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Effect of extreme high and low water pH on survival and behavioral changes of *Labeo rohita*

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Abstract
A microcosm test was conducted to determine the lethal concentration for 50% mortality (LC_{50}) after 96 hours. The LC_{50} at alkaline pH range was from pH 10.4 to pH 10.6. and the acidic range was from pH 4.4 to pH 4.6. Microcosm tests were carried out to investigate how water pH affects aquatic ecosystems. Range-finding test was performed on fish, *Labeo rohita* (weight 5.05±0.02, length 7±0.03 cm) with thirteen different pH values such as 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 7.0, 10.0, 10.2, 10.4, 10.6, 10.8, and 11.0. Fish were fed pelleted feed with 35% crude protein. The control group (pH 7.0) showed better survival. However, pH 4 and pH 10 showed very low survival. Mucus secretion reached elevated levels, and opercular movements increased at both pH 4 and 10. Furthermore, extremely high and low pH levels led to rapid jerking movements and frequent surfacing compared to the control (neutral) pH of 7.

Keywords: Microcosm test, alkaline pH, acidic range, *Labeo rohita*, behavioral changes

Introduction

Aquaculture success depends on an understanding of the chemical and physical properties of water. Fish depend on water to breathe, feed, grow, maintain a salt balance, and reproduce. pH is an essential measure of the acidity of the water and is one of the most significant environmental elements influencing fish farming. The range of the pH scale varies from 1 to 14, 7 being neutral—that is, neither basic nor acidic. Values below 7 are acidic, while values above 7 are basic. The acceptable range for fish culture is typically between pH 6.5 and 9.0. Fish cannot endure long in waters with a pH value lower than 4 or higher than 11. Any alteration in this range triggers biochemical and physiological mechanisms to prevent death. The immature stages of aquatic insects and young fish are susceptible to pH levels. The variation in water pH from the normal range (6.5 to 9.0) will adversely affect fish growth, survival, and reproduction and cause high mortality in aquatic species. The optimum pH range differs among species. Fish can die from rapid pH changes, even if they are within a range where they would otherwise be able to survive. Although fish can alter their body chemistry in response to changes in pH in their surroundings, doing so requires energy that could be utilized for development and reproduction. Fish that are kept in harsh environments and have to maintain a constant internal pH experience stress, which increases their vulnerability to parasites and illness. Therefore, keeping the pH level within an acceptable range is a critical component of water, ensuring optimal fish production.

According to EIFAC (European Inland Fisheries Advisory Commission) criteria (1971), the pH of water safe for fish ranges from 6.5 to 8.5. Monitoring and control of pH are very critical. Indirect effects of pH changes on aquatic life can also be seen in other aspects of water chemistry. A buffering system is crucial in aquaculture as it prevents significant pH fluctuations. Pond pH levels can vary throughout the day from about 4 or 5 to over 10 if there is no way to store the carbon dioxide released during plant and animal respiration. Fish respiration in recirculating systems can cause carbon dioxide levels to rise, interfering with fish's ability to take in oxygen and lowering the water's pH. Without a buffering system, large amounts of weak acid (carbonic acid) will be formed by free carbon dioxide, which could lower the pH level at night to 4.5. The phytoplankton will absorb most of the free carbon dioxide during photosynthesis' peak times, raising the pH level above 10.

Water pH is one of the most critical limiting factors in intensive aquaculture. Commonly, freshwater fish farming occurs in waters at neutral pH or close to neutrality (Boyd, 1998; Copatti *et al*., 2011; Ghanbari *et al*., 2012) [2, 6, 17]. However, the responses to water pH values vary among fish species, and the fish's life stage also affects these responses (Copatti *et al*., 2011; Lemos *et al*., 2018) [6, 27]. This problem transpires where conditions of acidification or alkalinization of water may occur, resulting in the decline of fish populations in the environment and in fish farms. Acidic cations in the soil can produce sulfuric acid when oxygen is present (Zweig *et al*., 1999) [48]. Alternatively, acidic deposition from the atmosphere can cause water acidification (e.g., acid rain).

Aquatic animals, including fish, are susceptible to pH levels in water. Generally, fish require water pH ranges from 6.5 to 8.5, which is considered optimal for good fish growth. The growth of most fish species is affected at pH below 6.0 or above 9.0 (Parra & Baldisserotto, 2007) [36]. Roberts and Palmeiro (2008) [38] reported that pH fluctuations could result in lethargy, stress, skin irritation/lesions, behavioral changes, corneal edema, skin color change, gill irritation, increased mucus production, respiratory signs, and mortality. Changes in pH can disturb the acid-base balance, ion regulation, and ammonia excretion, harming their survival. A study on common carp found that they grow and survive best when exposed to a pH of 7.5-8.0. However, exposure to higher pH (pH 8.5) reduced the survival rate (Heydarnejad *et al.*, 2012) [20]. The embryonic and larval fish stages of fishes are susceptible to pH changes. It was observed that fish grew better at slightly higher pH (pH 7.0-8.0). Heydarnejad *et al*. (2012) [20] showed that the growth parameters of carp increase as water pH increases from 6.0 to 8.0, and the best growth performance occurs at water pH 7.5-8.0. The increase in weight and length of carp at higher pH values agrees with the findings of Menendez *et al*. (1976) [28], who reported that brook trout (*Salvelinus fontinalis*) grow larger at pH 7.1 than at a lower pH value. Common carp achieve optimal survival and growth when exposed to pH values ranging from 7.5 to 8.0 (Heydarnejad *et al*., 2012) [20].

Behavioral observation is a promising tool in toxicity assessments in many species, including fish. Fish behavioral alterations can provide important indices for ecosystem assessment. The behavioral endpoints serve as valuable tools to distinguish and evaluate the effects of exposure to environmental stressors (Kane *et al*., 2005) [24]. The most common symptoms of behavioral abnormalities in zebrafish include imbalance, accelerated respiration, loss of movement and coordination, fish lying at the tank bottom and moving in one spot, subsequent short excitation periods with convulsions and movement in circles, and listlessness before death. The same observations were also reported by Dobsikova *et al*. (2006) [7], Khoshbavar-Rostami *et al*. (2006) [25], and Velisek *et al*. (2009) [45].

Materials and Methods

Experimental Site

The experiment was carried out in the Department of Aquatic Environment Management's Toxicology Laboratory at the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata. The tap water was kept in a circular FRP tank with aeration. The selected fish species were advanced fingerlings of *Labeo rohita*, which were procured from Naihati Fish Market, Naihati, West Bengal.

Experimental Fish

The experimental fish were fingerlings of Rohu (*Labeo rohita*, Hamilton; 1822). The body was moderately elongated. Mouth inferior, and lips thick and fringed with a distinct inner fold. The dorsal fin ends in line with or slightly ahead of the anal fin after inserting anterior to the pelvic fins. Scales are cycloid and moderate in size. Lateral line with 40-44 scales. The body color is blue to brownish along the back and silvery on the sides and belly. During the breeding season, the fins turn black or grey, and each scale has a red mark on it.

Maintenance of Experimental Fish

Uniform size (weight 5.05±0.02 g, length 7.02±0.03 cm) of fingerlings of Rohu fish (*Labeo rohita*) were procured from the Naihati fish market and brought to the laboratory in oxygenated polyethylene bags by local transport. They were carefully transferred to a circular fiberglass reinforced plastic (FRP) tank (size 183.00 cm x 65.30 cm; capacity 500 L) with adequate aeration. During acclimatization, fish were fed pelleted feed twice a day at the rate of 4% of their body weight.

Toxicity studies

Experimental setup for range finding test

The experimental fish (weight 5.05±0.02 g, length 7.02±0.03 cm) were distributed in 40 L capacity glass aquariums (60.9 cm x 30.5 cm x 29.6 cm) covered with mosquito nets to prevent jumping out of the aquarium. The aquarium was first washed very well with potassium permanganate solution @ 5 mg/L overnight, and then they were washed with clean water. The total volume of the water in each aquarium was maintained at 20 L throughout the experiment. The aeration was provided around the clock through an aeration pipe. Observations were made on mortality, disease symptoms, or abnormal behavior of fish. Only healthy fingerlings of rohu of uniform size were used for the experiments, irrespective of sex. For the range finding test (96 hours exposure), 10 aquariums of 40 L capacity each with 20 L of water in each aquarium were arranged in columns and rows according to gradual increase in pH concentration. Ten different pH (pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0) were prepared using 0.5N HCl and 1N NaOH and measured through a pH meter (EU - Tech). pH 7.0 was treated as a control. In each aquarium, 10 fingerlings (weight 5.05±0.02 g, length 7.02±0.03 cm) were taken. There was no feeding for fingerlings throughout the experiment. The fingerlings were restricted to feeding one day earlier than the beginning of the experiment. Observations were taken after 6, 12, 24, 48, and 96 hours, respectively. The cumulative mortality percentage was recorded for 96 hours.

Experimental setup for acute toxicity (LC_{50}) test

The static acute toxicity test of extremely high and low pH was carried out as per the protocol developed by EPA (2002) [10] in a 40 L capacity glass aquarium maintaining 20 L water. Thirteen different pH along with a control (4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 7.0, 10.0, 10.2, 10.4, 10.6, 10.8, 11.0) were prepared based on the range finding tests using 0.5 N HCl and 1 N NaOH and measured by a pH meter (EU-Tech). In each tank, 20 fingerlings (weight 5.05±0.02 g, length 7.02±0.03 cm) were stocked. Feed was not offered to the fish throughout the experiment. This experiment was continued for 96 hours. The physical-chemical parameters of the water were analyzed twice within 96 hours of the experimental period using standard methods of APHA (2005) [1]. The study was carried out in an indoor experimental setup with a 12-hour photoperiod. Observations were taken after 6 hrs., 12 hrs., 24 hrs., 48 hrs., and 96 hours, respectively. The cumulative mortality percentage of fingerlings of rohu was recorded for 96 hours, and the behavioural changes of the fingerlings were also noted. Dead fingerlings were removed from the tank with the help of a scoop net immediately after death. The LC_{50} was analyzed following Probit analysis (Finney, 1971) for extremely high and low pH values on the fingerlings of rohu (*Labeo rohita).*

Water quality parameters

Water quality parameters, like temperature, pH, dissolved oxygen, free carbon dioxide, and alkalinity, were determined following the methods of APHA (2005) [1] during the whole experimental period.

Estimation of Water Quality Parameters

Periodic estimation of quality parameters such as temperature, pH, dissolved oxygen (DO), free carbon dioxide, and alkalinity in the water of the fish tank was estimated following the standard methods (APHA, 2005) [1], as stated below.

Temperature and pH

The water temperature was recorded using a dry bulb centigrade mercury-in-glass thermometer (ranges 0-50 °C) on the spot and expressed as °C. The temperature was estimated by dipping the thermometer below the water surface of each tank. The pH in water was measured by a digital pH meter (EU-Tech) for all the experimental tanks. Determination of pH was done by dipping the electrode of the pH meter into the tank water, and the readings were observed. The reading was taken when the reading was fixed.

Dissolved Oxygen (DO)

Dissolved Oxygen (DO) was estimated using Winkler's iodometric method. The water samples were collected on the sampling day and immediately fixed with manganous sulfate and alkaline-iodide-azide solution. Brown precipitation appeared, and the bottles were brought to the laboratory in this condition. The sample was titrated against 0.025 (N) sodium thiosulphate solution using starch as an indicator after the precipitation was dissolved by conc. H2SO4. The following formula was used to calculate the DO content in the water sample, which was then expressed as mg/l.

Free Carbon Dioxide

Free carbon dioxide was determined by the titrimetric method of APHA (2005) [1]. The 50 mL water sample was taken in a 100 mL conical flask. Then, 2-3 drops of phenolphthalein indicator were added to it. If the sample was colorless, then the sample was titrated rapidly against 0.02 (N) sodium hydroxide (NaOH). The endpoint was indicated by the change of the colorless sample to pink, and the result was expressed as mg/ l. The carbon dioxide was calculated using the following formula:

Total Alkalinity

The total alkalinity of the water sample collected from the tank/aquarium was estimated by acid-base titration. The 50 mL water sample was taken in a 100 mL conical flask. After that, 2-3 drops of methyl orange indicator were added. It was then titrated against 0.02 (N) sulphuric acids. After calculation, the endpoint was indicated by the change of color from orange to red-pink and expressed as mg/ l as $CaCO_3$.

Results

Acute toxicity [LC_{50}] of *Labeo rohita* exposed to low and high pH in water

Acute toxicity

Before conducting tests of acute toxicity on the rohu fingerlings (*Labeo rohita*) using extremely low and high pH levels, a range-finding test was executed with 13 diverse pH values such as 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 7.0, 10.0, 10.2, 10.4, 10.6, 10.8, and 11.0. This preliminary test identified the pH levels 4.4 and 4.6 as low pH, while 10.4 and 10.6 as high pH levels. During the subsequent acute toxicity tests, the cumulative mortality of the Rohu fingerlings was recorded under these extreme pH conditions. Results are presented in Figures 1 and 2, showing the LC_{50} values for extremely high and low pH.

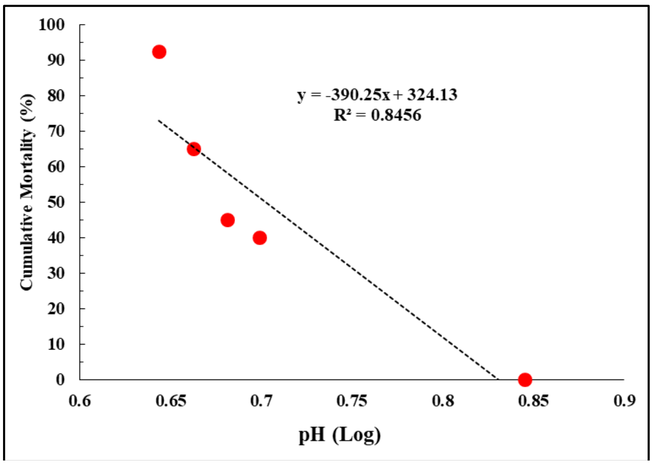

Fig 1: Graph showing the Probit line for determination of 96 h LC_{50} of *Labeo rohita* at low pH

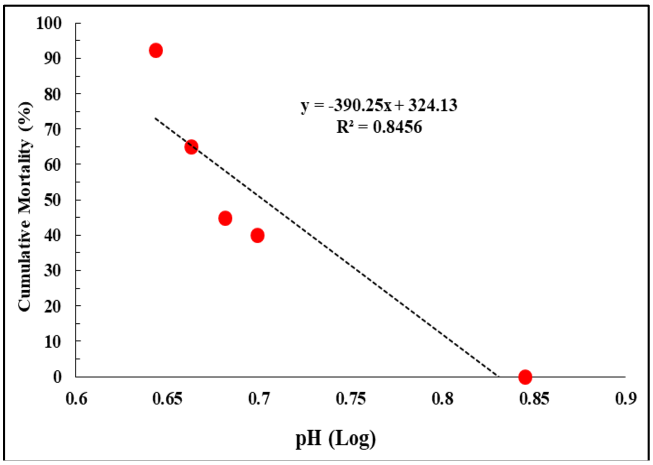

Fig 2: Graph showing the Probit line for determination of 96 h LC_{50} of *Labeo rohita* at high pH

Water quality parameters during acute toxicity test

In our current study, we maintained an identical temperature of 29 °C across all pH levels during the 96-hour acute toxicity test on the fingerlings of *Labeo rohita* (Table-1). Here are the key observations:

Dissolved Oxygen (DO) Content

- DO levels decreased in both acidic pH (4.5 and 5.0) and alkaline pH (9.5 and 10.0) compared to the neutral pH level (pH 7).

Alkalinity

- Alkalinity was higher at pH 9.5 and 10 compared to other pH levels after 96 hours of the experiment.

Ammonia-Nitrogen (s) and Free CO_2

- NH3-N and free CO_2 concentrations were higher at pH 4.5 and 10 than at other pH levels after the 96-hour acute toxicity test (as shown in Table - 1).

Table 1: Water quality parameter after 96 hrs of LC_{50} test of *Labeo rohita* at different pH levels

pH Values	Temperature (°C)	DO (mg/L)	Alkalinity (mg/L)	NH_3-N (mg/L)	Free CO_2 (mg/L)
pH 4.5	29 - 30	4.8 – 5.4	139 - 145	0.10 – 0.13	3.9 – 4.1
pH 5.0	26 -29	6.1 – 6.8	158 - 164	0.07 – 0.09	3.5 – 3.7
pH 7.0 Control)	26- 30	7.4 – 8.1	176 -187	0	0
pH 9.5	28 - 29	5.8 – 6.5	248 - 256	0.05 – 0.08	0.38 – 0.42
pH 10.0	29 - 30	5.2 – 5.9	257 - 265	0.25 – 0.30	0.64 – 0.73

Behavioural and morphological changes of test fish

During the acute toxicity test, the exposed fish exhibited noticeable clinical toxicity signs, varying severity depending on the high and low pH stresses. In our study, we observed several behavioural changes in the fish, such as increase in opercular movements, loss of equilibrium, frequent surfacing, increased mucus secretion, rapid jerking movements, erratic swimming etc., additionally, changes in body color were observed in the exposed fish. Notably, during the initial hours after exposure to high and low pH levels, the fish showed hyperactivity and erratic movements within the aquarium. Mucus secretion reached elevated levels, and opercular movements increased at pH 4 and 10. Furthermore, extremely high and low pH levels led to rapid jerking movements and frequent surfacing compared to the control (neutral) pH of 7 (Table 2 and 3). Interestingly, the fish's color also transformed at high pH levels, turning whitish due to alkalinity. As the extremity of pH increased (low and high), behavioral irregularities in the exposed fish became more pronounced, demonstrating a positive correlation. The control group of fish (maintained at pH 7) did not exhibit abnormal behaviors during the study.

- In a highly acidic environment, the fish assumed a diagonal posture, with its head facing the water's surface.
- The exposed fish became lethargic and occasionally jerked violently before succumbing.
- Fish subjected to lethal basic pH levels exhibited restlessness and rapid movements, often striking out with the tips of their tails.
- Fish exposed to extremely low pH displayed irregular swimming patterns.

These findings highlight the significant impact of pH extremes on fish behavior and health.

Table 2: Behavioural and morphological responses of *Labeo rohita* exposed to extremely low pH (pH 4 to pH 5) compared to pH 7.

Behavioural/ Morphological changes	pH exposure levels						
	pH 7	pH 4	pH 4.2	pH 4.4	pH 4.6	pH 4.8	pH 5
Opercular movement	-	+ + + + + + +	+ + + + + + +	+ + + + + +	+ + + + +	+ + + +	+ + +
Jumping tendency	-	+ + + + + + +	+ + + + + +	+ + + + + +	+ + + + +	+ + + +	+ + +
Erratic swimming	-	+ + + + + + +	+ + + + + +	+ + + + +	+ + + +	+ + +	+ + +
Mucus secretion	-	+ + + + + + +	+ + + + + +	+ + + + +	+ + + +	+ + +	+ + +
Loss of equilibrium	-	+ + + + + +	+ + + + +	+ + + + +	+ + + +	+ + +	+ + +
Rapid jerk movements	-	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + +	+ + + +	+ + +
Frequent surfacing*	-	+ + + + + + +	+ + + + +	+ + + + +	+ + + +	+ + + +	+ + +
Changes in body colour	-	+ + + + +	+ + + + +	+ + + +	+ + +	+ + +	+ + +

Table 3: Behavioural and morphological responses of *Labeo rohita* exposed to extremely high pH (pH 10 to pH 11 compared to pH 7).

Behavioural/ Morphological changes	pH exposure levels						
	pH 7	pH 10	pH 10.2	pH 10.4	pH 10.6	pH 10.8	pH 11
Opercular movement	-	+ + +	+ + + +	+ + + + +	+ + + + + +	+ + + + + + +	+ + + + + + +
Jumping tendency	-	+ + +	+ + + +	+ + + + +	+ + + + + +	+ + + + + +	+ + + + + + +
Erratic swimming	-	+ + +	+ + +	+ + + +	+ + + + +	+ + + + + +	+ + + + + + +
Mucus secretion	-	+ + +	+ + +	+ + + +	+ + + + +	+ + + + + +	+ + + + + + +
Loss of equilibrium	-	+ + +	+ + +	+ + + +	+ + + + +	+ + + + +	+ + + + + +
Rapid jerk movements	-	+ + +	+ + + +	+ + + + +	+ + + + + +	+ + + + + +	+ + + + + +
Frequent surfacing*	-	+ + +	+ + + +	+ + + +	+ + + + +	+ + + + +	+ + + + + + +
Changes in body colour	-	+ + +	+ + +	+ + +	+ + + +	+ + + + +	+ + + + +

Discussion

In a related study by Zahangir *et al*. (2015) [47], lethal toxicity tests were conducted on zebrafish using pH levels ranging from 2.0 to 12.0. The 72-hour LC_{50} value for low pH was 3.9, while for high pH, it was 10.8. Interestingly, no mortality occurred at pH 5.0 or higher; similarly, no mortality was observed at pH 9.0 or lower. These findings align closely with our present results. The zebrafish exhibited symptoms such as loss of balance, floating upside down, and 100% mortality at very low pH levels (pH 3.0 and below) and very high (pH 11.5 and higher). Respiratory distress was evident through gaping mouths, forced expansion of gill opercula, and reduced swimming activity at pH levels below 4.0 and above 11.0. However, at pH 5.0 and 10.0, these symptoms gradually subsided within 6 to 12 hours, and the fish returned to normal behavior during the experimental period. Exposure of eggs of Atlantic salmon to a range of high pH values showed no excess mortality for eggs exposed to pH 9.70 or pH 11.34, while total mortality was observed for eggs kept in water with pH 12.3 (Foldvik *et al*., 2022) [15]. Zweig *et al.* (1999) [49] recommended pH ranges for salmonid production were 6.4–8.4 and 6.7–7.5. Saleh *et al.* (2013) [41] showed high mortality in larvae exposed to high pH (9, 9.5). Reduced oxygen consumption in fish exposed to alkaline water was observed by Murthy *et al.* (1981) [30], and mortality was raised due to difficulties in oxygen uptake by gills and disturbances in oxygen transport by blood. Similar changes were observed by Jezierska (1988) [23] in fish exposed to an alkaline environment. Muniz *et al*. (1978) [18] reported that disturbances in ionic regulation, especially loss of sodium, were a direct cause of fish mortality. Wilson *et al*. (1999) [46] noted high mortality in larvae when exposed to low pH. The cause of mortalities that occur to fishes at low pH levels is the imbalance between the place where the fishes live and the content of body fluids (Wilson *et al.,* 1999) [46]. Most organisms possess a well-defined range of pH tolerance. Mortality of the fry, especially in an extremely low pH of 4 and an extremely high pH of 10, could be attributed to internal respiration practiced by the fry whereby there is direct contact between the solution and the internal organs of the fish, which led to the immediate destruction of the fry after 24 hours at pH10 and 192 hours at pH 4 (Ndubuisi *et al.*, 2015)) [32]. A similar study conducted on *Clarias gariepinus* hatchlings confirms the ability of *C. gariepinus* hatchlings to tolerate extreme pH of 4 and 10. This is achieved due to cutaneous respiration carried out by the hatchlings, which minimizes their contact with the toxic solution (Ndubuisi *et al.,* 2015) [32]. Meanwhile, Boyd (1982) [3] and Gaunder (2005) [16] observed that fish's acid and alkaline death points are about pH 4 and 11, respectively, with reproduction and growth diminishing with increasing acidity or alkalinity. The median lethal pH of 4.3 and 9.2 were recorded for the fry bluegill sunfish (*Lepomis machrochinus*), rainbow trout, roach, and goldfish in acidic and alkaline treatment, respectively (Ndubuisi *et al.,* 2015) [32].

Findings of current study align with a study by Sahu *et al.* (2018) [40], where they observed significant impacts on water quality parameters during acute toxicity tests on *Trichogaster lalius* exposed to different pH levels.

Prochilodus lineatus larvae died quickly when exposed to a pH range between 3.7 and 4.4, and no larvae survived after 42 hours (Ndubuisi *et al.*, 2015) [32]. Ferreira *et al*. (2001) [12] observed total mortality of jundia (*Rhamdia quel*en) eggs incubated at pH 4.0. After 72 hours, fish survival was practically zero at pH 4.4–4.6, demonstrating that *Prochilodus lineatus* larvae are susceptible to pH values lower than 4.6. Survival at 4.8–5.6 and 8.7–9.2 was like the reference pH. However, final survival was slightly different and directly related to mortality intensity in the initial 6 hours of the experiment. Norrgren and Degerman (1992) [34] observed that Atlantic salmon (*Salmo salar*) are sensitive to low pH values, and total larval mortality occurs at pH 5.1. Similarly, Jezierska and Witeska (1995) [22] observed total mortality in *Cyprinus carpio* larvae at pH 5.5. For C*atostomus commersoni*, yolk sac larvae mortality was registered at a pH below 4.99, whereas *Micropterus dolomieui* juveniles presented mortality below 4.98 (Holtze & Hutchinson, 1989) [21].

Most of the teleost exposed to acidic or alkaline waters showed higher survival in hard rather than in soft waters, as demonstrated for recently hatched *Oncorhynchus mykiss* larvae (Parra & Baldisserotto, 2007) [36] and *R. quelen* juveniles (Towsend & Baldisserotto, 2001) [44]. The critical pH points for acid and alkaline stress in fish were approximately pH 4 and 11, respectively. As acidity or alkalinity increased, both reproduction and growth were adversely affected. These findings align with Boyd's (1982) [3] and Gaunder (2005) [16] research. A study on common carp found that they grow and survive best when exposed to a pH of 7.5-8.0. However, exposure to higher pH (pH 8.5) reduced the survival rate (Heydarnejad *et al*., 2012) [20]. The embryonic and larval fish stages of fishes are susceptible to pH changes. It was observed that fish grew better at slightly higher pH (pH 7.0-8.0). However, very high pH levels had a detrimental effect on survival. Exposure to alkaline waters caused an increase in plasma ammonia, which is toxic to fish. Alkaline conditions (pH > 9) can contribute to fish mortality through gill damage, decreased plasma ion concentrations, and decreased NH3 elimination. Thus, the primary cause of observed death at an alkaline pH is the reduction of ammonia excretion and increased ion loss (Heydarnejad *et al*., 2012) [20]. Townsend and Baldisserotto (2001) [44] said fingerling deaths were not observed at pH 9.5. However, the mortality of fingerlings exposed to this pH and water hardness of 30 mg/L as $CaCO_3$ was 50%. Therefore, mortality as a function of alkaline pH can seem to vary according to the silver catfish population tested. Survival of silver catfish fingerlings at pH 10.0 and 10.5 was improved by increasing water hardness (Townsend & Baldisserotto, 2001) [44].

Behavioral observation is a promising tool in toxicity assessments in many species, including fish. The behavioral endpoints serve as valuable tools to distinguish and evaluate the effects of exposure to environmental stressors (Kane *et al*., 2005) [24]. The most common symptoms of behavioral abnormalities in zebrafish include imbalance, accelerated respiration, loss of movement and coordination, fish lying at the tank bottom and moving in one spot, subsequent short excitation periods with convulsions and movement in circles, and listlessness before death. Identical observations were also reported by Dobsikova *et al.* (2006) [7], Khoshbavar-Rostami *et al.* (2006) [25], and Velisek *et al.* (2009) [45]. Nagelkerken and Munday (2016) [31] and Leduc *et al.* (2013) [26] have previously shown that ocean acidification can differ animal behaviors that are related to cognition and physiology, such as lateralization, activity levels, swimming behavior, learning, boldness, schooling, reproduction, and foraging. The primary inhibitory receptor in the vertebrate brain is the GABAA (Y- Amino butryricacid) neuroreceptor, which has been linked as a driver of modified behaviors due to its altered functioning under elevated CO_2 (Smith, 1983; Nilsson *et al.*, 2012 and Hamilton *et al*., 2014) [43, 33, 19]. The sensory capabilities of fish are adapted to accommodate the unique characteristics of the aquatic environment. Fish use one or more sensory cues and different sensory mechanisms to gain information about their environment and guide their behavior (Simpson *et al*., 2011; Rossi *et al*., 2015; Ferrari *et al*., 2012; Chung *et al*., 2014; Munday *et al*., 2009; Pistevos*et al*., 2017; Caprio *et al*., 2014) [42, 39, 11, 5, 29, 37, 4]. The pH of the aquatic environment can be stressful to fish, and the alteration in pH can affect multiple sensory modalities in larval and post-settlement stage fishes, such as audition, vision, olfaction, and pH sensing (Zahangir *et al*., 2015) [47]. Roberts and Palmeiro (2008) [38] reported that pH fluctuations could result in lethargy, stress, skin irritation/lesions, behavioralchanges, corneal edema, skin color change, gill irritation, increased mucus production, respiratory signs, and mortality. Additionally, Sahu *et al*. (2018) [40] reported that juveniles of *Trichogasterlalius* exhibited gulping, erratic swimming, jumping, and increased mucus secretion at extremely high and low pH levels. The milky color of the water resulting from this mucus secretion ultimately led to the death of the fish.

Similarly, Ndubuisi *et al*. (2015) [32] observed mortality in the fry of *Clarias gariepinus* at extremely low pH (pH 4) and extremely high pH (pH 10) due to internal respiratory distress. During the study, there was an abrupt mortality of *Clarias gariepinus* fry due to direct contact between the solution and the fish's internal organs. This occurred 24 hours after stocking at pH 10 and 92 hours after stocking at pH 4. Another study involving *Clarias gariepinus* hatchlings confirmed their ability to tolerate extreme pH levels of 4 and 10.

Conclusion

The pH level of water is an essential parameter for fish survival. Changes in pH may alter the behavioral pattern of fish. Also, Changes in pH can influence other water parameters. For instance, pH affects the solubility of minerals, nutrient availability, and the toxicity of certain substances. Fish health and behavior are closely tied to water pH. Even slight fluctuations in water pH can directly or indirectly affect various physico-chemical parameters, creating a delicate balance. Therefore, maintaining an optimal pH in water is crucial for fish survival. The present investigations involving rohu fish (*Labeo rohita*) revealed critical pH levels. The LC_{50} occurred at pH 4.4–4.6 and pH 10.4–10.6. Extreme pH levels (both low and high) adversely affected the survival and behavior of fish.

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