Effect of Water Hardness on Egg Hatchability and Larval Viability of *Labeo rajasthanicus*

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VIJAY KUMAR

Thesis

Master of Fisheries Science

(Aquaculture)

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DEPARTMENT OF AQUACULTURE
COLLEGE OF FISHERIES
MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY,UDAIPUR – 313001 (RAJASTHAN)

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MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

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This is to certify that this thesis entitled**"Effect of water hardness on egg hatchability and larval viability of *Labeo rajasthanicus*"**submitted for the degree of **Master of Fisheries Science** in the subject of **Aquaculture**, embodies bonafide research work carried out by **Vijay Kumar** under my guidance and supervision and that no part of this thesis has been submitted for any degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on 28/06/2018.

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Date: (Vijay Kumar)
Place: Udaipur

DEDICATION

This thesis is dedicated to myFamily and Teachers

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Chapter: 1

INTRODUCTION

Aquaculture is one of the fastest growing food production sectors worldwide. Ithas become vital for nutritional security, rural employment and livelihood option for country's growing population. Aquaculture is practiced by both some of the poorest farmers in developing countries and by multinational companies in developed countries. Worldwide billions of people directly depend on fish as their primary source of animal protein. In terms of health benefits, it has an excellent nutritional profile. It is a good source of protein, fatty acids, vitamins, minerals and essential micronutrients.Fisheries are one of the most important sources of revenue to economy of a country and as an important food sector in human nutrition (Dwivedi*et al*., 2009).

Worldwide per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (FAO, 2016). Population growth, urbanization and rising income would lead to increase in the world fish consumption manifold.Inland finfish aquaculture is the most common type of aquaculture operation in India as well as world. In aquaculture carps such as Catla, Rohu, Mrigal, Grass carp, Silver carp and Common carp are the widely cultured species contribute to the majority of the national carp production. In India, three tiered carp culture system is practiced namely, nursery, rearing and grow-out production systems (Nandeesha*et al.* 2013). Indian government has established Fish Farmers Development Agencies (FFDA) in almost all districts of every state and provided special support to promote carp farming. As a result of government support, carp culture at commercial level is practiced in most of all states of India.

The supply of quality seed is the prime requirement of aquaculture production. Quantity of carp seed production has gone up to 49.5 billion fry in 2015-16. Fish seed accounts for approximately 27-30% of production costs and the stocking of poor quality seed results in poor growth, low production and less profit for farmers(Anon,2017).In freshwater aquaculture, theimproved varieties of rohu (Jayanti Rohu) has increased production considerably and similar efforts in the case of other species are also required.

Fish larvae are the smallest self-supporting vertebrates and in order to increase their chances of survival, they need to complete their morpho-functional system so as to escape predation and to obtain food (Osse*et al.,* 1997). The transition from endogenous to exogenous feeding is one of the most critical stage for a high rearing success on a commercial scale (Gulbrandsen, 1993;Jahnichen and Kohlmann, 1999). Success of larval rearing depends mainly on the availability of suitable diets that are readily consumed, efficiently digested and provide the required nutrients to support good growth and health (Giri*et al.,* 2002).

The production of marketable fish beginswith stocking of fry or juveniles in to a rearingenvironment that assured optimum and rapid growthto allow harvest in shortest possible time. The fishfarmer has to obtain adequate number of young fishto meet his production goal (Bisht*et al.,* 2013).Webber and Riordan (1976) state that availability of fry and fingerlings in the development of aquaculture are one of the main obstacles. Growth and survival of fry and fingerlings in nursery ponds depend on stocking density, type and quantity of fertilizers and supplementary feeds. It would be necessary to stock the ponds at appropriate stocking densities for optimum growth and survival of fingerlings to obtain maximum economic returns.

Temperature, dissolved oxygen, and pH are the more frequently investigated factors affecting the fertilization and hatching success of fish species. Water hardness is important to fish culture and is a commonly reported aspect of water quality. Fish can absorb calcium needed directly from the water or food. Total hardness is the concentration of all divalent cations in water, and Ca^{2+} and Mg^{2+} are the most common cations in almost all freshwater systems. Calcium carbonate hardness is a general term that indicates the total quantity of divalent salts present and does not specifically identify whether calcium, magnesium and some other divalent salt is causing water hardness(Wurts*et al.,* 1992). Calcium and magnesium are essential in the biological processes of fish bone and scale formation, blood clotting and other metabolic reactions (William *et al.,*1992). Kane *et al.*(1990) stated that hardness is a contributor of necessary ions for basic physiological functions during larval development of fishes. It has been reported that salt uptake by fish is affected by external concentration of calcium and magnesium(Fleming *et al.*, 1974). The suggested value for water hardness for fish cultivation in ponds is above 20 mg/l $CaCO_3$ (Kasiri*et al.,* 2011).

The hardness of the water is a serious problem in the fish production, which varies considerably from place to place. In general, surface water is softening than ground water. Excess amount of calcium in water is not a healthy sign. Its presence beyond certain limits not only creates problems for human life and industry but also to fish as well. Its limiting effects in terms on hatching of eggs and survival greatly vary with species, age, physiological state of fish and environmental conditions. The effect of water hardness on survival of fish eggs and preference for water hardness varies in oviparous and ovo-viviparous fishes (Gonzal*et al.,* 1987 and Ketola*et al.,* 1988).The swelling of newly fertilized eggs, with water hardness, has been exhibited to have a direct effect on this stage show egg swelling increases when water hardness decreases because low water hardness usually means low osmolarity and its swelling decrease when water hardness increases (Kasiri*et al.,* 2011). Incubation of eggs in calcium deficient water, which contributes significantly to the total hardness of natural water (Boyd, 1979), may result in poor hatchability and fry survival (Brown and Lynam, 1981). It is therefore necessary to control the ionic concentration of the medium to minimize premature bursting.

Agricultural limestone can be used to increase calcium concentrations in areas with acid waters or soils. Agricultural gypsum or food grade calcium chloride could be used to raise calcium levels in soft, alkaline waters. Expense may be prohibitive when large volumes of water need treatment. At a pH of 8.3 or greater, calcium will come out of solution as an insoluble carbonate (limestone). Likewise, agricultural lime will be insoluble in waters with that pH range.

Labeo rajasthanicus, locally known as ‘Sarsi’is an important food fish and considered as one of the most important indigenous minor carp species inSouthern Rajasthan. Among the minor carps, it is a very important alternate species for diversification in freshwater aquaculture in Rajasthanas well as in India and it has high market value in some region of India. This species has potential for inclusion in composite culture (Lal *et al.,* 2015). It has been reported from the two isolated rivers, Tidi and Chambal and also from Jaisamand Lake. Occurrence of Sarsiwas recorded from rocky substrates with shelter and higher depth (5-20 m), having low water velocity (Lal *et al.,* 2015).

In aquaculture lots of research has been conducted on effect of different water quality parameter on egg hatchability and larval viability in Indian major carps. Limited

research work has been reported on the effect of optimum level of water hardness for fish egg hatching and larval viabilityin minor carps& especially in *Labeo rajasthanicus.* In view of this, the present study was designed and conducted to investigate the effect of water hardness on breeding indices such as fertilization rate, hatching rate & larval survival of *Labeo rajasthanicus*. This study was basically conducted to achieve thefollowing two objectives.

To work-out the optimum level of water hardness for fish egg hatching.

To assess the effect of water hardness on fish larval viability.

Chapter: 2

REVIEW OF LITERATURE

The review of literature on the work done in the past is essential to understand the problem in depth, which provides necessary guidelines as well as feedback for the fulfillment of objectives in the study. Account of related work done on the water quality for fish culture and with special reference of seed production and fish growth is as follows.

Total hardness is the sum of the concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) in water, expressed as mg/l equivalent of $CaCO_3$. Other divalent cations contribute to hardness, but their concentration in natural waters is usually low. Total hardness is an aggregate property and can be precisely interpreted in relation to aquatic animal health and culture system management until and unless the concentrations of substances contributing to total hardness are known. In general calcium is of greater importance than magnesium in the management of water quality for aquaculture.

In carp breeding (Silver & Bighead carp), a premature hatching and poor survival of hatched larvae in soft water was reported byChung *et al.,* (1980).Brown and Lynam (1981) reported that a concentration of 1 ppm sodium and calcium is sufficient to

ensure the successful hatching of eyed ova and subsequent survival of the alevin. At pH 4.5 hatching were prolonged by the alevin passing through a temporary encapsulated stage.

The hardness also contributes to pH stability and stimulates phytoplankton and zooplankton blooms in ponds (Piper *et al.*, 1982). Furthermore, water with low hardness and correspondingly low alkalinity has a poor capacity to buffer against acidification, while water with moderate hardness tends to decrease the susceptibility of fish to toxicants (Sprague, 1985).

Grizzle *et al.*, (1985) reported that the addition of calcium chloride to pond 5 days before harvest to raise the calcium concentration from 8 to over 20 mg/l Ca^{2+} increased post harvest survival of striped bass and sunshine bass juveniles from 16% to 80%, even when the water used to hold fish after harvest was low in calcium (10 mg/lCa^{2+}). Further, increasein calcium concentration in holding tank water from 10 mg/l Ca^{2+} to over 100 mg/l Ca^{2+}has increased survival to nearly 100%.

Pascoe *et al.*, (1986*)* observed that the toxicity test with rainbow trout confirm that cadmium is less toxic in hard water (96 hr LC50=2.6 mg Cdl-1) than in soft water (96 hr LC50= 1.3 mg Cdl-1). Water quality studies indicate that this was not due to a chemical reduction of available cadmium in hard water and no significant differences in cadmium uptake were detected between fish from the two levels of hardness.

Angelito*et al.*, (1987) observedwater absorption at 100 - 200 mg/l $CaCO_3$ caused eggs to burst prematurely and minimal water absorption occurred at 600 mg/l $CaCO_3$. Chloride concentration at 0 and 6 h post-fertilization was significantly related to egg hatchability. Therefore, the higher water hardness is best for hatching of Silver carp egg. Further, they recommended 300 - 500 mg/l $CaCO_3$ for the successful hatching of Silver carp eggs.

Ketola*et al.*, (1988) have suggested that relatively small quantities of suitable hardened water were required to markedly improve hatchability of Atlantic salmon (*Salmo solar*), rainbow trout (*Onchorhynchusmykiss*) and brook trout (*Salvelinusfontalis*). This effect might be specific for Ca^{2+}, or due to bivalency, a characteristic that Ca^{2+} ions share with other elements, such as Mg^{2+}.

Gonzal*et al.*, (1987) reported that the effect of water hardness on survival of fish eggs and preference for water hardness varies in oviparous and ovo-viviparous fishes.Wurtz*et al.*,(1994) have reported that calcium hardness could affect channel

catfish tolerance to copper toxicity in low alkalinity environments and also observed that alkalinity concentration had the most pronounced effect on acute copper toxicity to juvenile channel catfish when calcium hardness and alkalinity concentrations were treated as independent variables.

Wurtz*etal*., (1988) have reported thatthe red drum stocked in freshwater with calcium concentrations 1.7 mg/1 or less performed poorly 0-33% survival after 96 h. The growth and survival were not significantly affected when calcium was between 9 and 407 mg/1. Further, it has been suggested that the water should contain a minimum of 25 mg/l Ca^{2+} and levels of 50-100 mg/l or more are desirable for best survival, growth, and feed conversion efficiency of red drum (Pursley and Wolter 1994).

William *et al.,* (1992) reported that Channel catfish can tolerate low calcium concentrations as long as their feed contains a minimum level of mineral calcium but may grow slowly under these conditions. Similarly, rainbow trout can tolerate waters with free calcium concentrations as low as 10 mg/l if pH is above 6.5.

Tucker *et al.*, (1993) suggested that survival, development, and stress resistance of channel catfish yolk-sac fry were adversely affected at calcium concentrations below 5 mg/l.

Whitaker *et al.*, (1993) reported that at fertilization, egg activation and the initiation of development were always triggered by an increase in intracellular Ca^{2+} concentration within the egg and in fertilization of certain species such as sea urchin, frog and some fish eggs, a single transient of Ca^{2+} is triggered during egg activation. In other animals however, including mammals, ascidians and nemertean worms, a distinctive series of intracellular Ca^{2+} oscillations are observed (Miyazaki *et al.*, 1993 andStricker, 1999).

Laitinen*et al.*, (1994) suggested that the information on the fish physiology in relation to environments with high alkalinity and hardness or manipulation of Mg^{2+} on the embryonic development of fish is scarce (Wilkie and Wood, 1994).

Wurtz*et al.*, (1994) suggested that the mortalities decreased as calcium concentrations increased when bicarbonate alkalinity was held constant at 75 mg/l $CaCO_3$ and calcium hardness varied from 20 to 250 mg/l $CaCO_3$, Copper related catfish mortalities displayed high variability and means ranged from 50 to 60%.

Seals *et al.,* (1994) showed that the environmental calcium concentration over the range 5-80 mg/l Ca^{2+} did not affect growth, feed conversion, condition factor, or

blood chemistry of sunshine bass when the fish were raised in anapparently non-stressful environment.

Tilapia larvae showed similar hatching rates and wet weights in either high or low calcium medium, indicating that neither the growth or larval survival were affected by environmental calcium (Hawang*et al.,* 1996).

Bijvelds*et al.,* (1996) suggested that the freshwater teleosts primarily depend on dietary rather than waterborne Mg^{2+}, with the gills being a secondary route for absorption.

Bijvelds*et al.,* (1998) reported that the function of waterborne Mg^{2+} has not been well established, but Ca^{2+} and Mg^{2+} are important for ionic regulation of freshwater fish because both ions influence the permeability of biological membranes, preventing diffusive flow out of tissues and high ion loss to water.

Perschbacher*et al.,* (1998) have suggested thatthe copper sulfate is routinely used as an algaecides in commercial and recreational fish ponds. It has also been used as an effective treatment for pathogenic protozoan parasites of fish. It is generally recognized that copper can be highly toxic to teleosts. However, several studies have reported that either calcium hardness or alkalinity concentrations have significant effects on copper toxicity. Therefore, recommendations for safe use of copper sulfate have been based on hardness (Sawyer *et al.,* 1989) and total alkalinity concentrations of water. The calcium hardness increased copper induced catfish mortalities decreased significantly from 90% at 10 mg/l $CaCO_3$ to 5% at 400 mg/l $CaCO_3$.

Molokwu*et al.,* (2002) reported that total hardness also interferes the incubation period in *Clariasbatrachus* eggs. The incubation time increased from 19 hr at total hardness of 10 mg/l to 23 hr at total hardness of 200-700 mg/l. The mean hatching rate fluctuated between 42.31% at hardness of 10 mg/l and 64.66% at 200 mg/l.Survival of larvae of *Clariasgeripienus* eggs was zero in 10, 500 and 700 mg/l and highest survival was reported at 60 mg/l water hardness.Kane *et al.,* (1990) stated that the hardness is a contributor of necessary ions for basic physiological functions during striped bass larval development.

Coward *et al., (*2002) studied a wide variety of animal and plant species and demonstrated that development at fertilization is triggered by an increase in intracellular Ca^{2+} concentration within the egg that occurs as either a single transient or a series of distinctive oscillations depending upon the species under investigation.

This increase in intracellular Ca^{2+} activates the egg and also appears to play an important role in later embryonic development.

Silva *et al.,* (2003) suggested that the increase in water hardness upto 70 mg/l $CaCO_3$ using either Ca^{2+} or Mg^{2+} improve hatching rate, but the increase of waterborne Ca^{2+} above 20 mg/l, irrespective of water hardness, is not indicated for incubation of silver catfish (*Rhamdiaquelen*) eggs because it reduces post hatching survival.Townsend *et al.,* (2003)have reported that the response to increased water hardness varies from species to species. When larvae of silver catfish, *Rhamdiaquelen*were treated with different levels of water hardness, higher larval growth, survival, and biomass were obtained at 30 and 70 mg/l $CaCO_3$.

Adhikari (2003) has studied the effect of calcium hardness on *L. rohita*survival. The mortality rate significantly decreased with increased calcium hardness. A100% mortality was observed in magnesium-based hardness treatments. As calcium hardness increased, copper-induced catfish mortalities decreased significantly from 90% at 50 mg/l $CaCO_3$ to 4% at 400 mg/l $CaCO_3$. The rate of survival at 50mg/l $CaCO_3$was only 7% which was significantly higher (90%) at 350 mg/l $CaCO_3$.

Mateenet*et al.,* (2004) havereportedhighly significant difference in growth at different hardness regimes. However, therohu showed maximum growth at 300 mg/l, it is interesting to note that its hybrid performed best at 450 mg/l, hardness level. A non-significant difference in terms of total gain in body weight and gain/unit of body weight of both species in different treatments was observed. Growth in terms of body lengths (total & fork) was also highly significantly, while it was also non-significantly different interms of total gain and gain/unit at different hardness levels.

James *et al.,* (2004) reported that when water hardness of culture media is increased (76, 316, 540 and 1018 mg/l $CaCO_3$), *Xiphophorushelleri*exhibited maximum growth parameter and reproductive performance was highest inwater hardness of 1018 mg/l $CaCO_3$. On the contrarily *Bettasplendens*elicited better growth, feeding parameters and fecundity in hardness of 316 mg/l $CaCO_3$.

Chanu*et al.,*(2010) have noticed that the larval survival rate was 12.62% in 5 mg/l hardness.Further, an increasing trend with increase in water hardness level up to 63 mg/l $CaCO_3$ was observed. If calcium and magnesium are not acquired adequately, fish shows nutritional diseases and poor growth (Lall, 1979). Incubation of eggs in

calcium deficient water, which contributes significantly to the total hardness of natural water (Boyd, 1979), may result in poor hatchability and fry survival.

Senfuma*et al.,* (2011)showed that both temperature and salinity accelerates egg incubation and hatching period of *C. gariepinus*eggs and the optimum temperature-salinity combination for egg incubation and hatching period was 31°C and 3ppt. Egg hatchability was higher in salty water than in freshwater and higher at high temperature than at low temperature.The best temperature-salinity combination for egg hatchability was 28°C and 3ppt. Such temperature-salinity combination is recommended for the improvement of catfish seed production which is key problem in catfish industry.

Bart *et al.,* (2012)have reported a positive effects of increased water hardness level (>132 mg/l) on yolk sac larvae and swim-up fry survival. The study also showed that seawater salinity of 4 g/l was the most appropriate salinity level for incubating Nile tilapia eggs.Ofor*et al.,* (2012) have observed that the hatching of all eggs was lowest in media of 300 mg/l^{-1}as $CaCO_3$ total hardness. *Clariasgariepinus*eggs showed a higher tolerance for hardness than *H. longifilis*eggs, which showed preference for soft water.

Valeta*et al.,* (2013) have noticedthe shortest hatching period of 7.3 days, for Tilapia at 29°C,while the longest hatching period was 14.7 days, at 25°C. Further the hatchability and fry survival were higher at higher temperatures.

Chatakondi*et al.,* (2014) have observed higher hatching success of hybrid catfish eggs at calcium hardness $CaCO_3$ of 75 mg/l in hatching waters than calcium hardness of 25 or 50 mg/l. However, further increasesin calcium concentration did not improve hatching success. A minimumwater hardness of $CaCO_3$ at 75 mg/l was recommended forincubating hybrid catfish eggs.

Rach*et al.,* (2014) have studied the effect of water hardness on egg swelling, hatching and larval survivability. They noticed higher swelling and hatching when the eggs were placed in 50ml/l water hardness for 24 hrs. Further, they suggested that, unlike the effect of water hardness during egg hardening, the water hardness during incubation appeared to have no effect on egg hatching success.

Chapter: 3

MATERIAL AND METHOD

As per the approved technical programme, the experiment on the evaluation of suitable water hardness for egg hatching of *Labeo rajasthanicus* was conducted at fish farm of Directorate of Research MPUAT Udaipur. The details of experimental procedure are described below.

Experiment Location

The experiments were carried out at Aquaculture Research & Seed Unit, Directorate of Research, MPUAT, Udaipur. It is situated in the main city of Udaipur (Rajasthan). (24.57 N longitude & 73.70 E latitude)

Experimental Fish

A minor carp, *Labeorajasthanicus*(Plate 3.1)was selected for the present study to evaluate the effect of water hardness on egg hatchability and larval viability. Taxonomical classification of this species is an under:

Kingdom	**Animalia**
Phylum	**Chordata**
Super class	**Osteichthyes**
Class	**Actinopterygii**
Order	**Cypriniformes**
Family	**Cyprinidae**
Genus	***Labeo***
Species	***rajasthanicus***

Plate 3.1: Experimental fish *Labeorajasthanicus*

This species has been identified from southern Rajasthan under NAIP (2014) project and published in Indian Journal of Fisheries (Lal*et al.*, 2015).

Description of *Labeorajasthanicus* characteristics

Theexperimentalfish,*Labeorajasthanicus*has 58 – 64 lateral line scale without tinged color margins; predorsal scale 18-20; ventral fin rays 09; dorsal fin ray 14-15; anal fin ray 6-7. Difference in cytochrome oxidase subunit I sequences is also an indication of species level separation of *L. rajasthanicus*with other species of the gonius group of *Labeo*genus. Both genetic and morphological pieces of evidence support its distinction as a separate species from *L. dussumieri.* It has elongated body and dorsal profile slightly convex than ventral. Small head and snout with few tubercles and slightly projecting beyond the mouth. Eye medium size, not visible from underside of the head. Mouth narrow and sub-inferior, lips thick. Dorsal fin inserted nearer to the snout tip than the base of caudal fin. Pelvic fin inserted below the middle of dorsal and does not reach to the anal fin. Anal fin is short and not reaching to the caudal fin. Caudal fin deeply forked with pointed lobes. Two pairs of barbells, both are same in length. Genital opening situated distant to anal fin origin and the maximum size recorded was 37.0 cm. (Lal et al, 2015).

Collection of experimental fish and brood-stock management

Active and disease free farm raised fish were collected from farm pond of Research Farm, MPUAT, Udaipur. Fishweretreated with 5 % $KMnO_4$ to disinfect and kept in FRP tanks (2mx 1mx 1m) for conditioning. These fish were fed with commercially formulated feed at 2-3 % of their bodyweight. Feeding was done twice in a day. First feeding was done in the morning time and second in the evening time.

Brood fish selection

Fully matured male and female fish were selected for breeding purpose. The potential brood stock was selected on the basis of secondary sexual characteristics. Mature fish showed sexual dimorphism; pectoral fin of the male become rough, pointed and narrow genital papilla with freely oozing milt when slight pressure is applied on to the abdomen. Genital papilla was swollen and slightly pinkish in color with the smooth pectoral fin. Abdomen of the female was bulgy.

Breeding

For the breeding experiment free oozing male and ripe female were selected in the ratio of 1:1 respectively and kept 4-6 hrs separately in nylon hapa under showering for conditioning. Induced breeding was done in nylon hapa fixed in cemented cistern pond and following prescribed techniques of hypophysation (Rath*et al*, 2011). The Gonopro - FH was used as inducing agent. Inducing hormoneGonopro - FH was used @ 0.2 and 0.3 ml/kg body weight to male and female respectively (Paliwal*et al.,* 2016). After 8 hrs of injection, dry stripping method was used to obtain ova &sperm, and water having different hardness was used for experimental fertilization for different hardness [200(T1),175(T2), 150(T3),125(T4),100(T5)mg/l] was maintained by mixing soft & hard water.

Incubation of eggs

The eggs were incubated in five different hardness levels[200(T1), 175(T2), 150(T3), 125(T4) and 100(T5) mg/l). For this purpose a simple hatching device was fabricatedusing 2000 ml plastic bottles (Fig. 3.2). This device was designed following flow through principle of carp hatchery. In each hatchery jar 5000 eggs ware incubated & each treatment was run in triplicate. The following breeding indices were recorded following standard methods (Rath*et al.*,2011).

- Fertilization Rate
- Egg diameter
- Hatching time
- Hatching Rate
- Hatchling survival

Plate 3.2 Simple hatching device fabricated for egg incubation

Fertilization Rate

By counting the number of fertilized eggs from the total number in given sample, (The unfertilized eggs were translucent whereas the fertilized eggs were transparent). The fertilization per cent wascalculated as below.

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no.of eggs in the sample}} \times 100$$

Egg diameter

The diameter of egg was measured using Leica microscope with computer based measuring facility.

Hatching time

The time taken for complete hatching was observed. For this time of fertilization and hatching were observed manually & total time duration was recorded in hrs.

Hatching per cent

The hatching percentage was calculated using following formula.

$$\text{Hatching \%} = \frac{\text{No. of hatched hatchlings}}{\text{no. of fertilised eggs}} \times 100$$

Hatchling Survival

$$\text{Hatchling survival(\%)} = \frac{\text{No. of individuals harvested}}{\text{total no. of larve}} \times 100$$

Physico-chemical Water Quality Parameters

Following standard methods of APHA (2005)selected water quality parameters such as Temperature, Dissolved Oxygen, Alkalinity, TDS, Electric conductivity, Salinity etc.were measured. The detailed methodology is described below.

Temperature: The water temperature of all the incubation bottleswas recorded using HACH water quality monitoring system (HACH Si 1).

pH: The pH was measured by a HACH water quality monitoring system (HACH Si 1)for all the experimental units.

Dissolved oxygen: The dissolved oxygen was measured using HACH water quality monitoring system (HACH 10).

Total hardness: Total hardness was estimated by titrimetric method using standard EDTA solution as titrant and Ereochrome black-T as indicator, and the values were expressed as mg $CaCO_3$/l (APHA, 2005).

Total alkalinity (TA): The total Alkalinity was estimated by titration of water samples with 0.02 N H_2SO_4 in presence of indicators (phenolphthalein and methyl orange) and values were expressed as mg $CaCO_3$/l.

Electric conductivity: Electric conductivity was recorded using HACH EC meter and concentration was expressed inmMoh/cm.

Total dissolved solid: Total dissolved solid was measured using HACH TDS meterand concentration was expressed in mg/l.

STATISTICAL ANALYSIS

Statistical analysis of the data obtained during this study was performed following one-way ANOVA (Steel *et al*, 1996). A significant difference among means was also determined by Duncan's multiple range tests. All data presented in the text, figures and tables are means ± standard error and statistical significance for all statistical tests were set at $p<0.05$. Further, second order polynomial regression analysis was also performed

between hardness levels and breeding parameters such as fertilization rate, hatching rate, ova diameter, hatching time & larval survival.

Chapter: 4

RESULTS

The present study was conducted to evaluate the effect of water hardness on *Labeo rajasthanicus*breeding indices. As such the results pertaining to water quality, rate of fertilization, ova diameter, hatching duration, hatching percent, etc are presented in Tables 4.1 to 4.11 and Figures 4.1 to 4.10.

The overall water quality status of different treatments is presented in Table 4.1.From this table it is evident that water quality parameters such as TDS, conductivity, salinity, hardness and alkalinity were significantly different among treatments.However, temperature and dissolved oxygen remained more or less same in different treatments.

The water temperature ranged between 27.5 to 28 °C. The lowest (27.6 °C) mean water temperature was recorded in T3, T4 and T5, while, the highest (27.67 °C) being in both T1 and T2. The mean concentration of dissolved oxygen in different treatmentsranged between 5.76 to 6.32 mg/l with lowest in T4 and highest in T5.In general, the values of dissolved oxygen remained always above 5 mg/l, which is congenial level for carp hatcheries.

In all the experimental unit/treatments the water remained alkaline with mean pH values of 8.09 to 8.31. The highest mean pH value (8.3) was in T5 followed by T3 (8.3), T1 (8.29) and T2 (8.22). The lowest mean value of pH was recorded in T4. In experimental treatments, total alkalinity varied from 128 to 230 mg/l with highest in T1 and lowest in T5.Similarly the lowest (140 mg/l) and highest (220 mg/l) mean value of alkalinity was noticed in T5 and T1 respectively.

The amount of total dissolved solids in different treatments rangedfrom 270 to 511 mg/l. Whereas, the mean values were between 271 to 509 mg/l, Both TDS and conductivity had positive relationship with treatments as these parameters showed an increasing trend with increasing hardness levels in different treatments (Table 4.1).

Breeding indices

To work out the optimum level of water hardness for carp hatcheries, the selected breeding indices such as fertilization rate, ova diameter, hatching duration & hatching percent and hatchling survival in different hardness levelswereobserved. From the result of these parameters (Tables4.2 to 4.11 and Figs4.1 to 4.10) it is evident that water hardness has significant impact in carp egg incubationas both lower and higher hardness levels in hatchery water are not desirable.

Fertilization rate

The per cent fertilization rate in different treatments ranged between 65.71 to 79.46 % (Table 4.2). The highest fertilization rate was recorded in T5 (79.46 %) followed by T4 (75.1 %), T3 (71.43 %) and T2 (70 %).The lowest fertilization rate (65.71 %) was recorded in T1. From Table 4.2 it is clear that fertilization rate significantly reduces with increasing water hardness levels. The data obtained for fertilization rate for different treatments were statistically analyzed using second order polynomial regression equation. From the results of this equation (Figure 4.2 and Table 4.3) it was noticed that a hardness level of 137 mg/l is optimum for better fertilization rate.

Ova diameters

The hardness of experimental water and ova diameter were found negatively correlated. As the size of ova diameter significantly reduced with increasing hardness levels, the smallest (3.33 mm) ova diameter was observed in T1. The biggest (3.54 mm) ova diameter was in lowest water hardness level (T5). The ova diameter was statistically significant among treatments.Further, the second order polynomial regression analysis suggested that the hardness between 125 to 150 mg/l is favorable for egg hardening.

Hatching duration

The water hardness has significantly impacted the hatching duration. The minimum time (15.45 hrs) was noticed in lowest hardness treatments (T5). The hatchingduration significantly increased with increasing hardness level and the highest hatching time was recorded in highest level of hardness. When hatching duration in relation to hardness was analyzed (Figure 4.5) through second order polynomial regression analysis (Table 4.6), it was noticed that a maximum hardness of 150 mg/l is favorable for optimum hatching duration.

Hatching percentage

From Table 4.8 and Figure 4.7 it would be seen that maximum hatching rate of 95 % was in T5 followed by T4 (91.33), T3 (82) and T2 (69.66 %). The lowest hatching rate of 69.33 % was recorded in T1. The hatching percent in different treatments was statistically significant except in T1 and T2. Further, to work out the optimum dose of water hardness in relation to hatching percent, second order polynomial regression analysis was performed between hatching rate and hardness (Table 4.8 andFigure 3.7). It is clear from the regression graph that hardness range between 100 to 125 mg/l is better for higher hatching rate.

Hatchling survival

The larval survival in different hardness treatmentsvaried between 65.71 to 79.46 %. The lowest was being in T1 and highest in T5. The larval survival significantly reducedwith increasing hardness level and among the treatments survival rate was significant except T3 and T4 (Table 4.10). The second order polynomial regression analysis of larval survival and hardness suggested that the water hardness maximum upto 125 mg/l is favorable for better survival of *L. rajasthanicus* larvae.

In general, the results of this study indicated that the water hardness has significantly impacted in hatchery waters. All the parameters (breeding parameters) indicated that the water hardness of 125 mg/l and less than 125 mg/l is better for fertilization, ova diameter, hatching duration and hatching rate. The statistical analysis and particularly second order polynomial regression equation indicated that hardness around 125 mg/l in hatchery water is most favorable for *L. rajasthanicus.*

Table 4.1: Range & mean value of selected water quality parameters in different treatments.

Parameters	Treatments				
	T1	**T2**	**T3**	**T4**	**T5**
TDS (mg/l)	507 - 511 (509.33)	444 - 445 (444.33)	387 - 388 (387.67)	351 - 354 (352.67)	270 - 272 (271)
Conductivity (mMoh/cm)	1036 - 1041 (1038)	907 - 310 (908)	792 - 795 (793.67)	721 - 726 (723.67)	570 - 572 (571)
Temperature (°C)	28 - 27.5 (27.67)	27.5 - 28 (27.67)	27.5 - 27.7 (27.6)	27.5 - 27.7 (27.6)	27.5 - 27.7 (27.6)
Salinity(ppt)	0.5 - 0.5 (0.5)	0.4 - 0.4 (0.4)	0.3 - 0.4 (0.37)	0.3 - 0.3 (0.3)	0.2 - 0.2 (0.2)
D.O. (mg/l)	5.28 - 6.7 (5.99)	5.47 - 7.05 (6.26)	5.25 - 6.85 (6.05)	5.08 - 6.45 (5.76)	5.45 - 7.2 (6.32)
pH	8.2 - 8.38 (8.29)	8.09 - 8.47 (8.22)	8.2 - 8.41 (8.3)	8.07 - 8.1 (8.09)	8.12 - 8.6 (8.31)
Hardness (mg/l)	200 - 200 (200)	175 - 175 (175)	150 - 150 (150)	125 - 125 (125)	100 - 100 (100)
Total alkalinity (mg/l)	210 - 230 (220)	170 - 212 (196.67)	152 - 208 (180)	142 - 168 (153.33)	128 - 146 (140)

Table 4.2: Fertilization rate in different levels of water hardness

S.No	Treatment	Fertilization (%)
1	T1	65.71±2.39
2	T2	70.00±3.16
3	T3	71.43±0.09

4	T4	75.71±2.07
5	T5	79.46±2.83

ANOVA *for fertilization rate*

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	339.357	4	84.839	1.968	0.176
Within Groups	431.193	10	43.119		
Total	770.55	14			

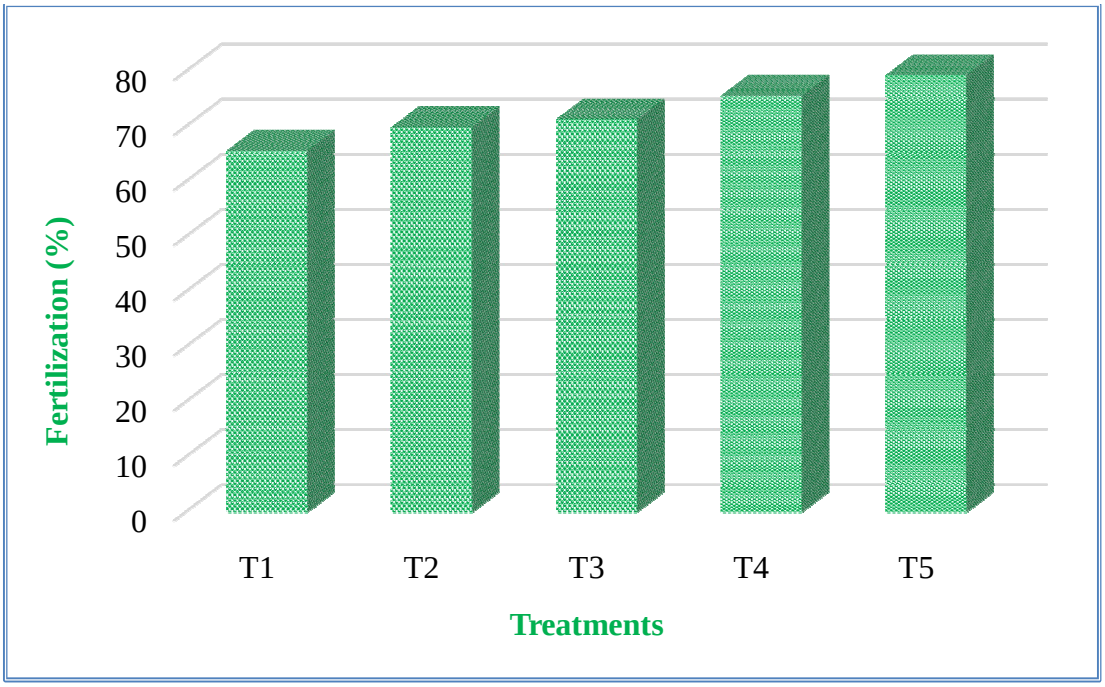

Fig 4.1: Showing mean of fertilization rate in different levels of water hardness

Table 4.3: Summary output of second order polynomial regression analysis between hardness and fertilization rate

SUMMARY OUTPUT	
Regression Statistics	
Multiple R	0.992082

R Square	0.984227					
Adjusted R Square	0.968453					
Standard Error	0.933448					
Observations	5					
ANOVA	***df***	***SS***	***MS***	***F***	***Significance F***	
Regression	2	108.7387	54.36933	62.3984	0.015773	
Residual	2	1.742651	0.871326			
Total	4	110.4813				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	97.14257	8.675117	11.19784	0.007881	59.81656	134.4686
X Variable 1	-0.20201	0.120328	-1.67879	0.235201	-0.71974	0.315726
X Variable 2	0.000234	0.000399	0.586948	0.616669	-0.00148	0.001952

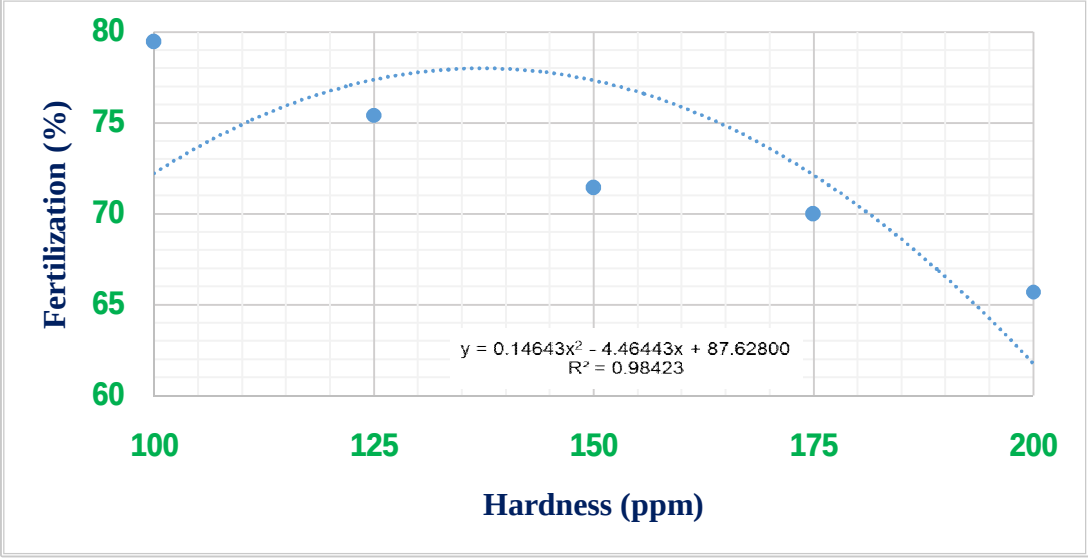

Figure 4.2: fertilization rate in different levels of water hardness

Table 4.4: Ova diameter changes in different levels of water hardness

Sr. No	**Treatment**	**Ova diameter**
1	T1	3.33±0.29

2	T2	3.35±0.53
3	T3	3.37±0.22
4	T4	3.38±0.30
5	T5	3.54±0.58

ANOVA *for ova diameter*

Source of Variance	***Sum of Squares***	***df***	***Mean Square***	***F***	***Sig.***
Between Groups	0.381	4	0.095	9.254	0.002
Within Groups	0.103	10	0.01		
Total	0.484	14			

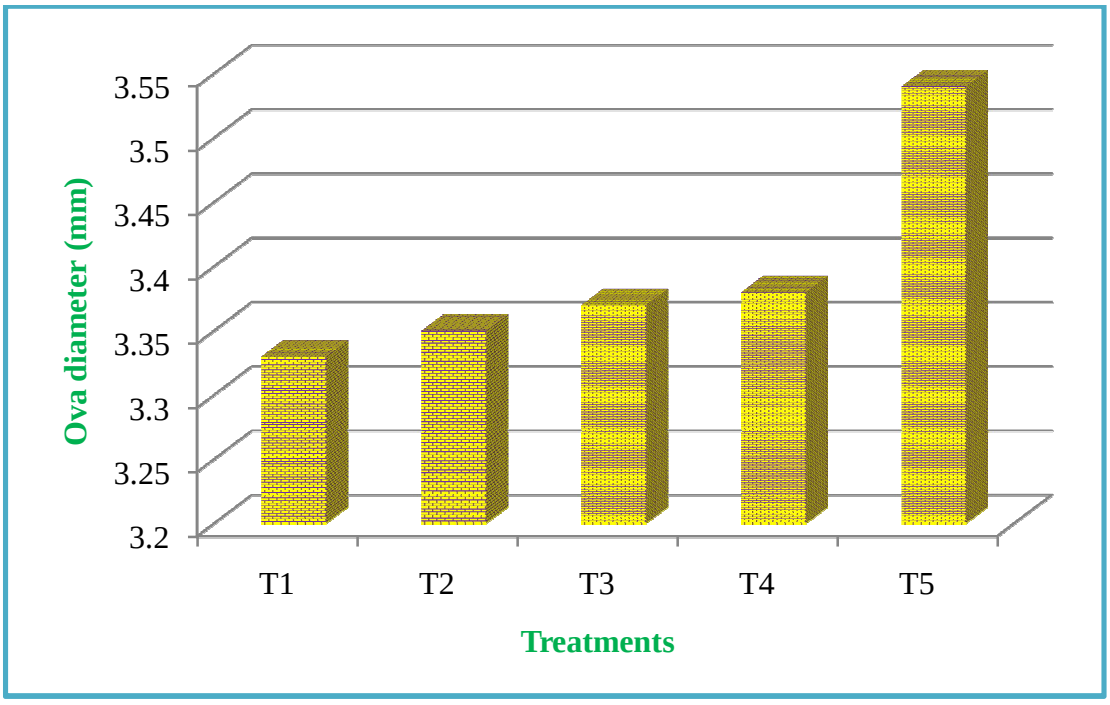

Figure 4.3: showing mean of Ova diameter changes in different levels of water hardness

Table 4.5: Summary output of second order polynomial regression analysis between hardness and ova diameter

SUMMARY OUTPUT
Regression Statistics

Multiple R	0.951475					
R Square	0.905304					
Adjusted R Square	0.810608					
Standard Error	0.036489					
Observations	5					
ANOVA	***df***	***SS***	***MS***	***F***	***Significance F***	
Regression	2	0.025457	0.012729	9.560086	0.094696	
Residual	2	0.002663	0.001331			
Total	4	0.02812				
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	4.319714	0.339113	12.73829	0.006106	2.86063	5.778798
X Variable 1	-0.01106	0.004704	-2.35075	0.143113	-0.0313	0.009181
X Variable 2	3.09E-05	1.56E-05	1.977611	0.186585	-3.6E-05	9.8E-05

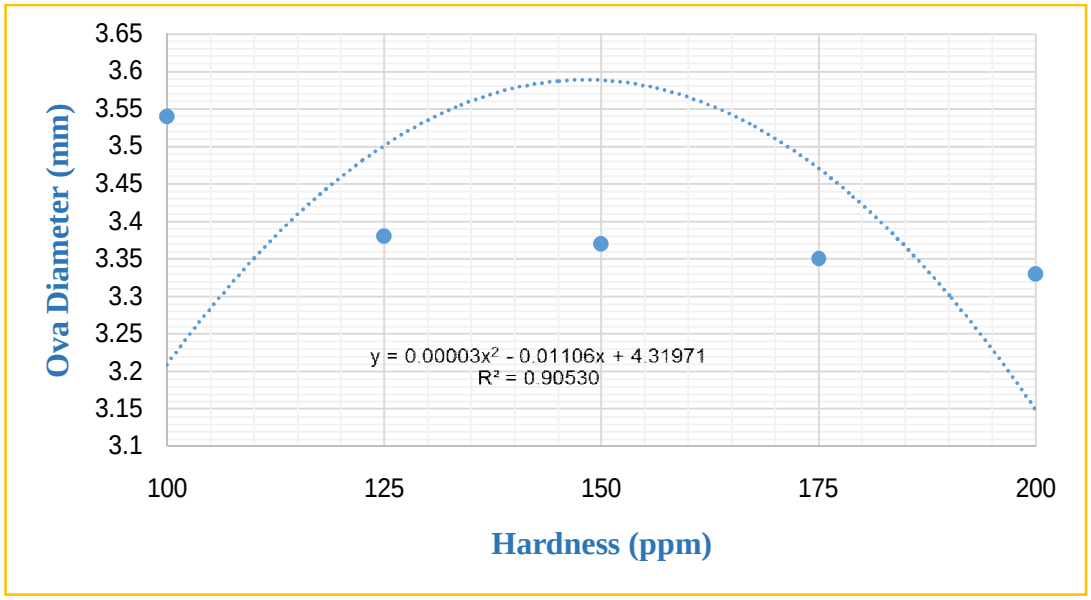

Figure 4.6: Ova diameter in different level of hardness

Table 4.6:Hatching duration in different levels of water hardness.

S.No	**Treatment**	**Duration (hrs.)**

1	T1	17.00±0.97
2	T2	16.40±1.12
3	T3	16.15±1.07
4	T4	16.05±0.77
5	T5	15.45±0.93

ANOVA *for hatching duration*

Source of Variance	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	2.884	4	0.721	0.721	0.597
Within Groups	10	10	1		
Total	12.884	14			

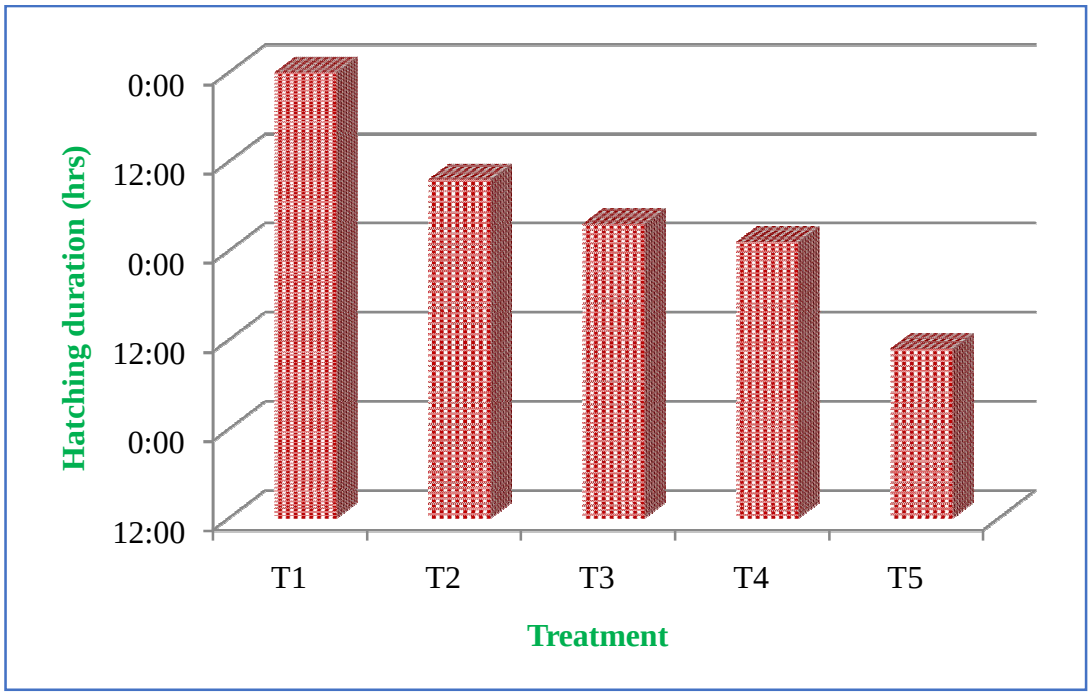

Figure 4.5: Showing mean of egg hatching time duration in different levels of water hardness

Table 4.7: Summary output of second order polynomial regression analysis between hardness and hatching time duration

SUMMARY OUTPUT

Regression Statistics						
Multiple R	0.888192					
R Square	0.788884					
Adjusted R Square	0.577769					
Standard Error	0.360896					
Observations	5					
ANOVA	df	SS	MS	F	Significance F	
Regression	2	0.973389	0.486694	3.736739	0.211116	
Residual	2	0.260491	0.130246			
Total	4	1.23388				
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	15.52257	3.354029	4.628038	0.043654	1.091351	29.95379
X Variable 1	-0.00513	0.046522	-0.11018	0.922328	-0.20529	0.195043
X Variable 2	5.83E-05	0.000154	0.37768	0.741982	-0.00061	0.000722

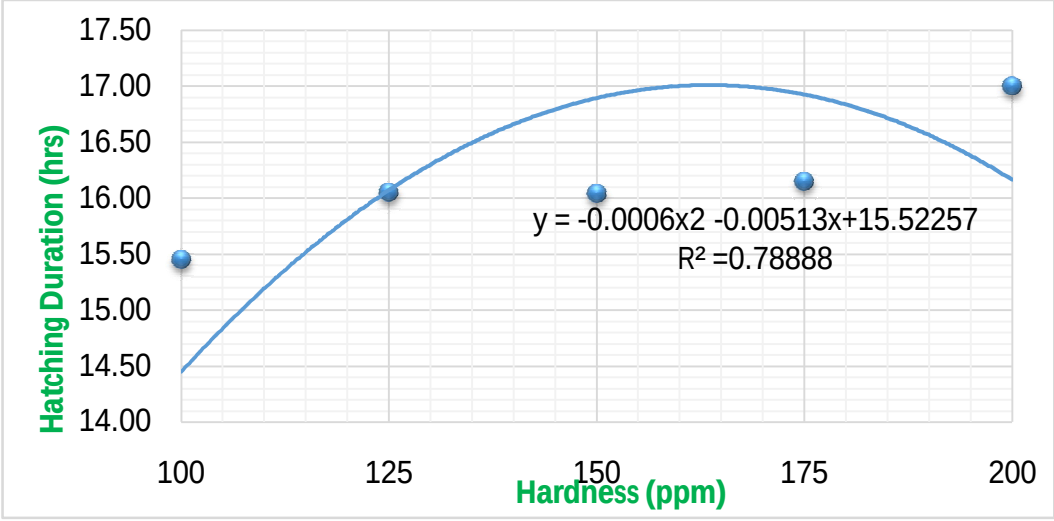

Figure 4.6: hatching time duration in different level water hardness

Table 4.8: Hatching per cent in different levels of water hardness

S.No	**Treatment**	**Hatching (%)**

1	T1	69.33±2.21
2	T2	69.66±3.02
3	T3	82±2.11
4	T4	91.33±1.98
5	T5	95±3.01

ANOVA *for hatching rate*

Source of Variance	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1702.251	4	425.563	6.931	0.006
Within Groups	614	10	61.4		
Total	2316.251	14			

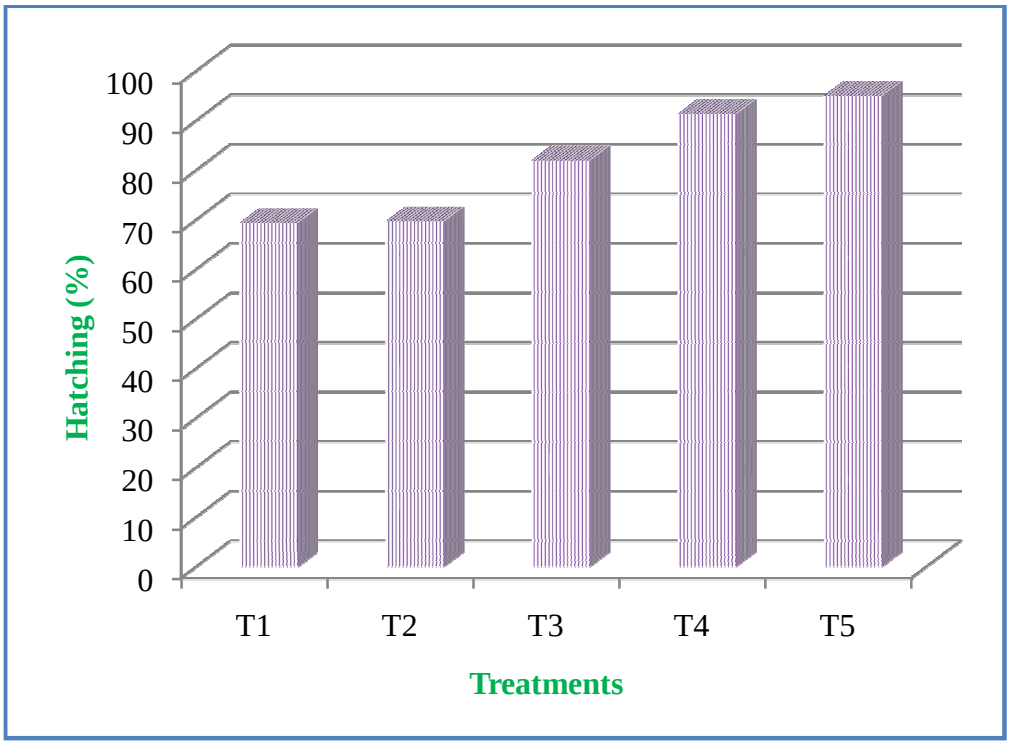

Figure 4.7: Showing mean of egg hatching per cent in different levels of water hardness

Table 4.9: Summary output of second order polynomial regression analysis between hardness and egg hatching per cent

SUMMARY OUTPUT						
Regression Statistics						
Multiple R	0.970114					
R Square	0.941121					
Adjusted R Square	0.882242					
Standard Error	4.087104					
Observations	5					
ANOVA	**df**	**SS**	**MS**	**F**	**Significance F**	
Regression	2	534.0081	267.004	15.98403	0.058879	
Residual	2	33.40885	16.70442			
Total	4	567.4169				
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	134.1829	37.98401	3.532614	0.071629	-29.2491	297.6149
X Variable 1	-0.41787	0.526858	-0.79313	0.510846	-2.68476	1.84902
X Variable 2	0.000419	0.001748	0.239986	0.832696	-0.0071	0.007939

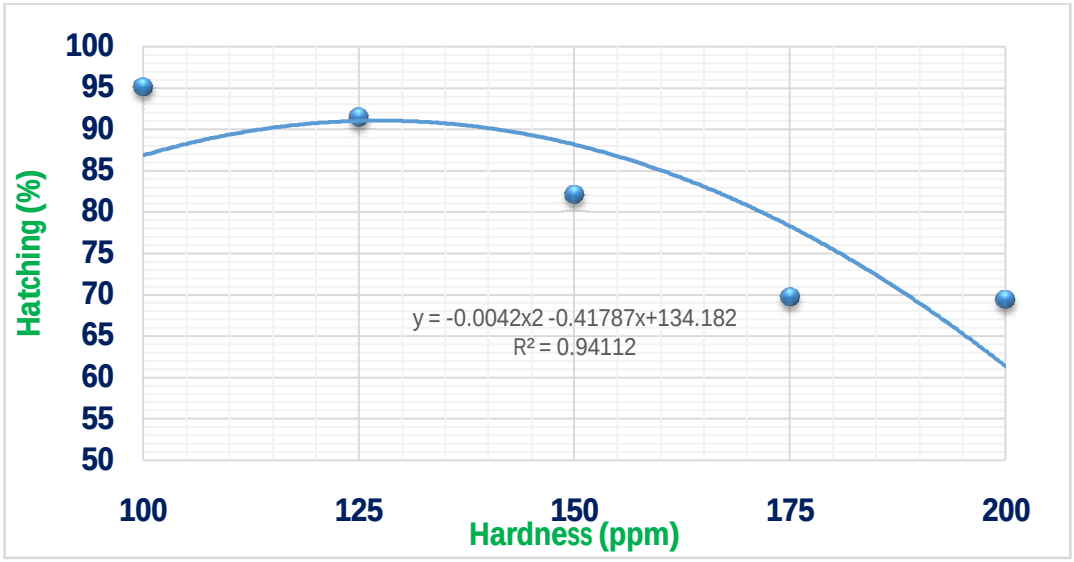

Table 4.8:Hatching per cent in different levels of water hardness

Table 4.10: Rate of larval survival in different levels of water hardness

S.No	Treatment	Survival (%)
1	T1	79.46±0.0.3
2	T2	75.71±0.02
3	T3	71.42±3.90
4	T4	70±2.40
5	T5	65.71±2.53

ANOVA*for larval survival*

Source of Variance	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	373.333	4	93.333	1.167	0.382
Within Groups	800	10	80		
Total	1173.333	14			

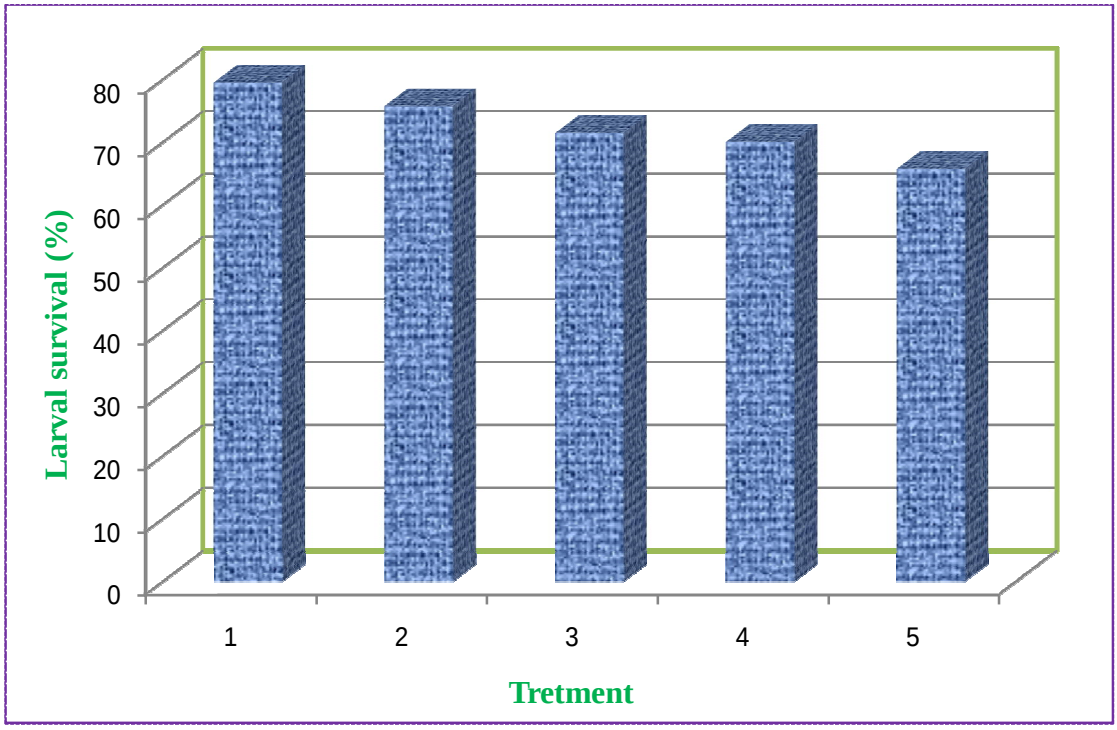

Figure 4.9: Showing mean of larval survival in different levels of water hardness

Table4.11: Summary output of second order polynomial regression analysis between hardness and larval survival

SUMMARY OUTPUT						
Regression Statistics						
Multiple R	0.992225					
R Square	0.98451					
Adjusted R Square	0.969019					
Standard Error	0.93245					
Observations	5					
ANOVA	df	SS	MS	F	Significance F	
Regression	2	110.5193	55.25964	63.55606	0.01549	
Residual	2	1.738926	0.869463			
Total	4	112.2582				
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	96.73314	8.665838	11.16258	0.00793	59.44705	134.0192
X Variable 1	-0.19421	0.1202	-1.61574	0.247525	-0.71139	0.322967
X Variable 2	0.000205	0.000399	0.513054	0.658964	-0.00151	0.00192

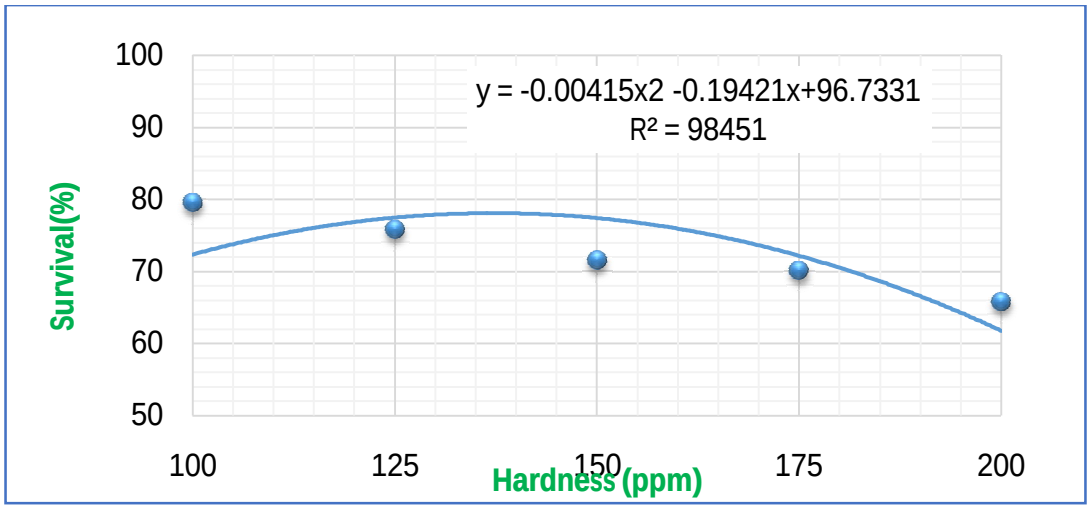

Figure 4.10: larval survival rate in different level of water hardness

Chapter:5

DISCUSSION

The environment of a fish can be defined in terms of the biotic and abiotic factors. The major objective of this study was to examine the effect of an abiotic factor (water hardness) on *Labeo rajasthanicus* egg development and larval survival, in order to define the water hardness requirements for their successful production. The results obtained on the effect of hardness on fertilization rate, ova diameter, hatching rate, eggs incubation duration and larval survival have been presented in chapter 4 of this thesis and the interpretations of these results is presented in following five sections:

- Effect of water hardness on fertilization rate
- Effect of water hardness on ova diameter
- Effect of water hardness on hatching rate
- Effect of water hardness on hatching duration
- Effect of water hardness on larval survival

1. Effect of water hardness on fertilization rate

The role of water hardness in fertilization and egg hardening has been studied by several researchers (Gonzal *et al.*, 1987; Whitaker *et al.*,1993; Miyazaki *et al.*,1993; Stricker 1999 and Spade & Bristow, 1999). In waters with very low hardness (soft water with hardness level <50 ppm), the premature bursting of the egg due to excessive water absorption was reported by Wurts and Durborow, 1992. On the other hand very hard water (hardness >300 ppm) reduces the fertilization percentage. In the present study, the impact of hardness on fertilization rate was significantly different in different hardness treatments (Table 4.2). The highest fertilization percentage and largest size of egg was noticed in lowest hardness treatment. Both, the egg size and fertilization rate reduced with increasing hardness levels. The reduction in egg size at higher hardness levels might be the result of less absorption of water due to higher osmotic pressure in outside environment (Alderdice, 1988).

Whitaker *et al.,* (1993) reported that at fertilization, egg activation and the initiation of development were always triggered by an increase in intracellular Ca^{2+} concentration within the egg and in fertilization of certain species such as sea urchin, frog and some fish eggs, a single transient of Ca^{2+} is triggered during egg activation. In other animals

however, including mammals, ascidians and nemertean worms, a distinctive series of intracellular Ca^{2+} oscillations are observed (Miyazaki *et al.*, 1993 and Stricker 1999).

Coward *et al. (*2002) studied a wide variety of animal and plant species and demonstrated that development at fertilization is triggered by an increase in intracellular Ca^{2+} concentration within the egg that occurs as either a single transient or a series of distinctive oscillations depending upon the species under investigation. This increase in intracellular Ca^{2+} activates the egg and also appears to play an important role in later embryonic development.

Ohta*et al.*, (1996) reported that the egg fertilization requires presence of small quantities of divalent ions (Ca^{2+} and Mg^{2+}) and once the egg has been activated, the micropyle is plugged. Fertilization initiates the second meiotic division in the egg, which at spawning contains two sets of maternal chromosomes. In rose bitterling (*Oryziaslatipes*), as with other teleosts, sperm penetrate the eggs via a defined sperm entry site, a region of plasma membrane just beneath the micropyle. In this fish, the sperm entry site transforms from a tuft of microvillii into a swollen mass that continues to plug the micropyle after sperm penetration.

2. Effect of water hardness on ova diameter

At low water hardness the increase in egg diameter is greater because the swelling process of flaccid newly shed eggs when they first contact water and absorb water is higher (Gonzal*et al.*, 1987; Spade & Bristow, 1999). Water hardness has been shown to have a direct effect on the swelling of newly fertilized eggs, which is an important process during the early development of the teleost egg (Spade and Bristow, 1999).The results of the present study has also indicated a negative relationship between egg size and water hardness as the egg size significantly increased with reducing hardness levels (Table 4.4).

The process of egg swelling is the uptake of extracellular water into the perivitelline space. The perivitelline space is located between the outer chorion of the egg and the vitelline membrane that surrounds the developing embryo. In a fertilized egg, the fluid filled perivitelline space provides room and protection for embryonic development (Eddy, 1974). The egg draws in extracellular water due to the fact that it has greater osmotic pressure than the extracellular water. The egg swelling increases when water

hardness decreases because low water hardness usually means low osmotic concentration. In the present study, the larger egg sizes observed in lower water hardness treatments were possibly due to more water absorption than those in higher treatment. These findings are in confirmation to earlier studies (Rach *et al.*, 2014).

3. Effect of water hardness on hatching rate

A water hardness of 300–500 mg/l $CaCO_3$ is recommended for the successful hatching of silver carp eggs (Gonzal*et al*., 1987). The increase of water hardness to 70 mg/l $CaCO_3$ increased hatch rate, but the highest Ca^{2+} level reduced post-hatch survival (Silva *et al*., 2003). However, the hatch rate of *Moronesaxatilis* (Striped bass) was 70 % at hardness range of 40 – 200 mg/l (Spade *et al*., 2017).It has been suggested that low water hardness may limit silver carp range expansions (Gonzal*et al*., 1987; Kolar et al. 2007). In our study, the highest hatching rates (95%) occurred in groups of eggs that were hardened in the softest water treatment (100 mg/l), whereas the lowest hatching rates (69%) occurred in groups of eggs that were hardened in the hardest water treatment (200 mg/l).

4. Effect of water hardness on hatching duration

Environmental calcium is required for “water hardening” of newly fertilized fresh water fish eggs and calcification of larval skeletal structure. Calcium also influences membrane permeability and is important in that regard for successful embryonic development. Carps like those of other freshwater fish, are hyper-osmotic to their medium. If the incubating medium has a lower ionic concentration hypoosmotic than the egg premature bursting of the egg from excessive water absorption may occur (Wurts and Durborow, 1992). In the present study, longest incubation period of 17 hr was at 200 mg/l hardness. Shortest incubation period of 15.45 hr was at 100 mg/l treatment. The hatching duration of *L. rajasthanicus* eggs increased with increasing water hardness levels in incubator. Kimmel *et al*., (1995) observed that, completion of rapid morphogenesis of primary organ systems; cartilage development in head and pectoral fin; hatching occurs within 48 hrs asynchronously in zebrafish. Similar observation were made by Chanu*et al*., (2010) where they reported that, as the water hardness increases from 5 to 315 mg/l, incubation period (zebrafish) also increases from 49 to 58 h. Alderdice (1988) has suggested that Brown trout (*Salmo truttafario*) need at least 10 mg/l $CaCO_3$ for incubation. However, incubation period were found

to increase with increase in the water hardness. Wurts and Durborow, (1992) revealed that a recommended range for free calcium in culture waters is 25 to 100 mg/l (63 to 250 mg/l $CaCO_3$ hardness).

5. Effect of water hardness on larval survival

Silver carp (*Hypophthalmichthys molitrix*) eggs incubated at 100 or 200 mg/l CaCO3 showed premature mass burstingof the eggs because of increasing water absorption and, therefore, water hardness of 300-500 mg/l $CaCO_3$ was indicated to improve hatching and larval viability of thisspecies (Gonzal*et al*., 1987). Uphoff, (1989) verified an increased hatching rate andlarval survival of striped bass in water hardness higher than 150 mg/l CaCO3. Hawang*et al.,* (1996) suggested that neither hatchrate nor the growth of tilapia larvae was affected by exposure to waters with 22 or 90mg/l CaCO3. In the present study the highest larval survival (79.46%) was recorded in 200 mg/l water hardness. However, the lowest larval survival rate (65.71%) was found in 100 mg/l hardness. Further, the survival rate had positive relationship as the rate of larval survival significantly increased with increasing water hardness. Tucker &Steeby (1993) have also noticed the similar trend for channel catfish, and*Ictalurus punctatus.*Molokwu&Okpokwasili (2002) recommended a water hardness range of 30-60 mg/l $CaCO_3$ for optimal normal hatching, viability and maximum larval development of *Clariasgariepinus.* However, a higher hardness level (100-200mg/l) for carp species has been recommended (Gonzal*et al*., 1987). The findings of this study further confirm that a water hardness level of 200 mg/l is favorable for better survival of carp and *Labeo rajasthanicus* in particular.

From the results (Tables 4.1-4.11 & Figs. 4.1-4.10) of present study and forgone discussions, it is marked that the hardness in hatchery waters has immense role in successful carp (especially *Labeo rajasthanicus*) seed production. Overall results suggest that the water hardness < 150 mg/l is the ideal for egg incubation of *L. rajasthanicus* for higher fertilization rate, better hatching percentage and more larval survival. These results may be a prelude to effectively utilize the benefits of hardness on better hatching rate and ultimately the cost of production in carp hatcheries. However, on the basis of second order polynomial regression analysis between water hardness and breeding indices (Fertilization rate, egg size, hatching rate and larval

survival percentage), the hatchery seed production of *L. rajasthanicus* is recommended between 125 and 150 mg/l hardness.

Chapter: 6

SUMMARY

The hardness requirement varies among species and even for various developmental stage (e.g., fertilization, hatching, larval survival, etc.) of fish.Calcium and magnesium are essential in the biological processes of fish bone and scale formation, blood clotting and other metabolic reactions.Fish can absorb needed calcium directly from the water or food. The hardness is also a contributor of necessary ions for basic physiological functions during larval development of fishes. Excess amount of calcium in water is not a healthy sign.The hardness of the water is a serious problem in the fish production, which varies considerably from place to place. In general, surface waters are soft than ground waters.Its limiting effects in terms on hatching of eggs and survival greatly vary with species, age, physiological state of fish and environmental conditions. The effect of water hardness on survival of fish eggs and preference for water hardness varies in oviparous and ovo-viviparous fishes. Considering this the present study was conducted to understand the influence of water hardness on egg hatchability as well as on larval survival rate in *Labeo rajasthanicus.* The principle objectives of this study were as below:

- To work-out the optimum level of water hardness for fish egg hatching.
- To assess the effect of water hardness on fish larval viability.

Considering of above objectives, the experiment was designed and conducted at Aquaculture Research and Seed Unit, Directorate of Research, MPUAT Udaipur.

Chapter I of this thesis, gives a general introduction of the subject including impact of water hardness on hatchability of egg and effect of water hardness on larval viability of fish. The need of this study has also been stated in this chapter.

Chapter II, the comprehensive review of researches conducted so far on related and allied field has been undertaken to have a general idea of the history of effect of water hardness on egg hatchability and larval viability.

Chapter III, of this thesis comprises of various standard method for selection of brood fish, breeding technique and various standard procedure followed for the analysis of water quality, study of egg fertilization rate, ova diameter, hatching duration, hatching percentage and hatchling survival. In this chapter, experimental setup techniques have also been described.

Chapter IV, explain the results obtained during experiments. The effect of water hardness on breeding performance of *Labeo rajasthanicus* are presented in Table 4.1 to 4.11. The highest fertilization rate was recorded in T5 (79.46 %), while the lowest (75.71 %)was in T1 (Table 4.2 and Fig. 4.1). The size of ova significantly reduced with increasing hardness levels. The smallest (3.33 mm) ova diameter was observed in T1. Whereas, the biggest (3.54 mm) ova diameter was in lowest water hardness level (T5).The minimum hatching time (15.45 hrs) was noticed in lowest hardness treatments (T5). The hatching duration significantly increased with increasing hardness level and the highest hatching time was recorded in highest (T1) concentration of hardness (Table 4.6 and Fig. 4.5).

The maximum hatching rate of 95 % was in T5 followed by T4 (91.33), T3 (82) and T2 (69.66) %. The lowest hatching rate of 69.33 % was recorded in T1. The larval survival in different hardness treatments varied between 65.71 to 79.46 %. The lowest being in T1 and hugest in T5. The larval survival significantly reduced with increasing hardness level and among the treatments survival rate was significant except T3 and T4 (Table 4.10).

The outcome of this research has been well discussed and explained in chapter V of this thesis.An exhausted list of references used in this study has been given Chapter VII. Based on the results of this study it was concluded that the water hardness hassignificant impacted in hatchery waters. For desiredfertilization, hatching rate and larval survival of*Labeo rajasthanicus*water hardness range of 125-150 mg/l is recommended.

"Effect of water hardness on egg hatchability and larval viability of *Labeo rajasthanicus"*

Vijay Kumar* **Dr. V. P. Saini****

Research Scholar Major Advisor

ABSTRACT

This study was conducted to assess the impact of water hardness on egg hatchability and larval viability of *Labeo rajasthanicus.*For this purpose,five hardness levels were tested (200, 175, 150, 125, 100 mg/l) in relation tofertilization rate, ova diameter, hatching duration, hatching per cent and hatchling survival of *Labeo rajasthanicus.* For incubation of eggsa simple hatchery device was fabricated. In each hatchery jar 5000 eggs ware incubation & each treatment was run in triplicate. The diameter of egg was measured using Leica microscope with computer based software. The smallest (3.33 mm) ova diameter was observed in T1(200 mg/l), whereas the biggest (3.54 mm) ova diameter was in lowest water hardness level (T5-100 mg/l).The Minimum hatching time (15.45 hrs) was noticed in lowest hardness treatments (T5),which was significantly increased with increasing hardness level and the highest hatching time was recorded in highest (200 mg/l) concentration of hardness. The highest hatching rates (95%) occurred in groups of eggs that were hardened in the softest water treatment (100 mg/l), whereas the lowest hatching rates (69%) was in T1 (200 mg/l). The highest larval survival (79.46%) was recorded in 200 mg/l water hardness and lowest larval survival rate (65.71%) was found in 100 mg/l hardness. Further, the survival rate had positive relationship with hardness as the rate of larval survival significantly increased with increasing water hardness.Overall results suggested that the water hardness 125 - 150 mg/l is the ideal for egg incubation of *L. rajasthanicus* for higher fertilization rate, better hatching percentage and more larval survival. These results may be a prelude to effectively utilize the benefits of hardness on better hatching rate and ultimately the cost of production in carp hatcheries.

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