Int J Adv Life Sci Res. Volume 8(2) 27-40
https://doi.org/10.31632/ijalsr.2025.v08i02.003

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

Assessing the Survival Challenges in Zebrafish Due to Acute Salinity Stress and pH Changes

Rutupurna Das and Gagan Kumar Panigrahi*

[1]*Department of Zoology, School of Applied Sciences, Centurion University of Technology and Management, Bhubaneswar, Odisha, India*

**Corresponding Author's Email: gagan.panigrahi@cutm.ac.in*

Abstract

Background: Water parameters are essential in maintaining the good health of aquatic organisms. Fluctuation in any of the parameters is a stress factor for the organism. Different stress agents' responses initiate the hypothalamus's activation and subsequent changes in the neuroendocrine system, metabolism, and physiology. The environment in various aquatic habitats heavily influences fish. **Objectives**: The purpose of this research is to analysis the survival and behavioural changes in zebrafish exposed to stress. **Methods**: In the current study, zebrafish were exposed to varying pH levels, and the resulting stress responses were observed. The acute toxicity and chronic effects of extreme pH 4.2, 8.2, and 10.2 are effectively seen on zebrafish gills. The acute toxicity and chronic effects of extreme salinity (6 g/L, 7 g/L, 8 g/L, 9 g/L, and 10 10g/L are effectively seen in zebrafish's heart tissue. **Result**: Zebrafish have substantial stress responses to variations in salinity, which cause changes in physiology and behavior. Salinity stress occurs when fish experience fluctuations in the salinity content of their aquatic surroundings. Excessive salinity can dehydrate freshwater fish and lead to an imbalance in ions; however, abrupt salinity reductions in saltwater fish can induce an influx of water and a loss of ions. Fish exposed to prolonged salt stress are more sensitive to illness, have lower general fitness, and have a lower chance of surviving. **Conclusion**: This study enlightens how water quality parameters like pH and salinity affect specific physiological and molecular events in zebrafish. In summary, exposure to sublethal pH and salinity concentrations influences zebrafish physiology, resulting in mortality.

Keywords: *fish health, mortality, pH, salinity, toxicity, Zebrafish*

Introduction

Stress is a condition that causes decreased fitness due to external factors that test an organism's homeostatic vitality and threaten species' survival (Avdesh *et al.*, 2012). The hypothalamus initiates the anti-stress response system by activating the neuroendocrine system, which in turn activates the metabolic and physiological systems. Stress negatively impacts behavior, development, reproduction, immunological function, and illness resistance (Das and Panigrahi, 2025). The degree of reaction in physiological and behavioral systems differs among animals and individuals (Sawant *et al.*, 2001). Adults, juveniles, and even fish larvae adapt well to environmental changes due to stress (Zahangir *et al.*, 2015). Because of their unique physiologies, all creatures, including fish, develop and grow differently. Endocrine, neurological, metabolic, and ecological connections all have a role in physiology. Physiological influences, alone or in combination, can influence or alter human physiology, particularly early development (Portz *et al.*, 2006). Salinity, among other external elements, is vital to fish because it influences osmolality and metabolism (Patro *et al.*, 2024). It alters activity, structure, and physiology, eventually influencing fish growth, habits, survival, and dispersion. Although some fish can give birth to new ones, most fish require in vitro fertilization and encounter several hurdles during fertilization and

Received on :9th July 2024; Revised version received on :22nd February 2025; Accepted: 15th March 2025

early development in their aquatic habitat. Furthermore, environmental risks, pollutants, and external variables contribute to lower egg production and juvenile survival rates (Ni *et al.*, 2019). The most common environmental stressors impacting ecosystem structure and function are abiotic stressors (temperature, cold, foreign substances, etc.) and biotic stressors (pathogens, predators, invasive species, etc.). Abiotic stress is caused by physical or chemical attacks from the environment in fish, whereas biotic stress is caused by biological attacks that a fish may encounter throughout its existence (Singleman *et al.*, 2014). Several physical, chemical, and biological stresses pose a potential hazard to freshwater ecosystems. As a result, various stressors continually endanger freshwater ecosystems. pH fluctuations represent a stressor for aquatic life like fish. Sudden pH changes can kill aquatic organisms, disrupting acid-base balance and ion regulation. Fish are highly stressed by pH fluctuations, which upset their internal physiological balance and general health. Fish species have developed to live in particular pH ranges suited to their natural environments. The fish are unable to maintain the body's homeostasis at sudden or drastic variations from these ideal pH levels (Chen *et al.*, 2017). Long-term changes in pH can upset the delicate equilibrium of the aquatic environment and cause various physiological and behavioral changes in fish. Fish can have extreme stress reactions when exposed to drastic pH changes. As a result, there are instant physiological changes due to disturbances in ammonia excretion, ion control, and acid-base balance. Fish having difficulty adjusting to the new environment may display unpredictable behavior, faster breathing rates, and changed swimming patterns (Sawant *et al.*, 2001). Zebrafish is one of the most popular model organisms used for research. It is a tropical fish that lives in rice paddies in Asia. Critical ethical concerns surrounding human experimentation have led to the use of animal models in medical research (Cleal *et al.*, 2020; Andrade *et al.*, 2017; Magalhaes *et al.*, 2012). In Latin, zebrafish are commonly known as "Danio rerio." It is commonly known as a zebrafish because its striped body resembles a zebra (Singh *et al.*, 2024). The stripe is dark blue and horizontal, flowing the length of the body from the caudal fin to the gills (Farhana *et al.*, 2019). Researchers widely use zebrafish due to their numerous benefits. Zebrafish are very robust and simple to care for. Since they occur naturally in ponds, they can easily grow in ideal conditions by changing the environment. Furthermore, they are very inexpensive (Mondal *et al.*, 2024; Sahoo *et al.*, 2024). However, it requires more space than other model organisms, such as flies (Panigrahi *et al.*, 2024). This model is also vertebrate, which makes it better than others (Zahangir *et al.*, 2015). Zebrafish have a high fertility rate, laying hundreds of eggs each week (Sampaio and Freire, 2016).

The generation time is also very short, giving scientists an infinite supply of this model (Patro *et al.*, 2023; Das *et al.*, 2025). This expedites the entire experimental process and is essentially a useful production method. Zebrafish share 70% of their genes with humans, and 84% of disease-associated genes have zebrafish counterparts (Killen *et al.*, 2013). The genome of zebrafish has been fully sequenced, with over 140,000 genes mutated to study their role in development and disease. Zebrafish are particularly useful as animal models due to their low cost, short breeding time, and relative similarity to humans (Schweizer *et al.*, 2022). Zebrafish share many of humans' genes, tissues, and organ systems. Because zebrafish are more like humans than invertebrate models (such as the nematode Caenorhabditis elegans or the fly Drosophila melanogaster), discoveries made in zebrafish are more likely than discoveries made in invertebrate systems to be directly applicable to humans. The fish are unable to maintain the body's homeostasis at sudden or drastic variations from these ideal pH levels (Sahoo *et al.*, 2023). Long-term changes in pH can upset the delicate equilibrium of the aquatic environment and cause various physiological and behavioral changes in fish. Fish can have extreme stress reactions when exposed to drastic pH changes. As a result, there are instant physiological changes due to disturbances in ammonia excretion, ion control, and acid-base balance. Fish having difficulty adjusting to the new environment may display unpredictable behavior, faster breathing rates, and changed swimming patterns (Chowdhury and Saikia, 2023). This study results in the exposure to sublethal pH and salinity concentrations influencing zebrafish's physiology, resulting in zebrafish mortality.

Material and Methods

Zebrafish facility

Hundreds of adult wild-type zebrafish (5-6 months post-fertilization (mpf)) were procured from the Central Institute of Freshwater Aquaculture (ICAR-CIFA). The wild-type adult zebrafish had a weight of 0.9 ± 0.1g. They were acclimated to laboratory settings for eight weeks in a 30L stock tank equipped with good-quality filters. The aquarium water conditions were maintained daily with a pH of 7±0.2, a temperature of 27±1°C, and a conductivity of 490-510 S/cm (Table 1). The aquarium was kept on a 14-hour light/10-hour dark cycle, with lights turned on at 9:00 AM (Avdesh *et al.*, 2012). They were fed commercial processed dry feeds (Optimum Tropical Fish Food—Mini Pellet) twice daily during the trial. We periodically clean the setup to prevent infection, once a week. After the acclimation period, the actual experiment began.

Feeding

Fish were fed twice a day with commercial floating feed.

Water quality parameters

Table 1: Different water quality parameters such as pH, temperature, salinity, dissolved oxygen.

Sl. No.	Parameter	Ranges	Device/ method used
1	Salinity	0.2 mg/L	Conductivity meter
2	pH	6.8-8.2	pH meter
3	Temperature	27-32 °C	Normal thermometer
4	Dissolved oxygen	3.9-8.5mg/L	Light and dark bottle method

Experimental design

pH stress

For the experiment, the seven 8-inch bowls with 3L water capacity were placed for the experimental setup. Three wild-type adult zebrafish weighing 0.9 ± 0.1g were placed in each bowl with the same circumstances as in the stock aquarium, along with an aerator pump. The first part of the experiment measured the zebrafish's tolerance to different pH water levels for 72 hours. There was a glass bowl with tap water as a control. In three glass bowls, acidic water concentrations of pH 4.2, 5.2, and 6.2 were built up with tap water using acetic acid. Similarly, sodium hydroxide was used to prepare alkaline water using tap water in another three glass bowls to create a range of concentrations, including pH 8.2, 9.2, and 10.2. The pH range of the tap water was 7.2–7.4, with 7.2 acting as the reference point. Three wild-type zebrafish were chosen at random and quickly moved into each of the experimental bowls that had aeration. CHEMI LINE Technologies, a portable pH meter, was used to measure the pH of water. The pH of the water was measured twice a day, in the morning and evening, and the pH of the basic and acidic baths was tested and kept within a ±0.1-unit range. Individual fish survival periods in the different pH concentrations were noted. The fish was considered dead based on its complete lack of response to external stimuli and its function of opercular beats.

Salinity Stress

Ten eight-inch glass bowls were taken. The bowls were named C, E1, E2, E3, E4, E5, E6, E7, E8, and E9, respectively, and 3 liters of water were filled in each bowl. The collected specimens (zebrafish) were in each bowl (3 adult fish per bowl). The fish were allowed to acclimate to their environment for 3–4 days. They were fed twice a day, and their behaviour was observed. After seven days, it was replaced with the following prepared solutions (the water in the controlled bowl was replaced with normal tap water). 3 liters of solutions were prepared in 3 different containers by adding 1g/L, 2g/L, 3g/L, 4g/L, 5

/L, 6g/L, 7g/L,8g/L,9g/L and 10 g/L to replace the water of E1, E2, and E3, E4, E5, E6, E7, E8, and E9, respectively. They were kept under observation for 7 days.

Result

Histology of Zebrafish

The dead zebrafish were dissected immediately after death. Then, the different parts of the zebrafish were seen under the microscope, and pictures were taken. Then, all the images were compared with the images of the control fish to distinguish the difference between each stress concentration level.

Behavioural study

There was no mortality of naïve fish during the acclimatization period before stress exposure. We observed the behaviour and morphology during the experimental days by comparing the stressed zebrafish with the control. We noted the information and recorded the survival period of fish post-stress (Table 2). According to the observations, the concentrations of acetic acid and sodium hydroxide that resulted in pH values of 4.2 and 10.2, respectively, were the top fatal limits of pH for zebrafish. Fish deaths were noted after exposure to different acidic and alkaline pH values (Table 2). Fish gills and skin were heavily coated in mucus, and the gills' respiratory epithelium was damaged mainly at acidic pH extremes. The fish showed hypoactivity while taking a diagonal position with its head toward the water's surface in the acutely acidic atmosphere (Table 2). It then became lethargic and occasionally shaken violently before passing away. When fish encountered a deadly basic pH, they showed restlessness, swimming quickly and slapping their tail area.

Table 2: Mortality record of fish after exposure to pH stress.

Experimental sets	pH	Mortality after stress (in hours)			
		Zebrafish (N=3)	**Fish 1**	**Fish 2**	**Fish 3**
Control	7.2	3	---	---	---
Set I	4.2	3	15	20	24
Set II	5.2	3	28	40	48
Set III	6.2	3	36	60	---
Set IV	8.2	3	40	65	---
Set V	9.2	3	28	36	45
Set VI	10.2	3	14	18	23

Additionally, it was observed that fish exposed to pH 4.2 and 10.2 initially had slow swimming patterns and a pale appearance after about an hour. There was decoloration of stripped line on the skin of zebrafish due to the extreme stress levels. Decrease the spontaneous activity of the fish towards taking feed. Fish showing higher opercular ventilator movements, with possible open mouth (Hyperventilation). Between 24 and 48 hours of exposure, all specimens died at pH 4.2 and pH 10.2. On the other hand, 70% of the fish perished at pH 5.2 and 9.2 within 48 hours of exposure. The zebrafish housed at all other pH levels 6 - 8 exhibited less appreciable behavioral changes than other extreme pH conditions.

During the observation period, some fish face mortality. We have given behavior post-stress information in the table below (Table 3).

Table 3: Effect of pH on the physiology of zebrafish.

Experiment Setup No.	Stress agent (pH)	Symptoms observed in zebrafish after stress
Control	7.2	Greyish skin color with striped lines, Normal behavior
Set I	4.2	Lighten skin color, hyperventilation, decrease in spontaneous activity, and swim slowly.
Set II	5.2	Lighten skin color, hyperventilation, Decrease in spontaneous activity, abnormal swimming behavior.
Set III	6.2	Slightly change in skin color, normal ventilation Decrease in feeding activity and abnormal swimming behavior.
Set IV	8.2	Slightly change in skin color, normal ventilation Decrease in feeding activity, abnormal swimming behavior
Set V	9.2	Lighten skin color, hyperventilation Decrease in spontaneous activity, abnormal swimming behavior
Set VI	10.2	Lighten skin color, hyperventilation Decrease in spontaneous activity, swimming at the bottom.

No fish fatalities occurred among the naïve ones in the acclimation period preceding stress exposure. Throughout the trial, we monitored their behavior and physical characteristics and contrasted the stressed Zebrafish with the control group. We recorded the fish's post-stress survival duration (Table 4). Our observations determined that the crucial limits for zebrafish mortality are higher salinity concentrations of 6g/L,7g/L, 8g/L, 9g/L, and 10g/L, respectively. Fish died after being exposed to different concentration levels, both alkaline and acidic (Table 5). Fish with extreme salinity values significantly damaged the cardiac system, as swollen heart tissue was seen. Fish in high salinity conditions were hypoactive, arranging themselves diagonally and pointing their heads toward the water's surface (Table 5). They then grew listless and occasionally trembled violently before passing away. By contrast, the fish were restless when exposed to a salty environment, which resulted in fast swimming and tail slapping.

Table 5: Mortality record of fish after exposed to salinity stress.

Experimental sets	Salinity	Zebrafish (N=3)	Mortality after stress(after exposure to stress)		
			Fish1	**Fish2**	**Fish3**
Control	0g/L	3	---	---	---
Set I	1g/L	3	---	---	---
Set II	2g/L	3	---	---	---
Set III	3g/L	3	---	---	---
Set IV	4g/L	3	---	---	---
Set V	5g/L	3	6th day(122hr)	6th day(128hr)	6th day(134Hr)
Set VI	6g/L	3	5th day(98hr)	5th day (106hr)	5th day(112hr)
Set VII	7g/L	3	4th day(76hr)	4th day(84hr)	4th day (92hr)
Set VIII	8g/L	3	3rd day(56hr)	3rd day(65hr)	3rd day(70hr)
Set IX	9g/L	3	2nd day(32hr)	2nd day(40hr)	2nd day(48hr)
Set X	10g/L	3	1st day(15hr)	1st day(20hr)	1st day(26hr)

Additionally, it was observed that fish exposed to salinity 8g/L, 9g/L and 10g/L initially had slow swimming patterns and a pale appearance after about an hour. There was decoloration of stripped line on the skin of zebrafish due to the extreme stress levels. Decrease the spontaneous activity of the fish towards taking feed. Fish showing higher opercular ventilator movements, with possible open mouth (Hyperventilation) and Mouth and opercular movements at the water surface, resulting in the intake of water and air (gulping), Rotation around a long axis; erratic movements, often in Bursts (Corkscrew swimming), Fast reflex expansion of mouth and operculate not at water surface assumed to clear ventilator channels (coughing) (Table 3). Between 24 and 48 hours of exposure, all specimens died at 8g/L, 9g/L and 10 g/L.

Table 6: Symptoms observed in zebrafish after exposure to different salinity concentrations.

Experimental sets	Salinity	Symptoms observed in zebrafish after stress exposure
Control	0g/L	Greyish skin color with striped lines, Normal behavior
Set I	1g/L	Lighten skin colour and hypoventilation, decrease spontaneous activity, and swim normally.
Set II	2g/L	Lightened skin color, hyperventilation, Decreased spontaneous activity, and normal swimming behavior.
Set III	3g/L	Slightly change in skin colour, normal ventilation Decrease in feeding activity and abnormal swimming behavior.
Set IV	4g/L	Slightly change in skin colour, normal ventilation Decrease in feeding activity, abnormal swimming behavior
Set V	5g/L	Lighten skin colour, hyperventilation Decrease in spontaneous activity, abnormal swimming behavior
Set VI	6g/L	Lighten skin colour, hyperventilation Decrease in spontaneous activity, swimming at the bottom.
Set VII	7g/L	Hyperventilation, Coughing, Abnormal skin pigmentation(lightened),
Set VIII	8g/L	Loss of schooling / shoaling behavior, Abnormal surface distribution/behavior
Set IX	9g/L	Abnormal skin pigmentation(lightened), Gulping, Corkscrew swimming, mucus secretion, Aggression
Set X	10g/L	Abnormal horizontal orientation, Loss of buoyancy control, Abnormal surface behaviour, hyperventilation

Comparative studies of zebrafish exposed to different stress

All the zebrafish exposed to different pH showed different morphological changes compared to the control. There were clear blue fluorescent lines on the body in control, while in highly acidic exposure, these lines were not seen in the body of the zebrafish (Figure 1a). In the alkaline water exposure, stripped lines were present on the bodies of zebrafish (Figure 1a). The black eye lens is discolored into fully white at a high acidic pH of 4.2 but less at pH 5.2 and 6.2 (Figure 1a). The discoloration of the eye lens is not seen in fish at pH 8.2, 9.2, and 10.2. In the red circle, there are also black spots on the gill area of the fish body at acidic pH, which shows that the gills may be highly affected by the acidic pH.

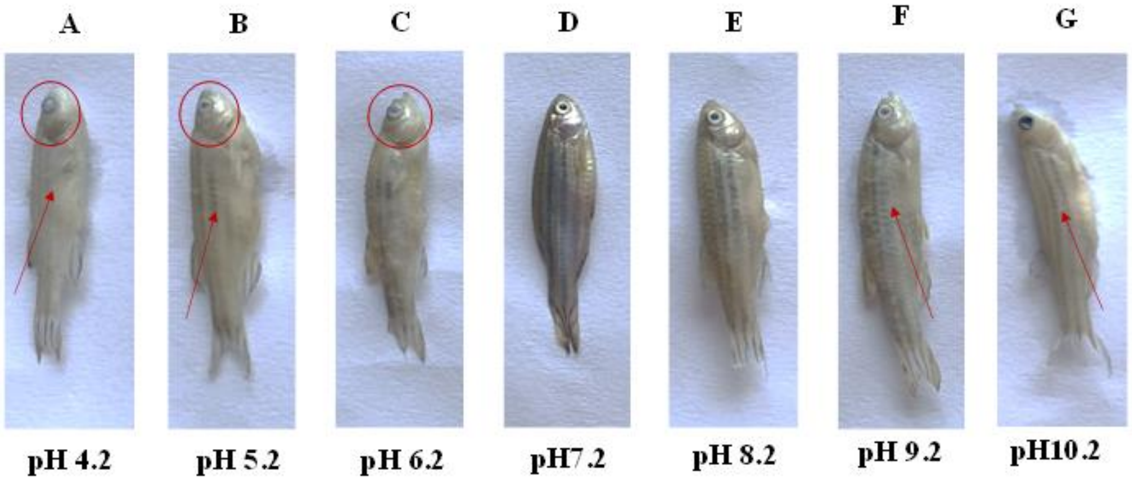

Figure 1(a). Dorso-ventral side view of fish The red arrow shows the discoloration of pigmented lines in the skin of zebrafish. The red circles show discoloration of the black eye lens.

All the zebrafish exposed to different salinity levels showed different morphological changes compared to the control. There were clear blue fluorescent lines on the body in control, while in highly acidic exposure, these lines were not seen in the body of the zebrafish (Figure 1b). In the alkaline water exposure, stripped lines were present on the bodies of zebrafish (Figure 1b). The black eye lens is slightly discolored into white at a high salinity. The discoloration of the eye lens is not seen in fish at lower concentration levels.

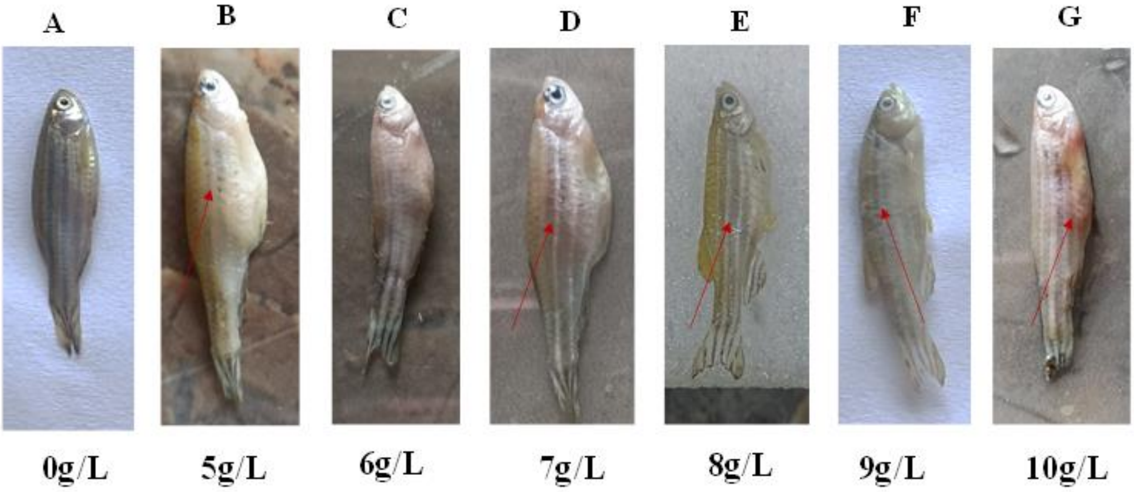

Figure 1(b). Dorso-ventral side view of fish the red arrow shows the discoloration of pigmented lines in the skin of zebrafish.

After the exposure to the stress environment, a brown colour was seen on the bodies of the zebrafish, which implies that the tissues present in that area were affected by stress-inducing factors (Figure 2a).

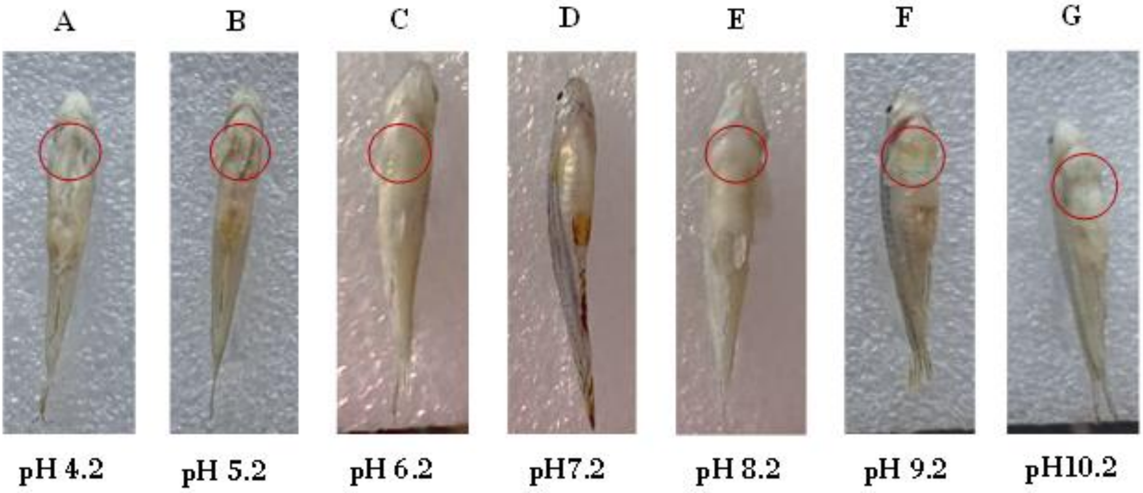

Figure 2(a). Ventral side view of fish: The red circle indicates the brown spots on the fish's body. The acidic pH shows that the gills and respiratory tract may be highly affected. The brown spots near the intestine area have an alkaline pH.

After the exposure to the stress environment, a brown colour was seen on the bodies of the zebrafish, which implies that the tissues present in that area were affected by stress-inducing factors (Figure 2b).

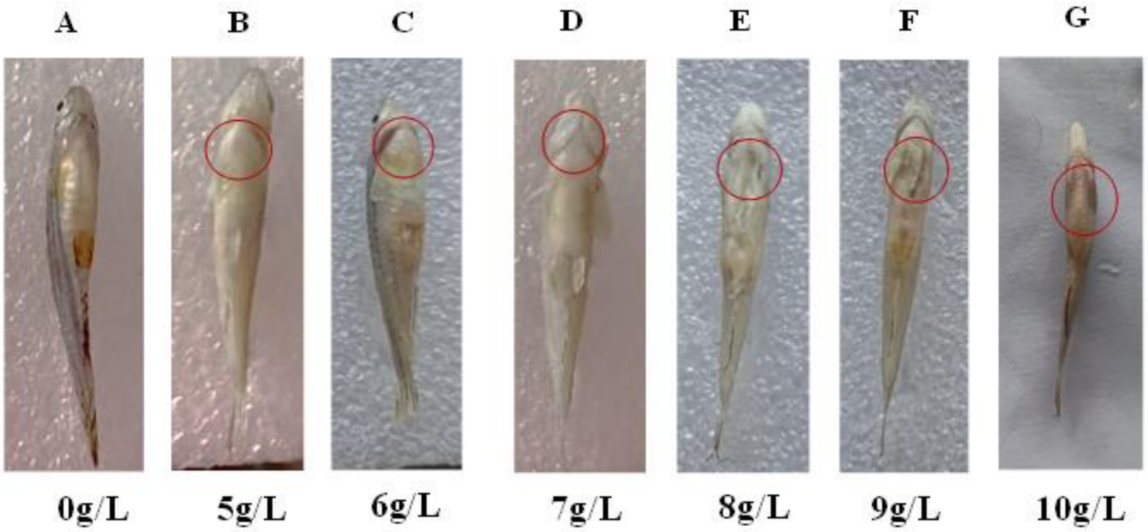

Figure 2(b). Ventral side view of fish: The red circle also has black spots in the fish after exposure to salinity.

During the observation days, fish showed different activities before death. When the fish was dead, it was identified by checking their response to stimuli and the movement of different parts, mainly gills. Once a fish was found dead, the immediately dissected fish was separated into different parts and generated pictures for analysis (Figure 3a). All the differences seen in the fish body are compared with the control zebrafish. A distinct difference was seen in the case of eyes, gills, and swim bladder in acidic pH 4.2, 5.2, and 6.2. In the case of alkaline pH 8.2, 9.2, and 10.2, the posterior intestine is highly affected as that tissue got rotten and is seen as black dead tissue (Figure 3a).

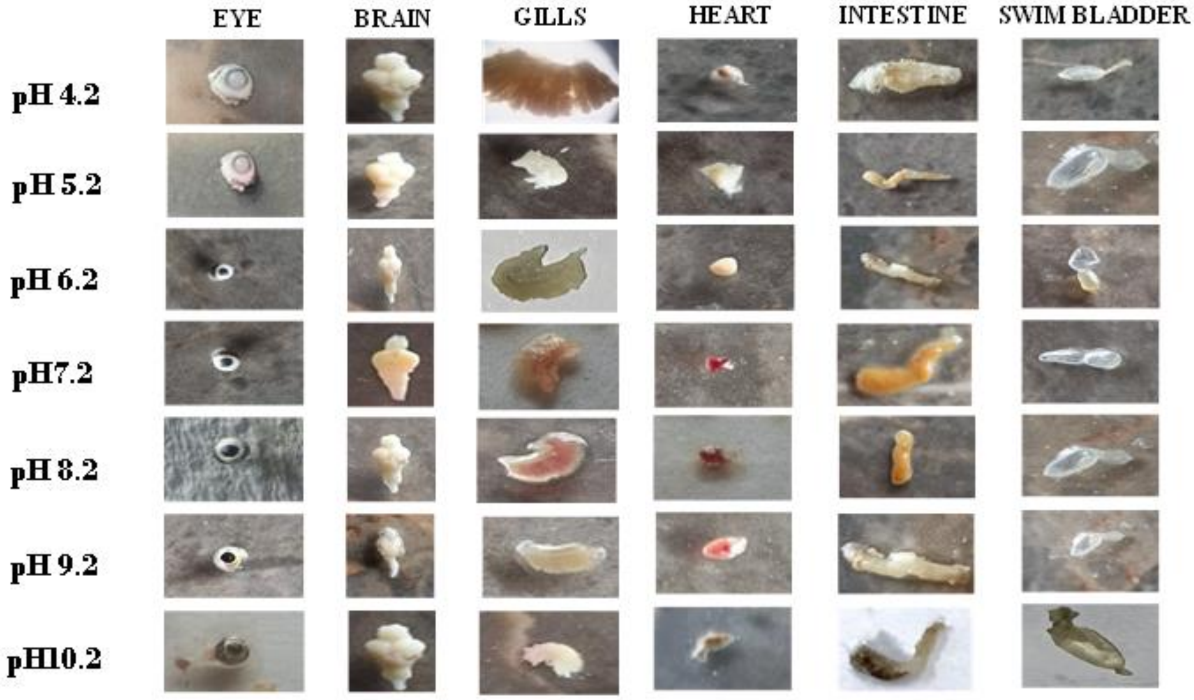

Figure 3(a). Comparative anatomy of zebrafish exposed to stress: The dead zebrafish (exposed to stress) were dissected and separated into different parts of the zebrafish, as shown in an image. Different body parts like the eyes, brain, gills, heart, intestine, and swim bladder of zebrafish are exposed to different pH stress.

During the observation days, fish showed different activities before death. When the fish was dead, it was identified by checking their response to stimuli and the movement of different parts, mainly gills. Once a fish was found dead, the immediately dissected fish was separated into different parts and generated pictures for analysis (Figure 3b). All the differences seen in the fish body are compared with the control zebrafish. A distinct difference was seen in the case of heart at higher concentration of salinity. (Figure 3b).

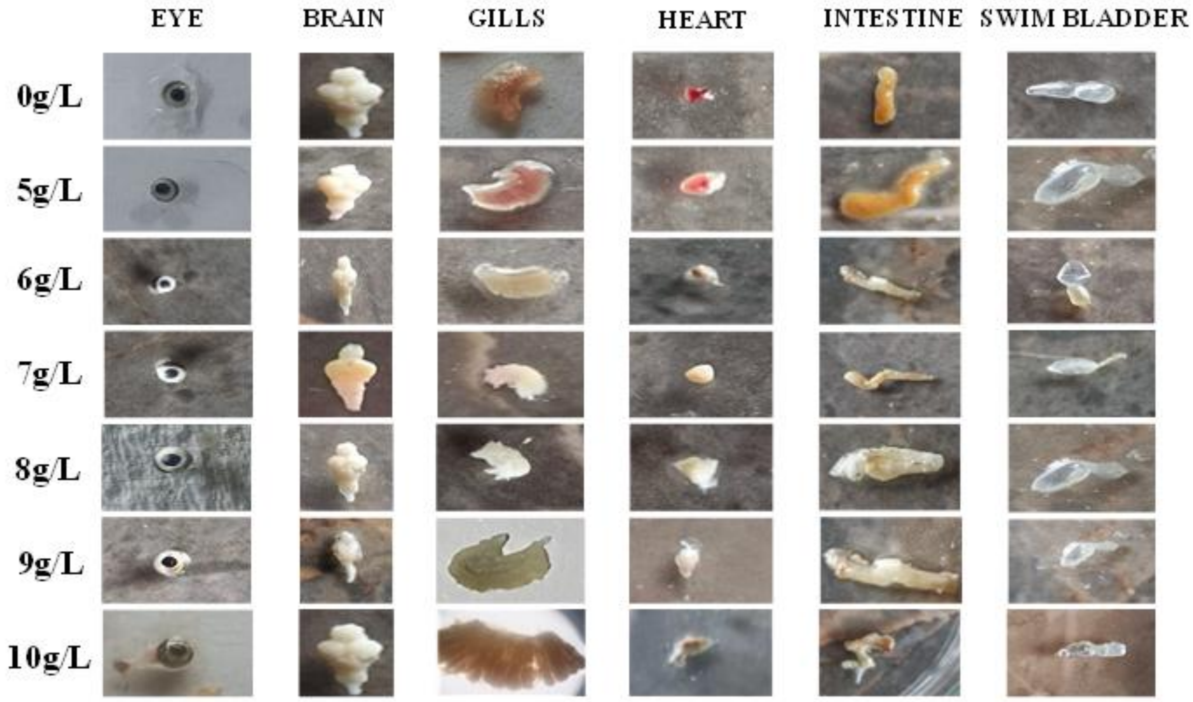

Figure 3(b). Comparative anatomy of zebrafish exposed to stress: The dead zebrafish (exposed to stress) were dissected and separated into different parts of the zebrafish, as shown in an image. Different body parts like the eyes, brain, gills, heart, intestine, and swim bladder of zebrafish are exposed to different salinity stress.

Subsequently, the staining images of the gills were compared for different pH. After the dissection, the collected gills were stained by the H&E staining process and seen under the microscope in the scale bar of 10x. In dissection, zebrafish's gills were highly acidic as the primary and secondary laminae were degraded (Figure 4a). From the images, it was identified that in highly acidic conditions, the primary and secondary laminae were degraded and attached to each other due to the effect of highly acidic conditions. The higher degeneration is seen in the case of pH 4.2 environments.

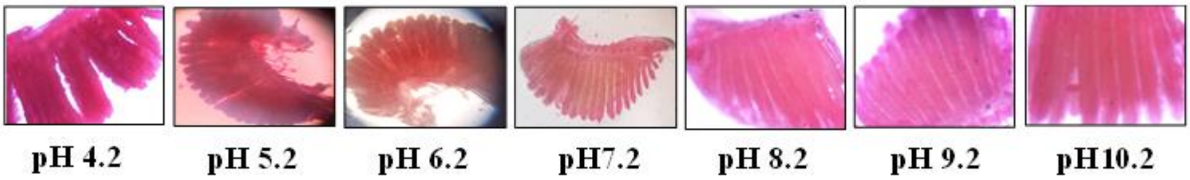

Figure 4(a). A histological study by H&E staining of zebrafish gill: Representative images are a view of zebrafish gill following hematoxylin and eosin staining. Magnification and scale bar 10x correspond to different pH stress.

Subsequently, the staining images of the gills were compared for different salinity. After the dissection, the collected gills were stained by the H&E staining process and seen under the microscope in the scale bar of 10x. In dissection, zebrafish's gills were highly acidic as the primary and secondary laminae were visible (Figure 4b).

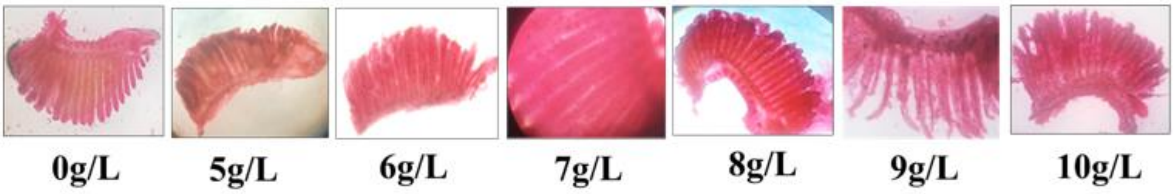

Figure 4(b). Histological study by H&E staining: Representative images are a view of zebrafish gill following hematoxylin and eosin staining Magnification and scale bar 10x correspond to different salinity stress.

The fish ended its density at the stage of mortality as the effect of exposure. Due to the stressor conditions, the fish died at different percentages and different pH levels. The mortality percentage is slightly higher in acidic conditions than in alkaline conditions (Figure 5a). There was no mortality in the case of control. Detailed information on mortality percentages in the different pH is described below (Figure 5a).

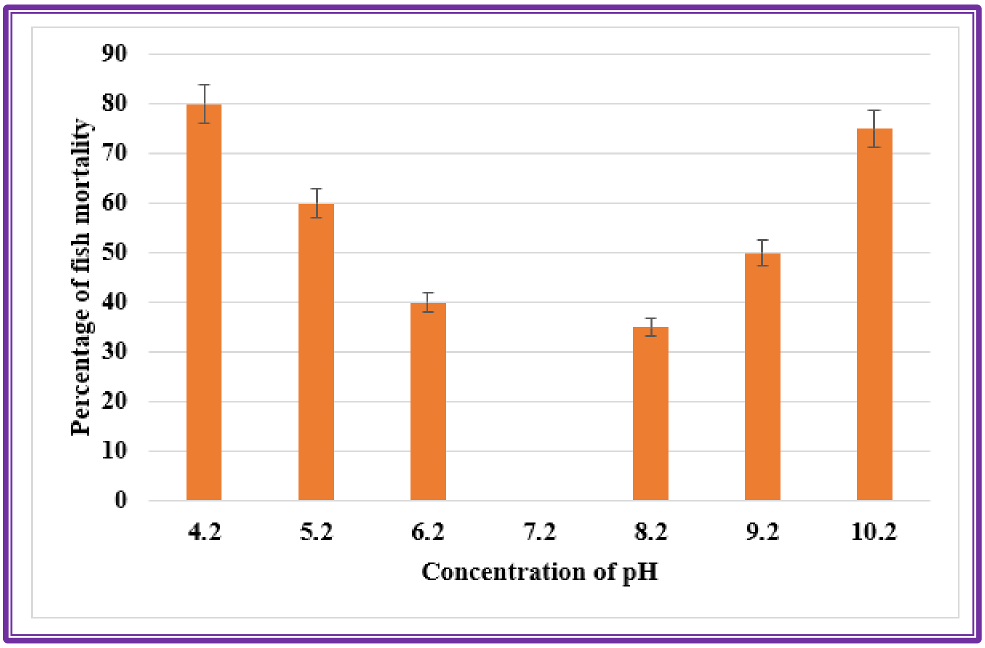

Figure 5(a). Showing the relation between concentration of pH and percentages of fish mortality

As an effect of exposure, the fish ended its density at the stage of mortality. Due to the stressor conditions, the fish died at different percentages and different salinity concentrations. The mortality percentage is slightly higher in 10ppm conditions than in comparison to control 0ppm (Figure 5b). There was no mortality in the case of control. Detailed information on mortality percentages in the different pH is described below (Figure 5b).

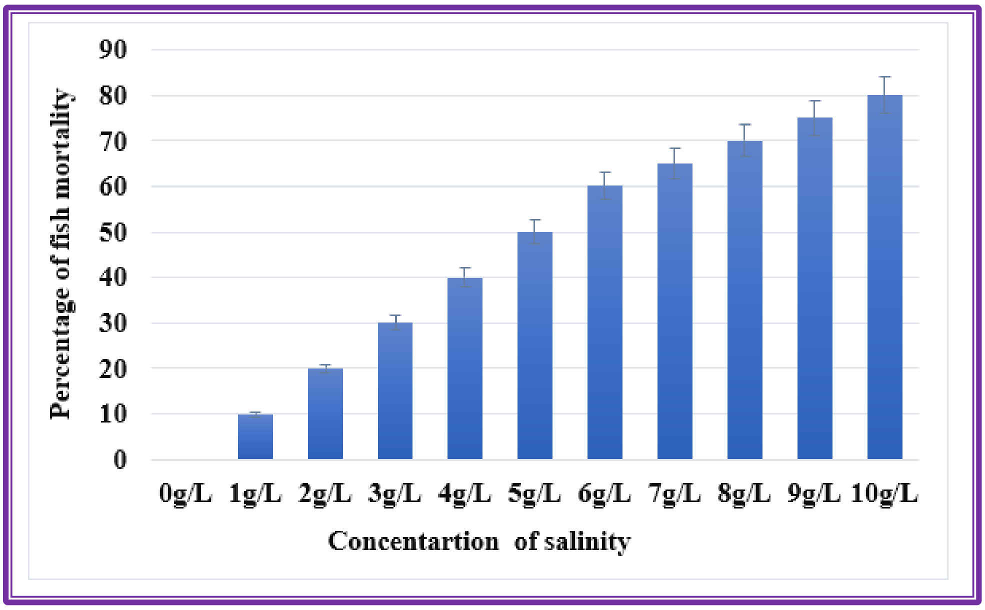

Figure 5(b). Showing the relation between the concentration of salinity and percentages of fish mortality

Discussion

Water quality parameters such as salinity and pH are crucial for maintaining the physiological stability and overall health of aquatic organisms. Fish, especially freshwater species like zebrafish (Danio rerio), are highly sensitive to changes in their aquatic environment, with even slight deviations in water chemistry capable of triggering significant stress responses. In this study, we investigated the impact of salinity and pH stress on zebrafish to see how environmental perturbation affects physiology, survival, and behaviour. The results indicate that about the stressors, both when they occur at sublethal or extreme levels, there are disruptions of homeostasis and tissue damage, changes in behavior, and mortality. One abiotic factor that influences osmoregulatory function in fish is salinity, or the concentration of dissolved salts in water (Das and Panigrahi, 2025a). Additionally, zebrafish are stenohaline freshwater species, and they are, therefore, adapted to environments with very low salinity. For that reason, they can’t deal with any major rise in salinity, which is going to cause them to lose the ability to keep in balance and water inside the cell. Under 6g/L, 7g/L, 8g/L, 9g/L, and 10g salinity conditions, acute and chronic physiological stress in zebrafish is apparent (Mondal *et al.*, 2024). Additionally, behavioral changes in zebrafish under salinity stress were evident, including hyperactivity, surface gasping, erratic swimming, and social disorientation, which are commonly regarded signs of environmental stress. These behaviors imply disturbances of neural signaling, perhaps associated with ionic imbalances or neurologic function from perturbed plasma osmolarity. Because of this, behavioral responses to environmental stress can often precede histopathological changes and are therefore, valuable early indicators of environmental stress in aquatic toxicology studies. External salinity stress disrupts the fish’s homeostatic systems which results in behavioral health and general fitness decline and the increased energy expenditure needed for coping with external stressors. As one of the main water quality determining factors besides salinity, pH is important for fish health as well. These zebrafish were exposed to three unique pH conditions: a low pH of 4.2 (acidic), a moderate, alkaline pH of 8.2, and a highly alkaline pH of 10.2. In order to evaluate the acute stress responses and long term physiological damage under the acidic and basic environments, these ranges were chosen (Zahangir *et al.*, 2015). Zebrafish are particularly sensitive to pH fluctuations because they cannot quickly adapt to acid or alkalinity fluctuations that would buffer the effects of the fluctuation. If the water has low pH levels, these can increase the concentration of the hydrogen ions in the water, which can prevent ion exchange across the gill membranes and then result in sodium and chloride ions loss, which are critical for neuromuscular and cellular function. Low pH also induces respiratory acidosis, reduces gill permeability, and increases the secretion of mucus, all at once, which individually reduces gas exchange and oxygen uptake. In contrast, exposure to high pH levels causes difficulty for the fish in divesting ammonia, and the toxic metabolites accumulate in the blood (Kwong *et al.*, 2014). Metabolic alkalosis can be induced in alkaline environments and this only compounds the problem further for enzyme activity and physiological performance. Gill and respiratory tract damage was recorded in this study especially under extreme pH conditions. These organs are imperative as their damage directly coincides with serious critical functions found in respiration and ion exchange as well as nitrogenous waste excretion. Epithelial lifting, lamellar fusion, and necrosis were confirmed as pH extremes harm respiratory and osmoregulatory tissues in histological analysis. These pathological changes show that the gill is the initial point of interaction with aberrant water and a site of primary pH-induced injury. These behavioral and physiological changes during varied pH levels in zebrafish reflect previous studies of elevated stress hormone (such as cortisol) levels, altered locomotor activity, and poor responses to an immune response. Both acidic and highly alkaline conditions reduced activity, altered swimming patterns, and increased mortality for zebrafish. The links of these behavioral changes to perturbations in central nervous system signaling are known to be correlated with sensitivity to extracellular pH. Finally, the stress effects are also compounded under environmental pH because the solubility and toxicity of most other waterborne substances are affected by their pH. It is important to note that stress is a biological mechanism used by fish to adapt to environmental challenges and hence, it is not solely a negative outcome. At high concentrations or prolonged exposure, zebrafish also exhibited reduced recovery following acute salinity and pH exposure, resulting in mortality, but both factors elicited acute stress responses. This work adds to a broader understanding of how water quality more particularly salinity and pH can impact fish at multiple biological levels from damage to cells, organs, behaviour, and survival. These results have significant consequences for environmental monitoring and aquaculture practices, and for ecological risk assessment.

Limitations

Although such a study offers important information about the physiological and behavioral effects of acute salinity and pH stress on zebrafish, some limitations of the study should be taken into consideration. The conditions for this experiment are highly controlled and do not necessarily replicate the complexity of natural aquatic ecosystems. The effect of environmental variables such as temperature fluctuations, the presence of other contaminants and biological interactions on the responses observed in the stress were not considered.

Future Scope

These limitations can be addressed by future research in which the study is expanded to include chronic exposure experiments of salinity and pH stress on the growth, reproduction, and lifespan of zebrafish. Such studies would enable us to know whether zebrafish can adjust or suffer permanent injury and long-term population effects of persistent stress. In future studies, the pathways activated during environmental stress also need to be explored using molecular and biochemical markers.

Conclusion

The outcomes of this experiment provide information that there was a direct relationship between mortality and concentration levels of pH; when concentration levels increased (both acidic and alkaline), the mortality rate also amplified. However, there was a negative relationship between the mortality time and different concentration levels; when the concentration level increased, the mortality time decreased. The current investigation results show that the fish responded to both the primary effects of pH stress and the secondary effects brought on by stress. Also, behavioral changes are seen prominently, along with increasing stress levels. The primary purpose of this study was to give preliminary data on the exposure of Zebrafish (Danio rerio) to the effects of salinity and pH stress. The findings of this study revealed a definite link between specific salinity exposures and changes in zebrafish physiology. Fish undergo genetic changes due to the impact of salinity. The primary goal of the current study was to present early findings about the susceptibility of Zebrafish (Danio rerio) to the effects of salinity stress. This investigation showed a clear connection between some salinity exposures and alterations in zebrafish physiology. Additionally, the degrees of these modifications depend on exposure duration and concentration. Fluctuations in the salinity concentration cause serious physiological issues that worsen fish health, cause secondary infections, and ultimately cause fish death. There are also noticeable behavioral changes, as well as increased stress levels. Further research will shed light on the molecular processes behind the interplay between abiotic stressors and the physiological mechanisms in eukaryotic model organisms, potentially addressing the Life Below Water challenges as framed by the United Nations Sustainable Development Goals (SDGs), such as SDG 14.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

The authors would like to thank the Vice Chancellor, Centurion University of Technology and Management, Odisha for providing financial support to GKP (grant approval letter no: CUTM/VC Office/45 to GKP).

Acknowledgement

Authors thank the administration and management of Centurion University of Technology and Management, Odisha, India for their heartfelt support. We apologize to all colleagues whose work could not be included owing to space limitations.

References

Andrade, T. S., Henriques, J. F., Almeida, A. R., Soares, A. M., Scholz, S., & Domingues, I. (2017). Zebrafish embryo tolerance to environmental stress factors-Concentration-dose response analysis of oxygen limitation, pH, and UV - light irradiation. *Environmental toxicology and chemistry*, 36(3), 682-690. https://doi.org/10.1002/etc.3579

Avdesh, A., Chen, M., Martin-Iverson, M. T., Mondal, A., Ong, D., Rainey-Smith, S., ... & Martins, R. N. (2012). Regular care and maintenance of a zebrafish (Danio rerio) laboratory: an introduction. JoVE (Journal of Visualized Experiments), (69), e4196. https://doi.org/10.3791/4196-v

Chen, Y., Zhou, J. L., Cheng, L., Zheng, Y. Y., & Xu, J. (2017). Sediment and salinity effects on the bioaccumulation of sulfamethoxazole in zebrafish (*Danio rerio*). *Chemosphere*, 180, 467-475.

Chowdhury, S., & Saikia, S. (2023). Evaluation of oxidative stress on Zebrafish under different pH and dissolved oxygen levels using MDA as biomarker: Oxidative stress in Zebrafish. *Journal of Fish Health*, 3(2), 33-44. https://doi.org/10.29303/jfh.v3i2.3249

Cleal, M., Gibbon, A., Fontana, B. D., & Parker, M. O. (2020). The importance of pH: How aquarium water is affecting behavioural responses to drug exposure in larval zebrafish. *Pharmacology Biochemistry and Behavior*, 199, 173066. https://doi.org/10.1016/j.pbb.2020.173066

Das, R and Panigrahi, G.K. (2025a). Messenger RNA surveillance: current understanding, regulatory mechanisms and future implications. *Molecular Biotechnology*: 1-18. https://doi.org/10.1007/s12033-024-01062-4

Das, R., & Panigrahi, G. K. (2025). Complexities of Salinity Stress on Zebrafish Survivability. *Toxicology International,* 32(1), 57-63. https://doi.org/10.18311/ti/2025/v32i1/45794

Das, R., Singh, S. P., Sahoo, A., Majumder, S., Sahoo, S. K., & Panigrahi, G. K. (2025). Synergistic stress of zinc oxide nanoparticles and pH triggers gill damage and mortality in zebrafish. *Nanotechnology for Environmental Engineering*, 10(1), 1-11. https://doi.org/10.1007/s41204-025-00418-8

Farhana, T., Haque, F., Amin, F. B., Zahangir, M. M., & Islam, M. S. (2019). Developmental pliability in zebrafish: an experimental enquiry of acute salinity stress on the early life of zebrafish. *Aquaculture Reports*, 14, 100189. https://doi.org/10.1016/j.aqrep.2019.100189

https://doi.org/10.1016/j.chemosphere.2017.04.055

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J., & Domenici, P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution*, 28(11), 651-658. https://doi.org/10.1016/j.tree.2013.05.005

Kwong, R. W., Kumai, Y., & Perry, S. F. (2014). The physiology of fish at low pH: the zebrafish as a model system. *Journal of Experimental Biology*, 217(5), 651-662. https://doi.org/10.1242/jeb.091603

Magalhaes, D. D. P., Buss, D. F., Da Cunha, R. A., Linde-Arias, A. R., & Baptista, D. F. (2012). Analysis of individual versus group behavior of zebrafish: a model using pH sublethal effects. *Bulletin of Environmental Contamination and Toxicology*, 88, 1009-1013. https://doi.org/10.1007/s00128-012-0608-9

Mondal, N., Kaur, J., Bal, A., & Ghosh, A. (2024). Effect of incremental salinity on survival and behaviour of zebrafish (Danio rerio). *Environmental and Experimental Biology*, 22(4), 167-177. https://doi.org/10.22364/eeb.22.16

Ni, H., Peng, L., Gao, X., Ji, H., Ma, J., Li, Y., & Jiang, S. (2019). Effects of maduramicin on adult zebrafish (Danio rerio): acute toxicity, tissue damage and oxidative stress. *Ecotoxicology and Environmental Safety*, 168, 249-259. https://doi.org/10.1016/j.ecoenv.2018.10.040

Panigrahi, G.K., Sahoo, A and Satapathy, K.B. (2024). The processing body component varicose plays a multiplayer role towards stress management in Arabidopsis. *Plant Physiology Reports*: 1-10. https://doi.org/10.1007/s40502-023-00778-w

Patro, A. K., Panigrahi, G. K., Majumder, S., Das, R., & Sahoo, A. (2024). Nonsense-mediated mRNA decay: physiological significance, mechanistic insights and future implications. *Pathology-Research and Practice*, 155677. https://doi.org/10.1016/j.prp.2024.155677

Patro, I., Sahoo, A., Nayak, B.R., Das, R., Majumder, S and Panigrahi, G.K. (2023). Nonsense Mediated mRNA Decay: mechanistic insights and physiological significance. *Molecular Biotechnology*: 1-15. https://doi.org/10.1007/s12033-023-00927-4

Portz, D. E., Woodley, C. M., & Cech, J. J. (2006). Stress-associated impacts of short-term holding on fishes. *Reviews in Fish Biology and Fisheries*, 16, 125-170. https://doi.org/10.1007/s11160-006-9012-z

Sahoo, A., Satapathy, K.B and Panigrahi, G.K. (2023). Ectopic expression of disease resistance protein promotes resistance against pathogen infection and drought stress in Arabidopsis. *Physiological and Molecular Plant Pathology* 124(101949): 1-7. https://doi.org/10.1016/j.pmpp.2023.101949

Sahoo, A., Satapathy, K.B and Panigrahi, G.K. (2024). Security check: Plant immunity under temperature surveillance. *Journal of Plant Biochemistry and Biotechnology*: 1-4. https://doi.org/10.1007/s13562-023-00846-0

Sampaio, F. D., & Freire, C. A. (2016). An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. *Fish and Fisheries*, 17(4), 1055-1072.https://doi.org/10.1111/faf.12158

Sawant, M. S., Zhang, S., & Li, L. (2001). Effect of salinity on development of zebrafish, Brachydanio rerio. *Current Science*, 1347-1350. http://www.jstor.org/stable/24105851. Accessed 10 Aug. 2024

Schweizer, M., von der Ohe, P. C., Graff, T., Kuhnen, U., Hebel, J., Heid, C., ... & Kohler, H. R. (2022). Heart rate as an early warning parameter and proxy for subsequent mortality in Danio rerio embryos exposed to ionizable substances. *Science of The Total Environment*, 818, 151744. https://doi.org/10.1016/j.scitotenv.2021.151744

Singh, S. P., Panigrahi, G. K., & Sahoo, A. (2024). Optimizing nanoparticles use for growth promotion in zebrafish: insights on concentration-dependent effects. *Nanotechnology for Environmental Engineering*, 9(4), 757-767. https://doi.org/10.1007/s41204-024-00393-6

Singleman, C., & Holtzman, N. G. (2014). Growth and maturation in the Zebrafish, *Danio rerio*: a staging tool for teaching and research. *Zebrafish,* 11(4), 396-406. https://doi.org/10.1089/zeb.2014.0976

Zahangir, M. M., Haque, F., Mostakim, G. M., & Islam, M. S. (2015). Secondary stress responses of zebrafish to different pH: Evaluation in a seasonal manner. *Aquaculture Reports*, 2, 91-96. https://doi.org/10.1016/j.aqrep.2015.08.008