Biological Considerations in Hatchery Management: A Case Study in a Few Hatcheries in Jessore, Bangladesh

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Declaration

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The results presented in this thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

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Dedicated to

My Beloved Parents

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Abstract

The study was carried out from July to November, 2015 to assay the present condition of fish seed production in 10 hatcheries in Jessore, Bangladesh. Questionnaire survey and personal interviews were carried out to collect data on the detail management of hatchery and broods. The average number of manpower at the hatcheries was found to be 8 including 1 Manager, 2 Technicians and 5 Hatchery Assistants. Ninety percent of hatchery owners attended 3-7 days training of DoF, BFRI or Worldfish on hatchery management. Total area of brood ponds used by Ma Fatema Fish Hatchery (MFFH) was 8 with the highest water area of 2310 decimal. However, the range of water areas of the 10 hatcheries surveyed was obtained at 297 to 2310 decimal; the pond number similarly was found to range from 4 to 17. Highest seed production was found in Ma Fatema Fish Hatchery (9000 kg/year) having the production capacity of 14000 kg/year and the lowest production was in Anan Fish Hatchery (733 kg/year) having the production capacity of 8000 kg/year. Brood fishes were mainly collected from Government brood bank and Worldfish as well as used from their own stock. Hatchery owners did not maintain the pedigree history of breeders thus relatedness in the brood was common leading to inbreeding. Stocking size ranged from 10-20 kg/decimal. The yearly exchange rate of brood fish was varied from hatchery to hatchery which was 20 to 40% in different hatcheries. Occurrence of disease, unsuitable weather, poaching, lack of finance and bad communication were the problems faced by hatchery owners. Mainly two types of inducing agents *viz.* pituitary gland (PG) and Ovaprim were used for induced breeding practices in the surveyed hatcheries. The peak breeding season was April to June. Mean fertilization rate was found to range between 80 to 96.67 % for rohu and 76.67 to 96.67 % for Catla; the mean hatching rates in the same hatcheries were ranged between 57.78 and 88.89 % for rohu and 56.67 to 83.33 % for Catla. The weight of the breeders used in spawning in the hatcheries studied was ranged from 3.25 to 8.5 kg female and 2.5 to 5.24 kg male for catla; in rohu the range was 1.79 to 4.17 kg for female and 1.73 to 3 kg for male. Highest N_e was found at 92.12 in Rupali fish hatchery whose average inbreeding percentage (F) was 0.54 for rohu. For the same species, the lowest N_e was 10.11 with average inbreeding percentage being 4.95 in Matri fish hatchery. Highest N_e for catla was 21.91 with corresponding inbreeding percentage 2.28 in Rita fish hatchery. The lowest N_e was only 2 with corresponding inbreeding percentage 25 in Haque fish hatchery. For assurance of quality seed production of the two species the standard of management of hatchery and broods has to be improved with regard to both non-genetic and genetic aspects and for that the government has to strengthen its training and extension services.

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CHAPTER ONE

Introduction

Introduction

1.1 Background of the study

Bangladesh is considered as one of the most suitable countries in the world for aquaculture, because of its favorable agro climatic conditions. Development of aquaculture has generated considerable employment through the production and marketing of fish and fish seed (Nedelea and Shamsuddoha, 2009). Fisheries sector is one of the major components of agricultural activities and plays a vigorous role in nutrition, employment, income generation and foreign exchange earnings. It is also well recognized for nutrition supply and employment generation of rural population as well as for foreign exchange earning in Bangladesh (Ali *et al.,* 2014). This sector directly contribute approximately 4.37% of the country's Gross Domestic Product (GDP), however, the indirect contributions from gross agricultural income are estimated at 23.37% (DoF, 2013).

Aquaculture of Bangladesh is involved with a significant number of indigenous and exotic carp species. There are at least 13 endemic and 8 introduced species of carps, which are of interest to aquaculture in Bangladesh (Hussain and Mazid, 2005).

At present the total number of fish hatcheries are 936 of which government fish seed multiplication farms are 77, other government hatcheries are 7 and private fish hatcheries are 852 (DoF, 2013).

The availability of fish seed is an essential prerequisite for fish culture. There are two major sources of seed of Indian major carps, i.e. hatcheries and natural sources such as rivers. Until the year 1978, the country was completely dependent on seed produced in rivers. Fishermen collect the eggs from the upstream of Halda River and incubate the eggs in earthen pits on the river bank for hatching. This unscientific hatching system is responsible for the low rate of hatching and high rate of fry mortality. With the course of time, the production of fry in the rivers has been reduced due to the destruction of many breeding grounds caused mainly by siltation. Production of fry is also hampered by the lack of broods as they are caught by extreme fishing pressure. Combining both reasons, the production of fry in rivers becomes critically low. For example, in 1984, the spawn production in the river systems in Bangladesh was estimated to be 24551 kg and all hatcheries produced 652 kg; whereas in 2003-2004, the spawn production in hatcheries was estimated to be 345227 kg and only 1577 kg was reportedly collected from natural sources (FRSS, 2003-2004).

Jessore is one of the richest districts of Bangladesh in respect of its vast, diverse and unique fisheries resources in the form of physical, biological and others. In Jessore district a large number of public and private hatcheries are present though now few private hatcheries were closed. Now-a-days due to the degradation of ecological balance, natural resources of fish seed production has become greatly reduced. So, hatchery becomes the main source of fish seed production.

The seed production is now almost completely dependent on the hatchery produced seeds because of the remarkable destruction of the riverine natural breeding grounds in the country. The term "Hatchery" is considered in broadest sense as the facilities where fish fry and fingerlings suitable for stocking in growth ponds are produced in artificial manner by the process of induced breeding technique. Induced breeding is a technique whereby ripe brood fishes are stimulated by treating them with inducing agents to breed in captivity. The stimulation promotes a timely release of eggs and milts from ripe breeders. It is now used as a widely accepted means of artificial propagation to overcome constraints in fish fry supply particularly for species that do not breed in captivity (Aktar *et al.*, 2014).

Broodstock or brood fish are parent fish from which fry and fingerlings are produced. The quality and reliable supply of healthy fry and fingerlings having a sound genetic base is largely depends on the successful stocking and rearing of brood fish. Therefore, brood fish collection, rearing and management are the most important parts of aquaculture activities.

Brood fish are usually sourced internally and reared from the seeds produced in the hatchery and grow out in the fish farm. Selection of closely linked breeders caused the inbreeding problem resulting in weak, unhealthy and deformed fish seeds as well as declining growth performance. Production of seeds from own stocked breeders and traditional management of hatchery systems for years together resulted in low quality seed with genetic deformities and slower growth of stocked fish in aquaculture in the region. Such problems are reported from all across Asia. Importance of broodstock management and hatchery system improvement are the major concern of aqua-culturists from most of the SAARC countries. Proper management, periodic replacement and genetic improvement of broodstock are being discussed widely in the context of these problems (Mustafa, 2012).

The significant increase in hatchery production has improved the availability of fry for culture. This has contributed to protection of wild stocks, as the price of hatchery-produced fry has dropped, while the availability of fry in the wild is reduced. Moreover, there is a serious concern about the genetic erosion of hatchery broodstock. Not less than 9 – 10 generations of Indian Major and Chinese carps and as many as 18 – 20 generations of common carps have been used for induced breeding in the private hatcheries. Proper selection has not been maintained, resulting in the use of undesirable small size breeders. Closely related stocks have been repeatedly used over many generations, and hazardous hybridization has been conducted in many hatcheries (Hussain and Mazid, 2001).

Little attention has been paid to the issues of broodstock management in the past, since this was not considered as being a critical issue. The result has been that the fish used for breeding in the hatcheries are usually not in an appropriate condition for production of good quality fry. The quality of fish seeds in Bangladesh has declined over the years. The quality reduction is mostly observed in private hatcheries. The principle reasons for this are: poor nutrition, inadequate spawning techniques, inbreeding problems, inter-specific hybridization and negative selection which lead to poor performance in aquaculture production (Sarder, 2007).

The danger of genetic degradation is extended to the wild stocks too. Although natural hybridization accidentally occurs among Indian major carps (Desai and Rao, 1970., Khan and Kowtal, 1989), these are usually eliminated through natural selection. On the other hand, a strong negative effect can be expected on the wild stocks by mass introduction of domesticated, genetically degraded stocks, as fingerling releases in floodplains, and large scale escapes of cultured stocks due to increased flooding (Rajts *et al.*, 2002).

Pedigree identification in fishes is not practiced in majority of the hatcheries mainly due to the high cost and delicate technical procedures involved in tagging. This makes it difficult to identify the individual fish and to estimate the individual inbreeding coefficient near impossible. Hill (1979) suggested that in the absence of pedigree information, inbreeding rate may be estimated based on the effective population size, which is defined as the actual number of individuals that contribute progeny to the next generation (Crow and Kimura, 1970).

Unintentional inbreeding is always harmful and the magnitude of its effects is difficult to predict. It is well documented that inbreeding lowers genetic quality, reduces the overall performance of the animals and increases anatomical deformity and susceptibility to diseases. A long-term breeding policy is essential to maintain the inbreeding rate at a desirable level, therefore suitable predictive models need to be developed to predict the future trends of inbreeding in the culture stocks and thereby minimize the inbreeding depression (Deepak *et al.*, 2005).

Therefore Broodstock should be maintained scientifically so that ripe broods could be obtained during the whole breeding season. Most of the government hatcheries have own broodstock and around 25 percent recruitments take place in every year. On the other hand, few private hatcheries have their own stock and maintain them more or less scientifically but there are many private hatcheries that do not have the required number of broods. During breeding season they instantly buy broods from others and produce fry from them to fulfill their target (Sarder, 2002).

Inbreeding is a genetic concept that most aquaculturists have heard about, but few understand what it is or know how it can be used or abused. Inbreeding is one of the few natural biological processes that evokes deep-seated prejudices and invokes legal sanctions;

While inbreeding is an important breeding program that can be used to improve a population when it is planned and directed, unplanned and uncontrolled inbreeding can ruin a population through something called "Inbreeding depression" which is a decrease in growth and viability coupled with an increase in abnormalities. Genetic aspects of fish stock management are still in their infancy. Many farmers are still raising stocks that are either wild or only a few generations removed from the wild. Most farmers worry only about the environmental aspects of management-nutrition, water quality management, disease control and prevention-and ignore the genetic aspects. Since the modern science of aquaculture is still in its infancy, these management decisions make sense, because the best way to raise a crop has often not been quantified. Vast and immediate improvements can be obtained by refinements in fertilization regimens, feeding practices, or water quality management. These changes are often very cost effective because the changes are inexpensive.

The genetic aspects of fish farming have received far less attention because they require experienced managers, long-term forms of management, extra facilities, and additional financial budget; furthermore, the results are often unpredictable. While great improvements are possible, they are usually more costly than environmental improvements, and the return is not immediate.

Furthermore, small population sizes produce genetic drift, which is a random change in gene frequency. Genetic drift can be as important as selection in altering a population's gene pool. The effects of genetic drift can be devastating. Genetic drift can irreversibly alter gene frequencies and eliminate alleles, which can decrease a population's ability to survive or to adapt to an altered environment, and it can preclude future selection. The effect of genetic drift on a population's gene pool can make many management goals impossible to achieve. The loss of genetic variation can make future selective breeding programs ineffective. Selection improves productivity by exploiting a population's genetic variance; whole genetic drift decreases a population's genetic variance.

While, Inbreeding can occur when a farmer conducts a selective breeding program; in fact, it is inevitable. Selection is a breeding program that improves a population by allowing only superior animals to produce offspring. When this happens, the size of the breeding population is reduced. When only the best are allowed to mate and the only criterion for mating is a certain phenotype, relatives are often mated. This causes unwanted inbreeding. While inbreeding will be produced during selection and while it can cause some problems, it is of secondary importance. The major genetic goal during this type of breeding program is to improve the population by selection. However, inbreeding should be moderated when conducting a selective breeding program, because there is no point in conducting selection simply to counteract inbreeding depression.

Over the last two decades, Jessore district of Bangladesh has experienced an intense growth of fish breeding industries. The success of hatchery mainly depends on quality broods rearing techniques, high quality fry, pond management, including liming, fertilization and feeding and water quality management. Hatcheries in Jessore faced problems related to negative selection of broodstock, inter-specific hybridization and inbreeding but the problems and how they are specifically related to seed quality and seed performance, have not been rigorously and comprehensively investigated. Therefore, hatchery owners, nursery operators, fry traders and stakeholders face economic loss to some extent. Moreover, scientific study on this problem is still lacking. Considering the contribution of hatcheries in Jessore region on our national aquaculture, it can be stated that extensive research work should be carried out to improve the quality of fry..

Scientists and extension agents feel most comfortable when they can provide an unambiguous piece of advice to solve or prevent a problem to a farm and hatchery management. Farmers and hatchery managers feel more comfortable when they receive unambiguous advice, because this means that there is only one way of doing something, and if they follow the advice they can solve or prevent the problem and become successful. To this end, a number of publications have recommended that aquaculturists or hatchery managers should maintain a specific N_e in order to prevent inbreeding or genetic drift-related problems. These recommendations have ranged the N_e from 50 to 1,000; the majorities suggest either 500 or 1,000.

Unfortunately, there is no single N_e and there is no simple set of genetic guidelines that can be prescribed for every farmer and every hatchery manager. Unlike disease prevention and treatment, the recommendations and protocols that are needed to prevent genetic problems vary from farmer to farmer, from farm to farm, and from program to program.

In the present study efforts was made to survey overall brood fish management practices as followed in Indian major carps brood rearing in Jessore. The study was based on questionnaire survey and interview of the hatchery owners. The study was focused on the characteristics of carp broodstock management practices and breeding in the hatcheries involving sources of broods, liming, feeding and fertilization, stocking, record keeping, sex ratio in breeding etc.

1.2 Objective of the study

The present investigation was related to the following objectives:

- To know the practice of carp broodstock and hatchery management in Jessore, Bangladesh.
- To suggest for improvement of hatchery and broodstock management for production of quality seed of rohu and catla.

CHAPTER TWO

REVIEW OF LITERATURE

Review of Literature

The availability of fish seed is an essential prerequisite for fish culture. The main sources of fish seeds in Bangladesh are spawn produced in government and private hatcheries, and some collected from rivers. In 2012-2013, the number of government fish hatcheries or fry production farms was 936 but most of them are private fish hatcheries (DoF, 2013).

With the success of fish seed production through induced breeding and the increasing demand for quality fish seed for aquaculture practices, the government of Bangladesh established a number of hatcheries in public sector in different parts of the country. In addition to production of fish seed, these public sector fish seed farms act as centers of technology transfer and train a large number of private entrepreneurs who are interested in establishing hatcheries on their own initiatives. Since 1980, the technology for production of Indian major carps and some other commercially important fish species has been transferred to private sector from public sector and due to its simplicity a large number of hatcheries have been established in private sectors. About 936 private hatcheries and public nursery produced 0.49 million fry (DoF, 2013).

Jessore is one of the most important districts of Bangladesh in respect of its vast, diverse and unique fisheries resources in the form of physical, biological parameters. In Jessore district the numbers of public and private hatcheries are many. But now a few private hatcheries are closed. Nowadays due to the degradation of ecological balance, natural resources of fish seeds are destroyed. So, hatchery is now the main source of fish seed production.

Broodstock is the major and the core input for fish seed production. Broodstock supply thus needs to be categorized as a separate value chain function because of its importance and the complexity in its production and distribution (World Fish, 2012).

Young immature brood fish, also called "brood fish candidates" are usually selected from market size fish. Market size fish population available for selection of broodfish candidates should be healthy, uniform in size and well developed. Population consisting of fish of different age and size or produced in strongly overstocked/underfed pond are not suitable for selection. Selected fish must be reared in well managed fish ponds where standing crop of fish should not exceed 1.6-2.0 ton/ha. To avoid difficulties of using broodfish of too big size, yearly recruitment rate of population should be 25–30%.

The brood fish candidates may be stocked in those ponds where matured broodfish are kept in off season period. As feed requirement of broodfish candidates is different, market size fish ponds are not suitable for rearing brood fish candidates. Ponds with large water surface area and water depth of at least 1.5 m are best for broodfish candidates. Stocking ratio of different species should be in accordance with the general principle of stocking of polyculture ponds. Filter feeders should not exceed 35–40% and in it the ratio of zooplankton consumers (catla, bighead) should remain under 30% (Peteri *et al.*, 1992).

Shah (2004) stated that, good quality seed guarantees good production and good returns of investment with the improved knowledge of genetic problem can be achieved. Breeding fishes throughout the breeding season increases the chance of breeding more fishes from different species. Running the hatchery for some specified time of the breeding season is not advisable from the point of view of conservation of genetic variation; repeated act of such practice for generations can create the incidence of negative selection in favor of restricted breeding season.

Rahman (2004) reported that technical knowledge in respect of pond preparation is not bad in the Jessore area. He found that technical knowledge of the nursery operators was poor. Most of them had no institutional knowledge. They are doing the job on the basis of their practical experience that they achieve from long time observation. Due to lack of their technical knowledge they cannot manage their problems.

Lack of sufficient training results improper hatchery operation and produced low quality seed due to various genetic problems. Due to the lack of awareness on the principles of genetics, the breeders tend to misuse fish gene pool due to improper breeding practices like mixed spawning or the use of low number of brood stock for breeding (Paddy, 2000).

Haque (1991) stated that selection of brood fish is the main task on which successful spawning mostly depends. If brood fish are not properly selected the fish may give either poor quality eggs or fish may not spawn at all. The successful assessment of the condition of gonadal maturation is an useful measure in the selection of brood fish.

Islam (1989) reported that in two stages of nursing, fry are reared for a period of 4-6 weeks at a density of 1 million/ha by this time they reach a size of 2-3 inch.

Hatchlings collected from natural sources or produced by induced breeding are reared for one to two months in earthen rearing ponds to a size of 5-7 cm before being sold to the pond cultists (Hasan, 1990).

Samad *et al.* (2013) present a survey report on the existing status of management of carp brood fish. The survey was conducted with 40 hatcheries at Chanchra in Jessore from June to November, 2012. They stated that production of brood fish depends on the combined interaction between feed and culture technique. Brood fish selection for induced breeding was done on the basis of their experience. Pond preparation was carried out properly but stocking density of brood fish was quite high and water quality was not maintained properly. Negative selection of brood fish was done in few hatcheries aiming at reducing the cost for collecting or purchasing good quality broods. The sources of brood fish were mainly own and other sources were Halda River, Padma River, Government brood bank and World Fish Center.

Hussain and Mazid (1999) describe the present status of broodstock management, identify problems and suggest some guidelines to control negative selection and inbreeding in hatchery stocks in Bangladesh. They stated that, the common practice in the majority of fish hatcheries in Bangladesh is to replenish broodstock from: (i) internal sources (from the hatchery itself or from nurseries that received hatchlings from that particular hatchery); or (ii) external sources (collecting fingerlings/breeder from grow out ponds which have received fingerlings from other hatcheries or from riverine sources). There has been unconscious 'negative' selection in some hatcheries as relatively bigger (and hence fast-growing) individuals from grow out ponds are sold and the remaining smaller (and hence slow-growing) fish are used for broodstock replacement. The survey revealed that some hatchery operators use carp broodfish of very small size, even less than 500g each in weight and this affects the quality of seed production.
He also said that, inbreeding depression in hatchery stocks results from a lack of knowledge of broodstock management practices, especially about the need for recruitment of new breeders into the stock at regular intervals, maintenance of proper stocking density of broodfish and their balanced feeding, selecting broodfish of a desirable size, injecting adequate hypophysation doses, mating unrelated female and male breeders, basic disease control, water quality maintenance and record keeping of broodstock and spawning.

They also suggested that, the government should impose restrictions on the minimum size and age of fish to be used for breeding in order to control indiscriminate use of inferior quality broodstock for seed production by the hatcheries. According to him, the desirable size, particularly for female breeders, should be as follows: *L. rohita*> 1 kg, age 2 years and *C. catla*> 3 kg, age 3 years.

Shaha and Shah (2010) conducted a survey on Indian major carp brood rearing techniques relating to feeding and fertilization practices in some hatcheries in Jessore and Mymensingh regions. They described that, the seed quality largely depends on brood fish management relating to feeding and fertilization regimes followed by the hatcheries. Feed with proper ingredients is a key factor for attaining desired brood quality. Fertilization of the ponds was found to be done with urea, Triple Super Phosphate (TSP), Muriate of Potash (MP) and mustard oil cake at a comparatively higher rate in Jessore than in Mymensingh. Cow dung was seen to be used higher in amount in Mymensingh than in Jessore region. Percentages of rice bran and fish meal were higher in Jessore region; while wheat bran was used comparatively at higher percentage in Mymensingh region. Mustard oil cake and vitamin premix were found to be used closely similar in amount in both the regions. Some traditional food items were seen to be used in both the regions.

Aktar *et al.* (2014) observed that, for producing quality fish fry, induced breeding techniques are applied in the hatcheries. Mainly two types of inducing agents *viz.* pituitary gland (PG) and the Human Chorionic Gonadotropin (HCG) were used for induced breeding practices in the surveyed hatcheries. PG was used for all species except the silver and bighead carps where HCG was used. The incubation period of different fish species varied from minimum 10 to maximum 72 hours. The hatching rate of fishes in the surveyed hatcheries ranged between 55% and 80%.

Sarder (2007) reported that, there are two major sources of seed of Indian major carps, i.e. hatcheries and natural sources such as rivers. Until the year 1978, the country was completely dependent on seed produced in rivers. Spawns are collected during the monsoon season (May-August) from different rivers of Halda, Padma, Jamuna, Brahmaputra and their tributaries. Carp fry are collected from Padma-Brahmaputra river systems while fertilized eggs are collected from the Halda River. Fishermen collect the eggs from the upstream of Halda River and incubate the eggs in earthen pits on the river bank for hatching. This unscientific hatching system is responsible for the low rate of hatching and high rate of fry mortality. Most of the government hatcheries maintain the stocking density of 2500-4000 kg/ha which is much higher (5000-6000 kg/ha) in private hatcheries. Usually seven carp species are stocked and reared together with the ratios of Silver carp 30%,Catla 5%,Rohu 20%,Mrigal 25%,Grass carp 10%,Bighead carp 5% and Silver barb (Thai Sarputi) 5%.

According to Demoulin (1999), Indian and Chinese carp are species that mature at a large size (about 2 kg) and need to be kept for at least 2 years in the pond before becoming productive. Broodstock ponds should be of rectangular in shape, width not exceeding 20 m for easy netting operations. They must be drainable in order to assist collection of all fish and correct pond preparation (application of lime and manure). Water depth should not be less than 1 m and should be preferably between 1 to 2.5 m. Grass carp and some Indian carp are however considered to require more space and should be kept in ponds not less than 0.2 or 0.5 ha. A common stocking rate is 1,000 kg of broodfish per hectare, which means 10 kg/100 m^2.

Preferably big size, healthy, good looking and matured males and females should be collected from the natural sources (rivers, lakes, and reservoirs) as brood stock. Brood collected from culture ponds might be of the same sized, matured and healthy. Collected broods should be transferred into the brood stocking pond with aeration or with the facilities for water exchange in the month of October to December (Kumar, 1992).

Sharif and Asif (2015) reported that the Hatchlings production of Indian major carps was 24720 kg, Exotic carps were 21754 kg, and other species were 2966 kg respectively in 2013 at Jessore sadar. Fry production of Indian major carps was 399596 kg, Exotic carps were 391272 kg, and other species were 41625 kg. In the year 2013 the maximum production was found 8,500 kg while the minimum was 700 kg.

Naeem *et al.* (2005) worked on induced spawning of *Catla catla* and found that the fertilization and hatching percentage 91.01 and 67.50 respectively by using Ovaprim-C. His another work was on rohu species where he found the fertilization and hatching percentage 77.50 and 81.39 respectively by using Ovaprim-C.

Dhawan and Kaur (2004) reported the mean fertilization rate for *Catla catla* 80.51 and 90.03 by using Ovatid and Ovaprim respectively. In *Labeo rohita,* this rate was 89.13 and 80.55.

According to Bhuiyan and Aktar (2011), the incubation period and hatching rate in different fish species varied from 10 to 72 hours and 55% to 80%, respectively.

According to Delince (1992), 30-35^0 C temperature is tolerable to fish. Where, Bhatnagar *et al.* (2004) suggested the levels of temperature as 28-32^0 C good for tropical major carps; <12^0 C – lethal but good for cold water species; < 20^0 C – sub lethal for growth and survival for fishes and > 35^0 C- lethal to maximum number of fish species. The DO level >5ppm is essential to support good fish production. He also suggested that 1-3 ppm has sub lethal effect on growth and feed utilization; 0.3-0.8 ppm is lethal to fishes and >14 ppm is lethal to fish fry, and gas bubble disease may occur. Turbidity range from 30-80 cm is good for fish health; 15-40 cm is good for intensive culture system and < 12 cm causes stress. He suggested CO_2 levels as 5-8 ppm is essential for photosynthetic activity; 12-15 ppm is sub lethal to fishes and 50-60 ppm is lethal to fishes. He also recommended the level of ammonia (<0.2 mg L-1) suitable for pond fishery.

The suitable water temperature for carp culture is between 24 and 30^0 C. Suitable pH range for fish culture is between 6.7 and 9.5 and ideal pH level is between 7.5 and 8.5 and above and below this is stressful to the fishes. The secchi disk transparency between 30 and 40 cm indicates optimum productivity of a pond for good fish culture. The free carbon dioxide in water supporting good fish population should be less than 5 mg/L. Maximum limit of ammonia concentration for aquatic organisms is 0.1 mg L-1 (Santhosh and Singh, 2007).

According to Ekubo and Abowei (2011) tropical fishes can tolerate CO_2 levels over 100 mg/L but the ideal level of CO_2 in fishponds is less than 10 mg/L.

The Indian major carp catla (*Catla catla* Hamilton 1822) is of high importance for aquaculture production in India, Bangladesh and Nepal. Fish used in aquaculture are produced at private or public rearing facilities, and fry are subsequently distributed to fish farms through a complex system of 'middlemen'. Eknath and Doyle (1990) expressed concerns that effective population size might be very low in hatchery-reared catla, and using demographic methods they estimated Ne to be as low as 3_8–16_0 in different hatcheries. More recently, studies employing allozymes (Simonsen et al., 2005) and microsatellite DNA markers (Alam and Islam, 2005) have revealed low levels of variation in catla from Bangladesh. Moreover, Alam & Islam (2005) included a hatchery population in their study and found it to exhibit lower variation as compared to wild populations. All together, these results indicate that effective population sizes in hatchery propagation of catla are low, leading to inbreeding and loss of variation.

Avoiding loss of genetic variation is an important goal in management and conservation of populations. First, it is essential to maintain heterozygosity; increased homozygosity, i.e. inbreeding, may decrease fitness of populations, so-called inbreeding depression, either through fixation of deleterious recessive alleles or due to absence of variation at loci showing over dominance (Hedrick and Kalinowski, 2000). Second, maintaining allelic variation is a prerequisite for maintaining evolutionary potential, both for enabling wild populations to adapt to changing environmental conditions and for ensuring that traits and properties of captive populations can be improved by selective breeding programs (Frankham *et al.*, 2002). The factor determining loss of genetic variation and increase of inbreeding is the effective population size, N_e. N_e is defined as the number of individuals in an idealized population, i.e. with equal sex ratio and variance in family size approximated by a Poisson distribution, that would cause the same rate of inbreeding or genetic drift as that of the actual population under study (Frankham *et àl.*, 2002). In real populations, sex ratios may be unequal, Ne fluctuates over time and there may be high variance in reproductive success among individuals. Due to these factors, the effective population size is nearly always lower than the census population size (Frankham, 1995).

Deepak *et al.* (2005) work on four hatcheries in which the effective population size for catla ranged from 55 to 431. It was 11 to 152 in mrigal and 48 to 268 in rohu. The effective population size was larger for both catla and rohu than mrigal in general in all the four hatcheries in all generations. The effective population size in the study was estimated assuming that 50 per cent of the brooders utilized for spawning contributed to the next generation. He observed that the number of male brooders used was higher than the number of female brooders in all the species in all the hatcheries. In such a situation, the effective population size tends towards the lesser number sex. This variation in brooder sex ratio reduces Ne and thereby increases the rate of inbreeding. One way to minimize the accumulation of inbreeding is to maintain the sex ratio of 1:1 wherever it is not practiced presently. The 1:1 ratio helps to maximize the N_e and thereby reducing the inbreeding rate.

Literature on the effects of inbreeding in fish populations is reviewed and the breeding approaches for controlling the rate of inbreeding accumulation in brood stock populations are outlined and discussed by Kincaid (1983). He Studied to measure depression at a series of inbreeding levels in rainbow trout populations using the method of inbred-outbred half-sib families are discussed. While the actual depression estimates varied widely between populations and inbreeding levels, significant levels of depression were found in many hatchery, field, and brood stock performance traits after only one generation of brother--sister mating.

Hansen *et al.* (2006) work on the loss of genetic variation in hatchery-reared indian major carp, *catla catla.* He was tested the hypothesis that effective population sizes are low in hatchery-reared catla (*Catla catla*) from Bangladesh, possibly leading to inbreeding and loss of variation. The study was based on analysis of seven microsatellite loci in three samples of hatchery-reared catla and four samples representing wild populations. His investigation suggests strong genetic drift in hatcheries. Genetic variation, both in terms of expected heterozygosity and allelic richness, was significantly lower in hatchery samples than in samples of wild catla. The results show that low effective population sizes leading to loss of variation and possibly inbreeding depression should be a matter of serious concern in aquaculture production of catla.

CHAPTER THREE

Materials and Methods

Materials and Methods

3.1 Study area

The study was conducted during July to November, 2015. There were forty five (45) hatcheries in Jessore Sadar Upazila and most of the hatcheries were situated at Chanchra area. At present 27 hatcheries are running there. Among them 10 hatcheries were surveyed for comparative study. The name of these hatcheries with their details is shown in table 1.

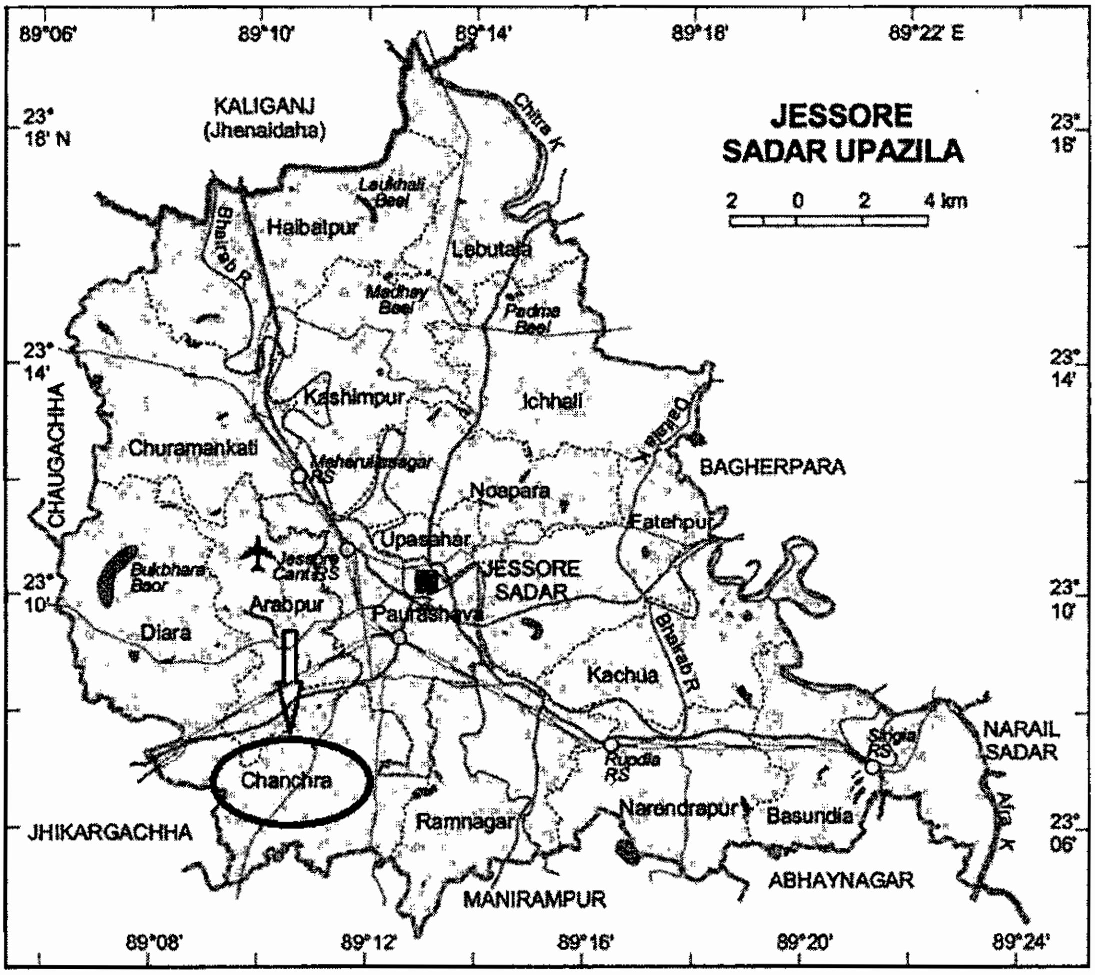

Fig. 1: Map showing the study area

3.2 Questionnaire preparation

The questionnaire was prepared emphasizing on the objectives mentioned above. Before finalization of the questionnaire, it was pre-tested through field visits. Then the questionnaire was finalized and prepared for survey work. The questions are specific and free from any kind of influence.

3.3 Determination of fertilization and hatching rates

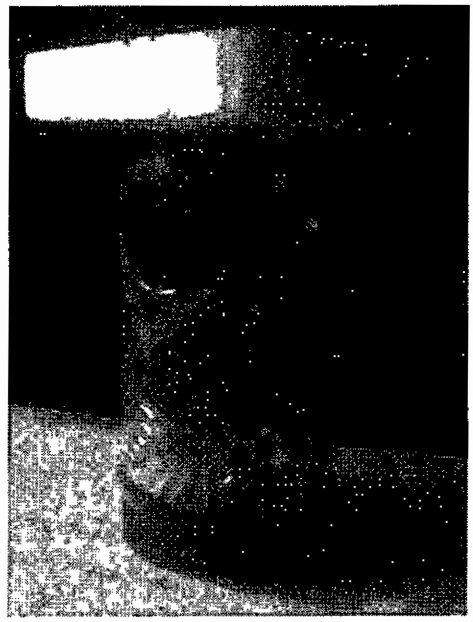

Fig. 2: A tool used for hatching fertilized eggs

Immediately after mixing the eggs with the milt, the fertilized eggs were kept in the round hatching jars of 50 liter water holding capacity. After 30 minutes of mixing, the eggs were examined to determine the fertilization rate with the help of a magnifying glass. Eggs were hatched after 18-20 hours of fertilization. To count hatching rate exactly, approximately 30 fertilized eggs were isolated and kept in three small hatching contrivances made with cut plastic bottles of 500 ml capacity with continuous water flow, floated within the hatching jar with the help of polystyrene foam. Then the mean hatching rate was determined by counting the number of hatchlings in the jars after completion of hatching after a day (Shah *et al.*, 2011).

The fertilized eggs were calculated by:

Fertilization rate (%) = no. of fertilized eggs / total no. of eggs x 100.

Percentage hatchling calculated by:

Percentage of hatchling = total no. of hatchlings / total no. of fertilized eggs x 100

3.4 Determination of the average inbreeding (F) and effective breeding number (N_e)

Effective breeding number (N_e) was determined by using the following formula:

$$N_e = \frac{4(\text{number of males})(\text{number of females})}{\text{number of males} + \text{number of females}}$$

Where, number of males and number of females are the number of male and female brood fish those produce viable offspring. If all offspring from a brood fish die, that male or female is not included when determining N_e (Tave, 1993). Once N_e has been determined, a simple formula can be used to calculate the average inbreeding value in the population:

$$F = \frac{1}{2N_e}$$

Where, F is the average inbreeding value in the population (Tave, 1990).

3.5 Data collection

Data were collected through a structured questionnaire. After finalizing the questionnaire, relevant data were collected by interviewing the hatchery owners and the farmers directly. The data were collected on two Indian major carps species bred at the hatcheries, viz., catla (*Catla catla*), and rohu (*Labeo rohita*).

In order to minimize errors, data were collected in local units. These were subsequently converted into appropriate units.

3.5.1 Secondary data collection

The existing data related to this study were collected from Freshwater substation, Bangladesh Fisheries Research Institute (BFRI), Jessore; Department of Fisheries (DoF); BRAC and others Government and Non-government organizations, different books, journals and websites.

3.6 Data analysis

The results obtained in the experiment were subjected to statistical analysis. Qualitative and quantitative analysis of all kinds of data were carried out. MS Excel was used to store all the data. MS Excel was also used for presentation of the tables and graphs obtained from different types of data.

Table 1: Name and addresses of the hatcheries under study area in Chanchra, Jessore district of Bangladesh

SL.	Name of the hatchery	Location
1	Anan Fish Hatchery (AFH)	Sabjibag, Chanchra, Jessore
2	Chowdhury Fish Hatchery (CFH)	Dalmil, Chanchra, Jessore
3	Haque Fish Production Centre (HFPC)	Chanchra Bzar More, Jessore
4	Kapotakkho Fish Hatchery (KFH)	Benapole Road, Chanchra, Jessore
5	Madhumoti Fish Hatchery (MAFH)	Benapole Road, Chanchra, Jessore
6	Ma Fatema Fish Hatchery (MFFH)	Dalmil, Chanchra, Jessore
7	Matri Fish Hatchery (MFH)	Chanchra Golder Bari, Chanchra, Jessore
8	National Fish Hatchery (NFH)	Benapole Road, Chanchra, Jessore
9	Rita Fish Hatchery (RIFH)	Dalmil, Chanchra, Jessore
10	Rupali Fish Hatchery (RUFH)	Sabjibag, Chanchra, Jessore

CHAPTER FOUR

Results & Discussion

Results and Discussion

Indian major carps are valuable and demanding fish species among the other freshwater fish species in Bangladesh. Recently, production of fish seed completely depends on the hatchery produced seed. Along with many other problems, genetic quality degradation of fish seeds and brood fishes is to be considered as a core problem in the hatchery management. This study was conducted in 10 carp fish hatcheries out of 27 hatcheries located in Chanchra, Jessore. The information about fertilization and hatching rate, broodstock size, average inbreeding value, effective breeding number, sex ratio, average weight of breeders was collected from the hatcheries which had been used to unclose the genetic problems occurred in these hatcheries. Here, a brief description is given below about the study carried out in the hatcheries.

4.1 Hatchery status

During the present study, total number of hatcheries located at Jessore Sadar was 45. Of which 27 hatcheries were situated at Chanchra region and out of them only 21 hatcheries were found active in operation.

The average number of manpower at the hatcheries was found to be 8 including 1 Manager, 2 Technicians and 5 Hatchery Assistants. Most of the hatcheries ensured a Manager and a Technician. The number of hatchery Assistant varied from 2 to 8 including the night guards. Table 2 shows the facilities of the hatchery surveyed.

Table 2: Present status of facilities of the hatcheries surveyed

Sl. No.	Hatchery Name	Area of Water body (decimal)	Brood ponds	Deep tube well	Rectangular tank	Hatching jar	Man power employed				
							Number of man power	Manager	Technician	Hatchery assistant	Night guard
01	Anan	495	4	4	10	31	5	1	0	3	1
02	Chowdhuri	528	5	5	16	32	12	1	1	8	2
03	Haque	1320	7	6	14	52	9	1	1	3	4
04	Kapotakkho	297	4	2	16	30	7	2	1	3	1
05	Madhumoti	1900	17	2	16	20	8	2	1	4	1
06	Ma Fatema	2310	8	2	17	36	14	2	1	8	3
07	Matri	990	12	2	14	48	11	1	2	6	2
08	National	990	7	4	7	14	7	1	1	3	2
09	Rita	1155	11	3	16	40	10	1	1	6	2
10	Rupali	500	8	2	17	40	10	1	2	5	2

It was found that the total area of brood ponds used by Ma Fatema Fish Hatchery (MFFH) was 8 with the highest water area of 2310 decimal; the second highest water area 1900 decimal was with Madhumati hatchery having highest total number of ponds standing at 17. However, the range of water areas of the 10 hatcheries surveyed was between 297 to 2310 decimal; similarly the pond number was found to range from 4 to 17. The number of deep tube well was ranged between 2 to 6 and the number of rectangular and circular tanks varied between 7-17 and 14 to 52 respectively. The manpower used in the hatcheries showed an almost similar composition of the categories of people, however, the total number employed was varied from 5 to 14. The limitation of the brood holding capacity restricts the number of broods in the hatcheries thereby increasing the likelihood of relatedness of the broods leading to the incidence of inbreeding (Shah, 2004); some of the hatcheries had fewer number of ponds with insufficient water areas for holding the broods.

4.2 Production capacity and species bred

The study revealed that the capacities of the hatcheries are not being fully used (Figure 2). The annual production capacity (Kg) of some of the surveyed private hatcheries was ranged from 2500 to 14000 kg and the total production of seed of some of the surveyed hatcheries in the study year (2015) was found to range from 733 to 8500 kg. Almost similar observation was reported by Sharif and Asif (2015).

The species of fish that were bred in these hatcheries had been commonly the rohu, catla, mrigal, silver carp, grass carp, common carp, Thai sharpunti, pangus, kalibaus, bata and often the black carp, bighead carp, koi and tilapia.

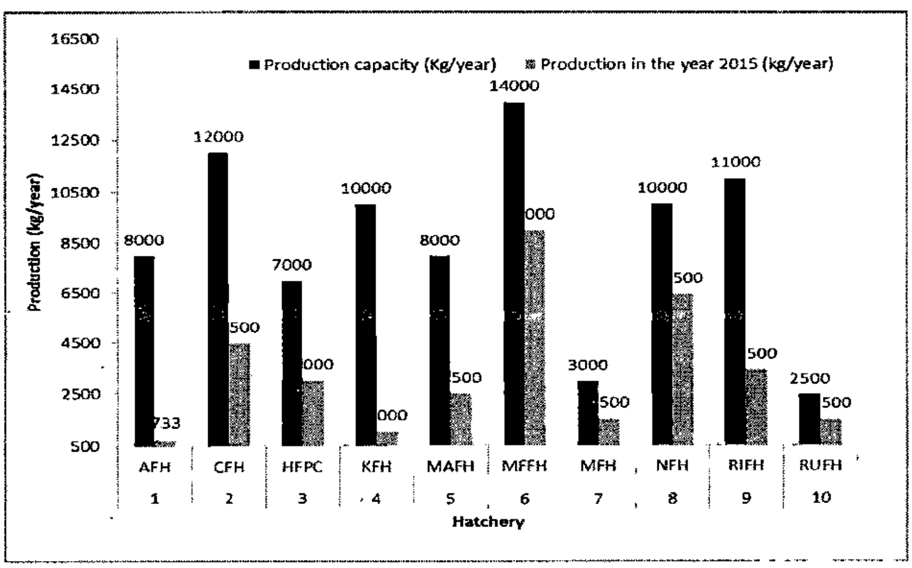

Fig. 3 : Present seed production scenarios of hatcheries surveyed in Jessore

4.3 Sources of brood and record keeping

Majority of the hatcheries surveyed were found to collect broodstock from Jamuna river and also from government and NGO sources from Barisal, originated from nature. Some hatcheries had reared their own broods in their leased-in brood ponds; they did not either exchange broods among other hatcheries. The hatcheries each, therefore, could be referred to as an isolated, self-sustaining and genetically closed unit. Eknath and Doyle (1990) find a similar situation of self sustaining closed breeding units operating in 18 hatcheries during 1987-88 breeding season of Indian major carps in the Karnataka State of India.

Recently, Worldfish in Bangladesh provided broods of Indian major carps of natural sources with free of cost to the hatchery owners under the project named "Feed the Future". The Wordfish collects brood of fry fish from different natural sources such as Halda, Padma, Jamuna rivers; stocks the broods in their own management and distributed them among the fish hatchery owners. Until the recent past the natural sources of rivers such as Padma, Jamulna and Halda rivers were the major sources of carp brood and seed in Bangladesh; with the utter damage of the breeding habitats of the foregoing rivers, the mainstay of supply of seed for aquaculture in the country has been the hatcheries (DoF, 2012).

The present study revealed that none of the surveyed hatcheries keep the pedigree record of the broods; they keep some sort of information just in their mind without maintaining any effective record in black and white. Similar situation was observed by Penman and McAndrew (2015) where they found that most hatcheries kept some kind of records but these were fairly limited and associated with production targets, e.g. number of fish produced per season and number of fish spawned. They suggested, however, to monitor effective population size, other quantitative data, principally in relation to the replacement of broodstock, e.g. how many individuals of the current generation contribute to the next generation. They also suggested to introduce a reliable tagging techniques for close monitoring of broodstock within hatcheries and from where broodstocks are to be transferred to other hatcheries.

Pedigree identification in fishes was not practiced in majority of the hatcheries studied mainly because of the high cost and delicate technical procedures involved in tagging. This makes it difficult to identify the individual fish and estimate the individual inbreeding coefficient near impossible. Hill (1979) suggests that in the absence of pedigree information, inbreeding rate may be estimated based on the effective population size, which is defined as the actual number of individuals that contribute progenies to the next generation (Crow and Kimura, 1970).

Hussain and Mazid (1999) suggest that the brood fish should be marked (e.g., Prussian blue) or tagged (e.g., numbered plastic tags or more sophisticated AVID tags) for record keeping and hatchery produced seed of different selected stocks should be stocked separately in a pool. All the necessary records, like number of breeders used in each slot with their tag number, date of spawning, date of hatching, date of stocking and number of individuals stocked in each nursery/rearing pond, should be maintained.

4.4 Pond preparation

Pond management plans of the hatcheries were done by dividing into pre-stocking management and post-stocking management. These were done according to the needs and available low cost facilities of the culture system. The steps of pond preparation are described below.

4.4.1 Drying

Most of the ponds retained water round the year but some ponds were completely dewatered by pumping and allowed to dry by sunlight. The period of drying varied from 7-10 days.

Some of the brood ponds being bigger in size with huge quantity of water, dewatering become costly and thus the hatchery owners resort to the alternative way of using rotenone to kill the unwanted lives in the pond before liming and fertilization.

4.4.2 Water source

Underground water was the main source of water for all the hatcheries. As underground water contained significant level of iron, there were chances of reproductive problem in which case increased iron content creates a layer on eggs of fish. According to Bryan (1976), the sub lethal toxicity of iron may be in the form of change in morphology, histology, growth, development, swimming performance, respiration, blood chemistry, enzyme activity, reproduction, endocrinology, reproduction and behavioral changes. The higher concentration of iron in fish habitats may create a layer on fish gill that leads fishes in cardiovascular disease, heart disease, respiratory problems, making susceptible to microbes etc.

4.4.3 Liming

Though the rate of liming was depended upon pH of the pond water, most of the farmers were not aware of it. They usually spreaded lime over the pond surface 5-6 times in a year. Rate of liming varied from hatcheries to hatcheries. Some farmers used lime during drying however, others during culture period when they considered. The doses were found to be ranged from 500- 4000 gm/dec in the present study. Liming is normally done at a rate of 1000 g/dec (Haque, 1991 and Mazid, 1992). Chakraborty (1979) reports liming dose at 600-1200 g/dec in India. Shaha and Shah (2010) report that liming rate ranged from 500-700g/dec and 600-800g/dec in Jessore and Mymensingh regions respectively. All the above findings were similar to results of the present study.

4.4.4 Fertilization

Most of the farmers used fertilizer before stocking of fish and during culture period whenever they thought necessary. The differences in the rate of fertilizer application in Jessore region was ascertained to be based on the species combination, soil fertility, water quality and on the farmers' own experience on culture practices. Most of the farmers were seen using fertilizers at near optimum rates, only a few used at very low levels.

Table 3: Application of fertilizers in pond water

Serial	Fertilizer	Amount (gram/decimal)
1	Urea	200-300
2	TSP	500-1000
3	MP	100-300
4	M. oilcake	1000-4000
5	Salt	200-500
6	Cow-dung	2000-5000

The application rate of fertilization differ from region to region (Islam et al., 2002). Shaha and Shah (2010) reported that, in general, the amount of the fertilizers applied by the farmers in Jessore region was higher than that applied in Mymensingh region and this was ascribed to the differences in soil and water quality. Quality of soil and water of Jessore was not as good as in Mymensingh region and in Jessore area there is the big problem of iron in ground water. So the findings of the present study conformed to the results of Shaha and Shah (2010).

4.4.5 Water quality parameter

Water quality parameter is the most important factor for brood fishes' health, growth, survival and reproduction. In brood fish ponds, suitable temperature, DO, p^H and transparency are crucial for their expected performance. The study revealed that the hatchery owners maintained the suitable water quality parameters of the ponds following the guidelines as provided by the USAID, a USA based NGO in Bangladesh. Ekubo and Abowei (2011), Santhosh and Singh (2007), Bhatnagar *et al.* (2004) and Delince (1992) corroborate the water quality guidelines of the USAID.

Table 4: The water quality parameters of broodstock ponds in the surveyed hatchery in Jessore

Sl. no	Parameters	Desired level
01	Temperature (^{0}c)	20-30
02	DO (mg/L)	5-7
03	pH	7.5-8.5
04	Transparency (cm)	17
05	Carbon-dioxide (CO_2) (mg/L)	10-12
06	Ammonia (mg/L)	0-0.2
07	Iron (mg/L)	0.5
08	Water Depth (feet)	5-7

4.5 Food ingredients used in brood ponds in the study area

Different types of food ingredients were found to be used in the surveyed hatchery (Table 5). The most common type of ingredients were consisted of varied food items ranging from fish meal, oil cake, rice bran, wheat bran, soybean meal, maize, molasses, di-calcium phosphate and salt etc. The proportion of the items in the feed was not in fixed and depended on the experience of the individual hatchery owners.

Table 5: The ranges of different food ingredients used in surveyed hatcheries

Serial	Food Ingredients	Ingredients used (%)
1	Fish meal	10-20
2	Oil cake	20-45
3	Rice bran	10-30
4	Wheat bran	15-20
5	molasses	5
6	Soyabean	20
7	Probiotic	1-2
8	Di-calcium phosphate	1-2
9	Mixture of vitamin & minerals	1-2
10	Salt	1-2

In some cases, readymade feeds were supplied with 25-30 % protein contents. The rate and frequency of feeding of brood fish in the region were found to be variable. The fishes were fed at 3-4% of body weight, 5-6 days in a week. The feeding rate was followed starting from initial stage of brood preservation, however, as the breeding season approaches, the rate was decreased to 1-1.5% of body weight, 3-4 times per week. The feeding rates were found to depend on species combination as well.

4.6 Problems faced by hatchery owners

Inbreeding

Due to the high fecundity in Indian major carps, in most species, only a few spawners with superior traits are used as breeders. Inbreeding may occur due to the breeding method adopted during artificial fertilization. Thus, inbreeding depression in aquaculture has become a common phenomenon. Fry produced from a small number of breeders poses the obvious risk of reducing the total amount of genetic diversity. Schom and Bailey (1986) suggest that inbreeding can be minimized if forty to fifty spawners are chosen at random in each generation. But, information of the surveyed hatcheries was contrasting sharply with the above suggestion. What was seen in the hatcheries had been very threatening for our future potentiality of carp hatchery sector. This is because, only few spawners were selected for breeding purposes with unequal sex ratio which was not expected for a better genetically healthy population.

Theoretical approach to inbreeding:

Inbreeding in an infinitely large population is defined as the mating of individuals that are more closely related to each other than individuals mating at random within a population. However, the populations actually used in most aquacultural programs are finite populations because they possess a limited number of members. All finite populations experience some degree of inbreeding that is based on the number of individuals that contribute progeny to each succeeding generation. Inbreeding is measured by a value called the inbreeding coefficient which is normally represented by the symbol F. The inbreeding coefficient (F) is the probability that two alleles at any locus are identical and descended from a common ancestor. Since the inbreeding coefficient is a probability, it can assume only the values within the range from zero to one (Kincaid, 1983).

Inbreeding coefficients express the amount of inbreeding that has accumulated starting from a specific point in the ancestry of the population. Because the number of independent ancestors is limited in any finite population, all alleles of a single form would be identical and descended from a common ancestor if they were traced far enough into the past. Therefore, the inbreeding coefficient is meaningful only if a specific time in the past is chosen beyond which ancestries will not be considered, and at which time all alleles are considered to be independent. This point is called the base population, and by convention it is considered to have an inbreeding coefficient of zero. The inbreeding coefficient of any given generation therefore expresses the fraction or percent of heterozygosity that has been lost since the base population generation. When an F-value is given for a brood stock population, the specific base population is not always identified but the existence of a base population is always implied (Kincaid, 1983).

The primary genetic consequence of inbreeding is to increase the frequency of pairing of similar genes. All genotypic and phenotypic changes associated with the practice of inbreeding stem from this one primary consequence. In general, inbreeding results in an overall lowering in performance. It is most obviously reflected in poorer reproductive efficiency, including higher mortality rates, lower growth rates and a higher frequency of hereditary defects. Despite these generally harmful effects, inbreeding is a very useful tool in the field of animal breeding. It enables the breeder to uncover and eliminate harmful recessive genes within the population. It is also essential to the development of prepotent animals and is desirable in the development of distinct family lines. In addition, seed stock and commercial producers have successfully used linebreeding to maintain a degree of genetic relationship in their animals to some outstanding ancestor or ancestors.

Inbreeding can be avoided by not mating closely related individuals. This can be achieved by tagging and maintaining a pedigree for all broodstock. When tagging of broodstock cannot be done, increased effective breeding number can be used to minimize inbreeding. FAO (2008) recommends for stocking, inbreeding level should be maintained below 0.01. The number of broodstock required depends on the number of generations the hatchery is designed to reach production targets, the sex ratio used and successful spawning rates. For example, in a close breeding population, i.e. no new genetic materials are introduced for 5 generations, assuming pair mating is applied, it requires 250 males and 250 females with 100% spawning rate to achieve an inbreeding level of 0.01. In reality spawning rates are lower than 100% and the number of male and female broodstock required in such a case are much higher.

Eknath and Doyle (1990) estimated the rate of inbreeding in one generation in catla and rohu and it ranged from 3.1 to 13.2 percent in catla and 6.0 to 12.6 percent in rohu in different hatcheries of the state of Karnataka, India. In the present study, estimated rate of inbreeding in one generation in catla and rohu ranged from 2.28 to 25.00 percent and 0.54 to 4.95 percent respectively (Table 8).

Inbreeding depression

Inbreeding depression is the effect of inbreeding normally measured as a reduction in the expected performance of the affected trait. Inbreeding depression is measured as the average performance difference between an inbred population and the base population (Kincaid, 1983).

Small or declining populations are at increased risk of extinction because of stochasticity and allelic effects (Lande 1998, Courchamp et al. 1999, Stephens and Sutherland 1999, Bourbeau-Lemieux et al. 2011), and several genetic problems that include reduction in genetic variability, an accumulation of deleterious mutations due to random drift, and increased rates of inbreeding depression (Frankham et al. 2002, Hedrick 2005, Allendorf and Luikard 2007).

Genetic drift

Genetic drift is random changes in gene frequency due to some natural or manmade causes. Man made changes may be ascertained to the inaccurate sampling of the population during the formation of hatchery population. This mainly happens if the sample taken is small. The smaller is the sample, the greater the likelihood that inaccuracies in sampling will occur. In Bangladesh the hatcheries generally maintain small number of broods (Fig. 6), do not maintain any pedigree record of the brood, thus relatedness always become a factor for reduction of genetic variability. Also, because of limitations in the captive facilities; the broods maintained in small numbers potentially leads to genetic drift situation; whereby many potential genes that determine important fitness traits are automatically left out from the population causing incidences of inbreeding.

Negative selection

Negative selection occurs in the hatchery by the hatchery workers inadvertently. It happens every time when the hatchery workers handle their fish for breeding. Sometimes for some reasons they select the broods preferentially and thus selection takes place in favor of some traits, which are actually may not desirable ones, but this happen without their conscious. Selecting larger or better appearance fish will reduce the chance of smaller or less attractive fish passing their genes to the next generation, which reduces genetic diversity.

It is seen from the above that there should be no selection of any sort during the process of breeding and that appears that avoidance of selection often can be the best breeding method to apply in the hatcheries to protect the population from being lowered in performance. This breeding program is called no selection method.

Occurrence of disease

Sound health management practice is a key to success in any hatchery or nursery operation. In the study area hatchery operators mentioned several problems that they encountered in their operations. Those are: Occurrence of disease, unsuitable weather, poaching, lack of finance and bad communication.

When ranking their problems, all of the hatchery owners (100%) mentioned that they encountered diseases in their farms. However when they asked about the specificity of the problem, the owners said that they considered diseases to be an important issue in hatcheries. The diseases were reported to be such as, fish lice, tail and fin rot disease, sudden spawn mortality, gill rot disease, air gulping, deformed larvae etc. The main problems of hatchling production in the Jessore region was argulus infection. Ninety five percent of hatchlings mortality was caused by argulus disease. There were no pesticides to control the argulus disease. The farmers reported that nothing could help to prevent or control that fatal disease.

Other conditions like suffocation and fin rot were also reported but with lower incidence. Majority of the hatchery owners reported that they observed disease in brood fish mainly during winter season.

Unsuitable weather

Sometimes lack of suitable weather becomes a great problem for induced breeding. When weather becomes too hot or too cold, it was very difficult for the hatchery owners to complete a successful breeding cycle.

Poaching

Poaching of brood fish and hatchery equipment was an often reported problem in the hatcheries, if they were not properly taken care of.

Lack of finance

Seventy percent of the hatchery owners identified the lack of credit facilities as their problem. They were not satisfied with their hatchery business due to production loss. Some hatchery owners leased out their property to recover this loss and some were languishing very tough situation. Some hatchery owners claimed unplanned and mismanagement for this situation.

4.7 Stocking rate and stocking size of broods

Stocking rate of the brood fish was more or less similar in all of the surveyed hatcheries. Stocking size was ranged from 10-20 kg/decimal (Table 6). The findings corroborate to that of the observations of Sarder (2007).

Table 6: Stocking ration and size of brood fish in the surveyed hatcheries

Sl. No.	Hatchery Name	Species (%)									Stocking Size (Kg/Decimal)
		Silver carp	Catla	Rohu	Mrigal	Grass carp	Sarputi	Common Carp	Bighead Carp	Bata	
01	Anan	30	5	40	20	5	-	-	-	-	15-20
02	Chowdhuri	20	20	25	10	15	5	-	-	-	15
03	Haque	13	6	25	20	6	30	-	-	-	14
04	Kapotakkho	30	4	27	20	8	6	-	5	-	14-18
05	Madhumoti	30	3	35	25	3	2	2	-	-	12-16
06	Ma Fatema	8	10	25	32	10	15	-	-	-	12-14
07	Matri	15	3	15	15	7	30	-	-	15	12
08	National	15	3	15	15	7	30	-	-	15	20
09	Rita	10	10	30	25	10	15	-	-	-	10-15
10	Rupali	7	2	34	40	3	14	-	-	-	14-15

4.8 Exchange of broods

It was observed that all the hatcheries except Rita Fish hatchery (RIFH) had partially replaced their brood fishes every year. The exchange rate was varies from hatchery to hatchery being in the range of 20 to 40% annually. Highest rate of exchange found in Ma Fatema Fish Hatchery (MFFH) and comparatively lower rates were seen to practise by the Haque Fish Production Centre (HFPC), Kapotakkho Fish Hatchery (KFH) and National Fish Hatchery (NFH). Normally the hatchery owners used to sell their old brood fishes to nearer fish markets; some are exchanged with the other hatcheries around. Peteri *et al.* (1992) suggest a yearly recruitment rate of 25–30% to avoid genetic problems in breeding fishes in hatchery. For influx of new genetic material to the broodstock population regularly introduction of 10-15% new brood stock is suggested in each generation, the same can help drastic reduction of inbreeding in hatchery population. An example in this regard is the fish stocking programs in Myanmar, where hatcheries are licensed to recruit new broodstock from the wild periodically, based on the agreement with the government that a proportion of fingerlings produced should be released into public waters as part of the national stock enhancement programs (Aung et al. 2009). Yearly recruitment of brood fish may be considered as an important part of management because most of the hatcheries have only a limited number of breeders than they actually ought to have. This steps can help them to minimize the loss of some valuable genes. But in such case, hatchery operators need to maintain pedigree records to reduce or to avoid the chance of mating between closely related breeders.

4.9 Training status of hatchery personnel

In the present investigation, it was found that 90% of hatchery owners attended 3-7 days training arranged by Department of Fisheries, BFRI or Worldfish on hatchery management. Only a very few had certificate of international training. The rest 10% owners had no training experience at all.

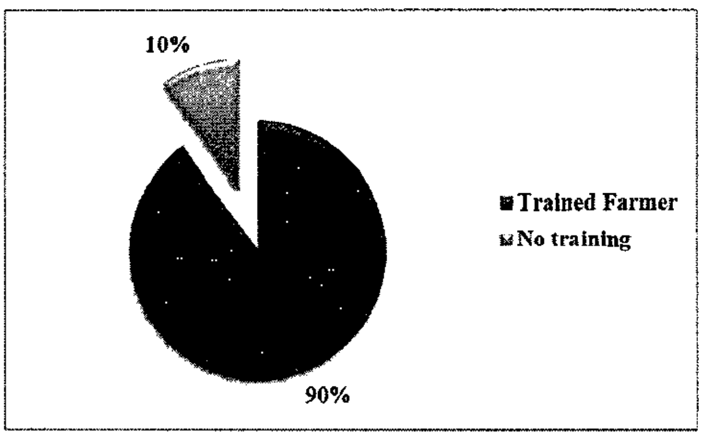

Fig. 4: Status of training of the hatchery personnel

Present study revealed that training attained was ineffective as the hatchery owners failed in applying their learning in hatchery management to protect or reduce the loss of genetic quality of broodstock. The owners as well as the personnel of most of the hatcheries in Jessore did not have sound knowledge about the genetic erosion of fish seed during breeding practices in hatchery. As a result they conduct breeding program only in view of commercial benefit. They did not maintain the pedigree record of the broods, did not either practice proper brood selection nor practiced proper sex ratio of the broods in the breeding program.

4.10 Breeding inducing agents

Success of induced breeding mostly depends on techniques applied in the hatcheries including the use of suitable breeding inducing agent. Mainly two types of inducing agents *viz.* pituitary gland (PG) and Ovaprim were used for induced breeding practices in the surveyed hatcheries. In case of female major carp, a preliminary dose of 0.2-2.0 mg/kg body weight was injected and after 5-6 hours, a second dose of 6-6.5 mg/kg body weight was injected. The first dose was called primer dose and second dose was called booster. The male, on the other hand, received a single dose of 1.0-2.0 mg/kg body weight at the time of application of second injection to the female. Besides, 0.1 – 0.2 ml/kg Ovaprim for male and 0.3 – 0.5 ml/kg Ovaprim for female were applied only as the 1st dose. Das and Khan (1962), Chaudhuri (1976) and Surnar *et al.* (1993) adopt a more or less similar practices for induced breeding of various species of fish. It was observed in the study that fishes when injected with the correct dose of hormone had bred easily with satisfactory results.

According to Surnar *et al* (2015), rates of fertilization and hatching are higher and size of eggs after water hardening always becomes considerably bigger in Ovaprim treated fish with the production of healthy hatchlings. It is more economical than pituitary, however, post-spawning mortality of fish is negligible with little or no effects on reproductive cycles and does not require refrigerated storage and preservation at ambient temperature.

4.11 Selection of brood fishes for spawning

The hatchery owners did not follow the proper sex ratio for induced breeding. Most of the hatchery owners follow some considerations for selecting brood fishes for artificial breeding based on some body parameters of brood fish, such as size of fish, shape of belly, anus and other body fitness. Ninety percent of hatchery owners discarded brood fish of inferior size or shape during induced breeding. The seasonal or market demand was the main consideration of the hatcheries in breeding fish. Eighty percent of hatcheries bred fish through out the season although production of seed was very low during the off peak season due to decrease of demand. The peak breeding season was April to June.

The desirable brood sizes, particularly for female breeders is said to be > 1.5 kg, age 2 years for rohu and > 4 kg, age 3 years for catla (Ahmed *et al.*, 2005). In the present study some of the hatchery owners had appeared to use underweight brood in both catla and rohu; in rohu the size was even as low as 1kg in many hatcheries. The weight of the breeders used in spawning in the hatcheries studied was ranged from 3.25 to 8.5 kg female and 2.5 to 5.24 kg male for catla; in rohu the range was 1.79 to 4.17 kg for female and 1.73 to 3 kg for male. Shah (2010) conducted a comparative study about the average weight and length of the Indian major carps between riverine population and hatchery stock. Where, he observe that the average size of the broods are significantly smaller than riverine stock due to the negative selection in favor of smaller brood size.

Table 7: Weight-wise selection of brood fishes for induced spawning

Sl. No.	Hatchery Name	Species	Average weight (kg)		Maximum weight (kg)		Minimum weight (kg)	
			Male	Female	Male	Female	Male	Female
01	Anan	Rui	3	3.75	2.5	3.5	1.5	2
		Catla	5.25	8.5	5.5	10	5	7
02	Chowdhuri	Rui	2.07	2.09	2.75	4	1	1
		Catla	4.5	6.25	5	7.5	3.5	4.75
03	Haque	Rui	1.88	2.67	2.5	3.75	1	1
		Catla	2.5	3.5	5	7.5	3.5	4.75
04	Kapotakkho	Rui	2.92	3.13	2.75	3.75	1	2
		Catla	3.5	5	4	5	3	5
05	Madhumoti	Rui	1.73	4.17	2.75	3.5	1	1.5
		Catla	4.67	5	5.5	10	4	7
06	Ma Fatema	Rui	2.12	2.86	2.75	4	1	1
		Catla	3.6	4.95	4	6	3	4
07	Matri	Rui	3	2.94	2.5	3.5	1.5	2
		Catla	3	3.88	3	4.75	3	3
08	National	Rui	2.5	4	2.5	3.5	1.5	2
		Catla	4	5	4	5	4	5
09	Rita	Rui	1.97	3.25	2.75	4	1	2.5
		Catla	3.32	5.06	4	8.5	3	3
10	Rupali	Rui	2.49	1.79	2.5	3.5	1.5	2
		Catla	4	4.67	5	7.5	3.5	4.75

4.12 Sex ratio and effective breeding number

Genetic problems are like drift, inbreeding and negative selection reduce the average viability of individuals from generation to generation, and they reduce evolutionary potential and therefore the long-term survival expectancies, especially of small populations (Frankham et al. 2002, Hedrick 2005, Allendorf and Luikard 2007). However, genetic problems are only indirectly linked to the census size (N_c). Instead, they are directly dependent on the genetically effective population size (N_e) that is defined as the size of an ideal model population that loses genetic variability at the same rate as the observed population. Usually,

N_e is significantly smaller than N_c because of variance in individual reproductive success, deviations from a 1:1 operational sex ratio, and other reasons. Risks of extinction are therefore increased if population sex ratios deviate from 1:1.

The typically expected sex ratio is 1:1 in natural populations because of strong frequency depended selection on the production of sons and daughters (Fisher, 1930). However, population sex ratios can be biased by non-random harvest as a consequence of, for example, sex differences in behavior, size, or morphology, or simply as a consequence of hunter preferences (Bunnefeld et al. 2009, Tryjanowski et al. 2009, Marealle et al. 2010).

Most of the hatchery managers used skewed sex ratios when they spawned their fish. This was done because one male could usually be used to fertilize eggs from several females or the sex ratios were sometimes significantly skewed towards too many females or too many males in these hatcheries (Table 8). This enables aquaculturists to use and maintain fewer males, which reduces production costs. This may be a great idea in terms of efficiency, but it is a bad idea if hatchery operators want to manage and control the genetic quality of the population. When a skewed sex ratio is used, the rarer sex has a disproportionate influence on the size of N_e. The factor determining loss of genetic variation and increase of inbreeding is the effective population size, N_e.

The present study revealed that only Ma Fatema Fish Hatchery (MFFH) maintained approximately 1:1 sex ratio for both the species during artificial breeding. A few hatchery operators followed this ratio only for catla. This scenario was more worst in rohu than in catla. Such unequal ratios of sex potentially increase the negative allelic effects and demographic stochasticity, sometimes leading to extinction. Non-equal sex ratios also reduce the genetically effective population size because $N_e = 4NmNf/(Nm+Nf)$, with Nm and Nf being the number of mature males and females, respectively (Hartl, 1988).

Therefore, populations with deflected sex ratios are expected to lose more genetic variability (i.e. evolutionary potential) and suffer more from the negative consequences of inbreeding than populations with equal sex ratios. The inbreeding that is produced by various combinations of males and females is shown in Table 8 which clearly shows that the best way to reduce inbreeding is to increase the number of males and females and to spawn a sex ratio that is as close to 1:1 as possible. The average inbreeding value of a population is determined from the population's effective breeding number (N_e). Effective breeding number (N_e) is one of the most important concepts in broodstock management, because it gives an indication about the genetic stability or genetic health of the population. This is because N_e is inversely related to inbreeding and to genetic drift. With decrease of N_e, inbreeding and genetic drift increase with the consequent increase in variance of gene frequencies in the population. Hatchery populations are usually small and are often closed. A closed population is one where immigration (the introduction of fish from another population) is not allowed; consequently, fish from other populations are not allowed to mate with or hybridize with fish from a closed population. Hatchery managers often maintain closed populations for various reasons; chief among them is to minimize the cost of keeping with respect to food and holding space; also a desire to minimize health problems by preventing the introduction of diseases that often accompany acquired fish (Tave, 1993).

Table 8: Percent inbreeding produced by various combinations of males and females. Inbreeding values were rounded to the nearest hundredth.

Sl. No.	Hatchery Name	Rui		Catla		Average inbreeding % (F)		Effective breeding num. (N_e)	
		Male	Female	Male	Female	Rui	Catla	Rui	Catla
01	Anan	6	8	2	2	3.65	12.50	13.71	4.00
02	Chowdhuri	14	14	4	3	1.79	7.29	28.00	6.86
03	Haque	20	30	1	1	1.04	25.00	48.00	2.00
04	Kapotakkho	12	16	2	2	1.82	12.50	27.43	4.00
05	Madhumoti	11	18	3	3	1.83	8.33	27.31	6.00
06	Ma Fatema	13	13	5	5	1.92	5.00	26.00	10.00
07	Matri	3	16	1	2	4.95	18.73	10.11	2.67
08	National	12	15	2	4	1.87	9.38	26.67	5.33
09	Rita	8	7	14	9	3.35	2.28	14.93	21.91
10	Rupali	37	61	3	3	0.54	8.33	92.12	6.00

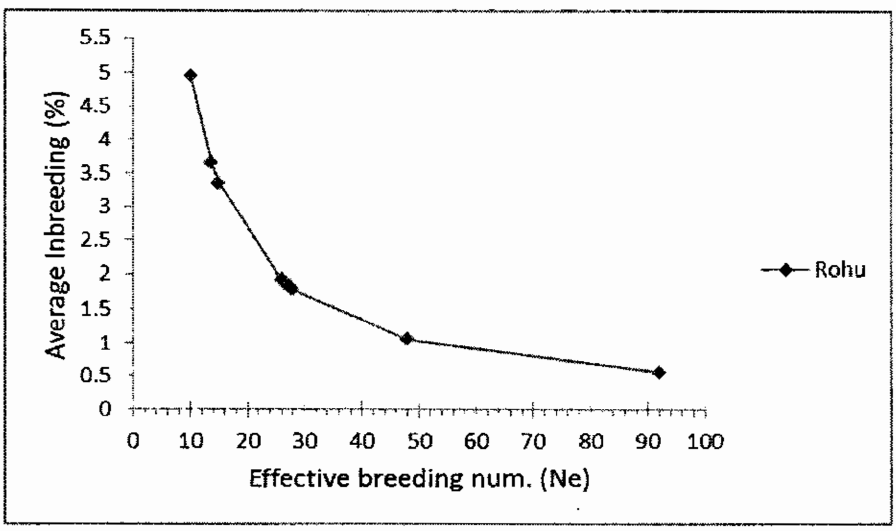

Fig. 5: The relationship between effective breeding number (N_e) and average inbreeding (F) among the hatchery population of rohu species

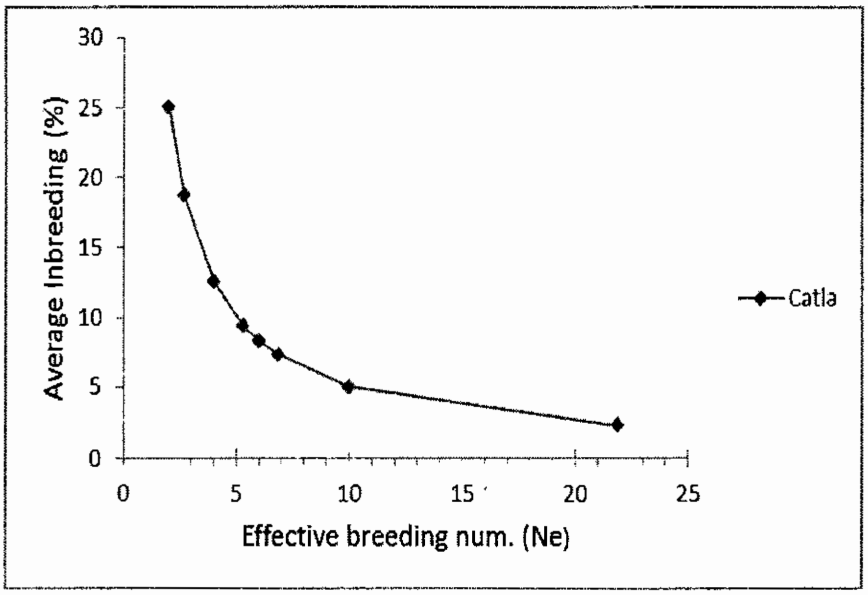

Fig. 6: The relationship between effective breeding number (N_e) and average inbreeding (F) among the hatchery population of catla species.

The highest effective breeding number (N_e) was found 92.12 in Rupali fish hatchery whose average inbreeding percentage was 0.54 for rohu. For the same species, lowest effective breeding number (N_e) was 10.11 with average inbreeding percentage being 4.95 in Matri fish hatchery (Table 8 and figure 4).

The highest effective breeding number (N_e) for catla was 21.91 with corresponding inbreeding percentage 2.28 in Rita fish hatchery. Effective breeding number (N_e) of catla, in general, in the hatcheries surveyed was lower than rohu which might lead faster rate of inbreeding in this species; the lowest value of effective breeding number (N_e) was only 2 with corresponding inbreeding percentage 25 in Haque fish hatchery (Table 8 & fig. 5). A farmer or hatchery manager who wants to manage the genetic aspects of his population must know how inbreeding can best be applied to improve productivity and profits.

Average inbreeding values should be determined for all hatchery populations every generation, and the acquisition and use of these data should be an integral part of hatchery management. The average inbreeding value is as important as average harvest weight or any other data that can be gathered.

Effective breeding number and the average inbreeding value are important population indices, because they help explain trends in yield, fecundity, and other important production phenotypes. These values also enable a farmer to predict if problems will occur as a result of inbreeding depression or the loss of genetic variance. Proper brood stock management cannot be accomplished without these information.

Since most farmers and hatchery managers do not mark fish, this usually means that N_e must be managed to minimize inbreeding. The management of N_e is meant to prevent genetic drift from robbing the populations' valuable alleles.

Unfortunately, there is no single N_e and there is no simple set of genetic guidelines that can be prescribed for every farmer and every hatchery manager. Unlike disease prevention and treatment, the recommendations and protocols that are needed to prevent genetic problems vary from farmer to farmer, from farm to farm, and from program to program. A number of studies have recommended that aquaculturists or hatchery managers should maintain a specific N_e in order to prevent inbreeding - or genetic drift-related problems. These recommendations have provided brood number ranging from 50 to 1,000; the majority, however, suggest either 500 or 1,000 (Tave, 1993).

4.13 Fertilization and hatching rate

The following figures (Fig. 4 and Fig. 5) depict the mean fertilization and hatching rate of catla and rohu among the surveyed hatcheries.

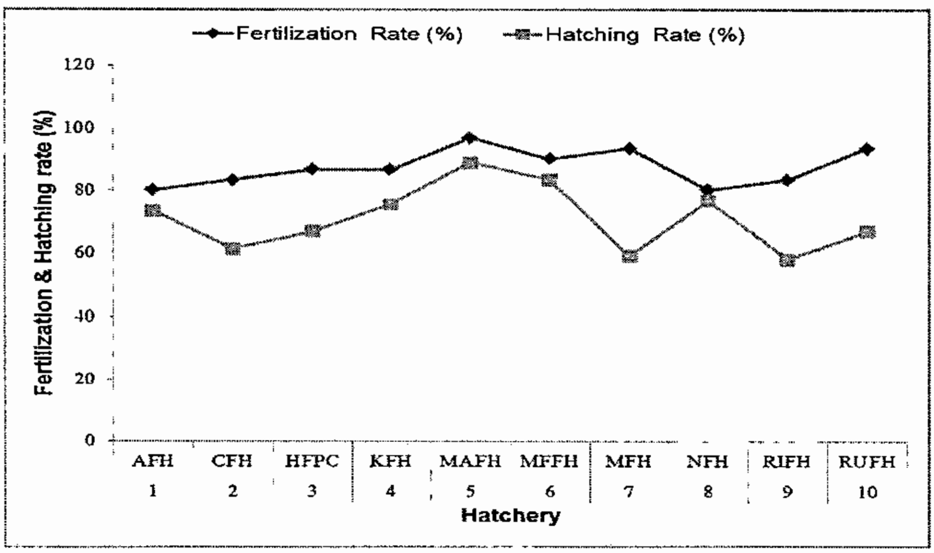

Fig. 7: Fertilization and hatching rate of Rui (*L. rohita*)

Naeem *et al.* (2013) found overall fertilization and hatching percentages of rohu were 0.78 and 0.81 respectively by using inducing agent Ovaprim-C; in case the catla the rates were 91.01% and 67.50% with the use of same inducing agent (Naeem *et al.* 2005).

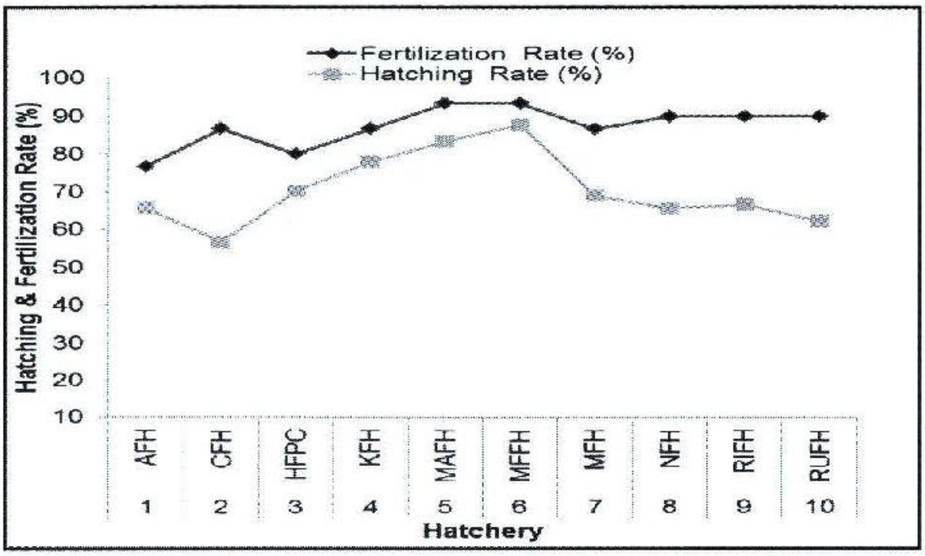

Fig. 8: Fertilization & hatching rate of Catla (*C. catla*)

Mean fertilization rate in 10 different hatcheries in the present study was found to range between 80 to 96.67 % for rohu and 76.67 to 96.67 % for Catla; the mean hatching rates in the same hatcheries were ranged between 57.78 and 88.89 % for rohu and 56.67 to 83.33 % for Catla. These findings also corroborate the observations of Dhawan and Kaur (2004), Bhuiyan and Aktar (2011), Alikunhi *et al.* (1964), Ali (1967) and Singh *et al.* (2000).

4.14 Population size

The factor determining loss of genetic variation and increase of inbreeding is the effective population size, N_e (Frankham et al., 2002). Figure 6 shows the total population size maintained in the hatchery ponds by species.

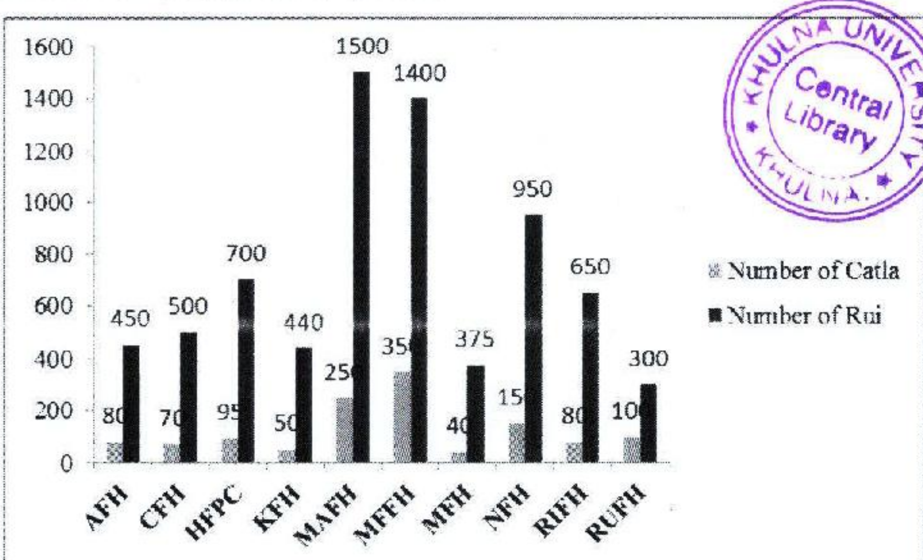

Fig. 9: Number of breeder maintain in the hatchery ponds

Hussain and Mazid (1999) indicate for stock deterioration due to accumulation of inbreeding and that can be avoided by keeping an adequate number of brood fish (a medium-sized hatchery needs to keep at least 3000 to 5000 breeders per species) in order to the harness the best performance of the broods in terms of size, maturation and breeding efficiency.

The problem arises when each hatchery maintains few number of breeders which may leads to a serious genetic loss in hatchery produced population. Hatchery owners operate their hatchery to meet their commercial target only. They don't care about the genetic quality of brood fish. In most cases they don't even know how to prevent different genetic problems like inbreeding, negative selection, genetic drift etc. In some cases they keep some knowledge about these problems but don't adopt any management plan to reduce the problems. In the present study, a hatchery was found only 40 breeders for catla and 300 for rohu. The maximum 350 broods were found for catla and 1500 for rohu.

CHAPTER FIVE

CONCLUSION

Conclusion

Aquaculture of Indian major carp is now almost entirely dependent on the hatchery produced seeds because of the phenomenal damage of the riverine natural breeding grounds in the country. There are now about 1000 hatcheries established under private and public sector. Jessore has been the prominent area for artificial production of fish seed; in Chanchra, Jessore itself, there were 27 hatcheries. The management of brood fish and hatcheries was not done with due consideration of the genetic norms and principles and as such the quality of seed produced could not be maintained; to date there are often the claims of poor growth performance of the hatchery produced seed. To find out the management problems of hatchery and brood fishes, the present study was undertaken. The management of 10 different hatcheries was assayed with respect to their facilities and practice of brood management, selection of broods and breeding of two species viz., *L. rohita* and *C. catla*. The overall management scenario of the hatcheries at Chanchra was not adequate at all. The hatcheries were seen to be highly variable with regard to all the different indices like breeding facilities, number of tanks, water supply, manpower employed, brood captivity facilities, feeding, fertilization, water quality, number of brood kept, sex ratio of broods, record keeping and adherence to the principles of mating broods to avoid inbreeding – a familiar problem in seed production in hatchery. Despite the significant backup of training and extension programs on hatchery management by the government and NGOs, the hatcheries were seen not to effectively follow the genetic principles of brood management and seed production. As a result, the hatchery population and seed produced were inevitably experiencing serious genetic erosion leading to the significant lower rate of fertilization and hatching; inbreeding due to relatedness of the broods was inevitably occurring in the process of mating as the owners did not maintain the pedigree records of the broods at the time of formation of the hatchery stocks. The size of brood fishes were seen to be smaller ranging from 1.0 to 2.75 kg for male and 1.0 to 4.0 kg for female rohu and 3.0 to 5.5kg for male and 3.0 to 10kg for female catla respectively. The effective breeding numbers (N_e) in the hatchery surveyed were calculated and found to range between 10.11 to 92.12 and 2.0 to 21.91 in rohu and catla respectively; the percentage of inbreeding (F), similarly were found to range between 0.01 to 0.05 and 0.02 to 0.25 respectively in the two species in the surveyed hatcheries. For quality seed production of the two species, the standard of practice of hatchery and brood has to be improved adhering to both the genetic and non-genetic principles. The government should strengthen its training and extension services to improve the situation.

CHAPTER SIX

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CHAPTER SEVEN

APPENDIX

Questionnaire for collection of data from hatcheries

No.	Item	Answer
1.	Date of interview:	
2.	Name of the hatchery:	
	Hatchery code:	
3.	Name of the owner:	
4.	Address:	
5.	Phone:	
6.	Establishment and area:	
	a) No. of ponds:	
	b) Area of water body:	
	c) Number of deep tube well:	
	d) Number of tanks and types: HJ:	BH:
	Number of manpower:	
7.	Category of people	
	a) Manager:	
	b) Technician:	
	c) Hatchery assistance:	
	d) Night guard:	
	e) Other:	
8.	Capacity of production per year:	
9.	Production this year:	
10.	Species bred:	
11.	Sources of brood fish:	
	a) World Fish Center b) BFRI c) Halda River d) Padma River e) Govt. Brood Bank	
	f) Own g) Other source:	
12.	Do you keep the record of the history of origin of brood? Yes/No	
13.	Brood pond preparation methods:	
14.	Stocking rate/ stocking ratio of brood fish:	

Silvercarp Catla Rohu Mrigal Grass carp Sarputi

15. Stocking size of brood fish:
16. Feeding:
17. Fertilizer used:
18. Water quality monitoring of the brood pond:
19. Brood fish conditioning:
20. Did you exchange brood? Yes/ No.
21. If yes,
    How much/ what percentage? From where?
22. Method of selection of brood:
    Sign/marks/body parameters/average size of brood fish bred in the hatchery
23. Do you have any considerations like cost of hormone, seasonal demands of seed throughout the season?
24. Do you breed throughout the season?
25. Peak breeding season
26. Do you discard fish of inferior shape/size/other qualities? Yes/No.
27. Do you have any problem with water quality/disease/parasites/mortality of fish? Narrate the problems
28. Did you attend any training in fish breeding/genetics? Yes/no
    If yes, on what topic, from where and for how long?
29. Can you mention any problem in fish breeding and management of the hatchery?