Fish seed production and hatchery management: A Review

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Abstract: Fish is the most important source of animal protein food for the human population and the potential of fish culture production from ponds, floating cages and various other small water bodies in India is great. The supply of quality fish seed is a key factor to the expansion of fish farming. Fish seed demand at present is strong, unsatisfied and expected to expand and fish seed business in India is profitable. This review, based on existing literature and field survey, provides clear understanding of the current status of freshwater fish seed resources for aquaculture development in Uttarakhand. The review includes information relating to: (i) fish seed resources and supply, (ii) fish seed production facilities and seed technology, (iii) fish seed management and seed quality, (iv) Induced breeding. Based on the above information, the emerging challenges in Uttarakhand freshwater fish seed sector are identified as: (1) broodstock management and species identification, (2) human capacity and training needs, (3) monitoring and evaluation, and indicators.
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INTRODUCTION

The production of marketable fish begins with stocking of fry or juveniles in to a rearing environment that assured optimum and rapid growth to allow harvest in shortest possible time. The fish farmer has to obtained adequate number of young fish to meet his production goal. These fish can come from wild capture. However there is little or no guarantee that adequate number can be captured and stocked in the time corresponding to optimum production conditions. The fish farmer than naturally turns to other means of obtaining his stock. By stimulation of conditions necessary for reproduction of fish, the farmer can spawn is only the beginning, however, the must hatch and these reared successfully to fry stage. The water quality parameters, feed for proper nutritive composition and particle size, resistance to diseases are some major factors which has to be taken in to account. A good appreciation of all these factors is needed for successful production of fish. Through artificial propagation the farmer can select, for desirable characteristics such as fast growth and resistance to diseases.

MATERIALS AND METHODS

Study Area: The government fish seed production centre at Baintwali Mandi Dhakrani (Uttarakhand) was established in the year 1972-1973 under crash scheme. The farm was established for Mahseer hatchery. The climatic conditions are very much favourable for Mahseer. This farm also involved in breeding of Mahseer especially in confined water conditions. For many years many scientists from reputed institutions like visited the farm and worked for Mahseer breeding and get some success also. This farm located near the Yamuna River. The breeding of common carp is also performed at this farm and fry are distributed to the farmers. The farm actively involved in the conservation of endangered fish Mahseer by stocking Mahseer fry in the nearby Rivers. The different species of fishes of composite culture are cultured in the centre are given below:

- Rohu
- Catla
- Nain
- Common carp
- Golden Mahseer

Brood Stock Management: The primary requirement of carp breeding is an adequate stock of good breeders. The excellent brood fish culture is the foremost essential material basis and a key factor for successful artificial propagation.

The brood pond: Ponds of size (0.5-1.0 meter) and depth (1.5-2.0 meter), preferably rectangular in shape with cemented uniform side slope (2:1/3:2) and flat bottom of compact clay – loam soil with bottom silt not exceeding 10-15cm and controlled pipe system for complete drainage or proper arrangements inlet and outlet of water and when required, were selected. The pond should be well protected from flood and drought and its surrounding should be get sufficient wind action and exposure to bright day light for 6-8 hrs in a day for at least 2-3 months from January to February onwards. This quantum of exposure and a moderate range of water temperature (27-32^{0}C) seem to be

optimum and quite inductive for rapid growth of gonad of fish.

Characteristics of ripe brood fish and their selection for stocking: Proper selection of brood fish, particularly females, is an important exercise in induced breeding. After using a catheter eggs of uniform shape and size suggest that the female is quite ripe. In grass and silver carps, the good ovarian eggs are mostly copper red and blue, respectively, though the colour varies in various ecological zones and also with age of female. Healthy breeders of catla, rohu, mrigal, grass carp and common carp of 2-4 years age group and 1-5 weight range are normally selected. However, catla spawners of 3-4 years and milters of 2-3 years are preferable.

Stocking Rate: The male and female fishes are desirable from separate stock source. Stoking of fish is normally initiated during October when their feeding intensity is, quite high. Sex composition of the breeders, is normally maintained as 1:1 the sex wise stocking of males and females in the two separate segregation tanks is not an essential measure, but it increase the affinity of mating of the pair during breeding experiment. Stocking is done 1500-2000kg/ha with species composition of 40 surface-feeder (catla 30-20 and silver carp 10-20) + 30 column feeder (rohu 20 and grass carp 10) + 30 bottom feeder (mrigal 20 and common carp 10).

Caring the Brooders: Fortnightly or weekly seining of the pond should be done to provide exercise to the breeders and also for checking general health and gonadal maturation. Frequent replacing of breeders from one pond to another is very much effective for properly utilizing the 'main food' available in that particular pond where the breeders are likely to be transferred, and also for gradation of maturity –stages. Care should be taken during netting. As the breeders may be gilled and damaged at netting, a small meshed net, preferably below 1.0 cm knot to knot, the fish. In case of infection, the fishes areb given a bath in 10ppm of potassium paramagnet or 1ppm of acriflavin (an orange brown coloured antiseptic) for half an hour and released back in to the pond.

Manuring and maintenance of water quality in brood – stocking pond: Initial manuring of brood pond is done 3 weeks prior to stocking of fish with cattle dung at 20kg/ha or compost manure (prepared) from land/aquatic weeds and farm animal dropping at 1:1 ratio, with 1% quick lime) at 10,000kg/ha. A prophylactic quick lime treatment at 200kg/ha is given to the pond 7 days before the initial fertilization. Monthly dose of raw cattle dung at 2000kg/ha is also followed. Constant application of manures and use of heavy supplementary feed causes repressive factor and this might inhibit the maturation to the point of ovulation and spawning of the fishes in captivity. Frequent change (month/fortnightly/ weekly) depending upon the state of contamination, of heavily metabolite loaded old-water is thus essentially required with the fresh ground (after proper oxygenation)/ canal/freshly accumulated rain water at the volume of 1-8th to 4th of the pond water in each operation. The additions of freshwater increase the dissolved oxygen level and improve the water quality. The lowering and the raising of water level in the pond by discharging old water and adding the cool ground water is so regarded as to maintain the optimum temperature. The sudden lowering of temperature associated with supply of fresh water is highly beneficial for maintaining good health of the breeders and quickening development of their gonads. The change of water also reduces the specific conductivity of the pond water on one hand, and increases the free electrolyzed ions.

Supplementary feedings: Though natural feed (phytoplankton) develops in the stocking pond yet well balanced supplementary feeding is given daily to breeders at a fixed schedule. A complete feed is proportionate vegetables, animal protein, carbohydrate and fat. The mixture prepared from half cooked rice and pulse (1:1, 50%) + oil cake (20%) + cattle dung (20%) + fish meal/silk worm pupae/or cooked slaughter house refuse (10%), is fed to catla, rohu, mrigal and common carp at 3-4% of their body weight daily from post-spawning months onwards and 1-2% for about one month before spawning and family with a stoppage of feedings for a week before the actual breeding. Fresh aquatic vegetation (preferably, Hydrilla, Vallisneria, Najas, Utricularia, soft young leaves of eichhornia) and napier grass are provide to grass carp 1.5-2.0 times of fish weight daily from post spawning months and till one week before its spawning is initiated, when the feeding is discontinued. Thick plankton concentration of Spriulina, Oscillatoria, and chlorella is cultured and feed to silver carp. Feeding with powered cotton-seed, containing vitamin-E shows better results in the gonadal maturity than feeding with mustard oil cake or ice bran. According to some workers Catla attains sexual maturity and responds well to hypophysation when feed on rice bran alone. However, the breeders well fed with balanced supplementary diet ensure better results.

Rate of Feedings: Initial higher rate of feedings is accordance with their high feeding intensity during post-spawning periods and found to be quite conductive to the quick development of general health and gonads. The subsequent feedings at a lower rate is in correlation with their decreasing feeding intensity due to the progress of their gonadal maturity. The complete stoppage of feeding about a week before spawning is for proper de-fattening and physical

tuning of the breeders in view of better response in the spawning activity. Excess feeding may result in heavy accumulation of mesenteric fat inhibiting the process of smooth ovulation.

Use of antibiotics during transport: The induction of infection disease and parasites in the fish consignment is a possibility that calls for adoption of prophylactic measures involving the use of antiseptic, antibiotics and germicidal chemicals. For the purpose of fish transport only short duration, chemical bath to fish prior to transportation may be desirable. An instant bath before transport may help in curbing further transmission of infections in fish consignments. A few of the more commonly used chemicals for fish bath are acriflavin, methylene blue, copper sulphate, potassium permanganate, chloromycetin, common salt etc.

Favourable conditions for induced spawning:

1. Breeding takes place both in clear and turbid water.
2. Accumulated fresh rain water promotes successful spawning.
3. Cool, rainy days are more conductive to spawning.
4. Flowing river water gives better results especially on warmer days, as the river water is cooler than pond water.
5. Spawning occurs when the temperature of water ranges from 24-31^{0}C, the most suitable temperature being around 27^{0}C in the plains of India.
6. That fishes breed at a fairy wide range of pH and dissolved oxygen content of water.
7. Breeding takes place both at day and night.

INDUCED BREEDING

For induced breeding, hypophysation is the first and foremost step requires the knowledge about location of pituitary and its dissection, preservation and storage, preparation fits suspension, dosage and method of its injection.

Location of Pituitary: pituitary is situated on the ventral side of the brain near optic chiasma just below the hypothalamus, contains gonad stimulating hormones viz. LH and FSH.

Source of Pituitary gland: collection is made only from fully ripe gravid fish. Pituitary obtained from immature and spent fishes do not give satisfactory sults. However, it has been observed that glands of induced breeding. Fishes collected immediately after spawning are also potent and can be used for breeding. A suitable period for the collection of glands for major carps is May to July when majority of fishes reach advanced stage of maturity. Gland from some species are more effective, though and from related species. Glands should be collected from living or killed specimen but not from rotten or semi-rotten specimen. Ice preserved pituitary can also be quite effective.

Dissection and removal of gland: Sharp knife or a hand saw is used for dissection of fish head, with oblique stroke; a portion of skull is at first removed. Gray matter and fatty substances are removed by a blunt forceps, when the brain is exposed. The entire brain is now lifted up by detaching olfactory and optic nerve. The pituitary gland is located ventrally just posterior to the optic chiasma covered by a thin membrane. When the gland is exposed, it is carefully picked up by a forecep.

Preservation and storage of pituitary: The gland is stored in 100% alcohol. After each 24 hrs, 100% alcohol is changed for further dehydration. The gland is weighed and stored in a refrigerator. The glands may be stored inside the desiccators containing anhydrous calcium chloride. Desiccators may be kept inside a refrigerator. Extract may also be preserved in glycerin (3ml extract+1ml water+2ml glycerin) The weight of gland shows variation from 7.0-18.8mg in rohu having the weight 1.0-3.6kg and 3.0-22.8 mg in mrigal having weight of 0.3-3.5kg.

Preparation of Gland suspension: The required quantity of gland is calculated from the weight of brooders to be injected. The gland is taken out on the filter paper left for a short while to allow the alcohol to evaporate and then homogenized glands are then diluted with same liquid. The gland suspension is centrifuged at about 100rpm for about 5 minutes. The supernatant fluid is diluted to desired dosage and drawn.

Dosage: Intramuscular injection on the caudal peduncle or shoulder region near the base of the dorsal fin with 2ml syringe was given.

Hypophyseal dosages depend chiefly upon the proper stage of sexual maturity of the breeders. Besides, potency of gland also vary depending of the stage of maturity of the donor fish and also on proper preservation of gland materials. A single injection of 5-10mg of pituitary gland per kg body weight of a female breeder arid 2-3 mg/kg of body weight of male breeder gives satisfactory results at optimum temperature. If first injection does not work, a second injection is given 8-10hrs after the first one. More satisfactory results were obtained when female alone was given a preliminary dose of 2-3mg/kg body weight and kept separately. After 6hrs a second dose of 5-8mg/kg body weight is given to the female and first dose of 2-3mg/kg body weight, is given to male. Both the injected males and females were put together in a breeding happa for spawning.

Collection, rearing and selection of brooders: Breeders were collected during winter months and stocked at the rate of 1000-2000kg/ha of water. These

fishes may be fed daily with artificial food like rice bran or oil cake.

Breeding Happa: The breeding happa is a rectangular, box shaped container made of fine meshed mosquito net cloth and are closed an all the side to prevent breeders from outside. It can be opened only from above by rolling the cloth, while introducing the injected breeders into it. The breeding happas are of variables sizes depending upon the weight of breeders. The fertilized eggs appear transparent, pearl like whereas unfertilized eggs are opaque and whitish. Fecundity rate of Indian major carp is about 2 lakh/kg body weight.

Factor influencing induced breeding:

1. Favourable climatic and hydrological conditions increase the chances of successful induced breeding.
2. Cool weather (24-31^{0}C) and rainy periods enhance breeding.
3. Flowing water is preferred by major carps.
4. Light drizzling heavy rain is seal for induced breeding.
5. Failures are mostly due to incorrect choice of breeders, wrong doses of pituitary extract and unfavorable climatic conditions. Hot sultry and sunny days are not suitable for undertaking induced breeding.
6. Dissolved oxygen should not be less than 5.0ppm.

Breeding Techniques

Indian Major Carps

Selection of breeders: Induced breeding is generally taken up on the onset of monsoon with the accumulation of fresh rain water, when the temperature goes down. The spawners are netted out carefully and arc weighted individually. The males are distinguished with the roughness of pectoral fin whereas in the females it is smooth. By pressing gently the belly near vent, if the milt oozes out gently and easily, the males are selected for injection. The females can be recognized by their more bulging abdomen if they possess a fully bulging abdomen which is soft to touch but not loose and having swollen pinkish vent, they can be chosen for injection under normal conditions. By using a catheter, a sample of eggs (oocytes) may be collected and examined to find out ripeness of Oocytes.

Dosage of Pituitary injections: Depending upon ripeness of gonads and prevailing climatic conditions, the dosage of pituitary to be administered is decided. When the recipient is in the prime condition, a very low dose is sufficient to precipitate spawning. The females are given the pituitary extract in increasing quantities of split doses of generally two whereas males receive only one injection. The two split doses in females may vary from 2-4 mg/kg body weight for first injection and 5-10 mg/kg body weight for second injection. The two injections are generally six hours and male gets the injection along the final injection of the female. The dosage will also depend upon the potency of the glands used.

Breeding: After the injection a set consisting of one female and two males is released in the breeding hapa. Breeding hapa is a rectangular cloth container stitched with close meshed cloth having opening one side through which breeders are introduced and taken out and can be securely tied. Breeding hapas are fixed in the marginal water ponds, canals, lakes and reservoirs while fixing the hapas water bodies having common carp and tilapia are avoided and so also ponds recently manured and having algal blooms. Breeding generally takes place within 3-6 hours after the final injection to females. Stripping method is not followed since the Indian major carps breed fully after injection with very high rate of fertilization.

Silver Carp and Grass Carp: Healthy males which readily oozes milt by a gentle pressure is prefer. In males the pectoral fin is rough at touch whereas in females it is smooth. Females with soft, bulging abdomen with pinkish genital opening is preferred. A catheter is helpful in selecting the females of silver carp and grass carp. Inserting the catheter some oocytes are taken out and examined in the field by keeping them in petri-dish. Silver carp female with uniform size of eggs very pale blue in colour but slightly pinkish with smaller ones are selected. The use of catheter is more helpful in selecting grass carp females to ensure that they gravid since well fed females may appear mature. Females having brownish or copper coloured eggs of uniform size are preferred.

Breeding Techniques: The breeding technique is same as in Indian major carps (Alkunhi and chaudhary in 1960). The females are given pituitary extract at the rate of 10-14 mg/kg body weight and male 3-4 mg/kg body weight. Two injections to female in increasing doses and one to male are given. The injected fishes, one male and two females are females are released in the breeding hapas. The females are examined 3-4 hours after the final injection to find out whether they are ready for stripping. Keeping the ventral side up by a slight pressure at genital opening if eggs are oozing out the fish is ready for stepping. Dry method of stripping is followed when the fishes are wiped by a towel to remove the excess water and the eggs are stripped into a well cleaned dry enamel basin arid immediately fertilization by milt. With the help of clean feathers eggs and milt are mixed for one or two mints and some water is added and subsequently washed for 3-4 times to remove the excess milt or any other foreign material. The eggs are then kept in breeding hapas for proper swelling and water

hardening. When grass carp are allowed to spawn themselves after injections high percentage of success is achieved whereas in silver carp rarely fertilized eggs are obtained and if fertilized the percentage is low. In silver carps quantity of milt is often insufficient and so extra males injected are kept separately and are used when needed. On cool rainy days free release of eggs have been observed in grass carp and silver carp even with a single low dose of 3-5 mg/kg body weight and eggs could be fertilized with un-injected males.

Breeding period: Natural conditions the Indian major carps breeded in flooded areas during monsoon seasons normally this period ranges from April to August/September in different parts of India. However, when examining the induced breeding conducted in India it was found that there are occasions fishes could be time, successfully bred for a longer period than generally thought possible. *Labeo rohita* could breed till the end of September in certain years (Alkuhmi et. al ;). Breeding of silver carp upto November in 1978 was reported by Singh et. al (1979), Sen Gupta et. al (1984) could breed successfully silver carp, grass carp and Indian major carps from last week of march to September. In 1984 Sukumarn and Rehman could breed Mrigal by hypophysation in the month of December.

Environmental factor for induced Breeding: In India injected breeders are normally released in hapas fixed in marginal water of ponds, lakes, reservoirs and canals for breeding. The water will be stagnant except in canal and water temperature could raise upto 35^{o}C or 38^{o}C in certain parts where it is not raining for 2 or 3 days. During breeding of Indian Major Carps in ponds by hypophysation spawning takes in clear and turbid water at temperature ranging from 24^{o}C to 30^{o}C. In silver carp and grass carp rain, sufficient accumulation of rain water sudden raise in water level and water temperature below30^{o}C are found conductive to spawning in hapas fixed in stagnant pond water. Water temperature above 33^{o}C affects spawning and hatching of eggs and brood fish kept in hapas. It is well known that fish move from water to high electrolytic level i.e. from sea water to fresh water, they gain water and thus gain weight and reverse is true when they come from low electrolytic level to higher level. In rivers and reservoirs there is a level decrease suddenly after monsoon rains in space between the follicular cells losing of adhesion between the cells and eggs and increase in diameter of eggs suggest that preceded by a remarkable absorption of water into ovary and ovarian eggs whether the conditions prevailing in ponds during spawning seasons favour such as absorption of water or not needs investigation especially because these carps do not mature I pond but not spawn there. The water in these ponds is perhaps not diluted that much with rain or flood water as that of river where they normally breed. Males spermiate in ponds but females do not.

Hatchery System: At present integration is developed wherein breeding and hatching eggs and rearing of hatchlings for a few days are taken up as a part of single system.

Modern Indian Hatchery: The system consist of a unit breeding tank of metal, cement or plastic pools with cooling tower or air conditioners to control the water temperature and a slow continuous flow of water \may or may not be maintained. The water may be filtered by the means of an in-situ filter and a pump is used to pump in water to the storage tank. The carp hatchery unit consists of sources of water where in-situ filter can be kept, pump, storage tank, aeration facilities with an air blower or compressor, vertical hatchery jars made of glass galvanized iron sheet or polythene, water supply system from the storage tank to hatching jars and spawnery.

Hatching: The hatching technique of carp eggs have undergone a lot of changes to get better hatching as survivals from the earthen hatchery pits and cement hatchery pits at spawn collection sites. Use of double hatching hapas is being replaced by running water systems with or without temperature control. The water hardened eggs are measured their number calculated and a sample is examined to find out percentage of fertilization. Under field conditions the eggs are kept in double walled hapas. Outer made of closed meshed cotton or nylon or inner made of thin round meshed mosquito net. The inner hapa is smaller leaving gaps of 15 cm on all sides and also at bottom between the inner and outer hapa. In an outer hapa of the size 2mx1mx1m and suitable size inner hapa a total of 50,000 – 100,000 eggs can be kept for hatching depending upon fertilization. The eggs are distributed in the inner hapa uniformly. The hatching time is 16-18 hours in case of Indian major carps and nearly 2 hours more in silver carp and grass carp at optimum water temperature range of 26^{o}C to 31^{o}C. The hatchlings pass through the inner hapa to outer hapa and inner hapa is removed. The floating hapas are also used for hatching purpose. In vertical jar hatchery, hatching jars with a capacity of 6.0 to 6.3 liter, 50,000 eggs can be kept and a rate of flow of water is maintained at 600 to 800 ml per minute for Indian major carps and 800 to 1000 ml for silver and grass carp. In hatching jars of high capacity more eggs can be introduced but the flow of water has to be suitably increased. The hatching time in the hatchery is nearly 2-3 hours less than in field.

Table-1: Recommended Dose of different drugs for selected fishes

S.N	Drugs	Recommended Dose
1.	Novacain	50mg/kg of fish
2.	Amobarbital sodium	85mg/kg of fish
3.	Barbital sodium	50mg/kg of fish
4.	Sodium amyta 1	52-1-72mg/kg of fish
5.	Methyl propyno 1	1-2ml/4.5lit
6.	Chloral hydrate	3-3.5g/4.5lit.
7.	Urethane	100mg/l
8.	Thinuracil	10mg/lit
9.	Quanidine	1mg/lit.
10.	Tertiary amy1 alcohol	2ml/4.5lit.

Result and Discussion:

Up to recent years it was possible to breed carps by hypophysation technique in India, almost throughout the year except in the mouth of January. However, the breeding success in February and December is exceptionally limited due to the prolific season continues to be south – west monsoon months i.e. from May – August/September in different parts of the country. Successful breeding of Indian carps in October to December is confined to south India where winter season is mild and consequently water temperature does not fall very low and day is not much reduced.

Multiple breeding of Indian major carps at different parts of country and Chinese carps to some extent also has been achieved. In China, grass carp is bred thrice in a year from April/August with appropriate care when water temperature is below 20ºC. Proper hatching facilities are needed to breed the fishes. Under natural conditions certain percentage of carps are seen to mature ahead of the season and with proper management of brood stock still higher percentage can be obtained. In the region where the influence of south west monsoon and north east monsoon is felt, there is every possibility of breeding all the cultured important species, during both the monsoons, though presently during north – east monsoon breeding is limited and confined to some species in south India. Separately rearing the offspring obtained from early breeding and late breeding and monitoring their growth and maturation cycle may culminate in evolving strains which can breed early and breed, whereby desired fish seed in adequate quantities are available throughout the year which may serve to tide over the paucity of the fish seed which hampers fish culture often.

Table-2: Comparison of egg, fry and fingerlings of selected fishes

Fish Seed	Catla	Rohu	Mrigal
EGG	Non-Floating	Non-Floating	Non-Floating
Diameter (mm)	5.3-6.5	5.0	5.5
Shape	Round	Round	Round
Colour	Yolk light red	Reddish	Golden
Hatchling Size	4.68mm Average	3.7mm Average	4.68mm Average
Yolk Sac	Both, the bulbous and narrower parts of yolk sac are equal in length	Same as Catla	Bulbous part smaller than narrower part
Somites	About 26 pre anal and 14 post anal myotomes	Same as Catla	28 pre anal and 14 post anal somites
FRY	Dorsal fin ray more than 11	Same as Catla	Same as Catla
Head	Head Small	Head Small	Head Small, body slender
Caudal region	No spot at Caudal	Transverse band at caudal	A triangle Dark spot
Barbal	No barbal	A pair of maxillary	No barbal
Lips	Lips thick, unfringed	Lips fringed	Lips thin, unfringed
FINGERLINGS			
Head	Head large	Head moderate	Head moderate
Caudal region	No spot on caudal peduncle	Dark transverse band at caudal peduncle	Spot become diamond shape
Barbals	No barbal	2 pair of barbals	Barbals not visible
Lips	Lips thick, unfringed,	Lips thin, fringed	Lips thin and not continued up to the corner of the mouth
Caudal fin	Dark grey in colour	Reddish tinge with dirty grey margins	Reddish

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