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Effect of water hardness on survival and growth of *Labeo rohita* (Hamilton) fry

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Abstract
The present study was conducted to assess the impact of water hardness on survival and growth of (*Labeo rohita*) fry. In each tank 100 fry ware introduced and the experiment was run in triplicate for a period of 28 days. The selected levels of hardness had significant impact on fish growth with a highest weight gain (2.019±0.036g) was observed in T_4 (Hardness 150 mg/l) and lowest T_5 (1.189±0.054g). The highest per cent weight gain (86.14%) was T_4 (Hardness 150 mg/l) while the lowest (49.66%) being in T_5. However, the highest gain in length (18.93±1.18 mm) was in T_4 and lowest (13.99±0.46 mm) in T_5 and per cent length gain was highest (130.33%) in T_4 and lowest (98.67%) in T5. Further the highest survival was in T4 (95.33%) and lowest (90.00%) in T5. Result of this study clearly indicated higher weight gain (2.019g), weight gain per cent (86.14%), length gain (18.93mm), length gain per cent (130.33%) and survival (95.33%) in the hardness level 150 mg/l. On the basis of these results a hardness level of 150 mg/l is recommended for *Labeo rohita* under local environmental conditions.

Keywords: *Labeo rohita*, fry, hardness, survival, growth

Introduction
Water is one of the most important vital resources for the living beings to survive and a number of physico-chemical properties that help the molecule to act as the best suitable medium for the aquatic life activities. Hence water is called "Elixir of life". Indeed it is a part of life itself since; the protoplasm of most of the living cell contains about 80 % of water. Most of the biochemical reactions that occur in the metabolism and growth of living cells involve water. Water quality refers to all physical, chemical and biological characteristics of water and plays an important role in the growth and survival of aquatic organisms. Relationship between water quality and aquatic productivity is a pre-requisite for obtaining optimum growth and production [14].
The water used for aquaculture would not give the desired production until and unless the prevailing water quality parameters are optimum for the organism under culture. Study of the physico-chemical parameters of an aquatic ecosystem is basic for understanding its biological productivity. Although each factor plays its individual role but it is the synergistic effect of various parameters which determines the composition and productivity of the flora and fauna. Conducive range of these factors is essential for obtaining optimum fish production. Physic & chemical water quality parameters which influence the aquatic productivity are temperature, pH, total alkalinity, hardness, dissolved gases like oxygen and carbon dioxide and dissolved inorganic nutrients like nitrate and phosphorus are considered to be important [13].
Water hardness is important to fish culture and is a commonly reported aspect of water quality. It is a measure of the quantity of divalent ions such as calcium, magnesium and/or iron in water. Hardness can be a mixture of divalent salts; however, calcium and magnesium are the most common sources of water hardness. It is traditionally measured by chemical titration. The hardness of a water sample is reported in milligrams per liter as calcium carbonate (mg/l $CaCO_3$). 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/or some other divalent salt is causing water hardness [19]. A low $CaCO_3$ hardness value is a reliable indication that the calcium concentration is low. However, high hardness does not necessarily reflect a high calcium concentration. But, since limestone is common in the soil and bedrock of the southern United States, it would be reasonably safe to assume that high hardness measurements reflect high calcium levels [20].

2. Material and method

2.1 Experimental fish

Fish use for the experiment were fry of rohu (*Labeo rohita*). The fry of rohu was procured from Aquaculture Research & Seed Unit, Directorate of Research, Maharana Pratap University of Agriculture and Technology, Udaipur.

2.2 Feeding

The *Labeo rohita* fry were completely fed with commercial carp feed (Growel) having 26 % protein at the rate of 04 % of body weight once daily at morning time.

2.3 Experimental set up

The experiment was conducted in the fifteen FRP tank (2x1x0.75m) filled with 1000 liters of water for a rearing period of 28 days at Aquaculture Research & Seed production Unit, DOR, MPUAT, Udaipur. The healthy fry of uniform size (0.0246g) and stocked in each treatment @ 100/tank were randomly distributed in five experimental groups having five different levels of hardness i.e (225,200,175,150,125 mg/l) was maintained. The graded level of hardness were maintained using soft water.

2.4 Evolution of fry growth and survival performance

The growth parameters of experimental fish viz., net weight gain, per cent gain in weight, specific growth rate, net length gain and per cent length gain were determined using following formulae:

Net weight gain = final weight (g) - initial weight (g)

Per cent gain in weight (PGW) = (Final weight – Initial weight)/ (initial weight) × 100

Net length gain = Final length – Initial length

Per cent gain in length (PGL) = (Final Length – Initial length)/ (initial length) × 100

$$\text{Survival (\%)} = \frac{\text{Total number of fry harvested}}{\text{Total number of fry stocked}} \times 100$$

2.5 Physicochemical water quality parameters

Selected water quality parameter such as Temperature, Dissolved Oxygen, Alkalinity, TDS, Electric conductivity and Salinity etc. will be observed following standard method of APHA [1].

3. Results

3.1 Physico-chemical water parameters

The open well water was used as source water during the whole experimental period, water temperature increased progressively during the experimental period due to changes in weather conditions. The minimum average water temperature of 26.90°C was recorded in T4 and maximum (27.14°C) in T2. Further, the minimum water temperature of 25.13°C was recorded in T3 and maximum 29.77°C in both T3 and T4 (Table 1). The minimum average value of the water pH value of (7.25) and maximum (7.74) were observed in T1 and T4 respectively. Whereas, the minimum mean pH values of 6.52 was noticed in T1. The maximum mean pH value (8.230) was in T5 (Table 1). During the experimental period narrow fluctuations in dissolved oxygen concentration were recorded. The minimum average value of dissolved oxygen (6.16 mg/l) was recorded in T2 and maximum (6.68 mg/l) in T4. Further, the minimum (5.05 mg/l) and maximum (7.76 mg/l) concentration of dissolved oxygen were recorded in T3 and T5 respectively (Table 1). The minimum value of alkalinity (140.0 mg/l) was in T5 and maximum (270.0 mg/l) was recorded in T1. The respective minimum (155.8 mg/l) and maximum (259.5 mg/l) average values of alkalinity were recorded in T5 and T1 (Table 1). The trends in electrical conductivity of treatments have been presented in Fig. 4.6. The TDS value minimum (340.6 mg/l) in T5 and maximum (581.0 mg/l) in T1. The average value of TDS was highest (573.9 mg/l) in T1 and minimum average value (352.1 mg/l) in T5 (Table 1).

Hardness is the measure of alkaline earth elements such as calcium and magnesium in an aquatic body along with other ions such as aluminum, iron, manganese, strontium, zinc, and hydrogen ions. Calcium and magnesium are essential to fish for metabolic reactions such as bone and scale formation. In the present study having five different level of hardness i.e (225,200,175,150,125 mg/l) was maintained by soft water in 5 treatments, Hardness range such as T1 225 mg/l followed by T2 200 mg/l, T3 175 mg/l, T4 150 mg/l and last one was T5 125 mg/l. in all the treatment best growth and survival was found at the level of hardness 150 mg/l.

Salinity is the measurement of the ionic composition of water. The minimum value of salinity (0.30 ppt) was recorded in T5 and maximum (0.6 ppt) was found in T1. Similar the mean value of salinity was lowest (0.31 ppt) in T5 and highest mean value (0.50 ppt) of salinity observed in both T1 and T2 (Table 1).

3.2 Fish growth performance

Fish growth data pertaining to weight gain, per cent weight gain, length gain, per cent length gain and survival rate are presented in Tables 2 to 4.5. The different levels of hardness treatments had significant impact on fish growth parameters. The different hardness levels significantly impact the growth of experimental fish. In all treatments, increment in growth was noticed as compared to initial weight in individual treatments. However, the highest weight gain was noticed in T4. (Table 2 and Fig 1). The highest gain in weight (2.0198±0.0366g) in T4 (Hardness 150 mg/l) was followed by T3 (1.77±0.11g), T2 (1.67±0.02g), T1 (1.48±0.06g) and lowest was recorded in T5 (1.18±0.05g). The net weight gain among treatments was significantly different ($p<0.05$).

The per cent weight gain was highest (86.14%) in T4 followed by T3 (72.08%), T2 (67.61%), T1 (59.43%). The lowest per cent weight gain was observed in T5 (49.66%) (Table 2 and Fig 2). The per cent weight gain among treatments significantly different ($p<0.05$). The different hardness levels significantly impact the growth of experimental fish. All treatments showed length gain but T4 showed more net body length gain in all treatments (Table 3 and Fig 3). The highest gain in length (18.9±1.18 mm) was in T4 followed by T3 (17.2±0.51 mm), T2 (16.1±0.13 mm), T1 (15.2±0.82 mm) and lowest was recorded in T5 (13.9±0.46 mm). The net length gain among treatments was significantly different ($p<0.05$).

The per cent length gain was highest (130.33%) in T4 followed by T3 (119.67%), T2 (108.0%), T1 (106.0%) Where, the lowest per cent length gain was observed in T5 (98.67%) (Table 3 and Fig. 4). The both net length gain & per cent weight gain among treatments ware significantly different ($p<0.05$). The fry survival in different hardness treatments varied between 90.0 % to 95.33%. The highest survival was in T4 (95.33%) followed by T3 (93.33%), T2 (92.67%), T1 (92.00%). The lowest survival rate being in T5 (90.00%). (Table 4 and Fig. 5)

Table 1: Range and mean values of selected water quality parameters during experimental period

Parameters	Treatments				
	T_1	T_2	T_3	T_4	T_5
Temperature (°C)	25.50-29.57 (26.99)	25.87-29.73 (27.14)	25.13-29.77 (26.93)	25.43-29.77 (26.90)	25.48-29.76 (26.99)
pH	6.52-7.76 (7.25)	6.96-8.03 (7.47)	7.36-8.15 (7.66)	7.33-8.26 (7.74)	7.24-8.30 (7.70)
Dissolved oxygen (mg/l)	6.27-7.02 (6.65)	5.18-7.25 (6.16)	5.05-7.16 (6.23)	6.25-7.12 (6.68)	5.86-7.76 (6.67)
Alkalinity (mg/l)	246.67-270.00 (259.53)	227.33-240.00 (234.00)	210.00-224.00 (216.67)	185.00-210.33 (195.27)	140.00-174.00 (155.80)
Conductivity (µS/cm)	1151.0-1186.7 (1165.8)	1011.3-1049.3 (1031.7)	951.3-1025.7 (989.3)	820.3-861.3 (842.1)	708.3-745.0 (724.4)
Total dissolve solid (mg/l)	567.33-581.00 (573.93)	496.67-517.00 (507.00)	467.33-504.33 (485.87)	400.33-420.67 (411.20)	340.6-363.33 (352.13)
Salinity (ppt)	0.5-0.6 (0.5	0.50-0.50 (0.50)	0.40-0.50 (0.48)	0.40-0.40 (0.40)	0.30-0.33 (0.31)

Table 2: Summary of net weight gain (gram) & per cent weight gain during experiment in different treatments.

Treatments	Initial (gram)	Final weight (gram)	Net Weight gain (g)	Per cent weight gain (%)
T_1	0.0252±0.0017	1.51^{b}±0.06	1.48^{b}±0.06	59.43^{ab}±3.69
T_2	0.0249±0.0009	1.70^{bc}±0.02	1.67^{bc}±0.02	67.61^{bc}±3.53
T_3	0.0246±0.0007	1.79^{c}±0.11	1.77^{c}±0.11	72.08^{c}±4.51
T_4	0.0235±0.0010	2.04^{d}±0.03	2.01^{d}±0.03	86.14^{d}±2.19
T_5	0.0240^{a}±0.0014	1.21^{a}±0.05	1.18^{a}±0.05	49.66^{a}±2.22

Table 3: Summary of net length gain (mm) & per cent length gain during experiment in different treatments

Treatments	Initial(mm)	Final(mm)	Net length gain(mm)	Per cent length gain
T_1	14.38±0.24	29.66^{ab}±1.06	15.28^{ab}±0.82	106.00^{a}±3.78
T_2	14.94±0.13	31.08^{abc}±0.25	16.13^{ab}±0.13	108.00^{ab}±0.57
T_3	14.36±0.16	31.60^{bc}±0.66	17.24^{bc}±0.51	119.67^{bc}±2.33
T_4	14.49±0.41	33.42^{c}±1.57	18.93^{c}±1.18	130.33^{c}±5.04
T_5	14.24±0.58	28.23^{a}±0.76	13.99^{a}±0.46	98.67^{a}±5.23

Table 4: Survival (%) of experimental fish in different treatments

Treatments	Stocked (No)	Harvested (Nos)	Survival (%)
T_1	100	92.00^{b}±0.57	92.00^{a}±0.57
T_2	100	92.67^{b}±0.33	92.67^{b}±0.33
T_3	100	93.33^{b}±0.33	93.33^{b}±0.33
T_4	100	95.33^{c}±0.33	95.33^{c}±0.33
T_5	100	90.00^{a}±0.57	90.00^{a}±0.57

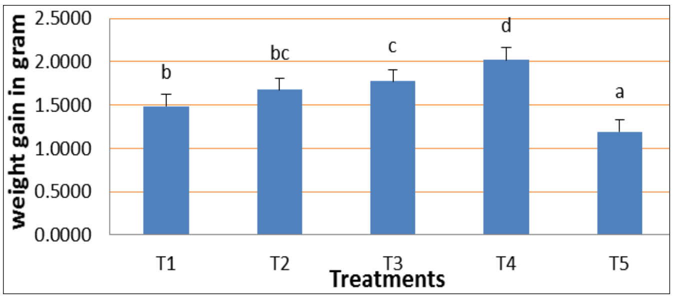

Fig 1: Weight gain (g) of *Labeo rohita* reared at different hardness levels

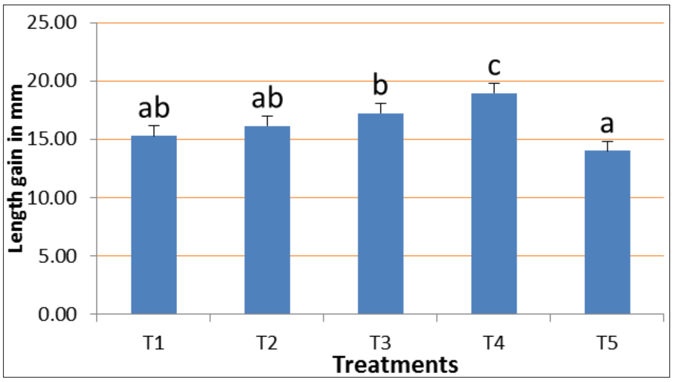

Fig 3: Length gain (mm) of *Labeo rohita* reared at different hardness level

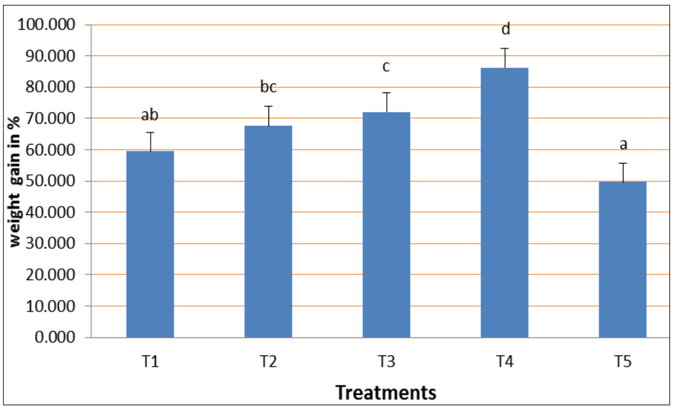

Fig 2: Per cent weight gain at in different treatments

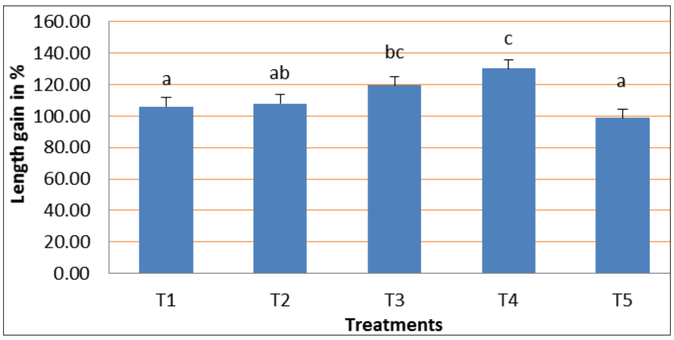

Fig 4: Per cent length gain in different treatments

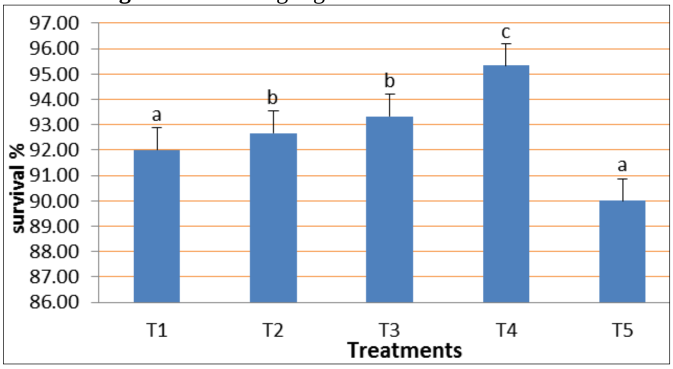

Fig 5: Per cent survival in different treatments

4. Discussion

In the present study, the impact of water hardness on growth and survival of *L. rohita* was evaluated from the results. It is evident that the hardness treatment levels had a positive impact on growth and survival of experimental fish. Further water quality parameters during the experimental period remained favorable for fish growth and survival.

The environment of a fish can be defined in terms of the biotic and abiotic factor. The major objective of this study was to examine the effect of an abiotic factor (water hardness) on *Labeo rohita* weight gain, length gain and survival, in order to define the water hardness requirements for their successful production.

The growth rate of rohu gradually increased up to 450 (mg/l). Further increase in water hardness did not favor in weight gain as growth gain decreased beyond this level [6]. Specific growth rate of 1.87±0.07 and 2.81±0.33 100 & 50 mg/1 $CaCO_3$ respectively [18]. Which were significantly ($p<0.05$) higher than other treatments. Among treatments, the best growth results were obtained at 100 mg/1 $CaCO_3$ hardness. Growth was significantly suppressed at 200 and 300 mg/1 $CaCO_3$ hardness. The results indicated that 100 mg/1 $CaCO_3$ is the optimal level for growth and survival of angel fish fry. The higher larval growth, survival, and biomass in 30-70 mg/1 $CaCO_3$ [18]. Therefore, this is the recommended hardness range for growth and survival of silver catfish larvae. Freshwater culture of striped bass, red drum or crawfish is being considered, free calcium concentrations in the 40 to 100 mg/l range (100 to 250 mg/l as $CaCO_3$ hardness) are desirable; value of 100 mg/l (250 mg/ l calcium hardness) matches the calcium concentration of fish blood [21]. Tests specific for calcium hardness should be performed on samples of the water source being considered for these animals. According to Stone and Thomforde [13] the desirable Range is 50-150 mg/l as $CaCO_3$ and acceptable range is above 100 mg/l as $CaCO_3$. Bhatnagar [4] have recorded hardness values less than 20 mg/l causes stress, 75-150 mg/l is optimum for fish rearing and >300 mg/l is lethal to fish life. Swingle [16] has suggested a hardness from of 150 mg/l or above as satisfactory for the growth of fish and do not require the addition of lime. In the present study the rohu fry gain highest weight (2.01±0.03g) was T4 (Hardness 150 mg/l) followed by T3 (1.7721±0.1127g), T2 (1.67±0.02g), T1 (1.48±0.06g) and lowest was recorded in T1 (1.18±0.05g). The highest gain in length (18.93±1.1863 mm) in T4 (Hardness 150 mg/l) followed by T3 (17.24±0.5122 mm), T2 (16.13±0.13 mm), T1 (15.28±0.82 mm) and lowest was recorded in T5 (13.99±0.46 mm). Thus the better growth rate was obtained in treatment having 150 mg/l water hardness. This level of hardness is quite within the safe and optimum level as suggested by above researchers.

Kasiri [5] have reported the highest survival (50.39 ± 6.23%) rate at the end of the experiment in water hardness of 100 mg/l $CaCO_3$. The results imply that too soft water (10 mg/l $CaCO_3$) and too hard water (300 mg/l $CaCO_3$) are not suitable for *Pterophyllum scalare* incubation and larval rearing [3]. These study demonstrated the positive effects of increased water hardness level (>132 mg/l) on yolk sac larvae and swim-up fry survival. Molokwu [7] found the highest larval survival of 71.05% in 60 mg/l water hardness. Based on statistics performed with analysis of variance (ANOVA) and further compared with Duncan's multiple range test ($p < 0.05$), the results imply that very soft water (0 –10 mg/l) and very hard water (300 mg/l and above) are not suitable for *Clarias* egg incubation and larval rearing. The suitable range of hardness (80 – 91) mgL^{-1} for rearing of *Clarias magur* larvae was recorded by Surnar [14]. A water hardness of 30–60 mg/l $CaCO_3$ is recommended for optimal normal hatching, high viability and maximum larval development of *Clarias gariepinus*. Silva [11] studied the survival of silver catfish and observed that a hardness of 20 mg/l $CaCO_3$ is growth for this fish, however water hardness of 70 mg/l CaCO3 was found unsuitable for this fish. Townsend [18] also obtained a higher survival with larvae of this species exposed to water hardness of 30 mg/l $CaCO_3$, followed by 70 mg/l $CaCO_3$ (increased with Ca^{2+}). The increase of water hardness with MgSO4 up to 400 mg/l CaCO3 (with MgSO4) reduced survival of *Ictalurus punctatus* juveniles to 0%, but when $CaCO_3$ was used to increase water hardness survival was higher (95%) [9] in the present study the highest fry survival (95.33%) was recorded in 150 mg/l water hardness treatment. However, the lowest larval survival (90.33%) was found in 125 mg/l hardness.

The pH of water is very important for the growth and survival of aquatic organisms, and the usual pH range for fish growth is 6.0 to 9.0 [8]. Increasing water hardness to 150-600 mg/l improves the survival of juveniles exposed to very acidic (pH 3.8) and alkaline (pH 10.0-10.5) environments [17]. The present study reveals that growth rates were high over a wide range of pH (6-9) [8]. In conclusion, water pH ranging from (6-9) seemed to be the most effective for growth of *Labeo rohita*. Table 1-4)

The results of the present study have revealed the significant impact of hardness on various water quality parameters and growth of *Labeo rohita* fry. Different water parameters, obviously the level of dissolved oxygen and pH, were found to have decreased with increasing temperature and alkalinity levels. However, the values of EC increased with increasing TDS levels. The levels of salinity were maintained till end of the experiment. In general, the status of experimental water quality indicates that all the observed parameters were within the tolerance limit of fish. Therefore, the variation in growth and survival rate in different treatments could not be assigned to the studied water quality parameters. Thus, the differences in growth rate in different treatment might be due to the variation in hardness levels.

It is evident from the results of this study that the treatments T4 (150 mg/l) has a great potential in aquaculture. In view of the above result and discussion it may be concluded that hardness level of 150 mg/l is more conducive for higher growth (net weight gain, net length gain and survival) of *Labeo rohita* fry. However, it is recommended to conduct further field studies. Dissolved oxygen is a most important parameter. It plays an important role in the respiration process, adequate DO is necessary for good water quality. Previous studies reported [2] stated that the DO concentration of about 5 mg/l is better for fish growth and survival. In the present study DO level 6.25-7.12 mg/l has also favored the better growth performance of *L rohita*, these result compliment previous researcher.

In the present study the total hardness of experimental water was found more effective in the range of 125 to 150 mg/l. The recommended ideal value of hardness for fish culture is at least 20 ppm [15] and a range of 30-180 mg/l [10]. The result of the present investigation has revealed the significant impact of hardness on survival and growth of *Labeo rohita* fry. Stone and Thomforde [12] have given the desirable range of 50-150 mg/l as CaCO3 and acceptable range is above 10 mg/l as $CaCO_3$. According to Bhatnagar [4] hardness values less than 20 mg/l causes stress, 75-150 ppm is optimum for fish culture and >300 mg/l is lethal to fish life as it increases pH, resulting in non-availability of nutrients. However, some euryhaline species may have high tolerance limits to hardness. In the present study reveal that growth rates were high over a wide range of salinity150 mg/l. these result compliments previous researcher.

5. Conclusion

This study clearly showed that the study of hardness level of 150 mg/l recommended for achieving optimum growth and survival of *Labeo rohita*. It is evident that the hardness treatment levels had a positive impact on growth and survival of experimental fish. Further water quality parameters during the experimental period remained favorable for fish growth and survival.

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