

**STUDY ON EMBRYOGENESIS OF
RAINBOW TROUT (*Oncorhynchus mykiss*) IN NEPAL**



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LETTER OF RECOMMENDATION

This is to certify that Mr. Hari Lama has completed this dissertation work "**Study on Embryogenesis of Rainbow trout (*Oncorhynchus mykiss*) in Nepal**" as a partial fulfillment of M.Sc. degree in Zoology(Fish and Fisheries) under my supervision. It is my pleasure to recommend this original work for the partial fulfillment of Master of Science in Zoology at Tribhuvan University.

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ACCEPTANCE

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I hereby declare that the work presented in this dissertation has been done myself and has not been submitted elsewhere for the award of any degree. All source of informations have been specifically acknowledged by reference to the author or institution.

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ABSTRACT

The study consisted of various stages of embryonic development of Rainbow trout (*Oncorhynchus mykiss*). Approximately, 2000 eggs were incubated in standard hatchery troughs supplied with spring water at Fisheries Research Division, Godawari, Lalitpur. Accepted fish cultural methods were followed to insure normal developmental patterns. Samples of these eggs were taken for study at 24 hours interval up to 5th day after fertilization; then once every 48 hours until they were 23 days old; then at 72 hours intervals up to 39th day. The embryos were fixed in 5 percent neutral formalin containing 0.95 percent sodium chloride, stained in Harris' hematoxylin and photographed.

Egg size affects both fecundity and offspring fitness and larger eggs result in larger fry. The size of trout was found larger, greenish in colour. The development of eggs of trout was found regulated by temperature, slightly alkaline pH and high dissolved oxygen. In present study, water temperature ranged from 10.4 – 11.7^oC, dissolved oxygen 7.21 – 7.37 mg/l and pH from 7.27 -8.0 respectively; these water quality parameters were recorded suitable for larval development of fishes. Rainbow trout embryonic and larval development required 10-12^o C but trout culture required an optimum temperature of 15-18^oC.

The hatching period, in present study, varied from 39-41 days at the water temperature of 10.4 – 11.7^oC. The alevins weighed about 40-50 mg and measured 1.3-1.8 cm in length. 50-60% of body weight was yolk sac. Large size of alevins of rainbow trout might be due to large egg sized. The yolk sac of the pro-larvae were recorded to absorb within 7-18 days and started to swim slowly on water surface. Slow growth of embryonic and post hatched stage could be attributed to very low water temperature required for the development and rearing of trout. The feeding started after 15-20% of larvae appeared swimming on water surface. The average weight of first swimming fry was 0.07- 0.1 gm and they were fed Starter Feed containing 45-50% protein at the rate of 5-10% of body weight at the interval of every 3/4hours.

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CHAPTER ONE

1.0 Introduction

1.1 Background

Nepal is an agrarian society. Agriculture shares 40% of GDP and about 88% of the population depends on agriculture for livelihood. Agriculture plays pivotal role for any attempts to increase income and improve living standard of the people in this country. Nepal is rich in water resources with abundant indigenous fishes of cold and warm water fishes. Fisheries and aquaculture are an integral part of the Nepalese farming system owing to the presence of large inland water resources in this country. Different water bodies of Nepal provide a suitable habitat for 232 indigenous fish species (Shrestha, 2008). Many local fishermen communities are depended upon capture fishery for the livelihood. Besides capture fishery, there is a great scope for the expansion of aquaculture in this country. Fisheries and aquaculture can meet the challenges of age-old poverty by opening the prospects of food security, employment, better income etc. The history of fisheries development dated back to 1952 with the introduction of traditional fisheries activities. Induced breeding of Chinese carps and Indian major carps started in Nepal from 1970/1971. A systematic aquaculture, particularly pond fish culture was initiated in 1981/82 with the implementation of "Aquaculture Development Project" supported by Asian Development Bank. During the project period, the production technology was successfully transferred to the private sector with the establishment of many private fish hatcheries to expand the fisheries activities. Warm water fishes like carps are the major items of aquaculture in the plain areas of Nepal. Due to poor valued commodity, semi-intensive polyculture of carp practiced in our country, it has contributed in strengthening subsistence economy of poor fish farmers only. The contribution of fishery sector to national AGDP is only 2.0%, so far.

The Agriculture Perspective Plan (APP) and Tenth Plan have emphasized diversification of agriculture to alleviate poverty, substitute import of agricultural products and maintain self sustaining economy. Nepal has a wealth of microclimates and agro-ecological conditions for the production of high valued products like fruits, vegetables, vegetable seeds, potatoes, tea, coffee, spices, medicinal plants, mushrooms, essential herbs,

oilseeds, pulses, fish and fishery products etc. The comparative advantages of climate, geography and ecology suitable for producing high valued items have been expedited only to a limited extent due to several structural deficiencies, weak infrastructure, lack of proper/advance technology and poor marketing system. The wealth of coldwater resources bears a great prospect for coldwater fish farming in Nepal as it comprises 15 % high hills and 68 % mid-hill. The cold-water fishery has gained increasing importance in recent years. There is a great prospect for commercial farming of exotic Rainbow trout in Nepal to expedite comparative advantage of topography and abundant coldwater resources.

1.2 Development of coldwater trout fishery

Trout is a native of North America and is world famous for its tastes. This fish was taken to California, Alaska and later on to Asia and Europe during 19th century. It is now present in all the continents of world except Antarctica. The commercial culture of trout began as early as 1853 in United States (Bardach et. al., 1972) and Rainbow trout was introduced in Japan in 1877. Among the countries in which trout is grown, Chile has led production followed by Europe and America. In the Asia-Pacific region, Iran produced the highest quantity of trout, followed by Japan and Australia. The fish loves high oxygen demanded cold and fast flowing hill streams with shade (16-17 hours of darkness), hard calcareous water and stony bottom or cemented raceway pond for their survival and growth. The growth rate decreases when the oxygen concentration falls below 7 ppm. Unlike normal fish (carp), trout fish is devoid of intramuscular 'Y' bone so consumers feel easy and comfortable to eat it. Trout is a high valued tasty fish with 70-80% soft edible part and contain all 10 'Essential Amino Acids'. Licospentoic acid present in muscle helps in lessening cholesterol in human blood. Trout is also rich in fatty acids including Omega-3 fatty acid that reduces the risk of coronary heart diseases. Initially two trout species, rainbow trout (*Salmo gairdneri*) and brown trout (*Salmo trutta*), was introduced in Nepal. The aim of introduction was to rear brood stock in Godavari and Trisuli fish farms and to release offspring's in the temperate rivers. In Nepal, late king Mahendra imported rainbow trout for the first time in 1969 from India. Later in 1972, Queen of England gifted 10 thousand fingerlings of brown trout to King Mahendra. In 1988, 50 thousand eyed eggs were again brought from Japan and kept in Fish Research Centre in Godavari and Trisuli. The attempt was successful this time to hatch rear and grow up to

brood size. In 1990 A.D., breeding of trout was successful and research works were continued in different aspects of trout aquaculture in government fish farms. The works lead to the development of a complete package of trout farming by 1997 A.D. suited to the condition of Nepal. From then onward, technical package of trout farming was disseminated to fish farmers and entrepreneur.

Trout is a carnivorous fish depending on small aquatic insects, crustaceans and small fish in the natural waters. But trout can be cultured in cemented raceways in flowing coldwater using artificial feed at all stages from fry to adults. The fish grows well at 10-13⁰C and shows optimum growth at 15-18⁰C (Yamazaki, 1991). Besides cold temperature, dissolved oxygen above 7.0 ppm and pH from 6.5 – 9.0 are very important for its culture. The raceways should be built at a slope of 1-3% for permitting adequate water flow. Trout can be cultured at high stocking density of 90-100/m² in cemented raceways. Rainbow trout attains commercial size (200-300 g) during second year (Marcel, 1975). The growth rate of the fish was the highest 1.5 g/day in raceway pond, 0.7g/day in cages and 0.69g/day the earthen pond respectively (FRD, Godawari, 1997/98). The growth rate was highest (1.89 g/day) in March and lowest in (0.68 g/day) September/October and survival rate was about 75%. The feed of young fish should be in crumble feed containing 45% protein content. The diet of growing fish over 50g should contain 35% protein in pellet form. The feed conversion ratio comes around 2:1 in Nepal. The feeding rate should be 5-6% of the body weight for young trout and 1.5 to 2% for bigger fish. The fish usually attain marketable size, viz.200 g after about 14 months of rearing in Nepal. The production rate of trout is 150-200 ton/ha. The trout fish farming was adopted by few farmers in Nuwakot, Rasuwa and Kathmandu districts with limit scale of production. The demand of table fish of trout is increasing day by day in the country particularly Kathmandu. Many farmers from different parts of country are interested and attracted to culture of rainbow trout. Due to the success and growing attraction of people in trout farming, Government of Nepal prioritized the policy to promote trout culture in mountainous areas especially in Rasuwa and Nuwakot districts of Nepal under a collaborative program 'One Village One product (OVOP)' of Federation of Nepal Chamber, Commerce and Industry (FNCCI)

CHAPTER TWO

2.0 Objectives

The main objectives of the present study are:

- To study the various stages of embryonic development of Rainbow trout.
- To study the behaviours and response of embryonic stages to different parameters.

CHAPTER THREE

3.0 Literature review

Brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) are generally used in sport and recreational fisheries. Rainbow trout (*Oncorhynchus mykiss*) are the hardiest salmonid and can tolerate wide environmental changes. For instance, they tolerate water temperature from 0-27°C (Uysal and Alpbaz, 2002b).

Studies on embryonic development and early life history of the different trout species were previously done (Grande and Andersen, 1990; Killeen *et al.*, 1999; Halacka, 1995; Başçınar and Okumuş, 2004). The early development and rearing conditions of *Salmo trutta labrax* and *S. t. abanticus* were investigated (Çakmak *et al.*, 2004; Uysal and Alpbaz, 2002a). Earlier researchers suggested that rainbow trout had higher survival rates than brown and brook trout at all hatchery stages (Başçınar and Okumus, 2004).

In cultured salmonids, larger eggs give rise to larger fry, which can survive longer without external feeding. Egg size affects both fecundity and offspring fitness and larger eggs result in larger fry.

Seasonal water temperatures and photoperiod play a role in the control of reproduction in this species (Titarev 1975; Billard 1985; Taranger & Hansen 1993; Pankhurst *et al.*, 1996; Davies & Bromage 2002).

According to Billard (1985), optimal temperatures for spawning of salmonids lie between 6 and 13⁰ C. Maintaining temperatures of 13⁰C or higher is reported to inhibit ovulation and to result in lower egg survival in Atlantic salmon (Taranger & Hansen 1993). Besides water temperature, Rainbow trout (*Oncorhynchus mykiss*) are relatively sensitive to hypoxia exposure (Gesser 1977). This sensitivity reaches a peak during early ontogeny, just before hatching (Rombough 1988). The physiological responses of salmonids to hypoxia are dependent on the species, age, and intensity of the hypoxia exposure (Jensen *et al.* 1993). Furthermore, responses to acute hypoxia may be different from those in chronic hypoxia. Several studies have focused on metabolic responses of salmonid adults

to hypoxia (Dunn and Hochachka 1986; Boutilier et al. 1988; Zhou et al. 2000), but few studies are available on embryonic stages. Previous studies have demonstrated that rainbow trout embryo survival within the redd is dramatically influenced by water O₂ levels. Youngson et al. (2004) reported that there was 83% mortality of rainbow trout embryos in redds where mean dissolved O₂ (DO) was at 28% saturation. Low DO concentrations have been shown to affect the rate of development of chum salmon (*Oncorhynchus keta*; Alderdice et al. 1958), lake trout (*Salvelinus namaycush*; Garside 1959), and Chinook salmon (*Oncorhynchus tshawytscha*; Silver et al. 1963) embryos.

Nepal has immense scope for the expansion of aquaculture in this country. The fish production from capture fisheries comprises 43 percent of total production in Nepal. But the production has become static; moreover, wild catch is declining in our nation and world wide due to environment degradation and/or over exploitation of the resources. Decline in capture fishery may also be due to increasingly vulnerable effects of global climate change affecting rural livelihoods in the tropics and subtropics badly with sudden profound changes in social and economic systems. To substitute capture fishery, aquaculture practices had been started in Nepal since last four decades. Warm water fishes like carps are the major items of aquaculture in the plain areas of Nepal. The wealth of coldwater resources bears a great prospect for fish farming in Nepal and the development of sustainable cold-water fishery has been gaining increased importance in recent years through three interrelated aspects - production technology, social/cultural and environmental aspects.

There is a great prospect for commercial farming of exotic Rainbow trout in Nepal. The primary requirement for trout culture is an abundant supply of clean and cold water. Rainbow trout culture requires a permanent supply of water with a temperature range of 10 to 20°C, and optimum temperature of 15-18°C (Yamazaki, 1991). The water should be clear, not turbid. A pH value of 6.5 - 8.0 and dissolved oxygen above 8 mg/L are considered suitable for trout culture (Huet, 1975). Calcareous water is preferable (Leitritz, 1963). Water supply of at least 5.0 l/sec is necessary to produce a ton of trout, although less may be sufficient, when temperature decreases (Pillay, 1993). Similar growth of rainbow trout was reported by Huet (1975). Detail technical package of trout farming was given in simple forms for trout farmers in Matsya Palan Shrinkhala 13 and 14 (DOFD, 2007 and 8). FRS Trishuli has confirmed that water temperature range of 10-20°C is

suitable for rainbow trout culture, if volume of supply is adequate and the water has a level of dissolved oxygen (DO) of 7-10 mg/L. Spring water is recommended for rearing alevins up to swim-up stage, because its temperature is warmer than that of a river. If the temperature of spring water is higher than 20°C it lowers the concentration of DO, therefore it should be mixed with cold water of snow and ice melt origin to adjust the temperature and DO. Water temperature should never exceed more than 23°C for rainbow trout culture (Sedgwick, 1985). Trout should not be kept for longer period in water temperature above 21°C as it stops feeding. It also stops feeding at temperatures less than 10°C. Sedgwick (1985) reported that a temperature of 18°C is regarded as the optimum for metabolism in rainbow trout. Higher temperature would assist higher level of metabolism and growth as well.

Rainbow trout was bred for the first time in 1990, culture experiments were initiated in 1993, and production of rainbow trout was started from 1995 and 1998 by government and private farms respectively in Nepal (Rai et. al. 2005). Hence trout farming in Nepal has a fairly short history. The research works carried out by NARC during past 10 years have demonstrated appreciable achievements in trout farming practices by developing suitable trout farming system in the country. Rainbow trout (*Oncorhynchus mykiss*) is a suitable fish for intensive aquaculture, sport fishery (Rai, 2002) and possess high economic prospect providing ample of opportunities for commercial production in mid and high hill area. In Trisuli fish farm, trout reached 200-500 g after 14-15 months of rearing from free swimming larval stage. Comparative study on Rainbow trout *Oncorhynchus mykiss* breeding and table fish production management (Govt.& Private farm) and various aspects of rainbow trout farming in Nepal was done by Basnet et al. (2007). Basnet et al. (2008) had carried scaling up of Rainbow Trout Farming in farmer field.

CHAPTER FOUR

4.0 Materials and methods

4.1 Study Area



Figure 1. Fisheries Research Centre, Godawari

The Godawary Fisheries Research Centre was established in 1962. This station is situated in central Nepal in the Kathmandu Valley, some 1,700 metres above the sea level and about 16 Km southeast of Kathmandu. The climate is sub-tropical and cool with water temperature falling to 8⁰C in winter. Pond waters reach about 26-27⁰C in summer but not more than 20⁰C in streams. The farm is fed by small streams from surrounding hills. The discharge rate of the stream is approximately 30 litres/second.

4.2 Care of Brood Stock

As in all animals, the time of sexual maturity is one of the most critical during the life of a fish, so brood fish should be handled with the utmost care. In order to obtain eggs of the highest quality, brood fish must receive an adequate diet. As more of the secrets of trout nutrition are learned, better diets and improved egg quality may be expected. Fish culturists are cautioned against using any diet containing regular cottonseed meal for

brood fish. If fed in more than slight amounts, it will adversely affect egg production in fish, just as it affects the reproductive system in other animals.

A female trout, heavily laden with eggs, cannot withstand the rough handling sometimes associated with poor hatchery practices. Great care should be taken during the sorting and spawning operation to dip up only two or three fish at a time. Never make a pass through a pen of nearly mature females and fill the bag of the dip net with fish. This can result in broken eggs, poor fertilization, and possibly permanent injury to the fish's reproductive system.

4.3 Selective Breeding

The general procedure used in selective breeding is as follows: The date when the selection should be made is determined by the period when eggs are desired to fit into the hatchery production program. In general, selections are made from desirable three-year-old fish that came from the best two-year-old stock, as there is more certainty of selecting rapidly growing fish with good conformity. After the males and females have been selected, they are spawned. The eggs from each female are kept separate. These individual lots of eggs are measured and counted and kept in separate trays or compartments of trays in the hatchery. Each lot receives an identification number. A record of losses is maintained throughout the incubation period. It is of interest to note that the losses in some lots are as high as 50 and 100 percent, suggesting that poor eggs encountered in production spawnings may come from a very few females. Before the eggs hatch, it is customary to eliminate all lots that are not of exceptional quality. The remaining lots are then hatched in separate compartments in hatchery troughs.

4.4 Sorting Frequency

The interval at which brood females should be sorted during the spawning season varies among hatcheries, and depends to a large extent on water temperature and season. Normally, it is not necessary to sort the females as often early in the season as during the

peak of the operation. To produce eggs of the best quality, it is necessary to watch the brood stock closely. The correct degree of ripeness must be attained in the females. Taking eggs before they are fully mature (ripe) is as bad as not sorting frequently enough, which may allow some of the females to overripen. Under the unnatural conditions associated with domestication, fish rarely deposit their eggs of their own accord. If they are not sorted often enough, overripe eggs are sure to be found. Such eggs are hard and glassy, probably as a result of contact with a serous ovarian exudation present in the fish, and are sometimes referred to as "moon-eyes". Besides being infertile, they may injure or even kill the fish.

One to three months prior to spawning (i.e. September 2007) , the male and female of 1⁺ age broods were separated by the appearance of external sign .The density rate of stocking of brood fish was 5- 10 kg/m² and water consumption per kg of live weight of brood fish was 1-1.5 lit/min .The dissolved oxygen in water was 8.25 mg/liter and pH value of water was 7.3 .The feeding rate was reduced from two times daily to 3-4 times per week for brood fish. The state of ripeness was examined two times at a week .

4.5 Collection of eggs

The eggs were collected in the container during 28th of December, 2007 at Fisheries Research Center, Godawari by applying mild pressure first on lower part of the ovary (near the vent) and upward the ovary over the ventral side of fish. If a fully mature (ripe) female trout is held by the tail, head down, the mass of eggs sags visibly toward the head and free eggs may settle into the forward end of the abdominal cavity outside the ova-containing membrane. Hence avoid holding a ripe female by the tail, head down. Forcible pressure in stripping may rupture the membranes and injure the ovaries, and result in a lowered egg survival. Inasmuch as trout, in the state of nature, do not emit all of their eggs at one time, no forcible attempt should be made to expel more eggs than those which flow easily under gentle pressure. It may take several manipulations to obtain the eggs. Since the eggs in the posterior part of the ovary, i.e., those nearest the vent, are the first to ripen, it is not necessary to apply pressure the whole length of the abdomen. Ripe female trout should be held tail down with the head high. This permits the eggs to flow or roll along the oviduct toward the vent.



Figure 2 Artificially spawning a female trout

4.6 Embryological studies

Eggs, when taken, are only slightly adhesive and on extrusion and absorption of water become firm and slick. After eggs extrude, these spend broods were bath in 3% common salt solution and kept in brood's pond for next year. Here, the salt solution holds the albumen from the broken eggs in solution and keeps the micropyles from becoming clogged. It also prevents agglutination of the sperm. The milt was mixed in the same container with stripped eggs in the ratio 3:1. Thereafter stirred gently with bird feather and allowed stand for one minute and individually, 0.9 percent salt solution about 1-2cm were poured carefully side of the container of eggs. Again those eggs were allowed to stand a few minute during which fertilization took place. Then the green eggs were washed with existing hatchery water, which was continuously, but gently shaken, the process continued until the water became transparent. Those green eggs were poured individually in the tray (local made 33-34 cm size). About 10 trays were put together into Atkins Capacity and made continuous and uniform flow of water at the rate of 2 lit/minute. The temperature of water was 10.5⁰C, level of dissolve oxygen in water was 8.0mg / liter an pH value was 7.3.



Figure 3. Hatchery with incubators in trays.



Figure 4. Microscopic studies and photography.

The eggs were washed by removing dead eggs. There is a wide variation in the rate of development in the first few days of incubation. The eggs should be shielded from light, especially from the direct rays of the sun as the eggs are killed when exposed to direct

sunlight for more than a few minutes at a time. The study consisted of various stages of embryonic development of Rainbow trout (*Oncorhynchus mykiss*). Samples of these eggs were taken for study of 24 hours interval up to 5th day after fertilization; then once every 48 hours until they were 23 days old; then at 72 hours intervals upto 39th day. The embryos were fixed in 5 percent neutral formalin containing 0.95 percent sodium chloride, stained in Harris' hematoxylin and photographed or hand drawing.

4.7 Water Quality parameters

Temperature - water and air, dissolved oxygen, pH of water were analyzed with the help of standard limnological heckkit and Laboratory method by APHA, AWWA,WPCF, 1985,Boyed 1991, 1994.

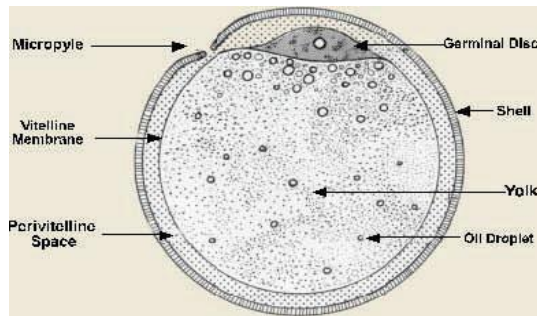
CHAPTER FIVE

5.0 Results and discussion

5.1 Structure of trout egg

The shell of trout egg was porous and minute pores could be seen in compound microscope. The shell was elastic and varied in strength and thickness among eggs from different females. The shell was highly transparent, which allowed a fairly good view of the developing fish. The thick shells were found whitish and opaque. The shell contained one larger opening called micropyle through which the spermatozoon entered to fertilize the egg. In response to some chemical attraction from within the egg, the spermatozoon entered the micropyle and found to penetrate the yolk membrane to unite with the nucleus of the egg. Though a number of spermatozoa may enter the micropyle, only one of them succeeded in uniting with the egg nucleus.

The yolk membrane was a thin protoplasmic layer surrounding the yolk to hold it together. But, it was not porous like the shell. In a trout egg, there was a space between the yolk membrane and the shell filled with a fluid called perivitelline fluid. When an egg was freshly stripped from the female, there was no water in this space. When the egg absorbed water, eggs became swollen and firm or turgid as they absorbed water. This swelling was called 'hardening' process and created a current through the micropyle to carry the spermatozoon into the egg. The outer shell became tough during the hardening period. This change was brought about through absorption of water into the material of the



shell.



Figure 5a. A Fertilized trout egg

Figure 5b. A Fertilized trout egg

5.2 Water quality parameters of water

The development of eggs of trout was found regulated by temperature. The good quality eggs were obtained from brood fishes kept at water temperatures not above 56⁰ F for a period of at least six months before spawning. Two years old rainbow brood stock showed good spawning when first 16-18 months of their life were kept at 60⁰ F and then transferred to about 54⁰ F to mature. The water temperatures above 56⁰ F and below 42⁰ F adversely affect incubation and egg development in rainbow. In an attempt to incubate eggs at 35⁰ F water temperature, mortality was recorded 100 percent. In present study, water temperature ranged from 10.4 – 11.7⁰C, dissolved oxygen 7.21 – 7.37 mg/l and pH from 7.27 -8.0 respectively (Table 1).

Table 1. Daily water quality study during experimental period.

No. of Observations	Days after Fertilization	Water Temperature	Dissolved Oxygen	pH
1	1	10.5	7.30	8.0
2	5	10.5	7.21	7.27
3	10	10.4	7.29	7.23
4	13	10.5	7.26	7.48
5	17	10.6	7.25	7.39
6	19	10.6	7.31	7.40
7	21	10.7	7.23	7.41
8	23	10.7	7.21	7.41
9	27	10.9	7.26	7.52
10	32	11.0	7.31	7.63
11	37	11.1	7.37	7.67
12	41	11.7	7.28	7.78

5.2 Effect of light on eggs and alevins

The light-exposed eggs hatched earlier than the eggs incubated in natural darkness. The total mortality of eggs and alevins was greater when they were light-exposed. The light-exposed alevins showed a decided negative reaction to light and constantly attempted to escape into a darker area but light-exposed alevins were extremely active, while the alevins in the dark environment were relatively inactive. The light-exposed alevins reached the "emerging fry stage" earlier than did the dark-exposed alevins.

5.3 Different embryonic stages of trout

The occurrence of cleavage was found to occur at about 7.0 -8.5 hours of fertilization. At 11 hours, the second cleavage appeared followed by third cleavage at 13.5 hours. During the third cleavage, a liquid filled cavity or blastocoel appeared. Most of the eggs were at 32 cells by 10 hours but several did not have 32 cells before 24th hours. Thus most of the embryos become blastoderms in the first day of life.

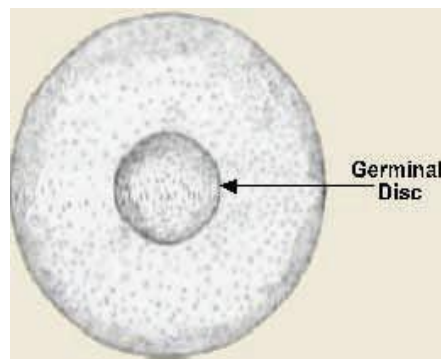


Figure 6. One day after fertilization at 56 average degree.

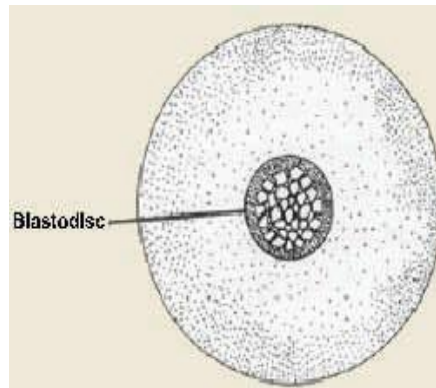


Figure7 . Two days after fertilization

After 30 hours, blastoderm did not increase in diameter, but the cells became smaller and more numerous arranged in three layers within 2 days.

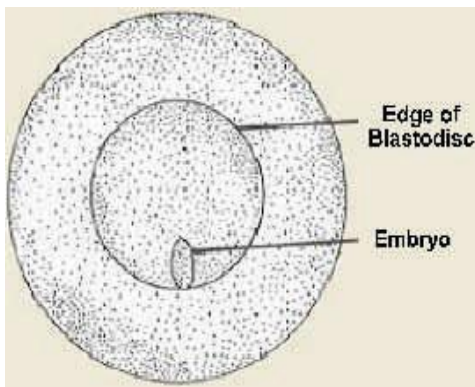


Figure 8a.

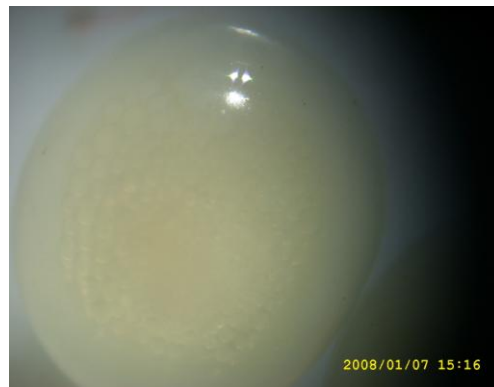


Figure 8b.

(Figure 8a. & 8b. Five days after fertilization)

At 56 hours, the blastula flattened spreading over the yolk surface. Three days after fertilization, the embryo was about 2 mm in diameter. At fifth day, formation of pole, overgrowth of yolk or process of epiboly was found to occur in the study.

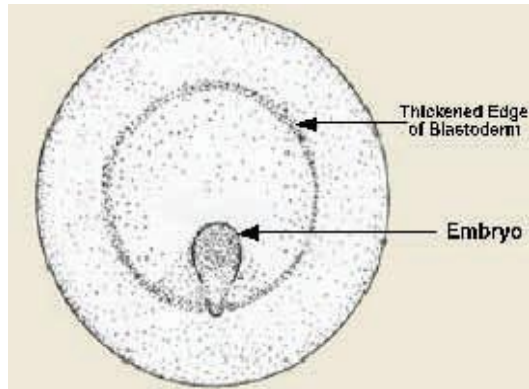


Figure 9. Six days after fertilization

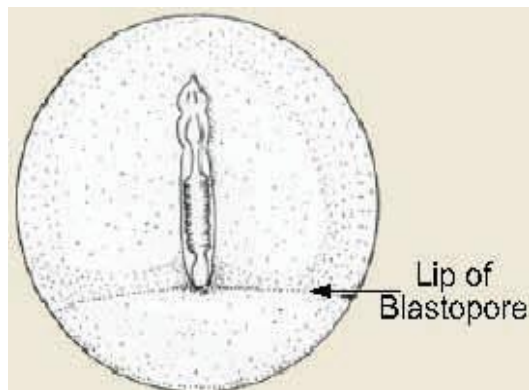


Figure 10. Seven days after fertilization

At sixth day, 17-24 somites were present on the developing embryo with optic tubercles and blastopore lip was found advancing rapidly to form the yolk plug. The blastopore closed at one week, eye lenses, forebrain, optic lobes, cerebellum and medulla distinguishable after 7days.

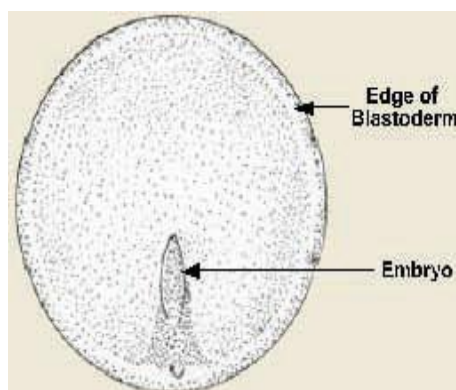


Figure 11. Eight days after fertilization.

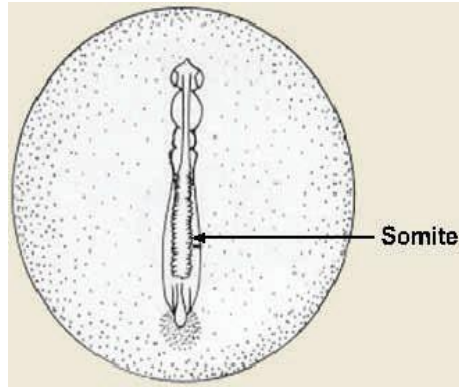


Figure 12. Nine days after fertilization.

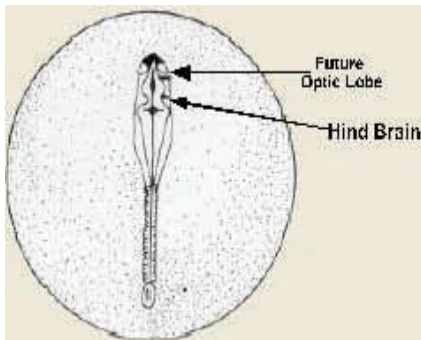


Figure 13a.



Figure 13b.

(Figure 13a. & 13b. Eyed stage after ten days after fertilization.)

On the 10th day, due to pigmentation eyes found visible through the chorion. This stage was called Eyed Stage.

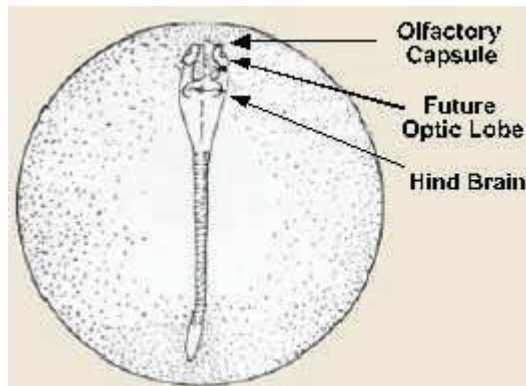


Figure 14. Eleven days after fertilization.



Figure 15. Development of opercle and elongation of embryo.(after 12 days)

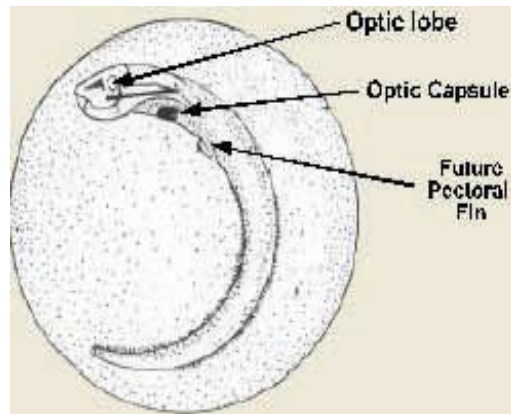


Figure 16.Thirteen days after fertilization



Figure 17. Eighteen days after fertilization

Development of opercle started to grow on the 16th day and by 18days with the elongation of embryo.

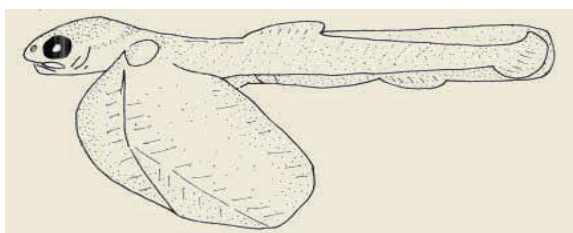


Figure 18 .Twenty days after fertilization



Figure 19a.

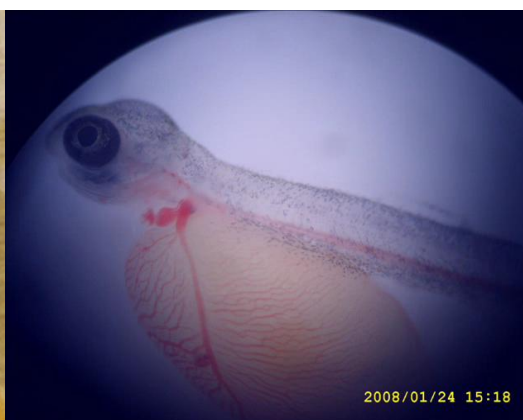


Figure 19b.

(Figure 19a. & 19b. Development of Vitelline Circulatory System after 22 days).

In 20 days, the dorsal fin exceeds the height of the fin fold and the embryonic body appears much darker because it is covered with many melanophores. Eyed stage became more distinct and development of vitelline circulatory system occurred.

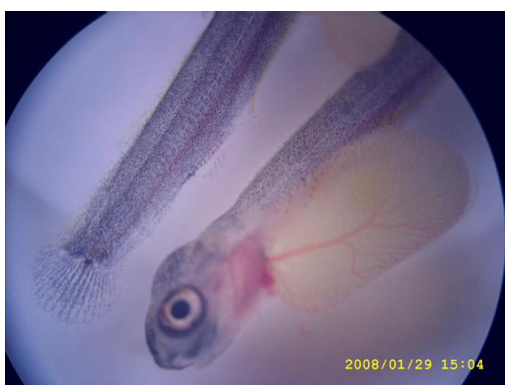


Figure 20a.



Figure 20b.

(Figure 20a. & 20b. Contraction of Yolk Sac 27 days after fertilization)

Hatching of egg of larvae occurred about 39-41 days after fertilization at the water temperature of 10.5 – 11.0⁰ C. The mortality rate of embryonic stages was ranged from 40-50%. The newly hatched larvae is called Alevins.

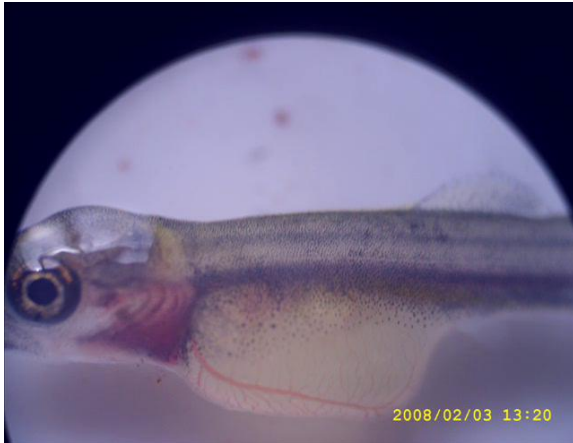


Figure 21a.

Figure 21b.

(Figure 21a. & 21b. Alevins after 31 days)



Figure 22. Alevins in feeding tray.

The alevins weighed about 40-50 mg and measured 1.3-1.8 cm in length. 50-60% of body weight was yolk sac. At this stage, the alevins were transferred to rearing trough in order to provide larger space and kept them by protecting from bright light. The water was supplied to trough as possible from the below at the rate of 0.3-0.5ml/min/1000 larvae. At this stage, the alevins remained at the bottom of trough and yolk sac provided nutrients

required for them about 15 days. The yolk sac of the pro-larvae were recorded to absorb within 7-18 days and started to swim slowly on water surface. The feeding started after 15-20% of larvae appeared swimming on water surface. The average weight of first swimming fry was 0.07- 0.1 gm and they were fed Starter Feed containing 45-50% protein at the rate of 5-10% of body weight at the interval of every 3/4hours.

CHAPTER SIX

6.0 Discussion

The size of trout was found larger, greenish in colour. The development of eggs of trout was found regulated by temperature, slightly alkaline pH and high dissolved oxygen. In present study, water temperature ranged from 10.4-11.7⁰C, dissolved oxygen 7.21 – 7.37 mg/l and pH from 7.27-8.0 respectively (Table 1). The mentioned water quality parameters was recorded suitable for larval development of fishes. Rainbow trout culture requires a permanent supply of water with a temperature range of 10 to 20°C, and optimum temperature of 15-18°C (Yamazaki, 1991). The water should be clear, not turbid. A pH value of 6.5 - 8.0 and dissolved oxygen above 8 mg/L are considered suitable for trout culture (Huet, 1975). Water supply of at least 5 L/sec is necessary to produce a ton of trout, although less may be sufficient, when temperature decreases (Pillay, 1993). According to Billard (1985), optimal temperatures for spawning of salmonids lie between 6 and 13⁰ C. Maintaining temperatures of 13⁰C or higher is reported to inhibit ovulation and to result in lower egg survival in Atlantic salmon (Taranger & Hansen 1993).

Besides water temperature, Rainbow trout (*Oncorhynchus mykiss*) are relatively sensitive to hypoxia exposure (Gesser 1977). Previous studies had demonstrated that rainbow trout embryo survival was dramatically influenced by water O₂ levels. Low DO concentrations have been shown to affect the rate of development of chum salmon (*Oncorhynchus keta*; Alderdice et al. 1958), lake trout (*Salvelinus namaycush*; Garside 1959), and Chinook salmon (*Oncorhynchus tshawytscha*; Silver et al. 1963) embryos. The eggs of rainbow trout will not incubate or develop normally if constant water temperatures above 56 degrees F were encountered or below 42 degrees F.

The alevins weighed about 40-50 mg and measured 1.3-1.8 cm in length. 50-60% of body weight was yolk sac. Size of alevins was also recorded to vary slightly in other fishes

also. The size of alevins of *Hilsa* also reported to vary from 2.3-3.1 mm in diameter. Large size of alevins of rainbow trout was due to large egg sized. The yolk sac of the pro-larvae were recorded to absorb within 7-18 days and started to swim slowly on water surface. Slow growth of embryonic and post hatched stage could be attributed to very low water temperature required for the development and rearing of trout. The feeding started after 15-20% of larvae appeared swimming on water surface. The average weight of first swimming fry was 0.07- 0.1 gm and they were fed Starter Feed containing 45-50% protein at the rate of 5-10% of body weight at the interval of every 3/4hours.

CHAPTER SEVEN

7.0 Conclusion and Recommendation

The farming of rainbow trout has opened up new area of highly productive high valued food commodity and it has high demand fetching good price in local and international market. Commercialization of products need a systematic process to enhance, support, establish a value chain from production level to market channeling for sustained livelihood and business of people associated. Major features required for trout commercial farming are – abundant coldwater, year round supply of trout seed, availability of dry pelleted feed, loan facility, concrete raceways, market, preservation and quality assurance. The major bottleneck in the trout industry is the lack of smooth supply of quality seeds to fulfill the overall demand of fish grower. The high rate of mortality of hatchlings after yolk sac absorption is also due to the lack of proper knowledge about the suitable conditions required during it. To expand trout industry in future, the area of seed development requires special attention. The fish growers must therefore pay due attention in hatchery development providing suitable conditions for embryonic and larval development.

CHAPTER EIGHT

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