

A review on induced breeding of cat fishes, murrels and climbing perches in India

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ABSTRACT

The breeding period of cat fishes, climbing perches and murrels in India is variable. Environmental factors play an important role in regulating reproduction in fishes. Choosing a successful synthetic hormone involves the selection of effective hormone formulations, proper duration of hormonal treatment and timing of the hormone administration. Induced breeding of cat fishes, climbing perches and murrels of India by various hormonal analogues is reviewed based on published literature. Synthetic hormones viz., ovaprim and ovatide are successfully being tested (in place of pituitary extract) for the induced breeding of fishes. Since, newly formulated inducing agents are also being tested for the induced breeding performance by various researchers in different parts of the country, under different climatic conditions, with varying degree of success. These synthetic hormones have following advantages over pituitary extract: ready to use in liquid form, consistent potency, stored at room temperature, stable with long shelf life and single dose requirement. Although natural spawning is the preferred method for breeding cultivated fresh water fishes, induced breeding is necessary to control timing and synchrony of egg production.

Key words: Murrels, Cat fishes, Climbing perches, Induced breeding, Synthetic hormones.

INTRODUCTION

Fish is one of the major components of the aquatic ecosystem and fish also form food for a variety of animals and human beings. The distribution of fish fauna is quite variable due to climatic and geological conditions. Biodiversity conservation necessitates knowledge on the diversity of animals and plants, their distribution status. The Western Ghats in India is one of the 25th richest mega biodiversity hot spot of the world. There is a need for the survey of biodiversity of fishes in different types of habitats in Karnataka and all over the country (Jayaram,1999; Talwar & Jhingran,1991).

Fishes form an important element in the economy of many nations as they have long been a stable item in the diet of many people. They constitute slightly more than one-half of total number of approximately 54,711 recognized living vertebrate species; there are descriptions of an estimated 27,977 valid species of fishes (Nelson, 2006).

Among the most significant advancements in the field of aquaculture during recent decades is the development of techniques to induce reproduction in fish. These techniques have allowed farmers to profitably breed and raise species that do not naturally reproduce in captivity, and to manipulate the timing of reproduction to suit production cycles. Some species will not readily breed in captivity due to environmental or culture conditions that are different from those found in nature, such as water temperature or substrate type. These conditions may cause stress or may not provide the cues needed to complete the reproductive process (Jeff Mittelmark and Anne Kapuscinski, 2008).

Aquaculture has assumed the status of fast expanding industry in India. India is basically being a carp country and the indigenous and exotic carps account for bulk of production. But now the culture of catfishes also received increased interest in recent years due to their high market price and hardy nature. Among the catfishes the air breathing species *Clarias batrachus* is a popular culturable fish in Asian countries. It has many advantages over other species. The hardy nature and tolerance to adverse ecological condition enable its high density culture with a high production per unit area (Sharma *et al.*, 2010).

The basic requirement of the controlled fish culture industry is the fish seed but now spontaneous captive breeding, short supply of quality seed and dependency on wild seeds, which is unreliable, time consuming and uneconomical are major constraints for culturing this fish. To overcome such problems, induced spawning is thought to be the only alternative method for quality seed supply and production (Sharma *et al.*, 2010).

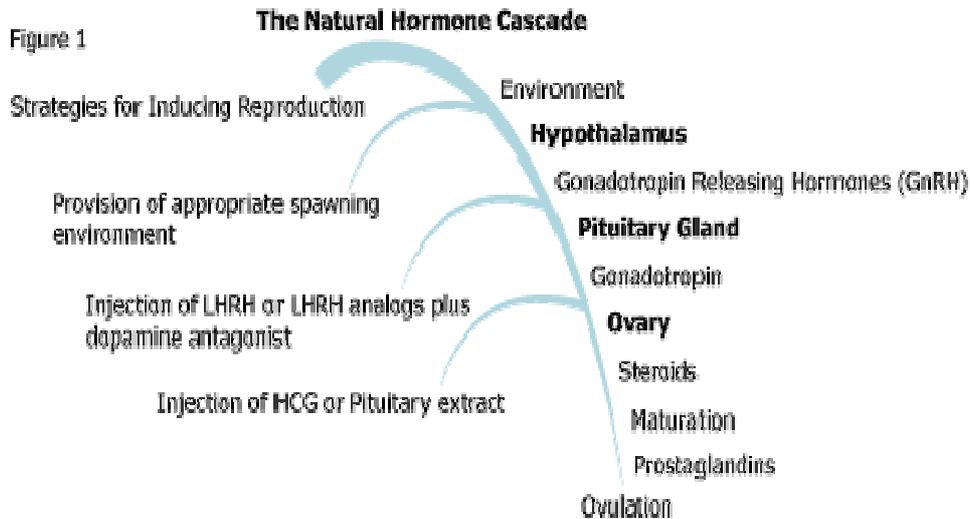
Among several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (sGnRH) or luteinising hormone releasing hormone (LHRH) analogues in combination with dopamine antagonists was found to be effective in fish breeding (Lin and Peter 1996). The use of synthetic inducing agents for successful ovulation followed by stripping in catfish is a common practice and has been studied at several occasions (De Leeuw *et al* 1985, Manickam and Joy 1989, Tan-Fermin *et al* 1997). There are associated problems in using these hormones, such as weighing and low quantity, preparation of these analogues and storage of these prepared solutions. On account of these difficulties, breeders are reluctant to use them in field conditions. However, the commercially available synthetic inducing hormones in readymade form containing GnRH and dopamine blocker receptor (Ovaprim, Ovopel, Dagin and Aquaspawn) are becoming very popular and found to be efficient in successful spawning of fishes (Peter *et al* 1988, Nandeeshia *et al* 1990, Brzuska and Adamek 1999 and Cheah and Lee 2000; Sharma *et al.*, 2010).

The murrels and cat fish resources are an important aspect of fishery potential of a water body. Catfishes and murrels due to their great demand and high market value and non-availability of stocking material largely hinder the organized culture of these fishes in our country. The small catfish species are noteworthy for their size, taste and market value. They are distributed in lentic and lotic water bodies and breed naturally in perennial rivers during monsoon. Though breeding and larval rearing of certain catfishes has been done successfully for their commercial production is yet to be achieved. Hence, maintenance of catfishes and murrels plays a key role in achieving successful induced breeding.

Fish in captivity may not always reproduce at the most advantageous time, and alteration of the spawning cycle may be desirable. This allows a farmer to:

1. Obtain fish outside of the normal spawning season either to lengthen time for grow-out or to produce hybrids with other species;
2. Improve efficiency by getting fish to spawn on a predetermined date; and
3. Maximize survival by fertilizing and incubating eggs under hatchery conditions. Where successful, techniques for altering the spawning cycle of fish have become a valuable tool.

In fish, as with all higher animals, hormones play a critical role in the reproductive process. Hormones are chemical messengers released into the blood by specific tissues, such as the pituitary gland. The hormones travel through the bloodstream to other tissues, which respond in a variety of ways. One response is to release another hormone, which elicits a response in yet another tissue. The primary tissues involved in this hormonal cascade are the hypothalamus, pituitary gland, and gonads (Fig. 1) (Jeff Mittelmark and Anne Kapuscinski, 2008).



Fish have evolved to reproduce under environmental conditions that are favorable to the survival of the young. Long before spawning, seasonal cues begin the process of maturation. In many fish, this can take up to a year. When the gametes have matured, an environmental stimulus may signal the arrival of optimal conditions for the fry, triggering ovulation and spawning. Examples of environmental stimuli are changes in photoperiod, temperature, rainfall, and food availability. A variety of sensory receptors detect these cues, including the eye, pineal gland (an organ in the dorsal part of the forebrain that is sensitive to light), olfactory organs, taste buds and thermo receptors (Jeff Mittelmark and Anne Kapuscinski, 2008).

The hypothalamus located at the base of the brain is sensitive to signals from sensory receptors and releases hormones in response to environmental cues. Principal among these hormones are gonadotropin releasing hormones (GnRH), which travel from the hypothalamus to the pituitary gland. The pituitary is responsible for a wide variety of functions including growth and reproduction. Certain cells of the pituitary receive GnRH and release gonadotropic hormones into the bloodstream. The gonadotropic hormones travel to the gonads which synthesize steroids responsible for final maturation of the gametes (Jeff Mittelmark and Anne Kapuscinski, 2008).

Maturation of the egg is a long process that involves complex physiological and biochemical changes. One important step, vitellogenesis is a process in which yolk proteins are produced in the liver, transported to the ovary and stored in the egg resulting in tremendous egg enlargement. The yolk is important as a source of nutrition for the developing embryo.

Also critical are germinal vesicle migration and germinal vesicle breakdown (GVBD). Before it migrates the germinal vesicle or nucleus is located at the center of the egg in an arrested stage of development. At this stage, the egg is physiologically and genetically incapable of being fertilized even though it has the outward appearance of a fully mature egg. When conditions are appropriate for final maturation, nuclear development resumes and the germinal vesicle migrates to one side. Finally, the walls of the germinal vesicle break down, releasing the chromosomes into the cell.

The maturity of eggs can be determined using biopsy techniques. Eggs are removed from the ovaries cleared with a prepared solution and viewed under a microscope. In mature eggs the migration of the germinal vesicle to the side of the cell will be complete.

After the egg has matured, a class of compounds called prostaglandins is synthesized. These stimulate ovulation which is the rupture of the follicle cells that hold the egg. The egg is then released into the body cavity or ovarian lumen, where it may subsequently be released to the outside environment. Following ovulation, the viability of the eggs can decrease rapidly. Fish with gametes that have not yet been released by the gonads are called "green." The term "ripe" is used to describe fish with gametes that have been released from the ovary into the ovarian lumen. Ripe fish can be stripped, green fish cannot.

CAT FISH BREEDING TECHNIQUES

Clarias batrachus

Asian catfish *Clarias batrachus* is considered as a potential aquaculture species in Indian subcontinent. The Asian cat fish, locally known as Magur fish, is an important air-breathing cat fish with good markets especially in North-Eastern parts of India where it fetches a higher price than the major carps. The scarcity of marketable fish as well as seed from the natural ground has been felt in this catfish. The potential to obtain magur seed from natural sources has become low due to the increasing use of pesticides in the paddy fields-which are the main breeding grounds of this fish. The breeding performance is an important parameter to evaluate the breeding success in captive condition which depends on the type of hormone used and its potency, dose of hormone and maturity status of the fish Human chorionic gonadotropin (HCG) at 14-23 h latency in combination with 3000-4000 IU HCG dose is one among them and is reported successful in catfish during induced ovulation. Appropriate combinations of the proper dose of inducing agent and stripping time always yield maximum egg output during induced breeding. A single injection of 0.6ml/kg body weight of ovaprim was the most effective. The males were given a single dose of 0.1-0.2 ml/kg body weight. Again administration of fish PG of dosage varied from 12 to 30 mg/kg weight of fish given in two doses, a provocative dose of 5-10 mg /kg and a final dose of 8-20 mg/kg, 5-6 hours interval was found successful in spawning. In hapa nursing of magur, we achieved an average survival of 51% while feeding with rice bran and mustard oil cake mixture at 1:1 ratio and termite twice daily (Datta,2013).

Before the female are stripped male fish with gravid testis are to be sacrificed and testes are taken out and macerated in normal saline (0.9% NaCl). The spermatozoa become inactive in this medium and this extract can be maintained for few hours in refrigerator. After 16 hours of latency period female fish is stripped and ova are collected in to dry enamel tray. Before fertilization milt (spermatozoa) extract medium is activated by addition of fresh water. Sperms become active and motility of sperms can be confirmed in microscope. Sperm preparation thus obtained will be sufficient to fertilize the ova stripped from 2 females. Sperm extract is sprinkled over the ova and gametes are mixed gently with bird feathers and allowed to 2 to 3 minutes for fertilization. After repeated washing with fresh water fertilized eggs are transferred to hatching trays for incubation (Datta, 2013)

Breeding of *Heteropneustes fossilis*

This is a common catfish found in freshwater swamps, ponds and tanks throughout the country. It is also suitable for pond culture. It reportedly attains a length of 200 mm at the end of the first year and a maximum length of about 450 mm. This fish breeds in ponds and confined waters almost throughout the year, peak season being monsoon. During rainy days, fishes move from wells to shallow inundated areas of paddy fields for breeding. The eggs are greenish in colour and are usually found attached to weeds. This fish has been successfully induced to breed in India by administering in one injection 75-100 IU of human chorionic gonadotrophin. It is also possible to induce this fish to breed 8 to 10 weeks ahead of peak spawning season by administering pituitary gland injections. By hypophysation these fishes can be induced to breed several times in the same season. Even the spawning season could be prolonged by 2-3 months when the fishes are kept exposed to light during this period for about 12 hours every day. The incubation period extends from 18-20 hours and the newly hatched larvae measure about 2-7 mm in length. At this stage, the larvae feed voraciously on zooplankton. Therefore, it is suggested that before stocking the larvae, the nursery ponds have to be prepared to have abundant zooplankton to get better survival of hatchlings (Datta,2013).

Breeding of *Aanbas testudineus*

The adults are solitary and aggressive. It can gain weight up to 52gm in one year. Maturity occurs at the age of one when the fishes reach a size of 10-12cm in total length. The sexual dimorphism in *A. testudineus* is more apparent during breeding season. The mature male acquires a reddish hue on the body, particularly on the pectoral and ventral fins. The female shows only a faint reddish color. Further in the male a distinct diamond shaped black spot appears in the caudal peduncle. In the female this black spot is oblong and somewhat diffused. Moreover, the female in contrast to the male, has a prominently bulging abdomen. The ventral distance between the bases of the two pectoral fins in the female is significantly greater than the male. In the breeding season, the female exhibits a prominent bulge at the vent, resembling the genital papillae while in the male this structure is absent. Mature males ooze out white milt and mature females ooze out ova even at a gentle pressure at the abdomen during breeding season. In nature the eggs are scattered in open water at the onset of the rains without any nest. The male wraps itself in the female body, fertilizing the eggs as they are laid. Each time 200 colorless eggs are released until about 5000 numbers are laid. The fecundity varies from 5000-35000 nos. The eggs rise to the surface and float. The eggs hatch in 24 hrs and the fry are about 2-3mm long. They are free swimming within two days of hatching.

While in artificial breeding with pituitary or synthetic hormone, a single dose of injection for both the male and female spawning actively and courtship behavior starts after 6 hrs of injection. The water temperature to be maintained at 28° C + 1°C. Good spawning was reported with 8.0-12.0 mg/kg PG to female and 4.0 mg/kg of body weight to male administration in hapa 7-8 hours after second injection in the temperature of 27-30°C. Fertilized eggs float on the surface of the water. It takes 18-19 hrs for hatching after spawning and newly hatched larvae measure 1.9-2.0 mm in length without any movement. Yolk sac completely absorbs on third day after hatching and settles at the bottom. Egg custard, plankton and Artemia are supplied as artificial feed for those fry up to 20-25 days. The survivability varies from 70-75% (Datta, 2013).

Breeding of *Pangasius sutchi*

Pangasiidae (order: Siluriformes) is a family of fresh water catfish common to southern and south-eastern Asia. Catfish of the genus *Pangasius* have been cultured in earthen ponds and other waters on the Indo-Chinese Peninsula since ancient times. The most important of these is *Pangasius sutchi*, which is commonly known as shark catfish or Thai pangas and reaches weights of 3 kg and lengths of 150 cm within 2 years of birth.

The fertilized eggs are adhesive and spherical with a yellowish or greenish-brown egg capsule. The yolk sac is yellowish-brown in color and 1.20-1.80 mm in diameter. Nine hours post-fertilization, the first cleavage stage, embryonic shield, head, tail region, neural grooves and somites were evident. The incubation period ranges from 24-36 h at a temperature of 20-30°C. The newly hatched larvae are quite transparent and light yellowish in color with a body length of 2.98-3.10 mm. Eye pigments appear and the heart starts to work within 12-14 h of hatching. In 1-day-old pro-larvae, the mouth becomes well developed; barbules are elongated, prominent and look like tiny threads. The yolk sac is fairly well absorbed and the palatine teeth are fully developed during the 3 day pro-larval stage. At the end of 12 days of larval development, the stomach becomes functional and aerial respiration starts. After 2 weeks, the young fry is well-developed, and is of an adult appearance, that is, measuring up to 13.56 mm in length (Datta, 2013).

Breeding of *Ompok* spp

Ompok pabda & *O. bimaculatus* (Ham.) popularly known as 'butter cat fish' or pabda is a freshwater species native to India especially in North East States of India. Open beels/mauns connected with rivers are common habitats. Pabda has fine flesh with a soft meat texture, good taste and high nutritional value. Presently *Ompok spp* has been listed as an endangered fish species in India due to its decrease in abundance and restricted distribution. Causes of the decline are likely to be indiscriminate fishing during the breeding season, wide use of pesticide and insecticides from agricultural fields causing pollution and siltation in habitat. The fish attained maturity at the end of the first year. Males matured earlier than females, which became mature at 30 -40 g in weight. Fertilization is external and spawning occurs once a year during the monsoon season (June- August) with a peak in July. Fully ripe females and males were segregated and used in induced breeding. Females can be distinguished by a rounder, fuller abdomen, reddish vent colour and rounded genital papilla. Males have an elongated and pointed genital papilla. Used Ovaprim to promote induction of spawning. Ovaprim was applied at 1-1.5 ml/kg body weight for females and 0.5-1.0ml/kg body weight for males, applied in a single injection. Females were stripped for spawning 8-10 hours after hormone injection and the eggs were collected in a tray. Milt was obtained from males by surgically removing the testes, which were macerated to produce a suspension to be mixed with the eggs for fertilization. Eggs were subsequently washed thoroughly with clean water and transferred to a fibreglass/cement tank for hatching, with constant aeration (Datta, 2013).

Breeding of murrels

Among freshwater fish species, murrels (snakeheads) and catfishes secure the top rank economically. Murrels have long been commercially cultured in Thailand, Taiwan and Philippines. But fish farmers in India are not much familiar with murrel culture due to want of breeding, feeding and culture techniques. The common species are Therefore it is imperative to make murrel culture popular among fish farmers and unemployed youths for income generation. Females with soft and swollen bellies are suitable for breeding. However a gentle pressure on the belly for oozing of eggs could check prime maturation. Males are selected by external examination of genital papilla since they do not ooze milt by pressure. The fecundity of *Channa punctata* ranges from 2,200 in a specimen of 12.1 cm TL to 33,873 in a specimen of 22.3 cm TL. Murrels are induced to spawn by injecting natural (pituitary, human chorionic gonadotropin) or synthetic (ovaprim, ovotide) hormones intramuscularly. Among the hormones tested a synthetic product of SGnRH α marketed by Glaxo Ltd. is recommended at a single dose of 0.5 ml/ Kg. Each breeding set consists of one female and two males. Immediately after injection each breeding set is introduced into a

breeding tank (6m x 5m x1m) having sides pasted with cement and bottom filled with clay. Aquatic weeds (e.g. water hyacinth) are introduced into the breeding tank for hiding purposes. Spawning activities are observed after 6-10 hrs of hormone injection. Courtship behavior continues till the complete release of eggs and milt (24-30 hrs). Fertilization is external and the fertilized eggs are usually floating. An egg mass of about 6-14 cm diameter consists of 5000-10,000 eggs (diameter 1.2mm -1.5mm). The rate of fertilization ranges between 70-90%. Hatching takes place 24-30 hrs after fertilization and the hatchlings (2.8mm - 3.2mm in length) are guarded by the parents especially by the male parent. From a single spawning 4000-8000 hatchlings/female are obtained.

Comparative efficacy on induced breeding of cat fishes, murels and climbing perches by carp pituitary extract (CPE) & various synthetic hormones in India by various researchers is depicted in Table 1.

Table 1: Comparative study on induced breeding of cat fishes, murels and climbing perches with carp pituitary Extract (CPE) & synthetic hormones in India by various authors

Sl. No.	Species	Hormone	Dose	Fertilization %	Hatching %	References
1	<i>Channa punctatus</i>	Ovatide	0.4 ml	90.6	91.33	Marimuthu et al., 2009
2	<i>Clarias batrachus</i>	Ovaprim	1.0-2.0ml	70.6-72.8	60.7-55.3	Srivastava et al., 2012
3	<i>Clarias batrachus</i>	Ovatide	1.0ml	83	71	Sahoo et al., 2005
4	<i>Channa striatus</i>	LHRHa+ pimozide	40-60µg+5mg	75-84	complete	Haniffa et al., 2000
5	<i>Channa striatus</i>	Ovaprim	0.3-0.7 ml	95.3-98	complete	Haniffa et al., 2000
6	<i>Channa punctatus</i>	Ovaprim	0.3-0.5ml	73.5-75	50-65	Haniffa & Sridhar., 2002
7	<i>H. fossilis</i>	Ovaprim	0.3-0.7ml	70-75	50-60	Haniffa & Sridhar., 2002
8	<i>Pangasius sutchi</i>	CPE	1.5-9.0 mg	85-98	96	Chattopadhyay et al., 2002.
9	<i>Clarias batrachus</i>	Ovaprim	1.0-1.5ml	-	-	Sahoo et al., 2007
10	<i>Clarias batrachus</i>	Ovatide	1.0ml	82.33	55.35	Sharma et al 2010
11	<i>Clarias batrachus</i>	SGnRHa +domperidone	20-30µg 10-15µg	High	High	Sahoo et al., 2005
12	<i>Anabas testudineus</i>	Ovaprim	1.5 ml	100 %	High	Kuldeep kumar et al.,2010
13	<i>Horabagrus brachysoma</i>	Ovaprim	1.0	92.01	63.4	PadmaKumar et al.,2011
14	<i>H.brachysoma</i>	CPE	50-60 mg/kg	78.95	26.67	PadmaKumar et al.,2011
15	<i>Anabas testudineus</i>	Ovatide	0.3 ml	90.2	92.3	Singh et al., 2012

INDUCED BREEDING OF CAT FISHES AND MURRELS

Ompok bimaculatus is induced to spawn by a single intramuscular injection of ovaprim 0.5ml/kg. Spawning is observed 5-6 hr after the injection (Sridhar and Vijayakumar,1997). Successful spawning of *C.punctatus* is observed at 0.3 and 0.5ml/kg and 3000IU/kg body mass of HCG. For *H.fossilis* successful body spawning is observed at 0.3, 0.5 and 0.7ml/kg body mass for ovaprim and 1000, 2000 and 3000 IU/kg body mass for HCG (Haniffa, 2002). The endangered riverine catfish, *Pangasius pangasius* administration of 10mg/kg body weight of pituitary gland extracts demonstrated the best result in consideration to fertilization and hatching rates of eggs. Hatching of fertilized eggs occurred between 28 and 32 hours (Khan and Mollah, 2004; Sudha, 2012).

Haniffa et al. (2000) were injected natural hormones (pituitary extract and human chorionic gonadotropin) and synthetic hormones (luteinizing hormone releasing hormone analogue and ovaprim) to murrel *Channa striatus*. When compared to the LHRHa and ovaprim, the latency period was long in pituitary- (24 h) and HCG-injected (26 h) fish. In the pituitary injected *C. striatus* the percentage of fertilization was the lowest (60-68%) but the duration of hatching was longest (39-43 h) followed by HCG- (36-38h), LHRHa- (34-36 h) and ovaprim-injected (21-23 h) individuals. In terms of fertilization (95-98%) and hatching, ovaprim yielded better results. Ova reached the highest diameter (1.34-1.45 mm) in *C.striatus* injected with ovaprim, followed by HCG (1.22-1.30 mm) and pituitary (1.21-1.27 mm). The lowest ova diameter (1.07-1.09 mm) was observed in *C. striatus* injected with LHRHa.

The effect of luteinizing hormone-releasing hormone analogue (LHRHa), pimozide (PIM) and ovaprim on oocyte maturation, ovulation and spawning of *Heteropneustes fossilis* has been evaluated by Nayak et al., (2001). Both LHRHa and pimozide when tested alone, failed to evoke any ovulation response, although both drugs resulted in the advancement of oocyte maturation in catfish as evidenced from germinal vesicle migration. On the other hand LHRHa + PIM (0.05 µg + 5 µg/g/ body weight respectively) when administered in combination intraperitoneally, caused high rate of ovulation and produced an average of 10±2.3 g eggs with high rate of hatching (93.5 ± 1.42%) and high yield of normal larvae (87.3±3.3 %). A single dose (0.6 - 0.8 ml/kg) of ovaprim injected intramuscularly resulted in an average production of 13.75 ± 2.9 g eggs having 96.3± 1.7% hatchability and yielded 92.5 ± 1.5% of normal larvae. The latency period (interval between the time of injection and spawning) ranged between 15-18 hrs

in LHRHa + PIM and 10-12 hrs in ovaprim treated fish. The eggs produced after induced spawning were viable and fertilized *in vitro* using homogenized testes and produced higher yield of normal larvae, minimizing deformed and crawled larvae.

Haniffa and Sridhar (2002) reported Induced spawning of the spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) was successfully carried out using ovaprim and human chorionic gonadotropin (HCG). Breeders were administered a single intramuscular injection of the hormones at varying dosages. Fecundity in *C. punctatus* was 3273 ± 75 for ovaprim and 1253 ± 126 for HCG, whereas in *H. fossilis* it was 6692 ± 790 for ovaprim and 82922 ± 5432 for HCG. Successful spawning of *C. punctatus* was observed at 0.3 and 0.5 ml/kg body mass for ovaprim and at 2000 and 3000 IU/kg body mass for HCG. For *H. fossilis* successful spawning was observed at 0.3, 0.5 and 0.7 ml/ kg body mass for ovaprim and 1000, 2000, and 3000 IU/kg body mass for HCG. Sahoo *et al.* (2004) and Rath *et al.* (1995) reported that induced breeding of *Clarias batrachus* and carps during premonsoon season might cause ovulation of immature eggs and might lead to the abnormal development of the larvae. But Teji and John Thomas (2006) reported the percentage of malformed embryos was high during monsoon period, especially in detritivorous, bottom living, soft-bodied catfishes. Normal ovum maturation and ovulation is controlled by episodic release of gonadotrophins. The surge of gonadotrophic hormones that follows the ovaprim injection might be one of the reasons for the observed deformities in fish larvae.

Marimuthu and Haniffa (2007) elucidated the embryonic and larval development of snakehead *Channa striatus* from fertilization until metamorphosis. The snakehead was successfully bred by injecting ovaprim, a synthetic hormone (0.5 mL/kg body weight). Spawning took place 24-26 hrs after the hormone injection. The fertilized eggs were floating, non-adhesive and straw yellow in color. The average diameter of fertilized eggs ranged from 1.20-1.40 mm. Incubation periods was about 23-24 hr at a temperature of $29 \pm 1^\circ\text{C}$. The percentage of hatching varied from 80-85. The newly hatched larva was 3.4 ± 0.2 mm in length. The yolk absorption was completed within three days after hatching. The larvae metamorphosed into juveniles within 20 days after hatching

A study was conducted by Sharma *et al* (2010) during June 2007 to evaluate Ovatide doses (0.6, 0.8 and 1.0 ml/kg body weight of female) on breeding performance of *Clarias batrachus* in the sub tropical region of Hisar. The breeding performance was judged on the basis of the total weight of stripped eggs, net fecundity, fertilization, hatching and survival. To judge the egg quality, the per cent fertilization, hatching and survival of fry were considered. The results indicated that the total weight of stripped eggs and spawning fecundity were the highest ($p < 0.05$) when females were injected 1 ml of Ovatide per kg body weight (BW) compared to those injected with other dose levels. The lowest stripping response was observed with injection of 0.6 ml Ovatide per kg BW of female brood fish. At the 1 ml dose, the percentages of total fertilized egg and hatching were 82.33 and 55.35% respectively, which were the highest ($p < 0.05$) among all treatments. The net survival of fry was found to be 98.52% at 1 ml Ovatide per kg BW. Therefore, it has been recommended that one ml of Ovatide per kg BW of female brood fish was found optimum among the three experimental doses for best breeding performance and egg quality in *Clarias batrachus*.

Marimuthu *et al.* (2001) were reported spawning and parental behavior in murrels *Channa punctatus* and *C. striatus*. Brood fishes ranging from 600 to 800g of *C. striatus* and 50 to 90g of *C. punctatus* were injected intramuscularly with a single dose of pituitary extract (50 mg/kg body weight) and HCG (2 IU/g body weight) respectively. Immediately after hormone injection, the breeding sets (one female and two males) were introduced into the breeding tanks. Aquatic macrophytes like *Hydrilla verticillata* and *Eichhornia crassipes* were introduced into the breeding tanks for hiding purpose. Spawning behaviour was observed at one hour interval after the hormone injection until egg laying. Spawning activities of *C. striatus* and *C. punctatus* were first noticed after 6 and 4 h of the hormone injection respectively. Spawning was preceded by active male movement below the female in opposite direction. Then the mating pair made a slow upward and downward movement of approximately 10 to 20 cm within the water column. After 12 h, the mating pair jumped frequently above the water column to a height of 30 to 90 cm and occasionally out of the breeding tank. In both the species, the male was more actively involved in the courtship and was found to hit frequently the female snout and vent, which culminated in the release of gametes. Courtship behavior continued till the complete release of gametes. (30 h for *C. striatus* and 28 h for *C. punctatus*). The unpaired male was driven out by the active male when it disturbed the mating pair. The impaired male was passive and idles at a corner of the breeding compartment. The fertilized eggs are usually buoyant in nature and adhered to each other forming an egg mass of 6-14 cm diameter (containing 2,500-4,000 eggs). The unfertilized eggs were not adhesive and were found scattered in the tank. In both the species, the male guarded the fertilized eggs. After hatching, the

male moved around hatchlings and ventilated them with its pectoral fins. It was always aggressive and kept the young ones under vigil.

No spawning behaviour or performance was observed in control group. Partial spawning was observed with the Ovotide dose of 0.2 mL · kg⁻¹ BW, and complete spawning was noticed in the medium Ovotide dose (0.4 mL kg⁻¹ BW), and the higher dose (0.6 mL · kg⁻¹ BW) administered fish. The highest total spawning fecundity ($P < 0.05$) was recorded when the females were injected with 0.4 mL of Ovotide · kg⁻¹ BW than those injected with other doses. The latency period and the number of spawned eggs were ranged from 25 to 31 h and from 1080 to 5814, respectively. The highest fertilization- (90.6%) and hatching (91.33%) rates were also observed at the medium dose ($P < 0.05$). With regard to hatching rates, no significant difference was noticed between the medium- and higher doses of Ovotide-treated groups. The synthetic gonadotropin-releasing hormone with a dopamine antagonist at the dose of 0.4mL kg⁻¹ BW could be used as an appropriate stimulating agent for successful spawning and induced breeding of *C. punctatus* under captive conditions (Marimuthu *et al.*, 2009).

Effects of low doses of salmon gonadotropin (SG-G100) and three steroids-17 α ,20 β -dihydroxyprogesterone (17 α ,20 β -diOHprog), deoxycorticosterone (DOC) and progesterone -individually, or combinations of steroid with SG-G100, on ovulation and hatching in *Clarias batrachus* (L.) were investigated. None of the steroids at any of the three different dose levels (1 μ g, 1.5 μ g/g and 2 μ g/g BW) could induce ovulation when injected alone. SG-G100 at a dose level of 10 μ g/g BW was not effective but at the dose level of 15 μ g/g BW it could induce ovulation. All the three steroids at their lowest doses (1 μ g/g BW) when injected in combination with SG-G100 (10 μ g/g BW) were significantly effective in inducing ovulation. When hatching percentage was taken into account, 17 α , 20 β -diOHprog in combination with SG-G100 was found to be the most effective combination in comparison with other treatments (Haider and Rao, 1994).

Sahoo *et al.*, (2005) conducted experiments to evaluate Ovotide doses (0.5, 1.0, 1.5 and 2.0 ml·kg body weight of female) on breeding performance and egg quality of *Clarias batrachus*. The results indicated that the total weight of stripped eggs and spawning fecundity were the highest ($p < 0.05$) when females were injected 1 ml of Ovotide per kg body weight (BW) compared to those injected with other dose levels. The lowest stripping response was observed with injection of 0.5 ml Ovotide per kg BW. There was difficulty in stripping at 0.5, 1.5 and 2.0 ml doses, but at 1 ml dose, it was smooth. At the 1 ml dose, the percentages of fertilization and hatching were 83 and 71 % respectively, which were the highest ($p < 0.05$) among all treatments. Increasing Ovotide doses above 1 ml led to over ripening of ova, which resulted in increased percentage of deformed larvae. More normal larvae were produced from the females when injected with 1 ml dose. One ml of Ovotide per kg body weight was found optimum for best breeding performance and egg quality in *C. batrachus*.

Three freshwater fishes, namely *Heteropneustes fossilis* (stinging catfish), *Anabas testudineus* (climbing perch), *Mystus vittatus* (striped dwarf catfish) were induced bred and morphological studies of the larvae were carried out by Teji and John Thomas (2006). Morphological and behavioral abnormalities were noticed among larvae produced through induced breeding techniques in all the three species. Morphological abnormalities were seen in head, trunk and tail region of the larvae. Under-developed head, deformed trunk, enlarged yolk sac, underdeveloped barbel, curved tail and vertebral abnormalities were observed. Tunicate larvae (larvae with undetermined growth) were common in these species. Induced spawning of catfish, *Clarias batrachus*, was attempted by Yadav *et al.*, (2011) using different doses of ovotide and ovaprim at varying latency period (interval between the time of injection and spawning). In both the sGnRH-based drugs, decreased doses with increased latency period gave better results of fertilization and hatching. Preparatory dose of ovaprim (male 0.1 ml/kg; female 0.5 ml/kg) administered intramuscularly 45 days prior to spawning for gonadal maturity resulted in higher rate of fertilization and hatching success. Optimum doses of ovaprim and ovotide were found to be 0.8-1.0 and 0.6-1.0 ml/kg body weight with latency period between 14-16 hr.

The catfish *Ompok pabda*, was successfully bred during 2008 by inducing with ovotide at two different doses (0.4 and 0.6 ml/kg of body weight of female) in the live gene bank facility (LGBF), Guwahati, Assam. The fish spawned 8-10 hours after single dose of injection. The spawning time, fertilization rate, hatching rate and survival rate were quantified in each set of -1breeding experiment. The latency period was 6-8 hours with 0.5 ml, where it was 8-10 hours with the dose of 0.4 ml kg⁻¹ of body weight. All the female spawned successfully and the average rate of fertilization (75.5 %) and hatching (60.5%) was higher with dose @ 0.6 ml kg⁻¹, while lower fertilization (65.1%) and hatching rate (45%) was observed @ 0.3 ml Kg⁻¹ of body weight. The fecundity ranged from 80-130 eggs gm

body weight. Eggs hatched 12-14 hours after spawning at water temperature of 28-32°C. The mean egg diameter was 0.95 ± 0.15 mm (Purkayasta et al., 2012).

Sahoo et al. (2005) demonstrated that *C. batrachus* could be successfully induced to spawn with an injection of sGnRHa in combination with domperidone. Administration of 20–30 μg sGnRHa kg^{-1} body weight of female and stripping at 14 and 17 hr post injection resulted in the highest rate of fertilization, hatching and normal larval production. This information would be of value for commercial catfish hatcheries, in order to ensure collection of the maximum quantity and optimum quality of eggs. Figure 2a showing injecting hormones and figure 2b showing stripping of eggs in cat fishes.



Fig. 2a: Intramuscular injection given to female brood fish *Clarias batrachus* for induced breeding. (Source: Sharma et al., 2010). Fig. 2b: Stripping of eggs in cat fish

Ovaprim has been successfully employed for induced spawning of fishes in a number of commercially important food as well as ornamental and threatened species [Lakara et al., 1996; Pandey et al., 1998, 1999, Sridhar et al., 1998; Nayak et al., 2001; Sarkar et al., 2006; Rath et al., 2007; Hill et al., 2009]. The product has been reported to be an efficient inducing agent for oocyte maturation and ovulation in *C. batrachus*. The latency period of 16-18 hours after the injection of Ovaprim (dose 1-2 ml Kg^{-1} bw to female and 0.5-1.0 ml Kg^{-1} bw to male) to subjected fishes was found suitable for the ovulation of this species. Similar findings were also reported by Sahoo et al. [2005] in the same species while using sGnRHa in combination with domperidone (14 to 23 hours). However according to Sahoo et al. [2005], the suitable latency period for final maturation of ova is also dose dependent when using sGnRHa and domperidone combinations on spawning performances and deduced that latency period of 14-17 hours and dose of 20 μg sGnRHa along with 10 mg domperidone and 30 μg of sGnRHa & 15 mg domperidone per kg of female was found to be suitable for best spawning and larval production (Srivastava et al., 2012).

A study was conducted by Srivastava et al. (2012) to observe the breeding and larval rearing of Asian Catfish, *Clarias batrachus* fed with live and/or artificial feed for 21 days in an indoor hatchery. The brooders of *C. batrachus* (Av. wt of female 160 ± 10.5 g; Av. wt of male 120 ± 6.75 g) were procured from outside ponds and stocked in a pond near the experiment site 2-months prior to spawning. The fishes were successfully induced bred using ovaprim @ 1.0–2.0 ml/kg body weight (bw) to females and 0.5–1.0 ml/kg bw to males. Fertilization, hatching and survival percentages at spawn stage were respectively recorded 70.6 - 72.8, 60.7 - 55.3 and 54.3 - 56.2. After yolk-sac absorption, fry of three age groups 7, 14 and 21 days were subjected to feed trial using *Artemia nauplii* followed by laboratory made feed for 21 days.

Nayak et al. (2000) reported the plasma steroid profiles during oocyte maturation and LHRHa pimoziide induced ovulation in the Asian catfish, *C. batrachus* and opined that the levels of estradiol-17 β and estrone rapidly increased reaching a peak during the vitellogenic phase, while a decline was observed during the spawning phase.

The results of Sharma *et al.* (2010) indicated that the total weight of stripped eggs and spawning fecundity were the highest ($p < 0.05$) when females *C. batrachus* were injected 1 ml of Ovatide per kg body weight (BW) compared to those injected with other dose levels. The lowest stripping response was observed with injection of 0.6 ml Ovatide per kg BW of female brood fish. At the 1 ml dose, the percentages of total fertilized egg and hatching were 82.33 and 55.35% respectively, which were the highest ($p < 0.05$) among all treatments. The net survival of fry was found to be 98.52% at 1 ml Ovatide per kg BW. They recommended that one ml of Ovatide per kg BW of female brood fish was found optimum among the three experimental doses for best breeding performance and egg quality in *Clarias batrachus*.

Many researchers who have worked on the breeding of *Pangasius sutchi*. While Meenakaran (1986) has reported successful induced breeding of *P. pangasius* with a mixture of carp pituitary extract and HCG, Gupta *et al.* (1998) have successfully bred the same species with fish pituitary extract alone. Mishra *et al.* (2001) conducted a study for induced spawning of cat fish, *Mystus cavasius* by administration of ovatide.

Breeding of channel cat fish by administration of LH-RHA was reported Busch and Steeby (1990). Rahman, Akhter, and Halder (1993) induced spawning with carp pituitary extract and human chorionic gonadotropin at dosages of 8 mg and 3.000 IU per kg body weight of female, respectively. Successful induction of *P. sutchi* using carp pituitary extract was also reported by Chattopadhyay and Mazumder (2002), with a initial dose of 2–3 mg/kg body weight of fish followed by a resolving dose of 9.0 or 10.0 mg/kg of body weight for females. Percentage of fertilization was 77% and 90%, and hatching rates were 87% and 92%, respectively.

Chand *et al.* (2011) was conducted breeding experiment on *Pangasius sutchi* from July 2008 to September 2008 in a hatchery in West Bengal, India, using three different inducing agents, each injected at two different dosages: carp pituitary extract (CPE) and two synthetic hormones, Ovaprim and Ovatide. The fertilization rate was 82% to 91%. Hatching took place within 34 to 36 hours from the time of fertilization, with a hatching rate 73% to 79%. The response to spawning with CPE was best with Ovatide and Ovaprim in terms of percentage of fertilization, high hatching rate, and ease of stripping.

Breeding of catfish *Horabagrus brachysoma* was successfully accomplished in captive condition by PadmaKumar *et al.* (2011) as this fish popularly known as golden catfish, is an endemic species in the Western Ghat rivers of peninsular India. Induced fishes responded well and spawned naturally in 8–14 h and the fertilized eggs hatched in 22–29 h. Artificial fertilization by stripping was also successful when carried out within 1–2 h of the latency period after hormonal manipulation. Seed rearing was successfully accomplished in earthen ponds. This opened up opportunities for mass production of seeds of this species for restoration, stocking and conservation. Consequent to introduction of hatchery reared seeds produced by these techniques into natural waters, the species is now on a comeback trail. Induced ovulation was successful in 92% of the trials using ovaprim and 79% with CPE (Table 1). Hatching percentage was also high in ovaprim induced trials as compared to CPE. However, there was enormous variability in the latency response of females even at similar temperature regime and was observed to range from 8–14.30 h with ovaprim and 8–12 h with CPE.

Sarma *et al.* (2012) has conducted to provide detailed information about the embryonic and larval development of *Ompok pabo* during December 2007 to November, 2010. Artificial breeding of *Ompok pabo* (Hamilton-Buchanan) were carried out at Goalpara College, Assam, India. They examined fertilized eggs till the end of larval developmental period to each and every stage of embryonic and larval development.

INDUCED BREEDING OF CLIMBING PERCH

Although *Anabas testudineus* are believed to be 'difficult to breed' in laboratory conditions, reports of success with the induced breeding of this species are also available, though scanty (Banerjee and Prasad, 1974b; Khan and Mukhopadhyay, 1975a; Banerjee and Thakur, 1981 and Doolgindachabaporn, 1994).

Comparative efficacy of pituitary gland and Ovaprim as inducing agent was studied in *Clarias batrachus* by Basu *et al.*, (2000). They opined that Ovaprim yielded better result with higher percentage of fertilization and hatching. Induction with Ovaprim yielded 80% fertilization of eggs and 60% of their hatching, whereas induction with pituitary gland extract resulted in 45% fertilization and 25% hatching (Basu *et al.*, 2000). However, percentage of fertilization achieved was 73.11% and percentage of hatching was 92.06% in breeding experiments with Ovaprim in induced breeding of *Anabas*.

Induced breeding of Murrel, *Channa striatus* with various inducing agents was reported by Francis *et al.* (2000). They reported that among the different hormones used, Ovaprim showed better performance in terms of higher fertilization rate (93%) and lower latency period (21 hrs.). Latency period on Ovaprim induction in the present experiments was comparatively lower in *Anabas* (10-12 hrs). A similar spawning time (10-14 hrs) was reported by Nandeesh *et al.* (1990b) when *Labeo rohita* was induced bred with Ovaprim. Among the hormones studied by Nandeesh *et al.* (1993), the highest percentage of fertilization (93%) was observed in Ovaprim induced fish. The hormonal dose of Ovaprim recommended for carp is 0.3 ml to 0.4 ml kg⁻¹ (Nandeesh *et al.*, 1993). However for *Anabas* as per Bhattacharyya and Homechaudhuri (2009) a much higher dose of 2ml kg⁻¹ body weight was required. Nandeesh *et al.* (1990b) also reported that *Labeo rohita* responded to higher dose of Ovaprim and presumed that this was because dopamine activity is higher in rohu. Peter *et al.* (1986) had reviewed dopamine activity in fish species and indicated that it may vary considerably between species. These supporting reports may suggest that higher dopamine activity of *Anabas* requires higher dose of Ovaprim. Unlike the carp pituitary stimulation, both males and females of *Anabas* were injected with Ovaprim only once in the present experiment. This is supported by Nandeesh *et al.* (1990b) who reported positive response of both male and female *Labeo rohita* to a single simultaneous injection of Ovaprim. In fact single simultaneous injection is very significant from the point of view of commercial fish seed production, as it saves a considerable amount of time and avoids excessive handling of brood fish. Single injection of another synthetic fish hormone, ovotide (GnRHa and a dopamine antagonist) resulted in successful induction of spawning in *Heteropneustes fossilis* (Marimuthu *et al.*, 2000). The applicability of Linpe method on induced breeding of *Anabas* has not been much exploited. Halder *et al.* (1991) elucidated the efficacy of murrel GnRH (in the 'Linpe' method with Ca⁺⁺) in inducing the spawning of *Anabas testudineus*. Results indicated that use of GnRH in the Linpe method with Ca⁺⁺ is satisfactory for induced breeding and final maturation of *Anabas*. Ghosh *et al.* (1999) reported successful maturation and ovulation of *Anabas testudineus* when injected by GnRH from *Channa punctatus*. The minimal effective standard dosage of 37.5 mg 100g⁻¹ body weight was administered to the female fish in two instalments. An additional dose of 25 mg 100g⁻¹ body weight of GnRH to the female fish was required for complete maturation and ovulation. The male fish on the other hand, received single injection of GnRH (25 mg 100g⁻¹ body weight) at the time of second injection to the female. According to several authors (Nandeesh *et al.*, 1993; Haniffa and Sridhar, 2002), ovaprim was the most potent in induced breeding of fish. However, in spite of good breeding results, the present experimental results indicated mortality of brooders from stress due to treatment of ovaprim containing Domperidone which may decrease haemoglobin in fish blood as in mammalian systems (Bhattacharyya and Homechaudhuri, 2009).

Induced breeding experiment on climbing perch, *Anabas testudineus* was conducted by Sarkar *et al.* (2005) using synthetic hormone Wova-FH in the intensity level of 0.1, 0.2 and 0.3 mL kg⁻¹ of body weight respectively. The brooders were injected one time and left to spawn in the spawning hapa in the sex ratio between male and female as 2:1. It was found that at all the intensity level hormone Wova-FH could enhance the fishes to breed and lay eggs whereas no breeding was observed in control set. The spawning time, quantity of the brooder spawn, fertilization rate, hatching rate and survival rate were quantified in each set of experiment. The egg output/female was significantly higher in 0.3 mL in comparison with 0.1 and 0.2 mL kg⁻¹ of body weight. The statistical analysis showed significant ($P \leq 0.05$) effect between hormone dose on fertilization rate, egg output and hatching rate. Their experiment suggests that Wova-FH at the dose of 0.3 mL kg⁻¹ body weight of fish is more effective which might be considered for raising captive population (Table 1).

The dosage of other synthetic hormones like Ovaprim in tropical air breathing fishes has been experimented by several authors. The doses of Ovaprim selected for induced spawning of murrels (*Channa* spp.) ranged from 0.3 to 0.6 mL kg⁻¹ body weight (Haniffa, Merlin & Shaik Mohamed 2000). Singh, Ram and Singh (2002) reported significant increase in ovulated eggs per fish in *Heteropneustes fossilis* injected at the dose of 0.2 mL kg⁻¹ of body weight after ovaprim treatment. However, no reports are available in standardizing the doses of Wova-FH in most of the freshwater food fishes. Longer latency period in low dose of synthetic hormone Ovotide was reported by Pandey, Koteeswaran and Singh (2002). The latency period of Ovaprim induced air breathing fishes are 18 h for *Channa punctatus* and *H. fossilis* (Haniffa *et al.* 2000). Pandey *et al.* (2002) reported varied interspawning period between 8 and 15 h in *H. fossilis* injected in the doses of 0.3–1.0 mL kg⁻¹ of body weight of synthetic hormone Ovotide.

Khan and Mukhopadhyay (1972) observed fecundity ranging from 10 002 to 36 477 in size range of 99–169 mm. However, Benerjee and Prasad (1974) reported the fecundity of 4588–34 993 in Bihar region in the fish size range 73–182 mm per 8.4–100.2 g. The fecundity data recorded at the Assam centre is 3812–28 490 eggs in the fish size

range of 74–138 mm per 7–57 g (Central Inland Fisheries Research Institute workshop Report (CIFRI 1982)). Chanchal, Pandey and Nath (1978) reported minimum 3481 to maximum 42 564 in the fish weight range of 9.0–53.1 g. Banerjee and Thakur (1981) reported shedding of 2000–13 000 eggs in seven sets of induced bred *A. testudineus* (24.8–40.1 g) in glass aquaria.

Kuldeep Kumar et al. (2010) conducted induced spawning experiments on *Anabas testudineus* during pre-monsoon and monsoon months showed that the fish could be induced for spawning from February through August. However, in spite of higher breeding response (80 - 100%) and egg production (295.7- 374.2 g-1) recorded during March to June, the higher larvae production (186.0 - 233.8 g-1) could be obtained only during May to July. Their study revealed that Ovaprim at the rate of 1.5 ml kg-1 body weight efficiently induced *Anabas* female for early and extended normal spawning. The period between May and July has been found most suitable for induced spawning and spawn production of captive reared *Anabas*.

Anabas testudineus was induced using 0.1, 0.2, or 0.3 ml/kg body weight of the synthetic hormone Ovotide and compared with fish injected with 30 mg carp pituitary extract (CPE) per kg body weight or 0.5 ml saline (control). Male and female brooders were injected once with an identical dose and left to spawn in tubs at a ratio of 2:1. No breeding occurred in the saline-injected control fish. There was partial spawning in the 0.1 and 0.2 Ovotide treatments and complete spawning in fish injected with 0.3 Ovotide. Spawning and number of eggs in fish injected with 0.3 Ovotide did not statistically differ from results in fish injected with CPE ($p \geq 0.05$). They suggested that Ovotide at 0.3 ml/kg body weight is optimal for seed production of climbing perch held in captivity and can be used for species restoration (Singh et al., 2012).

Growing fish in regular laboratory condition and releasing to the nature brings less viability in nature. Hence it's recommended to improve learning capacity and increased viability in nature by providing structured environment while rearing in laboratory. Practical pre-release training protocol that may be applied at the hatchery level in fishes to enhance survival ability hatchery reared fish in nature. Such a kind of pre-release hatchery training protocol, if applied in the restocking attempts of threatened fishes (as per IUCN red data book) can augment the success of conservation programs (Sheenaja, 2011).

CONCLUSION

Many species of fish will not readily reproduce under certain culture conditions. Others will but not necessarily when the farmer desires. In these cases, induction of spawning can be of great value. Two techniques are commonly used, sometimes in conjunction with one another. The first is manipulation of the culture environment to mimic some important quality in the fish's natural environment. The second is injection of hormones to stimulate spawning. The hormones may be natural hormones taken from fish or other animals, genetically engineered from bacteria or synthetic analogs of naturally occurring hormones. Methods vary from species to species and situation to situation. However, at least two generalizations can be drawn. First, brooders are very vulnerable to rough handling. Care should always be used to avoid damaging these valuable animals. Second, a fish that does not have mature gametes will not produce viable eggs or sperm no matter how many times it is injected with hormones. Ripeness is the result of environmental factors working over a period of time leading to maturation of the gonads and production of viable eggs. Many procedures have been developed for inducing fish to undergo the last steps of spawning. Farmers should thoroughly research the procedures that have been developed for their species of fish through experimentation and select those that best suit the circumstances. In addition, once the fish have spawned, there are many techniques involved in incubating and caring for the eggs and caring for the hatched fry. These too must be thoroughly researched.

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