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Egg Hatchability and Larval Viability of *Labeo rajasthanicus* in Relation to Water Hardness

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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(T1), 175(T2), 150(T3), 125(T4) & 100(T5) mg/l] in relation to fertilization rate, ova diameter, hatching duration, hatching per cent and hatchling survival of Labeo rajasthanicus. In each hatchery jar of two liters capacity, 5000 eggs ware incubation & each treatment was run in triplicate. 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), 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). 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. Further, the second order polynomial regression analysis between water hardness and breeding indices (Fertilization rate, ova diameter, hatching rate & larval survival) indicated that a water hardness of 125 mg/l is most suitable for Labeo rajasthanicus breeding.

Key words: *Fertilization, Hardness, Hatching, Labeo rajasthanicus, Ova, Survival*

INTRODUCTION

Inland finfish aquaculture is the most common type of aquaculture operation in India as well as world. In aquaculture carps 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[1]. The supply of quality seed is the prime requirement of aquaculture production.

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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[2]. 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 Ca2+ and Mg2+ 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[3]. Calcium and magnesium are essential in the biological processes of fish bone and scale formation, blood clotting and other metabolic reactions[4]. Kane *et al.*[5] 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[6]. The suggested value for water hardness for fish cultivation in ponds is above 20 mg/l $CaCO_3$[7].

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[7]. Incubation of eggs in calcium deficient water, which contributes significantly to the total hardness of natural water [8], may result in poor hatchability and fry survival [9]. It is therefore necessary to control the ionic concentration of the medium to minimize premature bursting.

MATERIAL AND METHODS

Experimental Site: The experiment on the evaluation of suitable water hardness for fertilization, egg size and hatching and survival rate of *Labeo rajasthanicus* was conducted during July-August, 2018 at Aquaculture Research and Seed Unit, Directorate of Research, MPUAT, Udaipur.

Experimental Fish: A minor carp, *Labeo rajasthanicus* was selected for the present study to evaluate the effect of water hardness on egg size, fertilization, hatchability and larval viability.

Collection of brood-stock and its management: The brood fish were collected from Aquaculture Research and Seed Unit, MPUAT, Udaipur. Fish were treated with 4 % KMnO4 to disinfect and kept in FRP tanks (2mx 1mx 1m) for conditioning. These fish were fed with commercially formulated feed (Growell) at 3 % of their body weight. Feeding was done twice (10:00 and 17:00) in a day. 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 colour with the smooth pectoral fin. Abdomen of the female was bulgy.

Breeding Protocol: 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[10]. The Gonopro - FH was used as inducing agent. Inducing hormone Gonopro - FH was used @ 0.2 and 0.3 ml/kg body weight to male and female respectively [11]. After 8 hrs. of injection, dry stripping method was used to obtain ova & sperm, and water having different hardness [200(T1), 175(T2), 150(T3), 125(T4) & 100(T5) mg/l] was used for experimental fertilization. The water different hardness was prepared by mixing soft & hard water.

Incubation of eggs: The eggs were incubated in five different hardness levels T1, T2, T3, T4 and T5. For this purpose a simple hatching device was fabricated using 2000 ml plastic bottles (Plate 1). 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 and following breeding parameters were calculated:

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 was calculated as below[10].

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

Egg diameter

The diameter of egg was measured using Leica microscope with computer based measuring facility[10].

Hatching duration

The time taken for complete hatching was observed. For this time of fertilization and hatching was observed manually & total time duration was recorded in hrs[10].

Hatching Rate

The hatching percentage was calculated using following formula[10].

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

$$\textit{Hatchling Survival rate} = \frac{\text{No. of individuals harvested}}{\text{total no. of larve}} \times 100$$

Physico-chemical Water Quality Parameters: Following standard method of APHA[12] selected water quality parameters such as Temperature, pH, Dissolved Oxygen, Alkalinity, TDS, Electric conductivity, Salinity etc. were measured.

Statistical analysis: Statistical analysis of the data obtain during this study was performed following one-way ANOVA [13]. 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. Further, second order polynomial regression analysis was also performed between hardness levels and breeding parameters such fertilization rate, ova diameter, hatching rate, hatching duration and survival rate.

RESULTS

The result of the present studies pertaining to water quality, rate of fertilization, ova diameter, hatching duration, hatching percent and survival rate etc. are presented in Tables 1 – 2 and Figs. 1- 5.

Water quality parameters: The overall water quality status of different treatments is presented in Table 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 dissolve oxygen in different treatments ranged 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 ranged from 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 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 survival rate in different hardness levels were observed. From the result of these parameters (Tables 2 and Figs 1 to 5) it is evident that water hardness has significant impact in carp egg incubation as 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 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 2 it is clear that fertilization rate significantly reduced with increasing water hardness levels. The data obtain for fertilization rate for different treatment were statistically analyzed using second order polynomial regression equation. From the result of this equation (Figure 1) it was noticed that a hardness level of 137 is optimum for batter 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. Whereas, the biggest (3.54 mm) ova diameter was in lowest water hardness level (T5). The ova diameter was statistically significant among treatments (Table 2). Further, the second order polynomial regression analysis suggested that the hardness between 125 to 150 mg/l is favorable for egg hardening (Fig. 2).

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 hatching duration significantly increased with increasing hardness level and the highest hatching time was recorded in highest level of hardness (Table 2). When hatching duration in relation to hardness was analyzed through second order polynomial regression analysis (Fig. 3) it was noticed that a maximum hardness of 150 mg/l is favorable for optimum hatching duration.

Hatching rate

From Table 2, 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 level of water hardness in relation to hatching percent, second order polynomial regression analysis was performed between hatching rate and hardness (Fig. 4). It is clear from the regression graph that hardness range between 100 to 125 mg/l is batter for higher hatching rate.

Survival rate

The larval survival in different hardness treatments varied between 65.71 to 79.46 %. The lowest being in T1 and highest in T5. The larval survival significantly reduced with increasing hardness level and among the treatments survival rate was significant except T3 and T4 (Table 2). The second order polynomial regression analysis of larval survival and hardness suggested that the water hardness maximum up to 125 mg/l is favorable for batter survival of *L. rajasthanicus* larvae (Fig. 5).

In general the results of this study indicated that the water hardness has significant impacted in hatchery waters. All the parameters (breeding parameters) indicated that the water hardness between 125 -150 mg/l is for batter 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.*

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 survival rate, in order to define the water hardness requirements for their successful hatchery production. The role of water hardness in fertilization and egg hardening has been studied by several researchers[14, 15, 16, 17, 18]. 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 [3]. 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 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[19, 20]. Whitaker *et al.*[15], 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. Coward *et al.* [21] 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. Ohta *et al.* [22] 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 (*Oryzia slatipes*), 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 micro villi into a swollen mass that continues to plug the micropyle after sperm penetration. 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[15,18].

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 [18].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 2). 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[23]. 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[24]. A water hardness of 300–500 mg/l $CaCO_3$ is recommended for the successful hatching of silver carp eggs. It has been suggested that low water hardness may limit silver carp range expansions[14].

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[3]. In the present study, longest incubation period of 17 hrs 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. Similar observation made by Chanu *et al*.[25], where they reported that, as the water hardness increases from 5 to 315 mg/l, incubation period (zebra fish) also increases from 49 to 58 h. Alderdice [20] Brown trout (*Salmo truttafario*) need at least 10 mg/l $CaCO_3$ for incubation. However, incubation period were increase with increase in the water hardness. Wurts and Durborow[3] 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).

Silver carp (*Hypophthalmichthys molitrix*) eggs incubated at 100 or 200 mg/l $CaCO_3$ showed premature mass bursting of 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 this species[14]. Hwang *et al.* [26] suggested that neither hatch rate 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[27] have also noticed the similar trend for channel catfish, and *Ictalurus punctatus.* Molokwu & Okpokwasili[28] recommended a water hardness range of 30-60 mg/l $CaCO_3$ for optimal normal hatching, viability and maximum larval development of *Clarias gariepinus.* However, a higher hardness level (100-200mg/l) for carp species has been recommended[14]. The findings of this study further confirm that a water hardness level of 125 mg/l is favorable for better survival of carp and *Labeo rajasthanicus* in particular.

Table 1: Range and mean value of selected water quality parameters in different treatments

Parameters	Treatments				
	T1	T2	T3	T4	T5
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)
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)
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)
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)
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)	270 - 272 (271)
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)

Table 2: Different level of water hardness for fertilization, ova diameter, hatching duration, hatching rate and survival rate

Treatments	Fertilization (%)		Ova diameter (mm)	Hatching duration (hr.)	Hatching (%)	Survival rate (%)
T1	65.71±2.39^{e}		3.33±0.29^{e}	17.00±0.97^{a}	69.33±2.21^{d}	79.46±0.03^{a}
T2	70.00±3.16^{d}	3.35±0.53^{d}	16.40±1.12^{b}	69.66±3.02^{d}	75.71±0.02^{b}	
T3	71.43±0.09^{c}	3.37±0.22^{c}	16.15±1.07^{b}	82.00±2.11^{c}	71.42±3.90^{c}	
T4	75.71±2.07^{b}	3.38±0.30^{b}	16.05±0.77^{b}	91.33±1.98^{b}	70.00±2.40^{d}	
T5	79.46±2.83^{a}	3.54±0.58^{a}	15.45±0.93^{b}	95.00±3.01^{a}	65.71±2.53^{e}	
The values superscript (column wise) with different letters are significantly different at 5% level of probability						

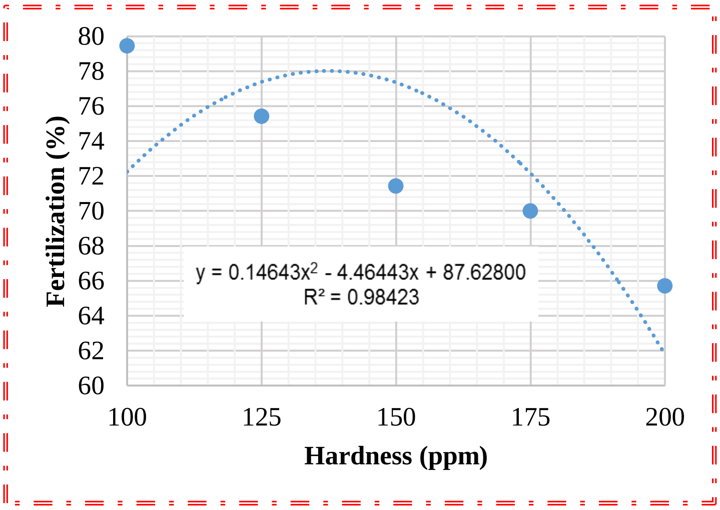

Fig. 1: The second order polynomial regression between fertilization and water hardness

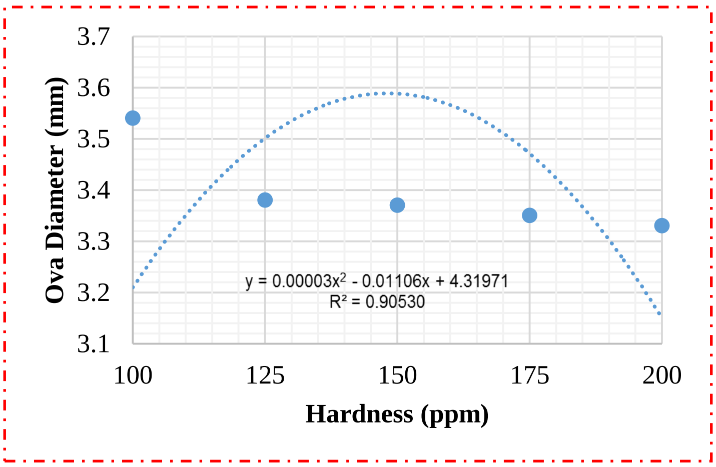

Fig. 2: The second order polynomial regression between ova diameter and water hardness

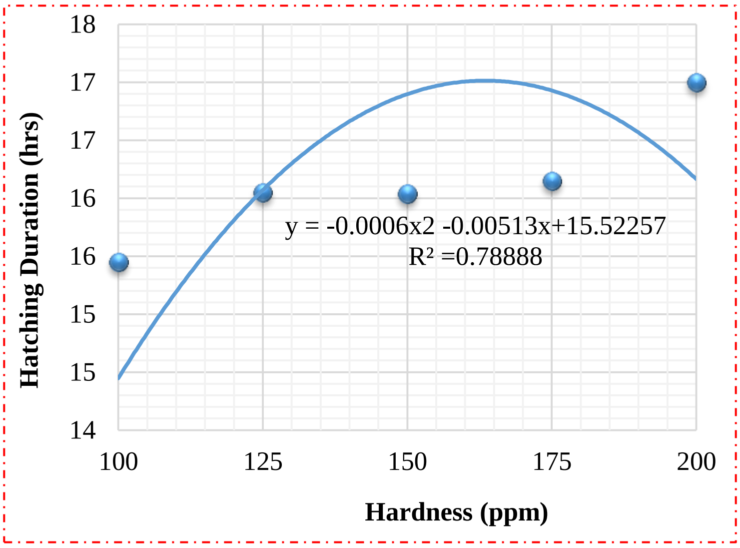

Fig. 3: The second order polynomial regression between hatching time and water hardness

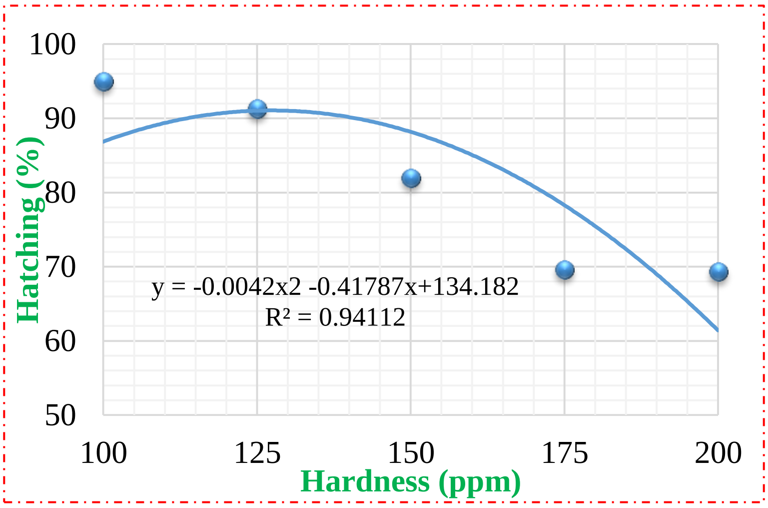

Fig. 4: The second order polynomial regression between hatching percent and water hardness

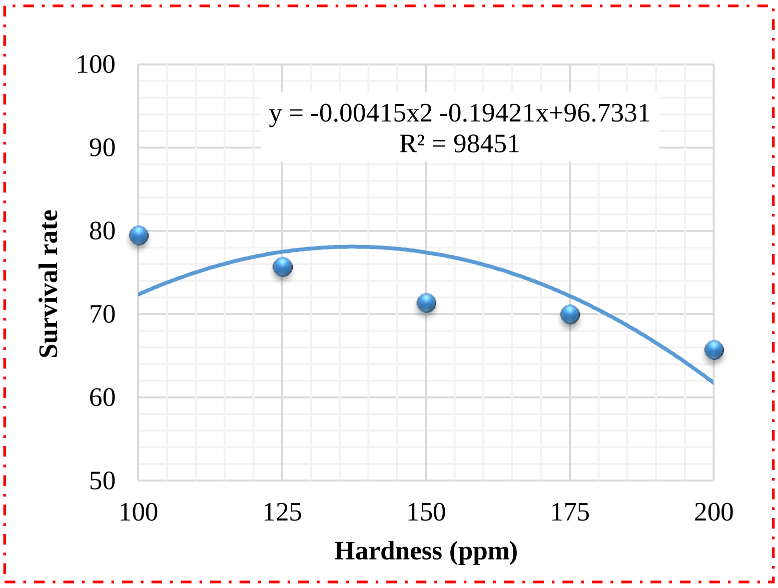

Fig. 5: The second order polynomial regression between larval survival rate and water hardness

Plate 1: Simple hatching device fabricated for egg incubation

CONCLUSION

From the results of present study marked that the hardness in hatchery water has immense role in successful carp (especially *Labeo rajasthanicus*) seed production. Overall results suggest that the water hardness below 150 mg/l is the ideal for egg incubation of *L. rajasthanicus* for higher fertilization rate, better hatching percentage and more survival rate. 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 survival percentage), the hatchery seed production of *L. rajasthanicus* is recommended 125 mg/l hardness.

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