T-2	T-1	T-2
Fertilization (%)	6	65-68	80-85	70-75	90-96
Hatching success (%)		50-52	70-72	55-60	80-85

Table 2 : Breeding responses of the Indian major carps with ovaprim.

	Species	Sets taken	April		May		June	
			T-1	T-2	T-1	T-2	T-1	T-2
Fertilization (%)	*Catla catla*	3	10-15	50-60	35-40	55-65	60-75	85-90
	Labeo rohita	3	10-15	55-60	35-40	60-70	60-75	85-90
	Cirrhinus mrigala	3	20-30	60-65	40-50	68-70	50-65	78-86
Hatching success (%)	*Catla catla*	3	Nil	18-20	22-25	30-32	35-38	78-80
	Labeo rohita	3	Nil	25-30	20-22	40-50	35-45	78-82
	Cirrhinus mrigala	3	Nil	30-32	28-31	46-52	40-46	74-80

extra-cost of broodfish rearing in colder parts of India. Since about 1 lakh eggs/spawn will be available from 1.0 kg female broodfish which on raising the spawn to fry would fetch good money. Thus, farmers can smoothly derive large profits from fish breeding and seed rearing.

Although the induced breeding techniques have solved the problem of breeding in several species of fishes under culture conditions, it must be taken at the proper time of gonadal maturity. If the prime time is missed or breeding is delayed for unavoidable reasons, the mature eggs will be resorbed and induced breeding of such fishes would not be successful. Furthermore, the rates of fertilization and hatching would also be affected adversely (Nayak *et al*, 2000a; Pandey *et al*, 2009).

One of the problems of induced breeding of fish is that the maturity of males and females may not synchronize. Although, the female fish attains good maturity but male may not be fully mature leading to non-fertilization of eggs. In order to overcome this situation, the "cryopreservation" of sperms has been developed so that the sperms are collected and preserved at very low temperature (-196°C) under liquid nitrogen in a suitable medium for use on demand by fish breeders. The seasonal breeding of carps and catfishes has also been overcome by advancing or delaying the maturity by hormonal (LHRHa and HCG *etc*) implants/injections (Kanungo *et al*, 1999; Nayak *et al*, 2000b) as well as dietary manipulations (Gupta *et al*, 1990; Somshekarappa *et al*, 1990; Pandey and Singh, 2003; Pandey *et al*, 2003; 2006a, b, 2007). The multiple breeding of carps and catfishes has been achieved when the first breeding of the fish is done much in advance of the natural breeding season (Bhowmick *et al.* 1977; Mahanta *et al*, 1998). All these modern developments in reproductive physiology, breeding and larval rearing of cultivable fishes

Table 5 : Amino acid sequences of the identified GnRHs of chordates.

Mammal	P-Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly-NH_2
Chicken I	P-Glu	His	Trp	Ser	Tyr	Gly	Trp	Gln	Pro	Gly-NH_2
Chicken II	P-Glu	His	Trp	Ser	His	Gly	Trp	Tyr	Pro	Gly-NH_2
Frog	P-Glu	His	Trp	Ser	Tyr	Gly	Leu	Trp	Pro	Gly-NH_2
Seabream	P-Glu	His	Trp	Ser	Tyr	Gly	Leu	Ser	Pro	Gly-NH_2
Salmon	P Glu	His	Trp	Ser	Tyr	Gly	Trp	Leu	Pro	Gly-NH_2
Whitefish	P Glu	His	Trp	Ser	Tyr	Gly	Met	Asn	Pro	Gly-NH_2
Medaka	P Glu	His	Trp	Ser	Phe	Gly	Ser	Asn	Pro	Gly-NH_2
Catfish	P-Glu	His	Trp	Ser	His	Gly	Trp	Asn	Pro	Gly-NH_2
Herring	P-Glu	His	Trp	Ser	His	Gly	Leu	Ser	Pro	Gly-NH_2
Dogfish	P-Glu	His	Trp	Ser	His	Gly	Trp	Leu	Pro	Gly-NH_2
Lamprey-I	P-Glu	His	Trp	Ser	His	Glu	Trp	Leu	Pro	Gly-NH_2
Lamprey-II	P-Glu	His	Tyr	Ser	Leu	Glu	Trp	Lys	Pro	Gly- NH_2
Tunicate-I	P-Glu	His	Tyr	Ser	Asp	Tyr	Phe	Lys	Pro	Gly-NH_2

Primary structure of the thirteen known GnRH molecules of chordates. (after Kah *et al*, 2007).

Table 6 : Effects of HCG and WOVA-FH administration on gonadosomatic index (GSI) and breeding responses of *Heteropneustes fossilis*.

Treatment	Sets taken	GSI	Fertilization (%)	Hatching success (%)
Control	6	4.24±0.26	20-25	10-15
HCG 25 IU	6	6.88±0.48	78-85	68-73
HCG 50 IU	6	8.72±0.32	82-88	75-82
WOVA-FH	6	6.54±0.18	72-76	65-69

(after Mani and Pandey, 2007).

Table 7 : Doses of ovaprim for induced breeding of carps and catfishes.

Species	Female Ovaprim (ml/kg)	Male Ovaprim (ml/kg)
Labeo rohita	0.30-0.4	0.1- 0.2
Catla catla	0.40-0.5	0.1-0.2
Cirrhinus mrigala	0.25-0.3	0.1-0.2
Ctenopharyngodon idella	0.40-0.8	0.1- 0.2
Hypopthalamichthys molitrix	0.4-0.7	0.1-0.2
Heteropneustes fossilis	0.5-0.9	0.3-0.4
Clarias batrachus	0.5-0.8	0.3-0.4
Ompok pabda	1.0-1.5	0.5-0.6

Composition of ovaprim: Each ml of ovaprim contains- (i) salmon gonadotropin releasing hormone (sGnRH)- 20 mcg and (ii) domperidone- 10 mg

has made it possible for the fish farmers to stock ponds and cultivable water areas with quality fish seed even during the off-season and grow more and more protein-rich fish in order to alleviate malnutrition and poverty in the country owing to the availability of fish at considerably lower prices.

Follicular atresia affecting fecundity

Follicular atresia is a highly regulated process in the vertebrate ovary which appears to be essential for maintenance of ovarian homeostasis (Wood and van der Kraak, 2001). The oocytes in different stages of growth are lost through atresia (or degeneration) affecting the fecundity/reproductive potential of the fish. Atresia in the fish ovary is of common occurrence during pre-spawning, spawning and post-spawning periods (Saidapur, 1978, 1982; Guraya, 1994; Miranda *et al*, 1999; Mani and Pandey, 2006). During the course of maturation process, some of the ova that fail to attain maturity or spawn undergo resorption and are called atretic follicles (Saidapur, 1978, 1982; Guraya, 1994; Khanna, 2006). All the four stages of follicular atresia, described in the teleost ovary (Belsare, 1962, 1975; Saidapur, 1978), were observed in the freshwater catfish. In *H. fossilis*, remnants of atretic follicles in the form of nodule of stroma tissue were encountered even in the immature ovaries during December-January. Previtellogenic atretic follicles in ovary of the catfish depicted excessive vacuolization of ooplasm towards periphery, flocculent appearance of ooplasm and hypertrophied granulosa cells penetrating the zona pellucida or oolema. Some previtellogenic atretic follicles of *H. fossilis* during March-April exhibited prominent granulosa cells, separation of ooplasm from zona pellucida and disorganization of ooplasm (Fig.7,8). Vitellogenic ovarian follicles of *H. fossilis* at the early stage of atresia (May-June) showed prominent granulosa cells, vacuolization of the ooplasm at periphery and ooplasm giving flocculent appearance (Fig.9,10).

Table 8 : Composition of feeds used for rearing of *Catla catla* fry.

Ingredients	Control diet (%)	Experimental feed (T-1) (%)	Experimental feed (T-2) (%)	Experimental feed(T-3) (%)
Fish meal	5	5	5	5
Groundnut oil-cake	35	35	35	35
Soyabean oil cake	25	25	25	25
Rice bran	24.34	24.34	24.34	24.34
Wheat flour	10	10	10	10
Supplivite M	0.66	0.66	0.66	0.66
Thyroxine	Nil	0.10	0.05	0.03
Crude protein	30.17	30.17	30.17	30.17

Table 9 : Details of the growth of *Catla catla* fry under hatchery condition.

Parameters	Control diet	T-1	T-2	T-3
Initial average weight (g)	0.52	0.49	0.51	0.54
Final average weight (g)	0.77	1.00	1.66	1.46
Weight increment (g)	0.25	0.48	1.14	0.94
Weight gain (%)	48.07	92.30	219.23	180.76

Table 10: Composition of feed for *Catla catla* and *Labeo rohita* fingerlings under field conditions.

Ingredients (%)	Control feed (T-1)	Experimental feed (T-2)
Fish meal	10	---1
Groundnut oil-cake (GOC)	40	40
Soyabean oil-cake (SOC)	25	35
Rice bran	14.27	12.27
Vitamin+mineral mixture	0.5%	0.5%
Calcium-hydrogen-phosphate	0.2	0.2
Vitamin C	0.03	0.03
Wheat flour	10	10
Lysomix	—	1.0
Methiomix	—	1.0
Composition		
Crude protein	34.5	32.50
Lysine content	5.04	5.72
Methionine content	1.39	2.18

Thickened zona pellucida and hypertrophied granulosa cells were recorded as the atresia advanced in the vitellogenic follicle of *H. fossilis* (Fig.11). During the advanced stage of atresia (September-October), the oocytes of the catfish depicted disorganized ooplasm, obscured germinal vesicle and hypertrophied granulosa cells. Phagocytic invading of granulosa cells in zona pellucida and ooplasm were also prominent (fig. 12). (Mani and Pandey, 2006).

Table 11 : Effect of lysine and methionine supplementation on growth and feed utilization of *Catla catla* and *Labeo rohita* fingerlings under pond conditions.

Period	T- 1		T-2	
	Catla (g)	Rohu (g)	Catla (g)	Rohu (g)
Initial	8.50	1.50	8.50	1.50
30 days	25.50	6.00	36.50	9.50
60 days	52.72	35.00	81.00	45.45
90 days	64.37	44.37	100.71	65.38
120 days	81.00	50.60	125.80	75.40
FCR	2.65	2.51	1.95	2.04
FCE (%)	37.73	39.84	51.28	49.02
PER	0.31	0.28	0.56	0.51
SGR (%)	0.38	0.32	0.52	0.45

Though the precise causes of follicular atresia in teleosts have not yet been clearly defined, several exogenous (photoperiod, temperature, rainfall, crowding, captivity, nutrition, physico-chemical characteristics of ambient water, pollutants/biocides *etc)* as well as endogenous (insufficient gonadotrophic hormone, imbalance of hormones and steroids) have been implicated in the process (Sundararaj and Goswami, 1968; Saidapur, 1978, 1982; Kling, 1981; Saksena and Raizada, 1984; Guraya, 1994; Rodriguez *et al*, 1995; Miranda *et al,* 1999; Wood and van der Kraak, 2001; Khanna, 2006). Further studies are required to resolve the causes and functions of follicular atresia for management of broodstocks to realize optimum fecundity of the fish. Mani and Pandey (2007) found that low doses of HCG and ovaprim delayed the onset of atresia in the captive broodstock of *H. fossilis*.

Hormonal and dietary manipulations in larval rearing for aquaculture and conservation

Larval survival is an important component of any aquaculture species (Phelps; 2010; Amano, 2010; Bobe and Labbe, 2010). Thyroxine plays important role in growth and metamorphosis of fish larvae (Lam, 1994; Power *et al*, 2001; Liu and Chan, 2002; Yamano, 2005). Even administration of low dose of thyroxime improved the larval growth and differentiation in coral reef fish (*Pomacentrus amboinensis*) and Nile tilapia (*Oreochromis niloticus*) (McCormick, 1999; Khalil *et al*, 2011). Experiments conducted under hatchery conditions have proved that low doses of dietary thyroxine (0.05 ppm) supplementation enhanced survival and growth of the fry and fingerlings of *Catla catla, Labeo rohita* and *Cirrhinus mrigala* (Pandey *et al*, 2002d, 2004a, b, c). Results of dietary thyroxine administration on growth of *Catla catla* have been summarized in Table 8-9. Better survival and growth were also observed in larvae of *H. fossilis* given immersion treatments in individual thyroxine (L-thyroxine 0.05 mg/l), cortisol (0.5 mg/l) and combined thyroxine (0.5 mg/l)+cortisol (0.05 mg/l) (Nayak *et al*, 2004).

Protein forms the major energy source in fish feeds and helps primarily in tissue build up and quality of protein in the feed is highly important for the growth and survival of commercially important teleosts (Cowey and Sargent, 1979; Halver 1989; Lovell 1989). For recording the dietary supplementation of lysine and methionine on growth of *Catla catla* and *Labeo rohita*, fingerlings reared in 0.04 ha ponds under high stocking density of 30,000/ha. They were given artificially pelleted (2 mm) diet containing ingredients such as fish meal, groundnut oil-cake, soyabean oil-cake, rice bran, wheat flour, vitamin mix, calcium diphosphate and ascorbic acid (Table 10). Fingerlings of all the groups were fed once daily @ 5% of their body weight for 120 days, samplings were carried out at the regular interval of 30 days and feed was adjusted accordingly. Cow dung manuring was done in order to ensure adequate supply of plankton. Physico-chemical parameters of the pond water were monitored regularly and optimum conditions were maintained. Feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio percentage (PER%) and specific growth rate percentage (SGR%) of the fingerlings of both the groups were calculated. It was observed that the addition of lysine and methionine @ 1% in the feed significantly ($P<0.05$) enhanced the growth of catla and rohu fingerlings as compared to those maintained on the control diet (Table 11).

Incorporation of plant ingredients in fish feed as protein source to replace fish meal is a good effort of feed manufacturer but while using plant ingredients, the essential amino acid content should be balanced (Rumsey *et al*, 1983; Ravi and Devaraj, 1990; Ronnestad *et al*, 1999; Takagi *et al*, 2001; Yang *et al*, 2010). Most of the feed stuffs of plant origin are deficient in some of the essential amino acid (Lovell 1989; Halver 1989; Lall 1991; Cho and Kausik 1990; NRC 1993). Viola and Lahav (1991) obtained significantly higher rate of carp production using feed with very low levels of fish/meat meal when the feed were fortified with synthetic amino acids (lysine and methionine), in the intensive fish culture operations. Further, they observed protein-sparing effect as well as reduction in pollution by lysine supplementation in practical carp feeds. The cost of feed was also reduced by 5% in these experiments. The synthetic amino acid supplementation has also been done in diets of rainbow trout for significantly higher rate of production (Fauconncau, 1988). These findings under hatchery as well as field conditions are having wider applications in enhancing growth and survival of fry and fingerlings of the commercially important as well as threatened fishes (Das and Pandey, 1999; Pandey and Das, 2002, 2004; Babin *et al*, 2007; Jakobsen *et al*, 2009).

CONCLUSIONS

Ovulation in teleost is regulated both by endogenous factors which initiate and mediate pre-ovulatory changes in the oocytes and by the exogenous factors that determine when the endogenous factors will become functional. Endogenous factors include GnRH, GtHs and local ovarian mediators of GtH action (steroids and prostaglandins). Elevation of blood GtH level is prerequisite for spontaneous ovulation. Pre-ovulatory GtH surge triggers two distinct ovarian processes *i.e.* final oocyte maturation, stimulated by 17α, 20β-P and follicular rupture (germinal vesicle breakdown, GVBD) which is evidently mediated by prostaglandins and maturation-promoting factor (MPF). Role of reproductive pheromones in gonadal maturation, synchronization of reproductive processes and spawning as well as reproductive containment of invasive species may not be overlooked (Stacey *et al.*, 1992; Stacey, 2003; Pandey, 2003, 2005, 2009; 2012; Sorensen and Stacey, 2004; Hubbard and Scott, 2007; Burnard, 2008).

With all these advancement of knowledge in the reproductive physiology, we are still far behind to understand the basic mechanism(s) involved in the process of fish propagation in nature. The knowledge of nutrition and reproductive endocrinology periodically refines the technology of production of quality gametes for the aquaculture. Till date, we are only in a position to advance or retard reproduction by a few months or weeks. The modern aquaculture practices demand from the endocrinologists and breeders to develop the techniques to mature and spawn the fish at any time of the year *i.e.*

crossing the seasonal reproductive cycle to produce gametes round-the-year. The targeted task is tremendous and needs well-coordinated, multidisciplinary approach from the fish breeder, endocrinologists and geneticists including nutrition and environment personnel.

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