EFFECT OF WATER QUALITY WITH SPECIAL EMPHASIS ON IRON IN GOLD FISH BREEDING AND INFLUENCE OF NATURALCOLOURING AGENT IN FEED FOR COLOUR AND GROWTH ENHANCEMENT OF GOLD FISH FRY

A Thesis

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in

AQUACULTURE

By

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Dedicated to :
Parents
&
Brother

West Bengal University of Animal and Fishery Sciences

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CERTIFICATE

This is to certify that the work embodied in the thesis entitled **"Effect of water quality with special emphasis on iron in gold fish breeding and influence of natural colouring agent in feed for colour and growth enhancement of gold fish fry."** *submitted by* Afrin Nahar *in partial fulfillment of the requirements for the degree of* ***Master of Fishery Science (Aquaculture)*** *in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bonafied research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.*

(Dr. T.K. Ghosh)
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APPROVAL SHEET

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF FISHERY SCIENCE

(AQUACULTURE)

We, the undersigned, have been satisfied with the performance of Miss. Afrin Nahar, in the Viva-Voce Examination, conducted today, the 25th Nov....., 2010, recommended that the thesis be accepted for the award of the Degree of Master of Fishery Science in Aquaculture.

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ABBREVIATION

mg/l	Milligram per liter
%	Percentage
^0C	Degree Centigrade
cm	Centimeter
L	Liter
Kg	Kilogram
g	Gram
h	Hour
DO	Dissolved Oxygen
CO_2	Carbon dioxide
sp.	Species
ANOVA	Analysis of Variance
APHA	American Public Health Association
ml	Milliliter
ft	Feet

CHAPTER -1

INTRODUCTION

Aquarium keeping as hobby started several hundred years ago in Eastern China. The ancient Rome was the first to keep ornamental fishes as pets at home. But keeping ornamental fish in aquarium as hobby became popular in England and Scotland in the eighteenth century. It appeared as a recent origin in India. It has been realized that aquarium keeping is not only a means of recreation but also a source of earning.

Ornamental fishes are called as "living jewels' due to their lucrative colouration, unique shape of the body and their adaptive behavior. Goldfish (*Carassius auratus*) are small ornamental freshwater fish that are commonly kept as pets. Gold fish is jack of all aquarium trades, easily adaptable for both aquarium and open outdoor cement cisterns.

Gold fish first originated in China and other country like Japan, Europe etc. They are fresh water fish, hardy in nature, live in a variety of habitats. It is an omnivorous fish and feeds on a wide variety of live feed and accepts artificial feeds also. Gold fish can be reared in high water hardness (1000 ppm) and very adaptable to various water hardness conditions but try to avoid extreme or fluctuating condition. They prefer shallow water having water temp around 28-31°c. Sexually dimorphic, females are larger than males having more bulging belly but males are more colourful and attractive than female (Chris Andrews *et al.*, 1997).

Due to intensive selective breeding and cross breeding, goldfish occurs in several varieties. Goldfish have been developed into many distinct breeds and are now found in various colours, colour patterns, forms and sizes far different from those of the original domesticated carp.

They exhibit various types of morphological characteristic such as divided caudal and anal fin, egg-shaped body due to shortened vertebrae, protruding eye, and thickening of skin on the top of head (called 'Wen'), absence of dorsal fin, transparent colour and brilliant colour.

Colour of gold fish ranged from red, orange, silver, black, brown, white and many more depending on the pigment in the skin. More than 120 varieties of gold fish are available. The colouration of the fish is due to presence of pigment cells in the skin underneath. Variation in fin shape such as fantail and veiltail among gold fish also appears. Gold fish has 3 single fins: dorsal, caudal and anal fins and two sets or paired fins: the pectoral and pelvic fins. For a beginner, this is a right fish to start with since its breeding procedure is quite easy compared to other egg laying ornamental fishes. Gold fish is worldwide adopted and accepted as indispensable ornamental fish.

One of the most important aspects of any living organism is to produce individual from the existing one, thus ensuring the continued existence of the organism concerned. Like other higher animals, fishes are also divided into males and females. Male produces sperms to fertilize the eggs of female, which develop into new generation. Most fishes release their eggs and sperm in water and fertilization takes place externally. Some fish species, however, have developed the system of internal fertilization where embryonic development takes place inside the body cavity of the fish.

There are numbers of factors that are responsible for successful breeding and spawning of ornamental fishes like physical and chemical properties of water. The most important physical factor that affects the breeding of fish is the temperature of the water. The optimum temperature range requirement is specific for each species. Therefore, they should be reared in the same temperature for proper gonad development and attaining their sexual maturity.

Natural breeding is influenced by several factors and by observing most of the factors in breeding, chances of failure in natural spawning are high. In natural breeding, activation for spawning takes very long time and even sometime it was observed that female did not spawn after attraction and may take more time in next spawning. It opens the scope of introducing artificial breeding (induced breeding), an alternative to conduct successful breeding, to produce more number of hatchlings and to achieve high survival rate.

Use of ovaprim in induced spawning offered good results (Nandeesha *et al.*, 1990, 1993; Alok *et al.*, 1993) in carps, cat fish and other commercially important fin fishes. Introduced ova prim released larger size eggs, which resulted in hatching and spawn production (Nandeesha *et al.*, 1990 and Singh *et al.*, 1998).

Ovaprim is a liquid peptide preparation that contains an analog of salmon gonadotropin releasing hormone (sGnRHa) and a brain neurotransmitter (dopamine) inhibitor. The sGnRHa in Ovaprim elicits the release of stored gonadotropins from the pituitary. The dopamine inhibitor (Domperidone) serves to remove other inhibition of GnRH release. Release of stored pituitary gonadotropins may aid spawning by stimulating ovulation and spermiation in sexually mature fish. Use of ovaprim for spawning of gold fish will provide GnRH, responsible for secretion of GTH. Release of GTH induces the secretion of MIH and it eliminates the dependency upon changing behavior of male during spawning.

Rearing of fish larvae primarily depends on size and quality of food (Leger *et al.*, 1986; Abi Ayad and Kestemmont *et al.*, 1995; James *et al.*, 1997). Use of green water for rearing fish larvae has given an excellent result in many fishes (Naas *et al.*, 1997; Bengston *et al.*, 1999). As hatchlings of fishes are sensitive to water quality, culture of green water and live food is ideal for larval rearing.

The aim of breeding ornamental fish is to obtain maximum number of young ones in controlled conditions as well as to develop new colours and strains or hybrid. Goldfish (*Carassius auratus*) are in demand in world markets due to their attractive golden colour. Carotenoids are the primary source of pigmentation on the skin of fishes. Pigmentation in the skin is responsible for coluration in the fish.

The improvement of breeding techniques of wild caught verities and rare species are of immense importance for the conservation and management of fishes in the natural environment, because it may no longer be possible to obtain the fish from the natural habitats. The existence of many species of fish will be dependent on artificial or semi-artificial breeding techniques. Recently much attention has been bestowed upon the ornamental fish production in our country due to its growing export potential. This industry could also be transferred in to a successful cottage industry in the rural sectors of our country.

Developing fresh water ornamental fish industry is believed to be one of the most important aquaculture activities. Means to diversify the agriculture practice that stimulates the stagnancy of local economy, approximately 75% of the fresh water aquarium fish imported in to the USA originated from South-East Asia (Shapman *et al.*, 1997). About 1500 different species of ornamental fish imported yearly. The largest market for ornamental fish is in USA, Japan, UK, Germany, Spain, Holland, Singapore and India (Kolkata, Chennai, Mumbai, Trivandrum and Kochi). Current ornamental fish production of world is 30,000 corers (Ziauddin *et al.*, 2007). On a global basis, India ranks one of top few countries with maximum fresh water and marine ornamental fish reserves. India's share in global ornamental fish trade is 1.23 million US$ (MPEDA, 2006-07) out of which West Bengal export 0.56 million US$ (MPEDA, 2006-07).

At present the ornamental fish export from India is dominated by the wild caught species. In India, there is a promising domestic market which is mainly based on domestically bred exotic species. The overall domestic trade in this field around 10 cores and is growing at the rate of 20 % annually. Ornamental fishes are now- a- days rapidly gaining importance because of their

aesthetic value and also due to their immense commercial value in the export trade world over. Attractive colouration determines the commercial value of ornamental fish.

The earning potential of this sector has hardly been understood and the same is not being exploited in a technology driven manner. Considering the relatively simple technique involved, this activity has the potential to create substantial job opportunities, besides helping export earnings. Attempt was made to develop a package of practice on induced breeding of gold fish using ovaprim and objective to select the present work was to study:

- Natural spawning of gold fish
- Induced breeding of gold fish using inducing agent-ovaprim
- Effects of water qualities especially hardness and iron on goldfish breeding
- Larval rearing, growth and colour enhancement of goldfish fry using natural colouring agents in feed.

CHAPTER -2

REVIEW OF LITERATURE

Carassius auratus, more commonly called Goldfish, a fresh water fish species whose history can be traced back over 1500 years to Ancient China (Chris Andrews, 2002). Goldfish culture believed to have originated in China where centuries of selective breeding produced many of the verities that are recognized today (Mitsukuri *et al.*, 1904). Goldfish is one the most popular and economically important of non-consumed fish in the world. They are attractive and colourful with peaceful nature (Jayasankar *et al.*, 1998) ;(Mukharjee *et al.*, 2000); (Singh and Ahmed *et al.*, 2005) with high reproductive potential (Pant *et al.*, 1981). Among ornamental fishes, goldfish (*Carassius auratus*) (Linnaeus, 1758) is the most admired ornamental fish though out the world and also in India.

Goldfish is a small to moderately-sized fish with a deep body and rounded in cross-section. It has large head and eyes with a small mouth and a forked tail. Scales are large and the single dorsal fin has 3-4 stout spines at the leading edge. Colour ranges from olive-bronze to deep golden along dorsal surface, fading to silvery-white along the belly (McDowall, 2000). Goldfish may grows up to 41cm in length, 2 kg in weight and live for 10 years in captivity (Fish Base, 2004).

In the wild, goldfish can be found in slow-moving, freshwater bodies of water. As with their close relative the carp, they thrive in slightly sludgy water. In an aquarium, bi-weekly water changes are a good idea as a goldfish tank is hard to keep clean. They thrive in a pond environment thus the addition of real plants is optimal if the owner is prepared to replace them fairly regularly; goldfish enjoy eating live plants. An aquarium with a dirt bottom is ideal but difficult to maintain. Small pebbles are a suitable substitute for the pond-like bottom.

2.1 Water parameters:

Typically, goldfish will survive in water temperatures ranging from freezing to 30°C . Fancy varieties (orandas, lionheads, ranchu, veiltailes) should be kept in water no cooler than room temperature. Goldfish become less active in colder temperatures, and more active in warmer. Fluctuation of temperature triggers spawning, which generally occurs from winter to spring (Axelrod and Burgess *et al.*, 1973). Temperature is especially important as goldfish prefer water temperature around 25^0C /77^0F (Boyd, 1977). They cannot tolerate rapid changes in temperature. Goldfish are very sensitive to temperature. Extremely high temperature (over

30^0C/86^0 F) can also be harmful of gold fish. Temperatures of 10^0C/50^0 F are dangerous to fancy varieties of goldfish. (Martty and Couto *et al.*, 1973) mentioned that *Carassius sp* developed well at temperatures 18 - 25^0C. Temperature influences growth and gonad ripeness (Huet *et al.*, 1973). At 14^0C, *Carassius auratus* maintain the last state of vitellogenesis and ovulation can be induced by increasing temperature to 20^0C (Harvay and Hoar *et al.*, 1980). The solubility of oxygen in water is reduced as temp increase. Because of an increased metabolic rate the oxygen requirement of a fish increases as water temperature increases (Cui and Wootton *et al.*, 1988).

Goldfish larvae require a relatively constant temperature and continuous food supply. Thus intensive grow out is best done in closed systems under controlled conditions using adequate diets (Kaiser *et al.*, 2003). Temperature has an important effect on feeding rate and hence growth of fish larvae (Verreth and Den Bieman , 1987). Since growth rate increases with an increases in temperature, until an optimal temperature for the culture of the species is attained (Kestemont *et al.*, 1995) reported that the highest specific growth rate for gold fish larvae was obtained with a water temp of 28^0c. Photo and light intensity are both important for larval fish growth. Thus extending photoperiod may give fish more time to feed and consequently improve their growth and survival (Cunha and Planas *et al.*, 1999). Feeding food items that were too small retarded the growth of fish (Dabrowski and Bardeg *et al.*, 1984). Effects of various environment factors will determine growth performance of fish (Kestemont and Baras *et al.*, 2001).

They are extremely tolerant to environmental stress (Abramenkko *et al.*, 1997) including high levels turbidity and fluctuation in pH and temperature (Spotila *et al.*, 1979; Balon, 2004). Goldfish are not particularly aggressive, thus combining sizes is not often a problem (Chris Andrews,1987).Goldfish are gregarious fish that show schooling behavior, as well as displaying the same types of feeding behaviors. Goldfish may display similar behaviors when responding to their reflections in a mirror.

Swingle (1961) reported that pH 6.5 - 9.0 is adequate for reproduction. The pH value reflect the acidity or alkalinity of water, and is measured on the scale from 0 – 14. Pure water has a pH of 7.0 and is said to be neutral. Low pH means more acidic and high pH value indicates more basic. For each change in pH value there is a 10^{th} fold change in the acidity or alkalinity.

Thus a small change in pH can have a far more drastic effect on many chemical and bio-chemical reactions occurring in the body of the fish and in the water medium.

Boyd, (1989) mentioned that *Carassius auratus* survived at low oxygen concentration.

Water hardness is related to the amount of dissolve salt present n the water. A vast variety of egg layers usually breed in soft and slightly acidic water. Excessive hardness may cause in absorbing the substances through its delicate membrane (Boyd,1989). When these fishes are raised in moderately or slightly hard water, it is observed that the ova in the ovary has been calcified or calcification occurs near the vent that blocks the vent and as a result the fish is unable to release the eggs. Fertilization rate is also poor in hard water.

2.2 Food:

Food is the most important and vital factor for the growth and survival of the living beings on the face of earth (Royce *et al.*, 1972). Food and feeding habits include a valuable portion in fish life (Joadder *et al.*, 2006). Proper knowledge about food preference, especially first food of fish larvae is vital for achieving good survival rates during fish culture (Ghosh *et al.*, 2003). In the scientific information regarding food preference of goldfish is scanty (Mitra *et al.*, 2006). Earlier work (Chatterjee *et al*,. 1997; Chandra *et al.*, 2008) has considered goldfish as a good consumer of mosquito larvae. A wide range of feeds is available to feed goldfish in aquarium but the cost does not make their use economical (Forster *et al.*, 1998).

According to Tocon (1993), feed ingredients such as rice bran, oil cake, fish meal and other source which have been used in traditional aquaculture as conventional feed can meet the nutritional requirements of cultivable species. Quality feed plays an important role in value addition to commercial goldfish by enhancing their growth, colouration and general health. Food restriction itself can seriously affect spawning success. Higher protein turnover and active metabolization have been further observed on addition of cod liver and vegetable oil (Bayar and Keshavanant *et al.*, 1993) due to protein sparing effect. The inclusion of fish meal in the diet of young goldfish with a maximum digestibility of 93% could promote their growth of young goldfish (Degani *et al.*, 1997).

Goldfish can survive for a lifetime on flake foods. However, it must be stressed that variation of diets will produce active, colourful and healthy fish. Goldfish will grow bored with flake food as their only source of nutrition. Flake food can be used as their staple food, but

attempt should be taken to substitute other foods several times a week to ensure the best possible result.

One of the reasons for varying the food intake of goldfish is to ensure that they have a balanced diet. The foods given to goldfish should supply them with the same essentials that all vertebrates need. Proteins, vitamins and minerals are obviously important for bones, etc. Fats and carbohydrates are, of course, necessary for energy. Fat storage is important for fish that live outdoors so that they can sustain themselves through the winter months. Fiber provides the diet with bulk, and is important for a healthy digestive system. They are omnivorous and do best with a wide variety of fresh vegetables and fruit to supplement a flake or pellet diet. Special goldfish food has a lower protein and higher carbohydrate content than conventional fish food.

The passage of cyanobacteria through the goldfish intestine stimulates cyanobacterial growth, which may result in algal blooms occurring. The bottom-sucking feeding methods of goldfish can also contribute towards algal blooms by re-suspending nutrients, which makes them available to algae (Morgan and Beatty, 2004). Goldfish have also been known to prey upon the eggs, larvae and adult of native fishes (Morgan and Beatty, 2004), as well as increasing water turbidity and depleting aquatic vegetation (Richardson *et al.*, 1995). They eat a variety of aquatic plants (including algae), detritus, crustaceans, worms, small insects and snails (Fish Base, 2004; McDowall, 2000).

It is vital to choose the potential parent fish or 'brood stock' with care, since these fish will pass on their best and worst characteristics to their offspring. One or two males are required for each female and care should be taken to avoid interbreeding among different varieties. Males and females are reared in separate tanks, or by keeping them on opposite side of the tank divider. The brood stock must be healthy. They should show good finnage and colouration and should swim normally.

Correct nourishment is vital for successful breeding of goldfish. This means that in the few weeks spawning the fish need a balanced and varied diet that includes both good quality prepared food and live food, such as earthworms and blood worms. Temptation to over feed must be resisted which may result in an accumulation of uneaten food in aquarium.

2.3 Selection of brood:

Male maturation observed when pressing the male abdomen, the milt would ooze out. The genital orifice in female is quite bigger, rounded and male having small opening. After selecting male and female by observing secondary sexual characters, spawning tanks were prepared. Water depth plays an important role of breeding tank. Water depth greater than 6-8 inches causes trouble to fry. Spawning mops and floating plant are used for breeding purpose.

Sexually active, male goldfish seek out, chase and 'nudge' individual ovulated females, attempting to position themselves beside them and aggressively pushing each other. Before and sometimes during this activity, sexually reproductive female will swim into floating vegetation (termed 'rising') seemingly to evaluate its merits for oviposit. Oviposition behavior characterized by female entering vegetation with pursuing males, positioning themselves side by side. Spawning occurs in shallow water amongst weeds, and up to several hundred thousand small eggs (1-2mm diameter) is laid at once (McDowall, 2000). Ovulated fish releases eggs at this time and males will release sperm in water. Each female lays about 2500-3000 eggs. Healthy eggs are transparent at first but as development progress, the transparent area decreases. Unfertilized eggs or those, in which growth has been stopped, will always remain transparent. Dead eggs become cloudy white and hair like aquatic fungus will grow out of them.

Goldfish often mature during their second year, but this will depend on diet, water temperature and other environmental factors. At the other end of the scale, use of fish older then four and five years for breeding purposes may not give good result as they are probably past their prime.

In many temperate regions, the breeding season is in the summer. However, it is perfectly feasible to breed goldfish outside this period in semi- tropical areas or in an indoor aquarium.

Goldfish is easier to breed in ponds rather than aquarium but both are possible. One of the difficulties for the amateur to breed this fish is difficulty in sexing. The shape of genital orifice in female is quite bigger, rounded and protruding out compared to male having a small opening. Immature fish or fish outside their breeding season can be difficult to sex reliably. During the breeding season, the body of the mature female will take on a full rounded appearance, especially when viewed from above. The mature male develops pale 'tubercles' – known as 'breeding tubercles' on their fins, head, gill covers and pectoral fins (R.D.Pauly, 2002).

When goldfish are ready for matting do not pair off. They are not particularly choosy about who they mate. At a time males will begin chasing the females around the tank. Male swims close to the female's abdomen for a long time. This process takes place during the morning and the gold fish will be seen swimming in the batches. The chases will intensify with the male getting aggressive and pushing against the female gold fish till she ejects her eggs and the male deposit the milt over the egg to fertilize then. Before introduction of female to male, weight of the female to be taken and recorded. Difference of weight of the female before and after spawning measured the weight of gonad. Fecundity of the fish was then calculated. At the end of each mating, female released eggs. No of eggs released in each batch fully depends on the pressing skill of the male during courtship. But decrease in no of eggs in between the spawning cannot be granted as an indication of end of spawning.

After each batch of spawning eggs are attached in the spawning mops or aquatic plant. Male and female fish are taken out the spawning tank because they eat their own eggs. Taken out male and female should be provided with live feed or dry or pellet feed. Goldfish eggs are round and regular in shape.

Khan, (1929) found that spawning usually begins early in the spring and occurs at frequent intervals from April to August over a period from 7.00 a. m. to 10.00 a. m. Innes, (1936) noted that spawning usually starts at daybreak and lasts till mid-afternoon. From early spring it may be repeated every few weeks until early August but the first spawn of the season is the largest.

The capsules of the eggs are of a mucilaginous character and adhere readily to aquatic plants to which they are usually attached singly, rarely in two or three and at intervals of one-half to one inch.

2.4 Natural breeding:

In natural breeding, activation for spawning takes very long time and even sometime it was observed that female did not spawn after attraction and may take more time in next spawning. It opens the scope of introducing artificial breeding (induced breeding), an alternative to conduct successful breeding, to produce more number of hatchlings and to achieve high survival rate.

Other factors include dissolve oxygen, turbidity, maturity stage of brood fish, behavior of male and female, proper feeding of brood fish, suitable spawning substrate etc., influence in natural breeding of fish.

2.5 Induced breeding:

Ovaprim is a new synthetic hormone has been used on a wide variety of fishes for aquaculture production (Mohd-zaini *et al.*, 1994). It consists of 20 mg sGnRH and 10 mg Domperidone in 1 ml solution (Peter *et al.*, 1993; Panday *et al.*, 1990). Ovaprim shown to stimulate male sexual behavior synchronizes male and female spawning, thus directly affecting the success in fertilization (Sorensen *et al.*, 1988). The recommended dosage of ovaprim is 0.5ml/ kg body weight for induction of spawning in fishes. Dose less than 0.5ml/ kg was found to be responsive in some fishes (McGovern – Hopkin *et al.*, 2002).

In case of gold fish, injecting GnRHa/LHRHa and dopamine antagonist in combination ('limpe method') was developed by (Peter *et al.*, 1986:1988) and (Lin, 1986). However, it is important that dopamine vary in their efficiency amongst different fish species (Zohar, 1988-89).

Gonadotropin (GtH) is the active ingredient in pituitary extract preparation responsible for inducing spawning in fishes (Donaldson and Hunter, 1983; Goetz, 1983; Nagahama *et al.*, 1994; Peter and Yu *et al.*, 1997). A surge of gonadotropin (GtH) associated with ovulation and its induction of final oocyte maturation by stimulating the synthesis of maturation inducing steroids (MID) by the follicular cells (Goetz, 1983; Kime, 1993; Nagahama *et al.*, 1986, 1988) has been observed in several teleosts.

Dopamine (DA) acts as the GtH-II release inhibitory factor in gold fish and a wide range of other teleosts, and has been thoroughly reviewed recently (Peter *et al.*, 1986, 1991; Trudeau and Peter *et al.*, 1995). Dopamine (DA) has been demonstrated to directly inhibit basal, as well as GnRH- stimulated GtH-II release (Peter *et al.*, 1986, 1991) with a Dopamine receptor antagonist, specifically Pimozide or Domperidone, to remove the inhibitory influence of Dopamine on pituitary GtH-II secretion (Peter *et al.*, 1988, 1991). This combination well simulates the normal ovulatory surge of GtH-II in gold fish (Sokolowska *et al.*, 1985) and common carp (Lin, 1987). A formulation of sGnRH-A and Domperidone has been marketed as a spawning kit ovaprim. Fishes, introduced ovaprim release larger size eggs which results hatching of healthier spawn (Nandeesha *et al.*1990 and Singh *et al.*, 1988).

In terms of fertilization and hatching, ovaprim yielded better results (Nandeesha *et al.*, 1990, 1993; Alok *et al.*, 1993). The highest percentage of fertilization (95-98%) was observed in ovaprim-injected *C. striatus*. In mrigal, ingected with ovaprim, 90% fertilization was observed (Azad and Shimray, 1991). In case of *Heteropneustes fossilis* it was reported that use of ovaprim caused increase in fertilization and hatching of eggs and development of deformities were negligible. This resulted in the higher yield of fry production (Nayak, P. K; Mishra, T. K; Singh, B.N; Pandey, A. K. and Das, R.C., 2001).

However, injection techniques can be difficult or prohibitively expensive for use in small species of relatively low fecundity, such as many other tropical ornamental fishes. Rainbow sharks (*Epalzeorhynchos erythrurus*) and red tail black sharks (*E. bicolor*) are commonly induced to spawn by using hormone injections. For such species, application of spawning hormones might result in less handling stress to the fish and in reduced labor costs for the producer (Hill, 2005).

2.3.1 Larval rearing:

Larvae goldfish fed upon phytoplankton and live feed. After 3 to 4 days of hatching larvae started swimming. Older larvae fed upon zooplankton. Twenty days old larvae eat mash feed. A diet is considered a complete one when it contains balanced level of all essential nutrients such as protein, lipid, carbohydrate, energy, vitamin and minerals, which promote their biological and physiological activities.

Feed is considered to be the major constituent of aquaculture impacts. The higher the culture technology applied, more the culture system relies on exogenous feed supply with natural food becoming less significant. Thus, the dependence switches away from natural food to supple feeding. Feed formulation is the process of combining feed ingredients to form a mixture that will meet the specific goals of production. These goals may be rapid growth rate, successful reproduction and induction of a vitamin deficiency or establishment of minimum dietary nutrient requirement (Mukhopadhyay *et. al.*, 1999).

A high requirement level for protein (53%) was found for goldfish larvae, in comparison to 29% for juvenile fish (Fiogbé and Kestemont, 1995). Guisande and Serrano, (1989) and Vijverberg and Frank, (1976) found protein content in zooplankton varied from 50 – 71%. In agreement with these data, larval goldfish were found to grow best on prepared diets containing about 50% protein (Sales and Janssens, 2003).

Small fingerling goldfish and koi grew best on prepared diets containing about 40% protein (Zeitler *et al.*, 1984; Rajan *et al.*, 1996; Bandyopadhyay *et al.*, 2005). Adult goldfish require only 29% protein (Sales and Janssen, 2003; Lochmann and Phillips, 1994), and they will continue to grow when receiving only 1% body weight of a diet containing 36% protein (Stone *et al.*, 2003). Koi, on the other hand require 30-35% protein, and N excretion was reduced when dietary protein content was 35% (Satoh, 1991), indicating more efficient nutrient use with the higher protein diet.

Commercial diets for goldfish and koi are available in a range of protein percentages. In general, diets with at least 30% protein will yield acceptable growth of fish. Diet digestibility will vary according to the primary nutrient sources. Goldfish can accommodate up to 70% carbohydrate in the diet (Zhou *et al.*, 2003) while koi have little ability to digest carbohydrates.

Das and Ray, (1989) used rice bran as feed ingredient on growth performance of the IMC. Bindhu *et al.*, (2002) utilized rice bran as feed ingredient in their experiment to report the impact of dietary protein on growth, feed utilization and body composition of *Puntius parraha*. Singh *et. al.* (2006) used rice bran for the development of supplementary fish feed from low cost indigenous materials. Mohanty *et al.* (1990) utilized rice bran for their experiment on protein utilization in IMC fry, *Catla catla, Labeo rohita and Cirrhinus mrigala* fed for protein diet.

Anjana, (2004) utilized rice bran as feed ingredients in formulated artificial feeds and evaluated protein requirement, growth and nutrient digestibility of *Labeo bata*. Victor,(2003) utilized ground nut oil cake and rice bran in their experiment conducted on *Cyprinus carpio, Catla catla and Cirrhinus mrigala* to observe the effect of dietary β-chitosan levels on survival and growth. Saroha and Garg, (2007) utilized rice bran as feed ingredients in their experiments conducted on *Cirrhinus mrigala* fingerlings to see the growth performance of the fish maintained on mixed feeding schedule of diets having different protein content.

Oilcakes are also widely used for substitution of fish meal in diets of fishes. Mustered oilcake is mostly used in fish diets due to its easy availability in market. Plant protein ingredients especially soya bean and ground nut oil cake have successfully substitute fish meal with nutritional and economic benefits (Fagbenro, 1999). Chakraborty and Kar, (1975), utilized mustard oil cake as feed ingredients in their experiment conducted on carp spawn and fry. Singh *et al.* (1979) used mustard oil cake as a feed ingredient to find out the feed intake, absorption and growth of fry fingerlings of *Labeo rohita*. Mustard oil cake was utilized as a feed ingredient by

Jafri and Anwar, (1995) to determine the digestibility of some low-cost feedstuffs in fingerling IMC.

Fish meal is one of the important ingredients in the formulated diets. Protein content of the fish meal usually around 50% to 70%, digestibility varied 85% to 95%. Fish meal protein has a high content of amino acids like lysine, methionine and tryptophan. It has about 20% mineral content which is high in calcium and phosphorus. However, fish meal based pelleted feed has become very expensive due to this high cost of the material. Different quality fish meal may affect feed intake and digestibility which consequently influence grow performance and aqua-waste (Akness and Mundheis, 1997).

2.3.2 Colouration of fish:

Ornamental fishes are recognized for their bright, brilliant and beautiful colouration and shape. Colour is one of the major factors which determine the price of the ornamental fish in the world market (Saxena, 1994). The most common and the most important natural occurring pigments are carotenoids responsible for colouration of ornamental fish. Apart from being the source of colouration, these natural pigments also perform a number of important biological functions. As fish cannot synthesize these pigments, they rely on dietary supply of carotenoids to achieve their natural skin pigmentation; one of the most important quality criteria regulates the market value of ornamental fish species. Presently, ornamental fish is an important component of the aquaculture industry and it is one of the most economically profitable areas of fish farming activities (Chapman, 2000). Algae, Yeast, fungus, vegetables, fruits, wide varieties of plant materials are rich in carotenoids and might be used to replace costly pure synthetic carotenoid pigments in ornamental fish feed (Gouveia *et al.*, 2003).

Carotenoids are a group of naturally occurring lipid soluble pigments occurring principally in plants, algae, and also photosynthetic bacteria. All living things procure their colour with a few exceptions from the natural pigments. Apart from being the source of colouration, these natural pigments also perform a number of important biological functions. Among the most common and the most important natural occurring pigments are carotenoids. They are not nutrient in classical sense of the term, but substances, which animals cannot synthesize. These compounds are not generally considered as essential but they can play various roles in both invertebrates and vertebrates, although these are still not perfectly understood, of

which pigment action is most obvious. They are also found to occur in some non photosynthetic bacteria, yeast, and molds where they perform a protective function against damage by light and oxygen. These pigments vary from a bright yellow to a deep red or even a violet or dark blue.

Owing to their ubiquitous occurrence, diverse functions and interesting properties, carotenoids are subject of interdisciplinary research in biochemistry, biology, chemistry, medicine, microbiology, physics and many other branches of science. Guibourt first suggested the existence of these pigments in green leaves in 1827. However, the crystalline yellow pigment called "carotene", was later isolated from carrots in, 1837, by Waken Oder (Pfander, 1992). Some 600 different carotenoids are known to occur naturally (Ong and Tee, 1992) and new carotenoids are still continue to be identified (Mercandante, 1999).

Feed pelleting leads to the loss of a significant fraction of the carotenoids pigments due to increase in temperature and abrasion of the coating during passage of the meal through the dies (about 20% in case of canthaxanthin). The damage caused to the pellets hastens the destruction of carotenoids as a result of the increased surface area exposed to the air. It would also increase loses during storage. Damaging pellets by poor handling and intake of humidity also lead to a decrease in their carotenoids content.

Attractive colouration determines commercial value of ornamental fish. Pigmentation of skin is responsible for colouration in the fish. Carotenoids are primary source of pigmentation of the skin of the fishes. In natural environment, the fishes meet their carotenoids requirements by ingesting of aquatic plants or through of their food chains. Carotenoids are responsible for many of red, orange, yellow hues of plant legumes, fruits, and flowers. The colour enhancing diets should contain additional natural pigments to enhance the colours of ornamental fishes. Goodwin (1980) established that fish do not possess the ability to synthesize carotenoids. The carotenoids pigmentation of fish results from the pigments presents in the diet (Hata and Hata, 1973; Steven, 1948). Thongrod *et al.*, (1995) stated that carotenoids influence the growth of fishes. Present work confirmed that carotenoids do play a role in the growth of goldfish. Similar observation made by Tveranger (1986) in rainbow trout. Peimin *et al.*, (1999) reported that induced growth and body colour of crusion crap *C. auratus*.

In biology pigment is any material resulting colour in plant and animal, which is the result of selective absorption, but some biological material has structural colour which is the result of selective reflection e.g., hair, skin furs etc. Pigments broadly are of two types such as i) chlorophyll ii) carotenoids. Of these two groups of pigments, carotenoids earn the distinction of not only being the most widespread of natural groups of pigments but also they play an important role in pigmentation.

In plants, carotenoids are considered as accessory pigment. Important example is fucoxanthin (brown) (Ong and Tee, 1992). Carotenoids is a factor which is responsible for many of the red, orange and yellow hues of plants leaves, fruits, flowers as well as the colour in some birds, insects, fish, crustaceans etc. Some of the familiar examples of carotenoids colouration are oranges of carrot, citrus fruits, red pepper and tomatoes and the pink of flamingoes and Salmon (Pfander, 1992). Among these the yellow, orange, and red carotenoids and the brown, grey or black 'melanin' are the predominant members.

According to Boonyaratpalin and Unparsert, (1984) the rate of colour development seemed to depend on the amount and nature of carotenoid present in the pigmentation source or ingredient. Fish use oxygenated carotenoids, one of the most important groups of natural pigments, for pigmentation of skin and flesh. Carotenoids commonly occurring in freshwater include beta-carotene, lutein, taraxanthin, astaxanthin, tunaxanthin, and zeaxanthin.

Marigold (*Targetes erecta*) is an herb very commonly found all over the world. Marigold is normally used for worship and decorating the gardens. The petal of a flower is known as corolla. The petals of a flower contain carotenoids and polyphenols and it is this which provides colour to the flower to attract pollinating insects. The principal pigments present in marigold flower are Xanthophylls and Lutein which are present in the form of esters of palmitic acid and myristic acid (Boonyaratpalin and Lovell, 1977). As marigold petals are potential sources of xanthophylls and lutein the used i.e. after using at worship, the petals of marigold flower either as dried form or as solvent extract can be used as feed additive of fish for enhancing colour of flesh and skin. Marigold flower have become a very popular source for pigmentation not only in fishes but also in other animals.

Rose petals contain anthocyanin which is the main colouring pigments responsible for orange to blue colour of the flower and other organs. The pigments which are present in the rose petals are water soluble flavonoid and non water soluble carotenoids.

China rose (*Hibiscus rosasinensis*) petals are also good source of carotenoids (Sinha and Asimi, 2007). Apart from astaxanthin additional carotenoid compound are the source of other bright colour.

To enhance colouration in ornamental fish a combination of synthetic and natural carotenoid pigments can be added at the rate of 0.04 to 2.00% of the diet (Chapmann, 2000). Gouveia and Rema, (2005) investigated the effect of different carotenoid sources and concentration on goldfish (*Carassius auratus*) skin pigmentation. Paripatananonal, (2007) determined the optimal dosage of astaxanthin for gold fish *Carassius auratus* by feeding a series of diets containing 0, 25, 50, 75, and 100 mg of astaxanthin/kg of diets for 4 weeks.

Marigold petal meal was used by Boonyaratpalin and Lovell, (1977) for the tiger barb (*Puntius tetrazona*) and found the tiger barbs were more brightly coloured than the fishes fed with control diet. Gocer *et al.*, (2006).

Used marigold flower along with red pepper and synthetic astaxanthin on pigmentation of *Penaeus semisulcatus* and they found marigold as a carotenoid source @ 2.4% in diet was as useful as the synthetic astaxanthin for the shrimp with the survival rate of 75%. Buyukcapar, (2007) reported that the most appropriate dietary dose of marigold flower for pigmentation of rainbow trout is 1.6%. Ezhil *et al.*, (2008) found that marigold as a carotenoid source improved pigmentation of red swordtail (*Xiphophorus helleri*) at 15% supplemental level on dry weight basis. Marigold petal meal could enhance pigmentation and make fish more colourful. It is an effective colour enhancer at a cheap price.

Due to presence of high fiber content carotenoid digestibility in carrot is less (25%), which limits the study on the use of carrot as carotenoid sources for enhancing the fish colour. High fiber content also may suppress growth of fish. Sherief and Mathew *et al.*, (1996) have demonstrated that the carotenoids pigment from carrot has satisfactorily produced a desirable golden yellow colour in goldfish. Carotenoids are the primary source of pigmentation in ornamental or tropical fish, responsible for various species-related yellow, red and other related colours. Normally these are obtained through carotenoid containing organisms in the aquatic food chain, but commercial feed ingredients such as yellow corn, corn gluten meal and alfalfa are used as source of carotenoids such as zeaxanthin and lutein (Lovell, 1992).

Astaxanthin is very common used carotenoid supplement in fish as it has been found to be essential for their proper growth and survival (Torrissen and Christiansen, 1995). A positive linear relationship has been established between the levels of dietary carotenoids and survival of shrimp (Kumlu, 1995). Feed additive, dried algae improve growth and feed efficiency in several species of fish (Mustafa and Nakagawa, 1995). A positive metabolic role of carotenoids in survival of fish larvae and fry was discussed by Shahidi *et al.*, (1998) and Lazo *et al.,* (2000).

Rema and Gouveia, (2005) used Chlorella vulgaris, Spirulina platensis and synthetic astaxanthin with constant level of pigment (45 mg/kg) along with a control diet without carotenoid supplementation for goldfish larvae during 28 days period and along with a first four diet a fifth diet containing *Haematococcus pluvialis* (45 mg pigment/kg) for goldfish juveniles during 12 weeks to observe the effect on growth and survival of gold fish larvae and juveniles and they found high rate of growth and survival in both larvae and juveniles. Sinha and Asimi, (2007) found that the China rose (*Hibiscus rosasinensis*) petal is a potent natural carotenoid source for goldfish, (*Carassius auratus*) to accelerate gonadal development at the supplemental level of 5 mg/kg diet.

Since goldfish and koi are ornamental fish and valued for their colouration, size and fin development, manufacturers of prepared diets developed colour-enhancing formulas to promote brilliant colours in the fish. The present experiment was carried out to find out the influence of botanical additives on the growth and colouration of goldfish (*Carassius auratus.*).

CHAPTER -3

MATERIALS AND METHODS

Carassius auratus is popularly known as Gold fish. They are relatively small member of the carp family. Moderately hardy fish except some varieties like telescopic eye. Gold fish are gregarious having schooling behavior (**Plate-1**). They produce adhesive eggs that attach to aquatic vegetation. They are bottom feeder and omnivorous in nature. They prefer shallow water having water temp around 28-31^{0}C. Sexually dimorphic (**Table-1**), females are larger than males having more bulging belly (**Plate-2**) but males are more colourful and attractive than female(**Plate-3**) (Chris Andrews,1997). They are not particularly choosy about who they mate. In spawning time, males chase the females at random (**Plate-4**). The chases will intensify with the male getting aggressive and pushing against the female gold fish till she ejects her eggs and the male deposit the milt over the eggs to fertilize them. Fertilization is external. No parental care is seen. The eggs hatch within 48 to 72 hours. If the parents are not removed from the breeding tank the eggs will be eaten. Larvae are fed with freshly cultured infusorians. Reproductive cycle of most gold fish is very short, like in many other ornamental fishes (James and sampath, 2003 and 2004). *Carassius auratus* is a continuous spawner with a short vitellogenic period.

3.1 SYSTEMATIC POSITION:

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Carassius*
Species: *Carassius auratus*

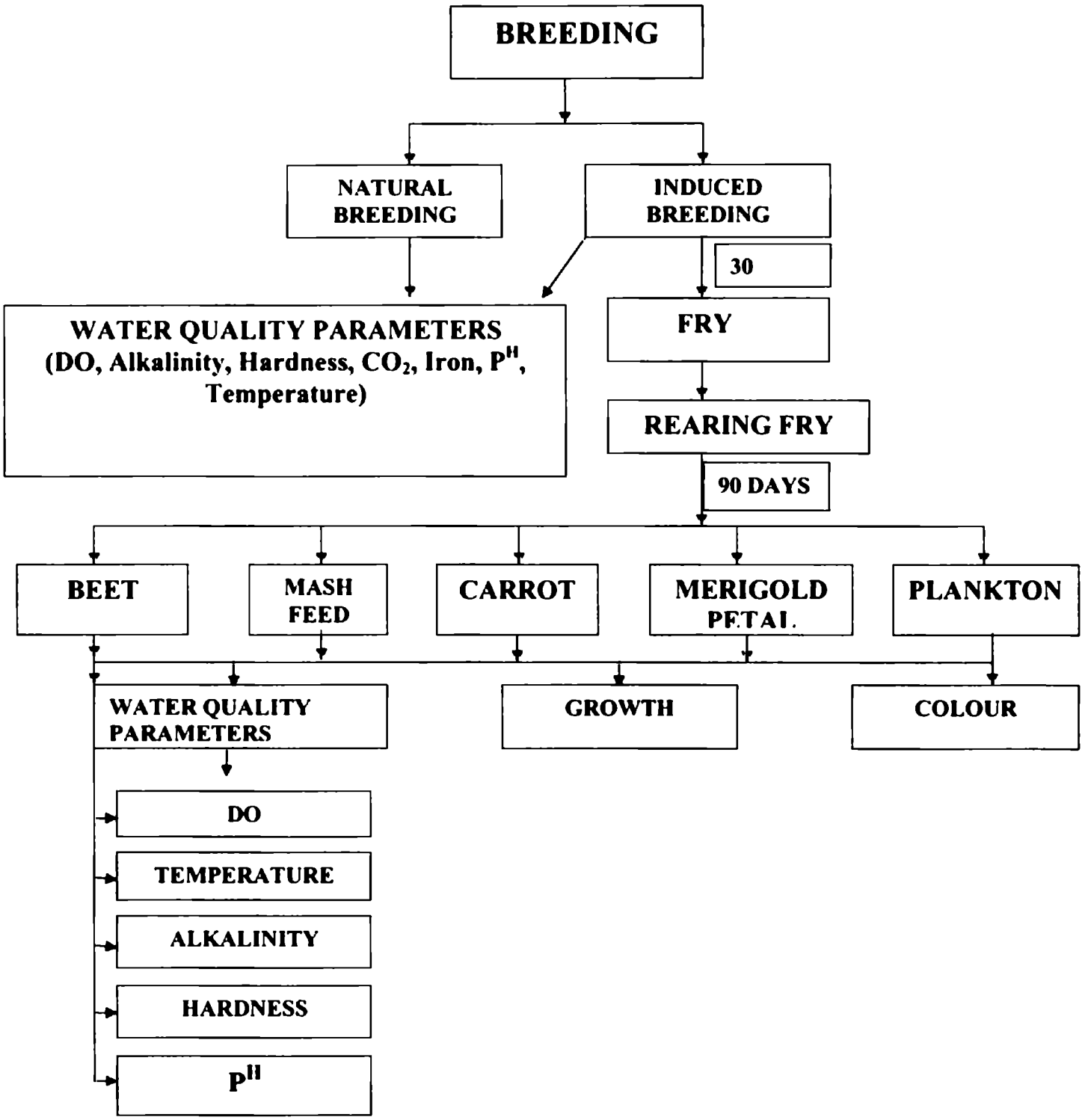
3.2 PROFILE OF GOLDFISH:

- Scientific name: *Carassius auratus*
- Common name: Goldfish
- Goldfish care level: Easy
- Breeding season: Spring and rainy season
- Maximum size: 41cm
- Maximum weight: 1.5kg
- Lifespan: 6 -7 years
- Origin: China
- Behavior: Schooling
- Breeding potential: An easily bred egg-laying species
- Compatible tank mates: Live bearers, Angelfish, shark species etc.

Table-1: Identification of male and female goldfish: (Plate- 5, 6)

Sl .No	Female	Male
1.	Tend to be more rounded.	Less rounded.
2.	No spot on operculum	Develop white spots on the head and operculum in mating season.
3.	Larger and taller.	Smaller than female.
4.	Mature female gold fish become rounder during breeding season	Male developed tubercles on operculum and head.
5.	Heavy abdomen.	Not so heavy.
6.	Vent round and convex.	Thinner and concave.
7.	Pectoral fin rounded.	Pectoral fin pointed.
8.	Less colourful and active.	Brighter coloured and more active
9.	Genital orifice quite bigger and protruding	Genital orifice smaller and non- protruding

EXPERIMENTAL PROTOCOL:



3.2.1 Experiment: I (Natural breeding Vs Induced breeding, November, 2009)

Premature gold fish of 2 years old were purchased from fish dealers in good condition and were acclimatized in separate tanks. Males and females were kept in separate chambers and reared in laboratory condition. They were provided with live and artificial feed daily @ 5% of their body weight. Fish reared in laboratory condition gradually attained sexual maturity and exhibited secondary sexual characters. Female were gradually separated in another tank as per maturation. Male developed tubercles on their heads, opercula and pectoral fins (**Plate-7, 8**).

The experiment was conducted in the month of November 2009 to evaluate the impact of water qualities on natural breeding especially the effect of alkalinity, hardness and iron concentration of water.

The ideal breeding pairs of Gold fish were selected based on desirable traits (colour, size and structure of their body and fins). A healthy fish should be active, colourful, vigorous and flaring. Selected brooders were free from parasites and devoid of abnormality. Male chased the female for several days before spawning occurs. Sick, injured or stressed fishes were not considered for breeding. Gold fish attains sexual maturity at the age of 2 years but those are 3 years old performs best.

3.2.2 IA- Natural breeding:

Natural breeding of goldfish was conducted in the month of November. Three rectangular PVC tanks T_1, T_2 and T_3 measuring (4.5 x 2.3 x 1.4 ft) each were selected (**Plate-9, 10**). Pond water having D.O: 5.8 mg/l, CO_2: nil, Alkalinity: 114.72 mg/l, Hardness: 282.0 mg/l, pH: 7.84, Iron: 2.6 mg/l and Temperature: 24°C was used as breeding medium. The water quality of the pond water was tested in a reputed laboratory in the month of November, 2009 to ascertain alkalinity, hardness and iron concentration of the pond water. Synthetic nylon threads were used as spawning mops in the breeding tanks (**Plate-11**). Spawning mops were used after proper disinfection. Two females and four males were release in each tank.

Females of 35.0 gm (avg. wt.) and males of 47.5 gm (avg. wt.) in T_1: females of 40.15 gm (avg. wt.) and males of 46.5 gm (avg. wt.) in T_2 and females of 37.5 gm (avg. wt.) and males of 42.5 gm (avg. wt.) in T_3 were introduced respectively. All the females were ripe with bulging belly and the males were brightly coloured and healthy having pale tubercles on the operculum.

The experiment continued for three days maintaining ideal situation in the breeding environment. The males chased females in all tanks. The chasing behavior continued for three days but no spawning occurred.

3.2.3IB- Induced breeding:

In natural breeding, activation for spawning takes very long time and sometime it was observed that female did not spawn after attraction and may take more time in next spawning. Long chasing by males caused failure in spawning. In the natural spawning, it was observed that long chasing caused damage of female, which may reduces hatchability and survival of larvae. It opens the scope of introducing artificial breeding (induced breeding), an alternative to conduct successful breeding, to produce more number of hatchlings and to achieve high survival rate. Other factors include dissolve oxygen, turbidity, maturity stage of brood fish, behavior of male and female, proper feeding of brood fish, suitable spawning substrate etc., influence in natural spawning. In the present study, induced breeding of gold fish (*Carassius auratus*) was carried out using ovaprim.

On seventh day of unsuccessful natural spawning, male and female brood fishes were selected using secondary sexual characters. They were introduced into the spawning tanks identical to natural breeding experiment. Prior to administered inducing agent, all necessary arrangements (weighing balance, ovaprim, hypodermic syringe, nylon cloth net, distilled water, 250 ml beaker, cotton etc.) were kept ready (**Plate-12,13**). Ovaprim @ 0.5 ml/kg body weight of fish (recommended dose) was injected to female and in case of male the dose was 0.2 ml/kg of body weight. Only single dose of ovaprim was applied in both sexes. In T_1 tank average weight of females was 35.0 gm and male was 47.5 gm, in T_2, average weight of female was 40.15 gm and male was 46.5 gm and in T_3, average weight of female and male was 37.5 gm and 42.5 gm respectively. Ovaprim requirement was very small and the inducing agent was diluted in distilled water.

Three rectangular PVC tanks T_4, T_5 and T_6 measuring (4.5 x 2.3 x 1.4 ft) each were selected. Pond water having (D.O: 6.0 mg/l, CO_2: nil, Alkalinity: 118.42 mg/l, Hardness: 290 mg/l, pH: 7.88, Iron: 2.62 mg/l and Temperature: 25°C was used in breeding purpose. Synthetic nylon threads were used as spawning mops in the breeding tanks. Spawning mops were used after proper disinfection. Two females and four males were release in each tank. Both male and female were injected at 5.30 p.m and released in spawning tanks T_4, T_5 and T_6.

The males chased females and chasing behavior continued for six hours. Female released eggs in spawning mops **(Plate-14, 15)**. In the early morning both females and males were separated from breeding tanks and mops were placed under aeration. Three separate breeding tanks were kept in laboratory condition. After 52 hours of incubation (at 24°C) hatching occur in all the three tanks (T_4, T_5 and T_6) **(Plate-16)**. Total 2010 spawns were obtained (T_4: 710, T_5: 910 and T_6: 390). Infusoria was added as live feed for the spawns but massive mortality was recorded in all three tanks. After three days only 240 spawns (11.94%) survived and they were released in specially prepared nursery tanks.

Table-2. Materials used in Experiments:

Experiment: I (Natural breeding Vs Induced breeding)

Natural breeding		Induced breeding	
MATERIALS (Goldfish)	**SPICIFICATION**	**MATERIALS (Goldfish)**	**SPICIFICATION**
Brood fish **Male: 12** **Female: 6**	a) Average weight: 40.50gm b) Average size: 8.25 cm. a) Average weight: 50.45gm b) Average size: 9.20 cm	**Brood fish** **Male: 10** **Female: 6**	a) Average weight: 40.5 gm b) Average size: 8.25 cm. a) Average weight: 50.45gm b) Average size: 9.20 cm
TANKS: **a) Stocking tank:** **b) Breeding tank:** **c) Larvae rearing tank (aquarium):**	 Number: 02 Size: 9.2 x 8.5 x 1.5 ft Number: 03 Size: 4.5 x 2.3 x 1.4 ft Number: 05 Size: 18 x 10 x 10 inch Capacity: 12 liter	**TANKS:** **a) Stocking tank:** **b) Breeding tank:** **c) Larvae rearing tank(aquarium):**	 Number: 02 Size: 9.2 x 8.5 x 1.5 ft Number: 03 Size4.5 x 2.3 x 1.4 ft Number: 05 Size: 18 x 10 x 10 inch Capacity: 12 liter
Water parameters	a) DO: 5.8 mg/l b) CO_2: Nil c)Alkalinity:114.72mg/l d) Hardness: 282.0 mg/l e) Iron: 2.60 mg/l f) pH : 7.84 g)Temperature:24°C	**Water parameters**	a) DO: 6.0 mg/liter b) CO_2: Nil c) Alkalinity: 118.42 ppm d) Hardness: 290.0 mg/l e) Iron: 2.62 mg/l f) pH: 7.88 g) Temperature: 25°C
Live feed	a)Tubifex worm b)Infusoria c)Zooplankton	**Live feed**	a)Tubifex worm b)Infusoria c)Zooplankton
p^{II} Pen	Range of 0 to 14	**P^{H} Pen**	Range of 0 to 14
Balance	Electronic balance: 0.02gm to 600 gm	**Balance**	Electronic balance: 0.02gm to 600 gm
Glass beakers	250 to 500 ml capacity	**Glass beakers**	250 to 500 ml capacity
Nylon cloth net	Soft Quality	**Nylon cloth net**	Soft Quality
Mops	Soft nylon threads	**Mops**	Soft nylon threads

Natural breeding		Induced breeding	
MATERIALS (Goldfish)	**SPICIFICATION**	**MATERIALS (Goldfish)**	**SPICIFICATION**
Plankton net	Collection of planktons	**Plankton net**	Collection of planktons
Scale	Measurement of length	**Scale**	Measurement of length
Aerator	For aeration purpose	**Aerator**	For aeration purpose
Siphoning pipe	Cleaning of aquarium	**Siphoning pipe**	Cleaning of Aquarium
Grinding Machine	Grinding of feed ingredients	**Grinding Machine**	Grinding of feed ingredients
		Hypodermic Injection syringe	1ml 1 capacity having 40 gradation
		OVAPRIM	sGn RH-A

3.2.4 Experiment: II (Natural breeding Vs Induced breeding, March, 2010)

Premature gold fish of 2 years old were purchased from fish dealers in good condition and were acclimatized in separate tanks. Males and females were kept in separate chambers and reared in laboratory condition. They were provided with live and artificial feed daily @ 5% of their body weight. Fish reared in laboratory condition gradually attained sexual maturity and exhibited secondary sexual characters. Female were gradually separated in another tank as per maturation. Male developed tubercles on their heads, opercula and pectoral fins.

3.2.5 IIA- Natural breeding:

Natural breeding of goldfish was conducted in the month of March, 2010. Three rectangular PVC tanks T_1, T_2 and T_3 measuring (4.5 x 2.3 x 1.4 ft) each were selected. Pond water having D.O: 6.0 mg/l, CO_2: Nil, Alkalinity: 126.0 mg/l, Hardness: 414.0 mg/l, pH: 7.5, Iron: 2.5 mg/l and Temperature: 26°C was used as breeding medium. Synthetic nylon threads were used as spawning

mops in the breeding tanks. Spawning mops were used after proper disinfection. Two females and four males were release in each tank.

Females of 188.33 gm (avg. wt.) and males of 131.66 gm (avg. wt.) in T_1; females of 190.15 gm (avg. wt.) and males of 137.5 gm (avg. wt.) in T_2 and females of 175.35 gm (avg. wt.) and males of 125.5 gm (avg. wt.) in T_3 were introduced respectively. All the females were ripe with bulging belly and the males were brightly coloured and healthy having pale tubercles on the operculum.

The experiment continued for three days maintaining ideal situation in the breeding environment. The males chased females in all tanks. The chasing behavior continued for three days but no spawning occurred. The females were exhausted. After three days of experiment the males and females were separated in other tanks.

3.2.6 IIB- Induced breeding:

On seventh day of unsuccessful natural spawning, male and female brood fishes were selected using secondary sexual characters. They were introduced into the spawning tanks identical to natural breeding experiment. Ovaprim @ 0.5 ml/kg body weight of fish (recommended dose) was injected to female and in case of male the dose was 0.2 ml/kg of body weight. Only single dose of ovaprim was applied in both sexes. In T_1 tank average weight of females was 188.33 gm and male was 131.66 gm, in T_2, average weight of female was 190.15 gm and male was 137.5 gm and in T_3, average weight of female and male was 175.35 gm and 125.5 gm respectively. Ovaprim requirement was very small and the inducing agent was diluted in distilled water.

Hypodermic syringe of 1 ml capacity having 40 units was used for pushing ovaprim. One unit of ovaprim in this syringe contains 0.025 ml of ovaprim. One unit ovaprim was diluted 10 times using double distilled water. Now the concentration of ovaprim diluted and one unit became 0.0025 ml. For example injection of half of a unit of diluted ovaprim will contain 0.00125 ml. of ovaprim suitable for 2.5 gram female.

Three rectangular PVC tanks T_4, T_5 and T_6 measuring (4.5 x 2.3 x 1.4 ft) each were selected. Pond water having (D.O: 6.8 mg/l, CO_2: Nil, Alkalinity: 182.0 mg/l, Hardness: 420.0 mg/l, pH: 7.5, Iron:-2.7 mg/l and Temperature:25°C was used in breeding purpose. Synthetic nylon threads were used as spawning mops in the breeding tanks. Spawning mops were used after proper disinfection. Two females and four males were release in each tank.

Both male and female were injected at 5.00 p.m and released in spawning tanks T_4-T_6. Two female and four male (1:2) were released in each tank. The males chased females and chasing behavior continued for six hours. Female released eggs in spawning mops. In the early morning both females and males were separated from breeding tanks and mops were placed under aeration. Three separate breeding tanks were kept in laboratory condition. After 48 hours of incubation (at 24°C) hatching occur in all the three tanks (T_4, T_5 and T_6). Total 3382 spawns were obtained (T_4: 1012, T_5: 1320 and T_6: 1050). Infusorians was added as live feed for the spawns but massive mortality was recorded in all three tanks. After three days only 520 spawns (15.37%) survived and they were released in specially prepared nursery tanks.

Table-3: Materials used in Experiments:

Experiment: II (Natural breeding Vs Induced breeding)

Natural breeding		Induced breeding	
MATERIALS (Goldfish)	**SPICIFICATION**	**MATERIALS (Goldfish)**	**SPICIFICATION**
Brood fish Male: 12	a) Average weight: 131.55 gm b) Average size: 10.47 cm	**Brood fish Male: 12**	a) Average weight: 131.55 gm b) Average size: 10.47 cm
Female: 6	a) Average weight: 184.61 gm b) Average size: 12.13 cm	**Female: 6**	a) Average weight:184.61gm b) Average size: 12.13 cm
Water parameters	a) DO: 6.0 mg/l b) CO_2: Nil c) Alkalinity:126 mg/l d) Hardness:414 mg/l e) Iron:2.5 mg/l f) pH : 7.5 g) Temperature:26°C	**Water parameters**	a) DO: 6.8 mg/l b) CO_2: Nil c) Alkalinity:182 mg/l d) Hardness:420 mg/l e) Iron: 2.7 mg/l f) pH : 7.5 g) Temperature: 25°C

3.3 Calculation of ovaprim dose:

Syringe capacity =1 ml.

Total no. of unit in syringe = 40

Volume of each unit = 1/40=0.025 ml.

So, one unit ovaprim diluted to 10 times = 0.0025 ml ovaprim /unit

Example:

Weight of fish: 2.5 gm, ovaprim required = (2.5/0.5/1000)=0.00125 ml.

Unit required to inject=0.00125/0.0025 = 0.5 unit.

After taking 1 unit of ovaprim in syringe it was diluted 10 times using double distilled water. Fish weight was taken and ovaprim requirement was calculated. Taking the fish in nylon cloth hand net, ovaprim was injected in the caudal portion of the fish carefully. Immediate after injection fish was released in water.

3.3.1 Experiment: III (Natural breeding Vs Induced breeding, July, 2010)

The experiment was conducted in the month of July 2010. It was repetition of previous experiments i.e. experiment in November 2009 and March 2010. The experiment was conducted to evaluate the impact of water qualities on natural breeding especially the effect of alkalinity, hardness and iron concentration of water. Almost same type of situation was created in laboratory and premature gold fish of 2 years old were procured, acclimatized in separate tanks and fed with live and artificial feed daily @ 5% of their body weight. Fish reared in laboratory condition gradually attained sexual maturity and exhibited secondary sexual characters. The ideal breeding pairs of goldfish were selected based on desirable traits (colour, size and structure of their body and fins).

3.3.2 III A -Natural breeding:

Natural breeding of goldfish was conducted in the month of July, 2010. Three rectangular PVC tanks T_1, T_2 and T_3 measuring (4.5 x 2.3 x 1.4 ft) each were selected. Pond water having D.O: 5.2 mg/l, CO_2: nil, Alkalinity: 130.0 mg/l, Hardness: 480 mg/l, pH: 7.5, Iron: 3.2 mg/l and Temperature: 31°C was used as breeding medium. Synthetic nylon threads were used as spawning

mops in the breeding tanks. Spawning mops were used after proper disinfection. Two females and four males were release in each tank.

Females of 137.5 gm (avg. wt.) and males of 124.3 gm (avg. wt.) in T_1: females of 180.15 gm (avg. wt.) and males of 132.5 gm (avg. wt.) in T_2 and females of 185.35 gm (avg. wt.) and males of 130.5 gm (avg. wt.) in T_3 were introduced respectively. All the females were ripe with bulging belly and the males were brightly coloured and healthy having pale tubercles on the operculum.

The experiment continued for three days maintaining ideal situation in the breeding environment. The males chased females in all tanks. The chasing behavior continued for three days but no spawning occurred.

3.3.3 Induced breeding- IIIB:

On seventh day of unsuccessful natural spawning, male and female brood fishes were selected using secondary sexual characters. They were introduced into the spawning tanks identical to natural breeding experiment. Ovaprim @ 0.5 ml/kg body weight of fish (recommended dose) was injected to female and in case of male the dose was 0.2 ml/kg of body weight. Only single dose of ovaprim was applied in both sexes. In T_1 tank average weight of females was 137.5 gm and male was 124.3 gm, in T_2, average weight of female was 180.15 gm and male was 132.5 gm and in T_3, average weight of female and male was 185.35 gm and 130.5 gm respectively. Ovaprim requirement was very small and the inducing agent was diluted in distilled water.

Three rectangular PVC tanks T_4, T_5 and T_6 measuring (4.5 x 2.3 x 1.4 ft) each were selected. Pond water having (D.O: 6.0 mg/l, CO_2: nil, Alkalinity: 138.0 mg/l, Hardness: 410 mg/l, pH: 7.5, Iron: 3.0 mg/l and Temperature: 32°C was used in breeding purpose. Synthetic nylon threads were used as spawning mops in the breeding tanks. Spawning mops were used after proper disinfection. Two females and four males were release in each tank except T_6 where 1:1 ratio was maintained i.e. 2 females and 2 males were released. Both male and female were injected at 5.30 p.m and released in spawning tanks T_4-T_6. The males chased females and chasing behavior continued for six hours. Female released eggs in spawning mops. After 40 hours of incubation (32°C) hatching occur in all the three tanks (T_4, T_5 and T_6). Total 3040 spawns were obtained (T_4: 1410, T_5: 1080 and T_6: 550). Infusorians was added as live feed for the spawns but massive mortality was recorded in all three

tanks. After three days only 220 spawns (7.23%) survived and they were released in specially prepared nursery tank.

Table-4: Materials used in Experiments:

Experiment: III (Natural breeding Vs Induced breeding)

Natural breeding		Induced breeding	
MATERIALS (Goldfish)	**SPICIFICATION**	**MATERIALS (Goldfish)**	**SPICIFICATION**
Brood fish **Male: 10**	a) Average weight:129.10gm b) Average size: 9.88 cm.	**Brood fish** **Male: 10**	a) Average weight:129.10gm b) Average size: 9.88 cm.
Female: 6	a) Averageweight:167.66gm b) Average size: 10.06 cm.	**Female: 6**	a) Average weight:167.66gm b) Average size: 10.06 cm.
Water parameters	a) DO: 5.2 mg/l b) CO_2: Nil c) Alkalinity: 130 mg/l d) Hardness: 480 mg/ l e) Iron: 3.2 mg/l f) PH: 7.5 g)Temperature:31°C	**Water parameters**	a) DO: 6.0 mg/l b) CO_2: Nil c) Alkalinity: 138 mg/l d) Hardness: 410 mg/l e) Iron: 3.0 mg/l f) PH: 7.5 g) Temperature: 32°C

Experiment –IV (Larval rearing and development of colour pigments):

3.4 Preparation of experimental diets:

3.4.1 Feed ingredients:

The experiment was conducted to study the growth performance and colour enhancement of gold fish spawns using various kinds of diets. Natural food like planktons and specially prepared artificial feed were used in this study. Natural colouring agents like beet, carrot and petals of marigold flower were added to artificial diet @ 10% to enhance pigmentation of fish spawn **(Plate-17, 18 and 19)**.

Five types of feed were selected- (T_1: artificial mash feed + beet powder; T_2: artificial mash feed; T_3: artificial mash feed + carrot powder; T_4: artificial mash feed + marigold petals; and T_5: planktons). The experiment was conducted in glass aquaria (18 x 10 x 10 inch) having three replicates each. Artificial feed were prepared using rice bran, mustard oil cake, fish meal, and maize and vitamin-mineral mixture. All the ingredients were procured from the local market.

3.4.2 Formulation feeds:

The artificial feed with 35% of protein was prepared using feed ingredients viz. rice bran, mustard oil cake, fish meal, maize and vitamin-mineral mixture. Protein requirement for goldfish spawn is 35%. The feed was prepared using Pearson's square method. The required quantity of feed ingredients was mixed and grinded in grinder. After proper grinding, the mixture was sieved through a fine mesh metallic sieve to get fine particles.

Beet, carrot and petals of marigold were collected from local market. Beet and carrot was cleaned properly, chopped into small pieces and dried in sun light. Marigold Petals also dried in sun light. After proper drying, all the ingredients were grinded in grinder separately and sieved through a fine mesh metallic sieve. Natural colouring agents like beet, carrot and petals of marigold powder were added to artificial mash diet separately @ 10% to enhance pigmentation of goldfish spawn. Vitamin-mineral mixture was added @1% in all five feeds.

3.4.3Test containers:

The present investigation was carried out in 5 glass aquaria (18 x 10 x 10 inch) having 12.0-liter bore well water (DO: 6.0 mg/l, CO_2: nil, alkalinity: 120.0 mg/l, hardness: 714.0 mg/l, pH: 7.5, temperature: 30°C). Among five treatments (T_1, T_2, T_3, T_4 and T_5), T_5 i.e. planktons was considered as control and the treatments were backed by triplicates to avoid experimental error. Each glass aquaria was stocked with 20 numbers of goldfish fry (30 days old). The average weight and length of fry was 0.4 gm and 1.0 cm respectively. The experiment was conducted for a period of 90 days to assess different growth parameters and colouration.

3.4.4 Water exchange:

In every 10^{th} day, 25% of water from each glass aquaria was drained off to remove accumulated fecal matter and replenished with well aerated bore well water. The exchange was done around 10.00 a.m after collection of water sample from each tank. A fixed level of water was maintained in the experimental tanks by periodic addition of ground water to compensate the losses due to the evaporation and sampling. The process continued till the termination of the experiment spreading over to a total of 90 days.

3.4.5Feeding schedule:

The goldfish fry were fed with test feed @ 4% of their body weight in two installments- morning and evening hours.

3.4.6Aeration:

Aeration facilities were provided to each aquarium throughout the period of investigation. Aeration was started at 4.00 p.m every day for a period of six hours (10.00 p.m) for maintaining good water quality parameters.

3.5 Sampling:

3.5.1 a) Water sampling: Water quality parameters like dissolved oxygen, alkalinity, hardness, pH and temperature was measured from each of the experimental tanks at an interval of 10 days following the standard procedure (APHA, 1985). Water samples were collected from each tank at around 8.00 a.m in every sampling days and standard collection procedure was followed. Hardness and alkalinity were determined by titrimetric method. The water temp was measured using a centigrade thermometer on spot and expressed as °C. The P^H of water was determined by pH meter. Dissolved oxygen was measured by Winkler's method (APHA, 1998).

3.5.2 b) Fish sampling: Sampling was done at an interval of 30 days i.e. 01, 30, 60 and 90^{th} day of experiment. Three fishes were caught randomly from each aquarium during every sampling to record their weight (gm) and length (cm) individually. The length of fish means the distance between snout and caudal peduncle. The appearance of colour in goldfish fry in different feeds was also examined at an interval of 10 days and recorded for calculation **(Table-10)**.

3.6 Statistical analysis:

All the results were subjected to statistical analysis. One way analysis of variance (ANOVA) was applied to test the significance. Linear regression analysis and F- value among the treatments were calculated. Standard deviation and mean value of all data were also calculation.

Plate-1: Schooling behavior of Gold Fish

Plate-2: Female Gold Fish

Plate-3: Male Gold Fish

Plate-4: Chasing Behavior of Gold Fish

Plate-5: Round Vent in Female Fish

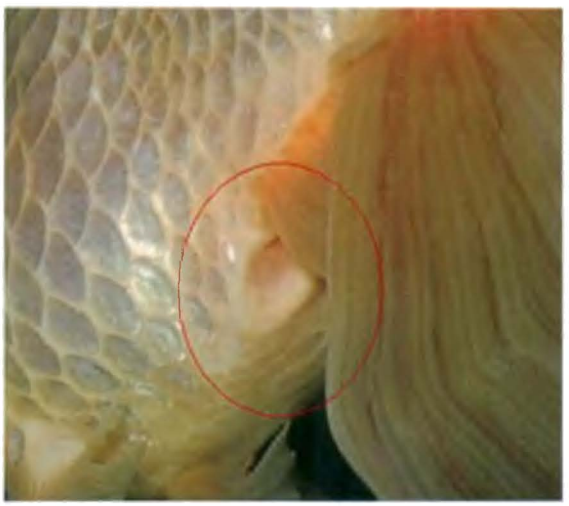


Plate-6: Vent in Male Fish

Plate-7: Tubercles in Male Fish

Plate-8: Pointed pectoral Fin in Male Fish

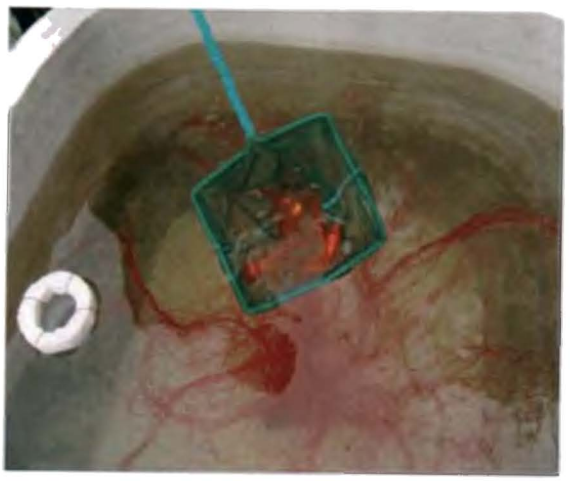


Plate-9: Releasing fish in Tank

Plate-10: Spawning Tank

Plate-11: Spawning Mops

Plate-12: Ovaprim taking in to syringe

Plate-13: Injection in Fish with Ovaprim

Plate-14: Eggs Attached in Mops

Plate-15: Fertilized Eggs

Plate-16: Hatchlings of goldfish

Plate-17: Beet Powder

Plate-18: Marigold Petal Powder

Plate-19: Carrot Powder

CHAPTER -4

RESULTS

The brood goldfish were selected based on of maturity level. The male and female ratio was 2:1 and they were kept in PVC tanks separately and reared for seven days. They were fed with tubifex worms @ 3% of their body weight.

Experiment-I

4.1 Natural breeding:

Goldfish breeds twice in a year-in spring and in rainy season. First breeding experiment was conducted in the month of November, 2009. Natural breeding was conducted in rectangular PVC tanks measuring (4.5 x 2.3 x 1.4 ft). Synthetic nylon threads were used as spawning mops in the breeding tanks. Spawning mops were used after proper disinfection. Three such tanks were used for breeding having two females and four males in each tank. All the females were ripe with bulging belly and the males were brightly coloured and healthy having pale tubercles on the operculum.

Goldfish, under the family Cyprinidae exhibit same breeding behavior like carps. Water qualities for breeding of goldfish are more or less similar to carp breeding. Ideal water qualities for carp breeding are in **(Table-5)**.

The experiment continued for three days maintaining ideal situation in the breeding environment. The fish never responded for spawning.

Table-5: Ideal water quality parameters of carp breeding and water quality of Experiment- I

Ideal water quality of carp breeding	Water quality of pond water used in goldfish breeding Experiment-I	
	Natural breeding	Induced breeding
DO: 4.5 mg/l	**DO:** 5.8 mg/l	DO : 6.0 mg/l
CO_2: <5.0 mg/l	**CO_2 :** Nil	**CO_2 :** Nil
Alkalinity: 50-300 mg/l	**Alkalinity:**114.72 mg/l	**Alkalinity:**118.42 mg/l
Hardness: 30-180 mg/l	**Hardness:**282.0 mg/l	**Hardness :** 290.0 mg/l
pH: 7.5-8.5	**pH:** 7.84	**pH :** 7.88
Iron: 0.01-0.3 mg/l	**Iron :** 2.6 mg/l	**Iron :** 2.62 mg/l
Temperature: 24-30°C	**Temperature:** 24°C.	**Temperature:** 25°C

Water quality of pond differed from ideal water qualities (**Table: 5**) in two parameters i.e. hardness and iron which are considered as critical factors in carp as well as goldfish breeding. Hardness value was 156% higher in natural breeding and 161.11% higher in induced breeding than maximum permissible limit of ideal condition i.e.180 mg/ l (**Experiment –I**). On the other hand, desirable iron concentration in breeding water is 0.3 mg/l but the concentration encountered during natural breeding of goldfish was 2.6 mg/l i.e. 866.66% and in induced breeding the value was 873.33% higher than ideal value.

Table-6: Ideal water quality parameters of carp breeding and water quality of Experiment- II

Ideal water quality of carp breeding	Water quality of pond water used in goldfish breeding Experiment-II	
	Natural breeding	**Induced breeding**
DO: 4.5 mg/l	**DO:** 6.0 mg/l	**DO:** 6.8 mg/l
CO_2: <5.0 mg/l	**CO_2 :** Nil	**CO_2 :** Nil
Alkalinity: 50-300 mg/l	**Alkalinity:**126 mg/l	**Alkalinity:**182 mg/l
Hardness: 30-180 mg/l	**Hardness:**414 mg/l	**Hardness:**420mg/l
pH: 7.5-8.5	**pH:** 7.5	**pH:** 7.5
Iron: 0.01-0.3 mg/l	**Iron:** 2.5 mg/l	**Iron:** 2.7 mg/l
Temperature: 24-30°C	**Temperature:** 26°C	**Temperature:** 25°C

Water quality of pond differed from ideal water qualities (**Table-6**) also in two aspects i.e. hardness and iron. Hardness value exhibited 230% higher in natural breeding and 233% higher in induced breeding than ideal value (**Experiment –II**). On the other hand, iron concentration in natural breeding was 2.6 mg/l i.e. 833.33% and in induced breeding the value was 2.7 mg/l i.e. 900% higher than ideal value.

Table-7: Ideal water quality parameters of carp breeding and water quality of Experiment- III

Ideal water quality of carp breeding	Water quality of pond water used in goldfish breeding Experiment-III	
	Natural breeding	Induced breeding
DO: 4.5 mg/l	**DO:** 5.2 mg/l	**DO:** 6.0 mg/l
CO_2: <5.0 mg/l	**CO_2 :** Nil	**CO_2 :** Nil
Alkalinity: 50-300 mg/l	**Alkalinity:**130 mg/l	**Alkalinity:**138 mg/l
Hardness: 30-180 mg/l	**Hardness:**480 mg/l	**Hardness:**410 mg/l
pH: 7.5-8.5	**pH:** 7.5	**pH:** 7.5
Iron: 0.01-0.3 mg/l	**Iron:** 3.2 mg/liter	**Iron:** 3.0 mg/l
Temperature: 24-30 °C	**Temperature:** 31°C	**Temperature:** 32°C

Water quality of pond in experiment III exhibited same trend **(Table-7)**. Hardness value increased 266.66% in natural breeding and 227.77% in induced breeding than ideal value **(Experiment –III)**. Iron concentration was higher 1066.66% (3.2 mg/l) in natural breeding and 1000% (3.0 mg/l) in induced breeding than ideal value.

4.2 Induced Breeding:

Ovaprim (sGnRHa + Dopamine) was introduced @ 0.5 ml/kg body wt. in female fish and 0.2 ml/kg in male fishes.

4.3 Experiment- I: November 2009

Induced breeding was conducted in rectangular PVC tanks measuring (4.5 x 2.3 x 1.4 ft). Synthetic nylon threads were used as spawning mops in the breeding tanks. Spawning mops were used after proper disinfection and kept in specially prepared nursery tanks.

Six hours after injection (Ovaprim) fishes in all the three tanks responded and females released eggs in nylon mops. After 52 hours of incubation (24°C) hatching occurred in all the three tanks (T_4, T_5 and T_6). Total 2010 spawns were obtained (T_4: 710, T_5: 910 and T_6: 390). In all the tanks (T_4, T_5 and T_6) infusoria was added as live feed for the hatchlings but massive mortality was recorded in all three tanks on 3rd day onwards. After seven days of rearing only 240 spawns (13.59%) were survived.

4.4 Experiment- II: March 2010

Male and female fish in all breeding tanks responded after six hours of injection (Ovaprim) and females released eggs in nylon mops. Male and female fishes were separated from the breeding tanks. Mops were placed under aeration for spawning in three separate tanks under laboratory condition. After 52 hours of incubation (24°C) hatching occurred in Tanks 4, 5 and 6. Total 3382 hatchlings were produced (T_4: 1012, T_5: 1320, T_6: 1050) but only 520 spawns (15.37%) obtained after seven days of rearing. Infusoria was added as live feed to the spawns. Survived ones were released in specially prepared nursery tanks.

4.5 Experiment- III: July 2010

Male and female fish responded and female released eggs attached to nylon mops for their sticky nature. After separating both males and females, nylon mops were kept in breeding tank under aeration for spawning. After 40 hours of incubation at 32°C, hatching occurred in all the three tanks. Total 3040 hatchlings were obtained (T_4: 1410, T_5: 1080 and T_6: 550). Survival of hatchlings was poor and after seven days only 220 spawns (7.23%) survived and were released in specially prepared nursery tank.

Experiment-IV:

4.2.1 Larval rearing:

One month old goldfish spawn (avg. wt.: 0.4 gm; length: 1.0 cm) were collected from nursery tanks. In all treatments (T_1, T_2, T_3 T_4 and T_5) 20 goldfish fry were release and different

types of feed provided as per experimental protocol. Length of the fish was measured randomly on different sampling days **(Table- 8).**

Table-8. Average body length (cm) of fish obtained in various days of sampling:

Treatment and Feed Type	Feeding rate	No of fish/Tank	Average body length (cm) of fish			
			Day 01	Day 30	Day 60	Day 90
T_1 (Beet powder) 10%	4%	20	1	2.5	3.6	4.26
T_2 (Mash feed)	4%	20	1.2	3.2	5.3	6.09
T_3 (Carrot powder) 10%	4%	20	1.25	2.7	3.8	4.5
T_4 (Marigold petals powder)10%	4%	20	1.1	2.6	3.55	4.58
T_5 (Control) Plankton	4%	20	1.3	2.8	3.34	4.61

One-way analysis of variance (ANOVA) tests of body length of goldfish fry in different treatments in different sampling days exhibited F- value between groups in various sampling days (Day 01, Day 30, Day 60 and Day 90) was 8.529, 12.529, 69.704 and 54.556 with high level of significance.

Table-9. Average body weight (gm) of fish obtained in various days of sampling:

Treatment and Feed Type	Feeding rate	No of fish / Tank	Average body weight (gm) of fish			
			Day 01	Day 30	Day 60	Day 90
T_1 (Beet powder) 10%	4%	20	0.4	0.78	0.91	1.15
T_2 (Mash feed)	4%	20	0.52	1.1	1.95	2.49
T_3(Carrot powder) 10%	4%	20	0.45	0.93	1.54	1.82
T_4 (Marigold petals powder) 10%	4%	20	0.49	0.99	1.62	1.98
T_5 (Control) Plankton	4%	20	0.5	1.2	1.87	2.32

One-way analysis of variance (ANOVA) tests of body weight of goldfish fry in different treatments in different sampling days exhibited F- value between groups in different sampling days (Day 01, Day 30, Day 60 and Day 90) was 3.439, 11.438, 62.016 and 77.005 with high level of significance.

4.3.1 Length-weight relationship:

The relationship between total length (L) and total weight (W) for nearly all species of fish is expressed by the equation:

$$W = a L^b$$

Values of W usually have been calculated from the logarithmic (base 10) equivalent: log W = log a + b·log L

A graph of log W against log L forms a straight line with a slope of b and a Y-axis (log W) intercept of log a. Invariably, b is close to 3.0 for all species.

In Experiment –IV, length-weight relationship of goldfish fry in all treatments (T_1, T_2, T_3 T_4 and T_5) during 90 days experiment was done treatment wise to evaluate the relationship **(Fig:-1, 2, 3, 4 and 5). Fig:-6** exhibited the length-weight relationship among all fishes exposed to various treatments on 90th day of experiment.

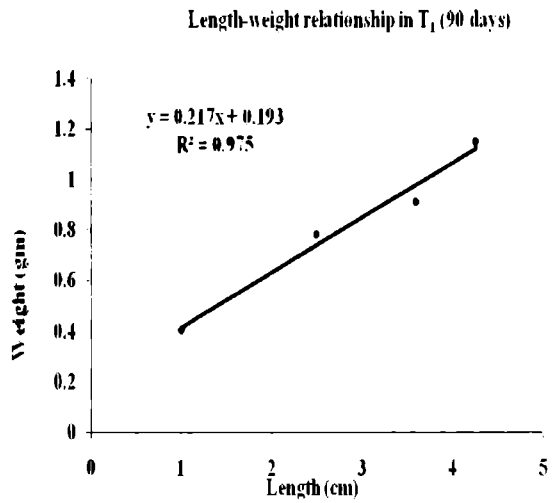


Fig-1

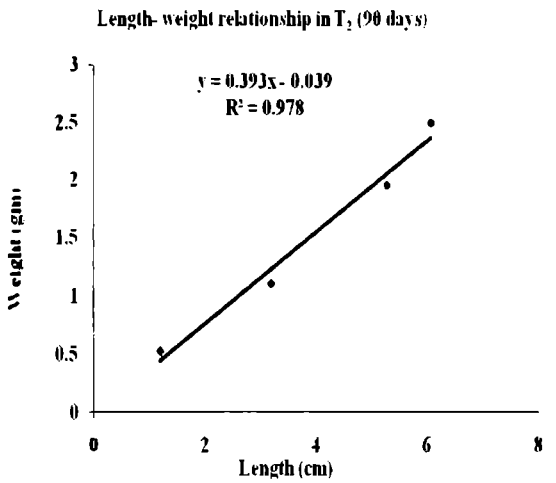


Fig-2

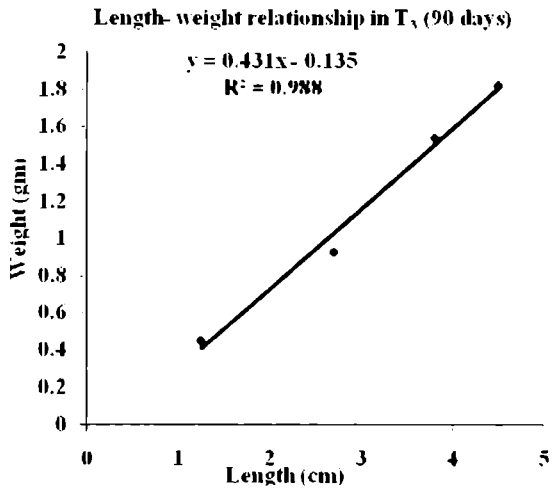


Fig -3

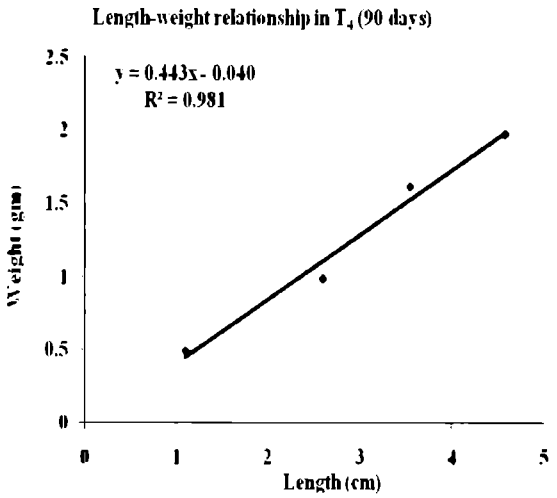


Fig-4

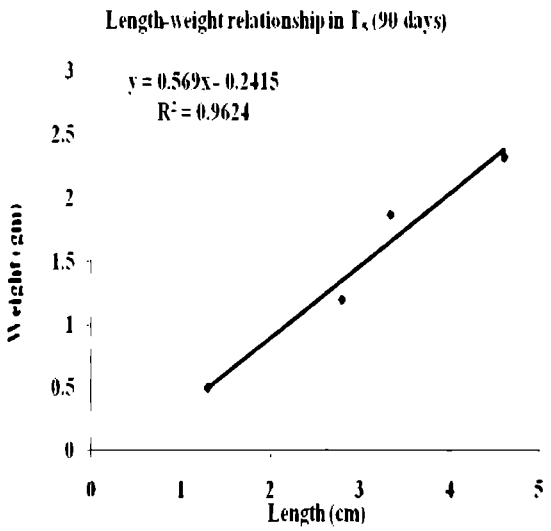


Fig-5

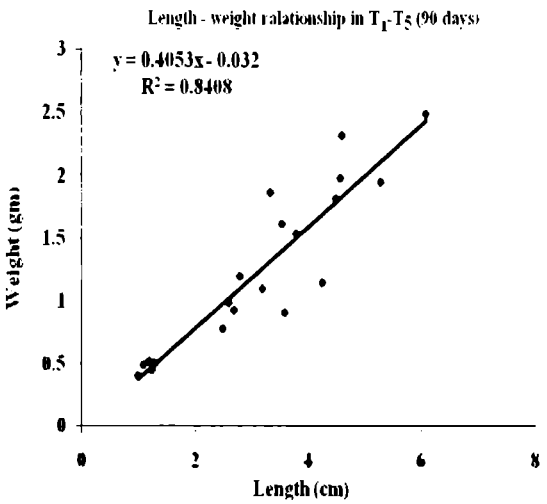


Fig -6

Length – weight relationship of goldfish fry at the end of 90 days experiment in all treatments exhibited linear relationship and such relationship was explained by 96 to 98 %. As a whole such relationship was explained by 84 % (**Fig. – 6**).

4.4.1 Colouration of fish: Goldfish fry were reared for a period of 90 days in different feeds (T_1, T_2, T_3 T_4 and T_5). In T_1, 65% of fish developed colour in day 80 and maximum 75% fish developed colour in 90th day which was lowest among all the treatments in 90 days period. In T_2, only 55% of fish developed colour in 80th day and100% colouration was achieved in 90th day. In T_3, 55% colouration was achieved on day 70 but only 85% was achieved on 90th day. T_4

exhibited 50% colour fish in day 60 which was highest among all treatments in the particular day of sampling but only 95% fish developed colour at the end of experiment. In T_5 (control), only 85% of fish developed colour at the end of experiment **(Table-10) (Plate 20-24)**

Plate20: Harvested goldfish (T_1)

Plate-21: Harvested goldfish (T_2)

Plate-22: Harvested goldfish (T3)

Plate-23: Harvested goldfish (T_4)

Plate-24: Harvested goldfish [T_5 (control)]

Plate: 20-24: Total number of fish developed colour at the end of 90 days experiment in different treatments

Table-10. Total Number of fish developed colour pigment and percentage of increase:

Treatment and Feed Type	Feeding rate	No of fish / Tank	Total Number of fish developed colour pigment and percentage of increase							
			20th day	30th day	40th days	50th day	60th day	70th day	80th day	90th day
T_1 (Beet powder) 10%	4%	20	1 5%	2 10%	4 20%	4 20%	4 20%	8 40%	13 65%	15 75%
T_2 (Mash feed)	4%	20	2 10%	5 25%	8 40%	9 45%	9 45%	10 50%	11 55%	20 100 %
T_3 (Carrot powder) 10%	4%	20	1 5%	31 5%	6 30%	7 35%	9 45%	11 55%	15 75%	17 85%
T_4 (Marigold petals powder) 10%	4%	20	1 5%	2 10%	4 20%	6 30%	10 50%	13 65%	15 80%	19 95%
T_5 (Control) Plankton	4%	20	2 10%	4 20%	4 20%	5 25%	8 40%	10 50%	12 60%	17 85%

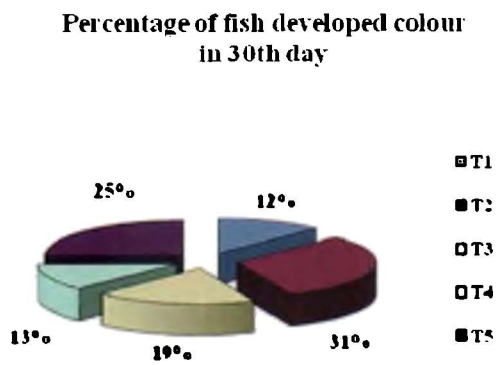


Fig: 7

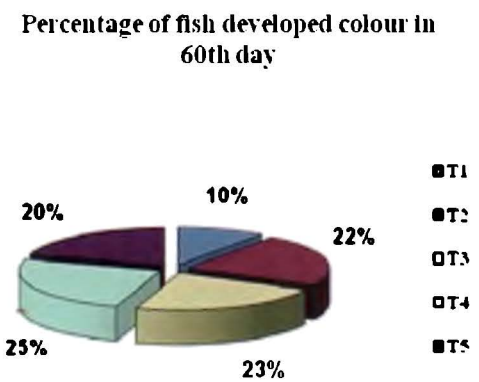


Fig: 8

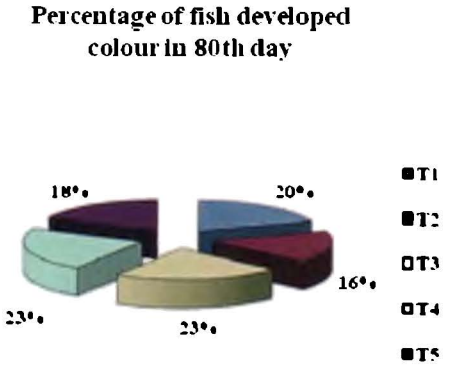


Fig: 9

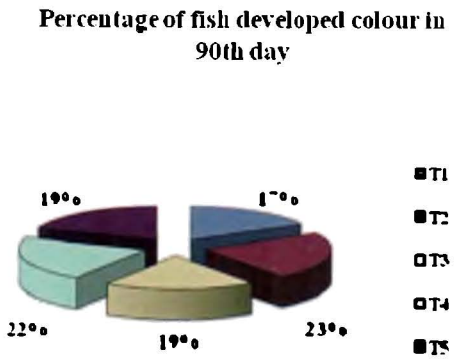


Fig: 10

Fig: 7-10 Percentage of fish developed colour in different days of sampling.

Goldfish fry were reared for 90 days in different feeds (T_1, T_2, T_3 T_4 and T_5). On 30th day of experiment maximum colour (30%) was developed in T_2 and minimum colour (13%) developed in T_1 and T_4 **(Fig: 7))**. On 60th day of experiment maximum (25%) fish developed colour in T_4 and minimum (10%) colouration developed in T_1 **(Fig: 8)** On 80th day, maximum (23%) fish developed colour in T_4 and minimum (17%) colouration was achieved in T_2 **(Fig: 9)**. On 90th day, maximum (23%) and minimum (17%) fish developed colour in T_2 and T_1 respectively **(Fig:10).**

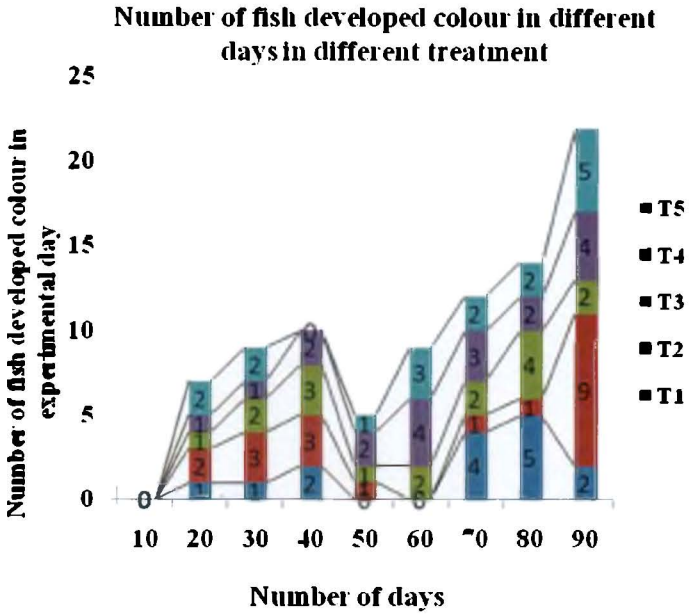


Fig: 11

Fig: 11: Number of fish developed colour in different days in different treatment. It is also expressed that total number of fish developed colour in particular treatment in the particular day of sampling.

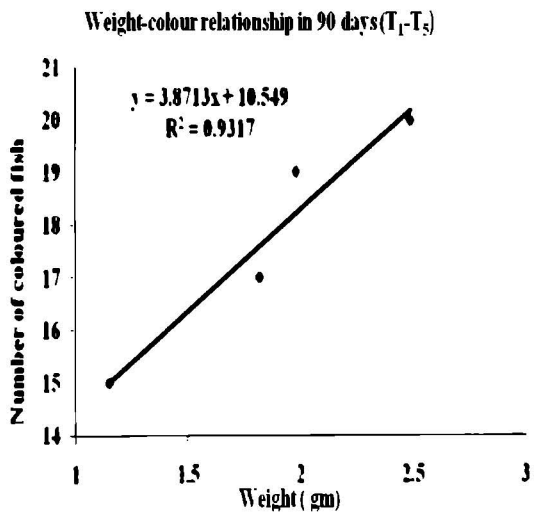


Fig: 12

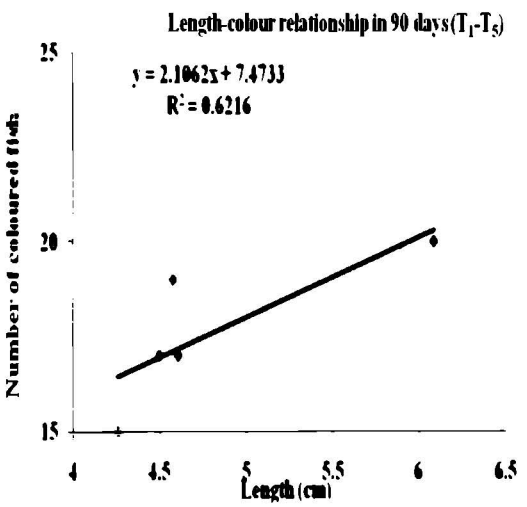


Fig: 13

In the present study relation between weight and number of colour fish was estimated and it was found that there was a strong significant relationship (R^2= 0.9317) between development of colour and weight of goldfish fry but the relationship between length of fish and number of colour fish was not significant (R^2 = 0.6216).

4.5.1 Water quality:

4.5.1.2 Dissolved oxygen:

Dissolved oxygen concentration mg/liter varied treatment wise in different sampling days. In T_1 highest value was recorded 5.72 mg/l on 80th day of sampling and lowest value was 4.0 mg/l on 50th day of sampling. In T_2 highest value was recorded 6.8 mg/l on 80th day of sampling and lowest value was 4.0 mg/l on 50th, 60th, 70th day of sampling. In T_3 highest value was recorded 6.52 mg/l on 10th day of sampling and lowest value was 4.0 mg/l on 70th day of sampling. In T_4 highest value was recorded 6.52 mg/l on 10th day of sampling and lowest value was 3.04 mg/l on 70th day of sampling. In T_5 highest value was recorded 7.04 mg/l on 10th day of sampling and lowest value was 3.6 on 60th day of sampling. On the 90th day the value of dissolve oxygen in T_1, T_2, T_3, T_4 and T_5 was 4.62, 5.72, 5.6, 4.8, 6.6 mg\l respectively **(Fig- 14)**.

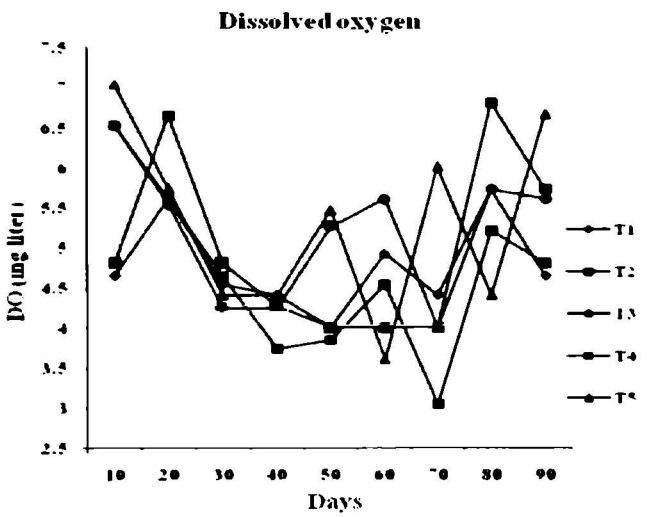


Fig: 14

One-way analysis of variance (ANOVA) of dissolved oxygen in different treatments (T_1-T_5) in different sampling days exhibited higher level of significance ($F \geq 4.571$; $P \leq 0.02$).

4.5.1.3 Alkalinity:

Alkalinity concentration mg/l varied treatment wise in different sampling days. In T_1 highest value was recorded 195.2 mg/l on 80th day of sampling and lowest value was 100.66 mg/l on 30th day of sampling. In T_2 highest value was 206.66 mg/l on 80th day of sampling and lowest value was 125.33 mg/l on 20thday of sampling. In T_3 highest value was 169.33 mg/l on 90th day of sampling and lowest value was 107.99 mg/l on 60th day of sampling. In T_4 highest value was 196.66 mg/l on 90th day of sampling and lowest value was 102.66 mg/l on 30th day of sampling. In T_5 highest value was recorded 183.86 mg/l on 90th day of sampling and lowest value was 88.6 mg/l on 60th day of sampling. On the 90th day the value of alkalinity in T_1, T_2, T_3, T_4 and T_5 was 176.66, 186.0, 169.33, 196.66, 183.86 mg\l respectively **(Fig- 15)**.

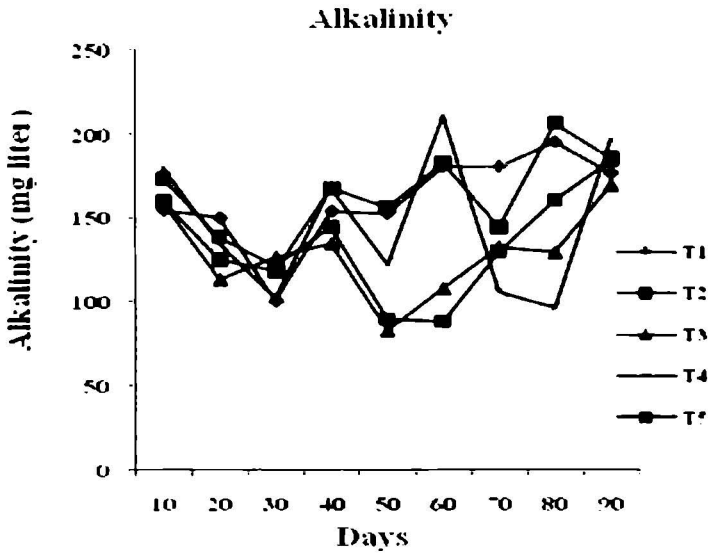


Fig: 15

One-way analysis of variance (ANOVA) tests of alkalinity in different treatments in different sampling days exhibited higher level of significance ($F \geq 54.676$; $P \leq 0.001$).

4.5.1.4 Hardness:

Hardness concentration mg/liter varied treatment wise in different sampling days. In T_1 highest value was recorded 972.0 mg/l on 80th day of sampling and lowest value was 746.66 mg/liter on 40th day of sampling. In T_2 highest value was recorded 984.6 mg/l on 90th day of sampling and lowest value was 686.66 on 24thday of sampling. In T_3 highest value was 998.66 mg/l on 80th day and lowest value was 600.00 mg/l on 40th day of sampling. In T_4, highest value was 998.66 mg/l on 50th day and lowest value was 680.00 mg/l on 40th day of sampling. In T_5, highest value was 987.2 mg/l on 90th day of sampling and lowest value was 676.00 mg/l on 40th day of sampling. On the 90th day the value of hardness in T_1, T_2, T_3, T_4 and T_5 was 964.0, 984.6, 986.6, 996.0, 987.2 mg\l respectively **(Fig- 16)**.

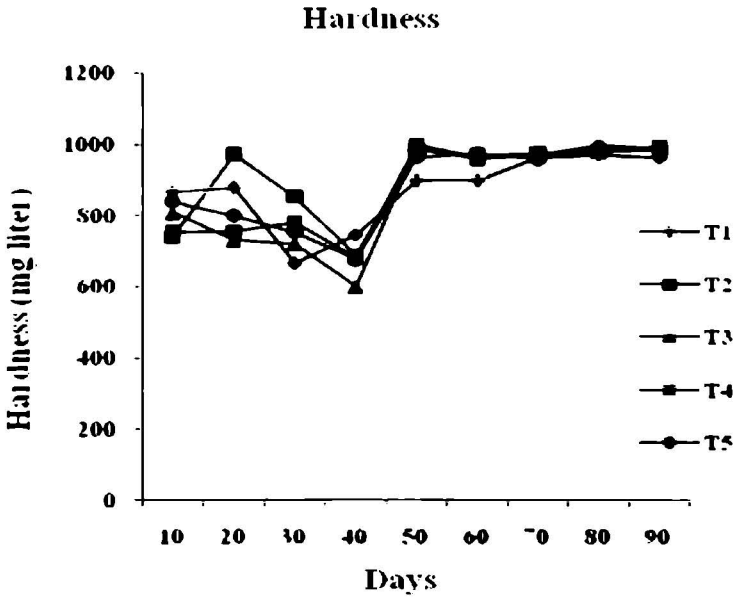


Fig: 16

One-way analysis of variance (ANOVA) tests of hardness in different treatments) in different sampling days exhibited higher level of significance ($F \geq 0.207$; $P \leq 0.001$).

4.5.1.5. P^{H}: There was no significance variation of pH among the treatments in various days of sampling. In T_1 highest value was recorded 7.50 on 80th day of sampling and lowest value was 6.00 on 40th day of sampling. In T_2, highest value was 8.00 on 90th day and lowest value was 7.2 on 60thday of sampling. In T_3, highest value was 7.90 on 80th day and lowest value was 6.8 on 40th day of sampling. In T_4, highest value was 7.95 on 50th day and lowest value was 6.50 on 60th

day of sampling. In T_5 highest value was recorded 7.80 on 90th day of sampling and lowest value was 6.80 on 30th day of sampling. On the 90th day the value of pH in T_1, T_2, T_3, T_4 andT_5 was 8.00, 8.00, 7.50, 7.80, 7.80 respectively.

4.5.1.6. Temperature: There was no significance variation of Temperature among the treatments in various days of sampling. Water temperature in all treatments of a particular day of sampling was same. On 10th day of sampling mean water temperature in all treatments (T_1, T_2, T_3, T_4 and T_5) was 32°C. On 20th day of sampling mean water temperature in all treatments was 30°C. The mean water temperature in all treatments was 34°C on 30th, 40th day of sampling and 35°C was on 60th day. On 70th day of sampling the temperature was 26°C. On 80th day mean water temperature rose up to 34°C but on 90th day mean water temperature was 32°C.

CHAPTER -5

DISCUSSION

Millions of people around the world keep aquarium fish as hobby. What is remarkable is that many of these enthusiasts will have started by keeping a gold fish (*Carassius auratus*), a fish whose history can be traced back over 1500 years to Ancient China. The goldfish was once a rather drab- looking carp but, after patient breeding and cross-breeding over the centuries, it is now available in many different forms. These range from the familiar common goldfish to over 100 fancy varieties. Gold fish is jack of all aquarium trades, easily adaptable for both aquarium and open outdoor cement cisterns.

One of the most important aspects of any living organism is to produce individual from the existing one, thus ensuring the continued existence of the organism concerned. Like other higher animals, fishes are also divided into males and females. Male produces sperms to fertilize the eggs of female, which develop into new generation. Most fishes release their eggs and sperm in water and fertilization takes place externally.

Goldfish are sexually dimorphic, females are larger than males having more bulging belly but males are more colourful and attractive than female (Chris Andrews , 1997).

Experiments on goldfish breeding (both natural and induced) were performed to study the effect of pond water having higher concentration hardness and iron than standard value. Breeding experiments were conducted thrice within a span of 12 months and it is very interesting to note that goldfish didn't respond in natural breeding though ideal breeding situation was created but same breeding pair when induced by ovaprim they all responded and spawning occurred.

Experiment-I, II and III (Natural breeding):

a) Breeding response and Spawning:

Natural breeding was conducted in rectangular PVC tanks measuring (4.5 x 2.3 x 1.4 ft). Synthetic nylon threads were used as spawning mops in the breeding tanks. Spawning mops were used after proper disinfection. Three such tanks were used for breeding having two females and four males in each tank. All the females were ripe with bulging belly and the males were brightly coloured and healthy having pale tubercles on the operculum. The experiment continued for three days maintaining ideal situation in the breeding environment.

Hardness and iron concentration of pond water was significantly higher than ideal water qualities (Table: 5). Hardness value was 266.66% higher in natural breeding and 227.77% higher in induced breeding than maximum permissible limit of ideal condition i.e.180 mg/ l (**Experiment –III**). Hardness and iron are considered as critical factors in carp as well as goldfish breeding. On the other hand, desirable iron concentration in breeding water is 0.3 mg/l but the concentration encountered during natural breeding of goldfish was 3.2 mg/l i.e. 1066.66% and in induced breeding the value was 1000.0% higher than ideal value.

Water hardness is related to the amount of dissolve salt present in the water. Water hardness plays a significant role in goldfish breeding. A vast variety of egg layers usually breed in soft and slightly acidic water. Excessive hardness may cause in absorbing the substances through its delicate membrane (Boyd, 1989). When these fishes are raised in moderately or slightly hard water, it is observed that the ova in the ovary has been calcified or calcification occurs near the vent that blocks the vent and as a result the fish is unable to release the eggs. Success of breeding depends on courtship between male and female fish.

In natural spawning, activation for spawning takes very long time and sometime it was observed that female did not spawn after attraction and may take more time in next spawning. Attraction between male and female allowed them to come closer and male press the females' belly to release eggs. Long chasing by male causes failure in spawning. In the natural spawning, it was observed that long chasing caused damage of female, which may reduces hatchability and survival of larvae. Calcification near vent blocked the passage and females were unable to release their eggs. As courtship continued for three days, the internal urge of releasing eggs was not enough to overcome the external blockage though male continued chasing. This observation indicated that hardness beyond the permissible limit poses acute problems in spawning.

Experiment-I, II and III (Induced breeding):

a) Breeding response:

Same breeding pairs were exposed to the inducing agent (ovaprim). Breeding environment was almost similar to natural breeding. Males chased females and chasing continued for few hours and female released eggs. There are numbers of factors that are responsible for successful breeding and spawning of ornamental fishes like physical and

chemical properties of water. Natural breeding is influenced by several factors and by observing most of the factors in breeding, chances of failure in natural spawning are high. In natural breeding, activation for spawning takes very long time and even sometime it was observed that female did not spawn after attraction and may take more time in next spawning. It opens the scope of introducing artificial breeding (induced breeding), an alternative to conduct successful breeding, to produce more number of hatchlings and to achieve high survival rate.

In three sets of induced breeding, total number of hatchlings obtained was 2010, 3382 and 3040 in Experiment I, II and III respectively. Fecundity of gold fish is 2000-3000 eggs. In each experiment, six mature females were introduced. So expected number of eggs in each experiment were 2500 x 6 = 15,000 but only 2010 hatchlings were obtained in Experiment-I which was 13.4% only. In **Experiment-II and III**, hatching percentage was 22.54% and 20.26% respectively. The poor percentage of hatching was due to high hardness and iron concentration of breeding medium.

b) Survival rate:

Survival rate of the hatchlings was very poor. Only 240 (13.59%) hatchlings in experiment-I, 520 (15.37%) in experiment-II and 220 (7.23%) in experiment-III were survived. Mortality of hatchlings started from 3rd day onwards and huge mortality was probably due to excessively high amount of iron (> 800% than ideal value) in water.

High concentration of metallic iron induces stress in the fish that resulted in significantly more oxygen consumption. Gold fish hatchlings take in water through mouth and expel it from the gill. High concentration of iron in water hampers gaseous exchange which leads to high mortality fish seed. In an experiment it was found that 30 days old fish spawns are highly sensitive to iron toxicity (5 mg/l). In the present experiment, 88% mortality of goldfish spawn was observed in high concentration of iron (Avg. 2.77 mg/l) in breeding medium.

It is also reported that sodium content of the test medium exhibited positive co-relation with iron, i.e. sodium showed its association to increase iron's toxicity in major carps. In the present experiment, the test medium was saline in nature (average salinity 4.5 ppt) which expedited the fish seed mortality. Both iron and manganese are detrimental to fish culture as the alkaline water favors the development of hydroxide which can precipitate on the gill filaments causing respiratory troubles for fishes. High mortality of goldfish larvae was due to the effect of iron in saline medium.

Experiment –IV (Larval rearing and development of colour pigments):

a) Growth of fish: Average body length (cm) of goldfish fry was measured in different sampling days and T_2 (mash) exhibited maximum value from 30th day onward. At the end of experiment, the value was 6.09 cm and minimum value on 90th day was recorded in T_1 (4.26 cm). Second highest value was obtained in T_5 followed by T_4 and T_3. From this observation it is clear that feed containing natural colouring agent exhibited lower body length. Lower body length in T_1, T_3 and T_4 may be due to 10% less fish feed replaced by natural colouring agent. Acceptability of feed is another factor for growth of fish. In T_1 (Beet) unconsumed portion of feed was higher than other feeds. This may be the reason of poor growth in T_1 treatment.

Average body weight (gm) of goldfish fry exhibited similar trend and maximum weight was recorded in T_2 (2.49 gm) and minimum in T_1 (1.15gm). Lower body weight in T_5, T_4 and T_3 on 90th day was 2.32 gm, 1.98 gm and 1.82 gm respectively. Due to presence of high fiber content carotenoid digestibility in carrot is less (25%), which limits the study on the use of carrot as carotenoid sources for enhancing the fish colour. High fiber content also may suppress growth of fish.

A diet is considered a complete one when it contains balanced level of all essential nutrients such as protein, lipid, carbohydrate, energy, vitamin and minerals, which promote their biological and physiological activities. Feed is considered to be the major constituent of aquaculture impacts. Feed formulation is the process of combining feed ingredients to form a mixture that will meet the specific production goals. According to Sales and Janssens 2003, larval goldfish were found to grow best on prepared diets containing about 50% protein.

The diet was selected primarily on development of colour. Parameters like weight and length was secondary consideration. Maximum number of fish developed colour in minimum rearing period will provide immense advantages to the fish breeder for early return. Length–weight relationship is another important consideration for rearing of fry.

b) Length–weight relationship:

The exact relationship between length and weight differs among species of fish according to their inherited body shape, and within a species according to the condition (robustness) of

individual fish. Length–weight relationship of goldfish fry on 90th day was measured and it was observed that in all treatments the co-relation was highly significant and value of R^2 varied from 0.9624 to 0.9881. In **Fig-6**, the value of R^2 on 90th day was 0.8408 and it was due to calculate all treatments together.

Linear regression of length –weight relationship is an indication of growth of fishes. In this experiment, the length –weight relationship exhibited highly significant value in all treatments.

c) Development of colour:

Ornamental fishes are recognized for their bright, brilliant and beautiful colouration and shape. Colour is one of the major factors which determine the price of the ornamental fish in the world market (Saxena, 1994). The most common and the most important natural occurring pigments are carotenoids responsible for colouration of ornamental fish. These pigments vary from a bright yellow to a deep red or even a violet or dark blue. Apart from being the source of colouration, these natural pigments also perform a number of important biological functions. As fish cannot synthesize these pigments, they rely on dietary supply of carotenoids to achieve their natural skin pigmentation. Algae, Yeast, fungus, vegetables, fruits, wide varieties of plant materials are rich in carotenoids and might be used to replace costly pure synthetic carotenoid pigments in ornamental fish feed (Gouveia *et al.*, 2003). Only natural food cannot support the colour development within a limited span of time in goldfish.

Development of colour pattern in different sampling days in different treatments exhibited an interesting trend. On 30th day of experiment maximum number of colour fish was obtained in T_2 (30%) but on 60th day T_4 produced maximum (24%) colour fish. On 80th day of sampling, T_4 maintained the same trend but on 90th day T_2 again occupied the highest position. From this observation it is clear that 70th day is the most suitable day for T_4 (marigold petals) where 65% of fish developed colour and selling of goldfish fry on that day will save 20 days rearing expenditure and fish breeder can harvest profit earlier.

Gold fish is jack of all aquarium trades because of its shape and beautiful colouration. Colour is an important criterion for selection of gold fish. Colouration of goldfish depends on presence of carotenoids in fish feed. Rearing of goldfish larvae with suitable diet produces colour in gold fish. Most of the artificial colouring agents which are available in the market are costly and causes serious health hazards of fishes.

Among the three natural colouring agents Marigold petals performed better than others. On 60th day on wards marigold petals exhibited higher percentage of colour fish in T_4 and maximum 95% of fish developed colour on 90th day. Carrot powder was the second highest performer and on 90th day 85% fish developed colour and beet developed only 75% coloured fish at the end of experiment. The difference of colour producing ability of three natural colouring agents mainly because of presence of carotenoids pigments. It has also been found that high concentration of carotenoids in food may not develop attractive colour in a certain period of time. There are several other factors which control the expression of colour in a fish. In the present study, carotenoids in Marigold petals exhibited quick and maximum colouration.

d) Relationship between colour and length:

Colour-length relationship was plotted on 90th day in all treatments and it was observed that the relationship was not highly significant. Value of R^2 in this experiment was 0.6216 only. Goldfish is a small to moderately-sized fish with a deep body and rounded in cross-section. As they are rounded in structure, length of fish does not play significant role. Length of a fish is the distance between snout and caudal peduncle. As gold fish exhibits various kind of caudal fin and the length of fin may be bigger than the fish. Thus the length of fin is not considered as body length especially in goldfish and the relationship was not significant.

d) Relationship between colour and length:

Colour-length relationship was plotted on 90th day in all treatments and it was observed that the relationship was not highly significant. Value of R^2 in this experiment was 0.6216 only. Goldfish is a small to moderately-sized fish with a deep body and rounded in cross-section. As they are rounded in structure, length of fish does not play significant role. Length of fish is the distance between snout and caudal peduncle, though caudal fin of goldfish may be various in shape and size.

CHAPTER -6

SUMMARY

Gold fish first originated in China and other country like Japan, Europe etc. They are fresh water fish, hardy in nature, live in a variety of habitats. Goldfish generally breed in natural water having normal water qualities. Fluctuation of water qualities like high concentration of hardness and iron inhibit natural spawning. Other factors include dissolve oxygen, turbidity, maturity stage of brood fish, behavior of male and female, proper feeding of brood fish, suitable spawning substrate etc., influence in natural spawning.

In natural breeding, activation for spawning takes very long time and sometime it was observed that female did not spawn after attraction and may take more time in next spawning. Long chasing by males caused failure in spawning. It opens the scope of introducing artificial breeding (induced breeding), an alternative to conduct successful breeding, to produce more number of hatchlings and to achieve high survival rate.

Water quality of pond differed from ideal water qualities in two parameters i.e. hardness and iron which are considered as critical factors in carp as well as goldfish breeding. Hardness value was 266.66% higher in natural breeding and 227.77% higher in induced breeding than maximum permissible limit of ideal condition i.e.180 mg/l. On the other hand, desirable iron concentration in breeding water is 0.3 mg/l but the concentration encountered during natural breeding of goldfish was 3.2 mg/l i.e. 1066.66% and in induced breeding 3.0 mg/l i.e. 1000% higher than ideal value.

After seven days of unsuccessful natural breeding, fish were exposed to induce breeding. Ovaprim @ 0.5 ml/kg of fish introduced. Female spawned within a short span but hatching rate was very poor. Mortality of spawn occurs on 3rd day onwards. High concentration of iron in water hampers gaseous exchange which leads to high mortality fish seed.

Three natural colouring agent viz. marigold petal, beet and carrot was used along with mash feed and plankton for larval rearing. The different colour producing ability of the three natural colouring agents varied due to various concentrations of carotenoid pigments presence. It was also found that high concentration of carotenoids in food may not develop attractive colour in a certain period of time.

In the present study, carotenoids in marigold petals exhibited quick and maximum colouration than other colouring agents. Higher growth rate of fish fry was observed in mash feed. Presence of high fiber content carotenoid in carrot reduces digestibility by 25%. High fiber content also may suppress growth of fish.

CHAPTER -7

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