Asian Journal of Fisheries and Aquatic Research

Volume 28, Issue 4, Page 85-97, 2026; Article no.AJFAR.156843
ISSN: 2582-3760

Impact of Temperature Stress on Adaptive Mechanisms in Zebra Fish (*Danio rerio*)

Madhurima Bag [a], Raja Saha [b], Madhumita Dubey [b], Tuhin Khaddar [b], Monjit Paul [a] and Sangita Maiti Dutta [b*]

[a] Department of Biological Sciences (Fisheries Science), Midnapore City College, Paschim Medinipur, West Bengal- 721129, India.
[b] Department of Biological Sciences (Zoology), Midnapore City College, Paschim Medinipur, West Bengal-721129, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author MB collected the samples and data and performed laboratory experiments. Author RS performed laboratory experiments, collected and curated data, and prepared the manuscript, including writing, reviewing, and editing. Author MD conducted stress treatment and created visualizations. Author TK validated the data and created visualizations. Author MP validated the data and performed data visualization. Author SMD supervised and conceptualized the study, created visualizations, validated the data, curated the data, and prepared the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajfar/2026/v28i41084

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/156843

Original Research Article

Received: 20/02/2026
Published: 16/04/2026

Abstract

Environmental temperature plays a crucial role in regulating physiological and biochemical processes in ectothermic organisms such as Zebra fish (*Danio rerio*). Thermal stress can disrupt homeostasis by elevating reactive oxygen species (ROS), leading to oxidative damage in vital tissues. The present study investigates the impact of gradual temperature stress on antioxidant and metabolic enzyme responses in Zebra fish, with a focus on key antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and metabolic markers such as acid phosphatase (ACP), alkaline phosphatase (ALP), B amylase, and

**Corresponding author: Email: smaitiduttazoo@gmail.com;*

Cite as: *Bag, M., Saha, R., Dubey, M., Khaddar, T., Paul, M., & Dutta, S. M. (2026). Impact of Temperature Stress on Adaptive Mechanisms in Zebra Fish (Danio rerio). Asian Journal of Fisheries and Aquatic Research, 28(4), 85–97. https://doi.org/10.9734/ajfar/2026/v28i41084*

Acetylcholinesterase (AChE) along with the lipid peroxidation marker malonaldehyde (MDA). Results demonstrated a significant increase in oxidative stress markers, with MDA levels rising by approximately 6.20-fold at 32°C and 6.48-fold at 38°C relative to control, indicating enhanced lipid peroxidation. Antioxidant responses were markedly elevated, with SOD activity increasing by 1.87-fold and 2.43-fold, CAT by 1.97-fold and 2.41-fold, and GSH by 1.97-fold and 2.02-fold at 32°C and 38°C, respectively compare to control. Metabolic enzymes exhibited temperature-dependent upregulation, with ALP increasing by 1.63-fold and 2.09-fold at 32°C and 38°C, respectively compare to control, ACP by 1.57-fold and 1.66-fold at 32°C and 38°C, respectively, and β-amylase by 1.20-fold and 1.70-fold at 32°C and 38°C, respectively as compare to control, suggesting enhanced metabolic turnover and energy mobilization. AChE activity also increased moderately (1.18-fold and 1.56-fold at 32°C and 38°C, respectively, indicating altered neurophysiological dynamics under thermal stress. These findings highlight the sensitivity of Zebra fish antioxidant systems to temperature fluctuations and underscore the importance of rate and duration of thermal exposure. This study contributes to understanding how climate-induced temperature changes can affect aquatic vertebrate physiology at the molecular and biochemical levels.

Keywords: Zebra fish (Danio rerio); temperature stress; Reactive Oxygen Species (ROS); superoxide dismutase; acetylcholinesterase; lipid peroxidation.

1. Introduction

Temperature is a primary environmental factor modulating physiological and biochemical processes in ectotherms like fish. Thermal fluctuations lead to shifts in metabolic rate and can generate excessive reactive oxygen species (ROS) from mitochondrial respiration and other redox reactions (Banh et al., 2016; Dutta et al., 2018). If unchecked, ROS such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH) can oxidize lipids, proteins, and DNA, disrupting cellular integrity and function. To counteract ROS, organisms employ antioxidant defence mechanisms (Guderley, 2004). Fish as Ectothermic organisms depend on external environmental temperatures to regulate their physiological processes. Even slight variations in water temperature can significantly affect their survival. Such fluctuations disrupt metabolic homeostasis, leading to energy imbalance and cellular stress, particularly during sensitive life stages such as embryonic development, early growth, and reproduction (Portner et al., 2007). Despite this sensitivity, fish play a crucial role in maintaining the survival, distribution, and overall health of aquatic ecosystems. Temperature directly impacts metabolic rate in fish, leading to increased oxygen consumption, heart rate, and ATP turnover (Guderley, 2004). This escalation is often accompanied by increased mitochondrial activity, resulting in the excessive generation of reactive oxygen species (ROS) (Saha & Dutta, 2025). Under normal conditions, ROS play crucial roles in cell signaling and homeostasis (Dutta et al., 2014). Thermal stress, either hypothermic or hyperthermic, can disrupt this balance, causing oxidative stress. Temperature stress can also modify membrane fluidity, alter ion gradients, and destabilize protein structure.

Zebra fish (*Danio rerio*), native to the warm, slow-moving freshwater streams of South Asia, are a crucial model organism in environmental biology and toxicology due to their genetic tractability, transparent embryos, rapid development, and physiological similarities to higher vertebrates (Basu et al., 2013). Their well-annotated genome and the availability of transgenic and mutant lines enable advanced studies in stress physiology, oxidative biology, and metabolism. The small size Zebra fish and ease of maintenance make them suitable for high-throughput assays and temperature-controlled experiments. Understanding their biochemical and molecular responses to thermal stress provides valuable insights into resilience mechanisms and potential tipping points in aquatic environments (Saha & Dutta, 2024). Their utility extends to conservation physiology, aquaculture, and ecotoxicology, where managing temperature stress has ecological and economic implications. Reactive oxygen species (ROS) are chemically reactive molecules produced by normal cellular metabolism, essential for cell signaling and immune responses (Gao et al., 2024). They can cause oxidative damage and cellular dysfunction under stress conditions (Saha et al., 2024). In aerobic organisms, mitochondria are the primary sources of ROS, while environmental temperature significantly affects mitochondrial production (Banh et al., 2016). Understanding these interactions is crucial for interpreting the physiological responses of Zebra fish to temperature fluctuations and for integrating oxidative stress biomarkers into ecological risk assessment and conservation physiology. Organisms have evolved intricate antioxidant defense systems to combat reactive oxygen species (ROS) and restore redox homeostasis (Liu et al., 2024; Saha, 2026). In Zebra fish (*Danio rerio*), the immune system plays a crucial role in maintaining cellular homeostasis under environmental stress.

Understanding the physiological and biochemical responses of *Danio rerio* to thermal stress is essential for integrating oxidative stress biomarkers into ecological risk assessment and conservation physiology (Wei et al., 2018). The present study evaluated the physiological responses of *Danio rerio* (Zebra fish) to varying degrees of thermal stress through a comprehensive biochemical analysis. Enzymatic assays were performed to quantify the activities of alkaline phosphatase (ALP), acid phosphatase (ACP), β-amylase, acetylcholinesterase (AChE), superoxide dismutase (SOD), catalase, as well as the levels of malondialdehyde (MDA) and reduced glutathione (GSH). The results revealed significant correlations between thermal stress and the modulation of stress-related enzymes and antioxidant defense mechanisms. These findings provide valuable insights for future research and practical applications across various subfields of fisheries science, including aquaculture management, environmental monitoring, and fish health assessment.

2. Materials and Methods

2.1 Experimental Animal

Selected fresh water fish species, *Danio rerio* after being collected from ornamental fish farm by hand net, immediately shifted to water filled bucket and acclimatize in laboratory aquarium for seven days. Samples size 3 ± 0.5 cm and weight 400 ± 50 mg. After that heat stress treatment (28-38^{0}C) was start in thermostatic aquarium for 30 days by increasing 1ºC in the interval of 3 days. 28ºC set as control temperature. All experimental procedures were conducted in accordance with Institutional Animal Ethical Committee (IAEC) guidelines. The fishes were sacrificed under ethical conditions. Triplicate the experimental data and whole-body tissues was collected from heat adapted fishes for further biochemical studies (Figs. 1 & 2).

Fig. 1. Experimental setup using 3 different aquariums for different temperatures

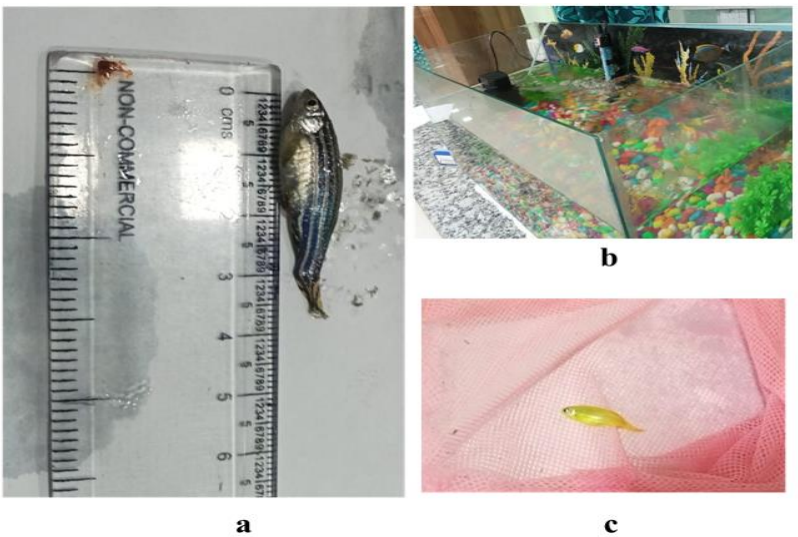

Fig. 2. Experimental animal in different conditions (a): Experimental animal; (b) aquarium setup for experiment; (c) sample collection

2.2 Protein Estimation

Total protein content was estimated by the standard method (Lowry et al., 1951). Bovine Serum Albumin was used as standard. The absorbance was recorded at 720 nm in spectrophotometrically against blank. Protein concentration was calculated and expressed as µg/ml wet tissue (Dednath et al., 2007).

2.3 Estimation of Malonaldehyde (MDA)

MDA was conducted following the protocol as in Buege and Aust, (1978). The MDA is measured at 540 nm in spectrophotometer against blank**.** Enzyme activity was calculated using the molar extinction coefficient of MDA ($1.56 \times 10^5\ M^{-1}cm^{-1}$) and expressed as nmol of MDA/mg protein (Osazee et al., 2024).

2.4 Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) activity was assessed by the ability of the enzyme to inhibit the auto-oxidation of pyrogallol (Marklund et al., 1974). The absorbance at 560 nm was recorded spectrophotometrically for 60 seconds against blank. Enzyme activity was calculated using One unit of SOD activity is defined as the amount of enzyme that inhibits the rate of pyrogallol auto-oxidation by 50% and express activity as units/mg protein (Kaur et al., 2014).

2.5 Catalase (CAT)

Catalase activity was estimated following the method of Aebi (1984), which involves monitoring the decomposition of hydrogen peroxide (H_2O_2) by catalase. The absorbance at 240 nm was recorded spectrophotometrically for 60 seconds against blank. Enzyme activity was calculated using the molar extinction coefficient of H_2O_2 (43.6 M^{-1} cm^{-1}) and expressed as µmol H_2O_2 decomposed per minute per mg protein (Hadwan et al., 2016).

2.6 Estimation of GSH

Reduced Glutathione (GSH) has been estimated according to the method of Davila et al., (1991). The absorbance at 412 nm was recorded spectrophotometrically against blank. Absorbance values were compared with a standard curve generated from known GSH concentration to compute tissue GSH levels. The enzyme activity was expressed as µmol of GSH/mg protein (Maiti et al., 2001).

2.7 Estimation of β-amylase

B-amylase activity was estimated by measuring the amount of maltose released from starch substrate followed by Miller (1959). The absorbance at 540 nm was recorded in spectrophotometrically against blank, Absorbance values were compared with a standard curve generated from known maltose concentration to compute tissue maltose levels. Enzyme activity was expressed as µmol maltose released/minute/mg protein (Araujo-Silva et al., 2018).

2.8 Estimation of Alkaline Phosphatase

The ALP activity will be determined by the method of Garen and Levinthal (1960). Then the absorbance was recorded at 410 nm in spectrophotometer against the blank. Absorbance values were compared with a standard curve generated from known p-Nitrophenol concentration. Enzyme activity was expressed as units/mg protein (Debnath et al., 2007).

2.9 Estimation of Acid Phosphatase

The procedure will be adopted for ACP activity same as for ALP activity except the buffer will be used in ACP assay is acetate buffer (0.2 M, pH 5.0) instead of bicarbonate buffer (0.2 M, pH 9.5) followed by Murmu et al., 2010. Then the absorbance was recorded at 410 nm in spectrophotometer against the blank. Absorbance values

were compared with a standard curve generated from known p-Nitrophenol concentration. Enzyme activity was expressed as units/mg protein (Yengkokpam et al., 2013).

2.10 Estimation of Acetylcholinesterase

The enzyme activity was measured by the method of Ellman's colorimetric method (Ellman et al., 1961). The absorbance at 412 nm was recorded spectrophotometrically blank. Enzyme activity was calculated using the molar extinction coefficient of DTNB (13600 $M^{-1}cm^{-1}$) and expressed as µmol/min/mg protein (Sinko et al., 2007).

3. Results

Malondialdehyde (MDA) levels were significantly elevated in Zebra fish tissues exposed to thermal stress compared to the control group. Activity was found almost 6.20 folds, and 6.48 folds increase than the control sample in 32ºC, and 38ºC respectively (Fig. 3). This increase in MDA concentration reflects enhanced lipid peroxidation, indicating greater oxidative damage to cellular membranes due to excessive generation of reactive oxygen species (ROS). ANOVA followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

Superoxide dismutase (SOD) activity showed a significant increase ($p < 0.05$) in thermally stressed Zebra fish compared to controls, indicating an enhanced antioxidant response to elevated levels of superoxide radicals. It was found almost 1.87 folds, and 2.43 folds increased than the control sample in 32ºC, and 38ºC respectively (Fig. 5). This upregulation suggests that *Danio rerio* mounts an initial defense mechanism against reactive oxygen species (ROS) during thermal stress. ANOVA followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

Catalase (CAT) activity was significantly elevated in Zebra fish exposed to elevated temperatures. Specifically, CAT activity increased by approximately 1.97 folds in the 32°C group and 2.41folds in the 38°C group compared to the control maintained at ambient temperature. This rise indicates an adaptive enzymatic response to elevated hydrogen peroxide levels generated under thermal stress. The enhanced catalase activity suggests an effective upregulation of antioxidant defenses aimed at minimizing oxidative damage caused by the accumulation of reactive oxygen species (ROS) during heat stress (Fig. 6). ANOVA followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

Reduced glutathione (GSH) levels exhibited a marked increase in response to thermal stress in *Danio rerio*. GSH activity was found to be approximately 1.97-fold higher at 32°C and 2.02-fold higher at 38°C compared to the control group maintained at ambient temperature. This elevation indicates the activation of non-enzymatic antioxidant defenses to counteract the rise in reactive oxygen species (ROS) induced by heat stress. The increased GSH concentration reflects its critical role in maintaining redox homeostasis and protecting cellular components from oxidative damage under elevated temperature conditions (Fig. 4). ANOVA followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

β -Amylase activity showed a notable increase in Zebra fish (*Danio rerio*) subjected to elevated temperature conditions. The enzyme activity was approximately 1.20 folds higher at 32°C and 1.70-fold higher at 38°C compared to the control group. This rise in activity suggests enhanced starch degradation and mobilization of energy reserves as part of the metabolic adjustment to thermal stress. The increase in β-amylase activity reflects a physiological adaptation to meet higher energy demands and maintain cellular homeostasis under heat-induced stress conditions. (Fig. 10). ANOVA followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

Alkaline phosphatase (ALP) activity significantly increased in Zebra fish subjected to elevated temperature stress. At 32°C, ALP activity was approximately 1.63-fold higher, and at 38°C, it rose to about 2.09-fold compared to the control group. This enhancement indicates increased membrane turnover and cellular metabolic activity in response to thermal stress. The elevated ALP levels suggest its potential role as a biochemical marker for tissue remodeling and stress adaptation under rising temperature conditions (Fig. 7). ANOVA followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

Acid phosphatase (ACP) activity showed a significant elevation in *Danio rerio* under thermal stress. The activity increased by approximately 1.57-fold at 32°C and 1.66-fold at 38°C compared to the control group. This increase suggests enhanced lysosomal activity and cellular turnover as a response to stress-induced tissue remodeling or damage. The rise in ACP levels under heat stress conditions indicates its involvement in intracellular degradation processes and serves as a potential biomarker of physiological stress in Zebra fish (Fig. 8). ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant.

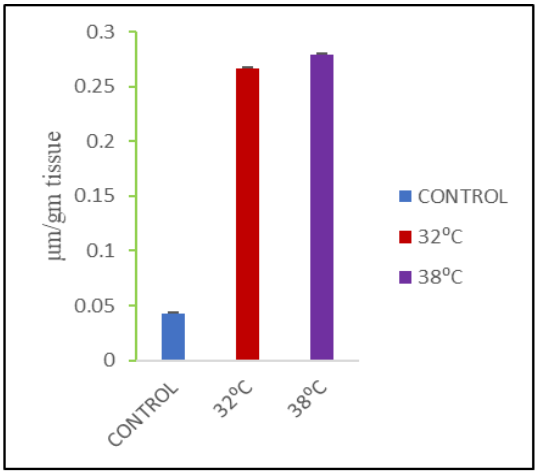

Fig. 3. Activity of Malonaldehyde (MDA) of *Danio rerio* in response to temperature stress and time-dependent manner. The value in the bar diagram denoted mean ± SE. ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant

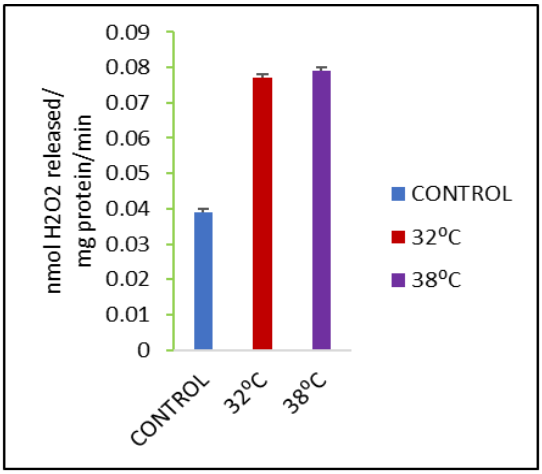

Fig. 4. Activity of Glutathione (GSH) of *Danio rerio* in response to temperature stress and time-dependent manner. The value in the bar diagram denoted mean ± SE. ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant

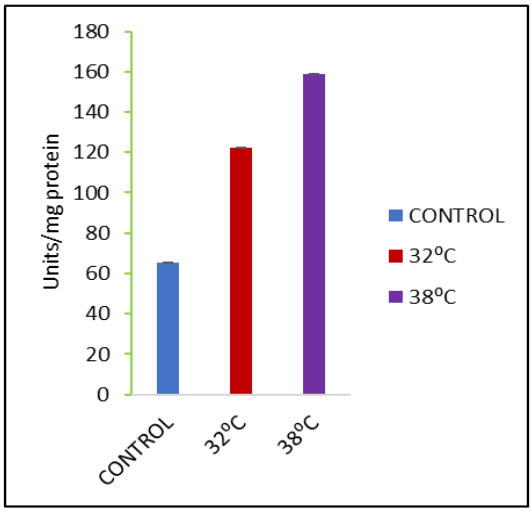

Fig. 5. Activity of Superoxide dismutase (SOD) of *Danio rerio* in response to temperature stress and time-dependent manner. The value in the bar diagram denoted mean ± SE. ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant

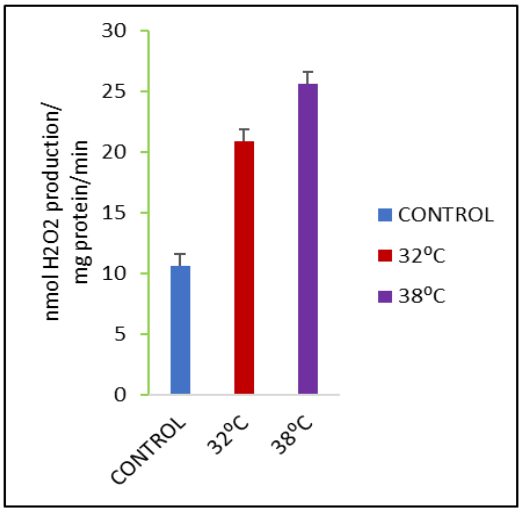

Fig. 6. Enzyme activity of Catalase (CAT) of *Danio rerio* in response to temperature stress and time-dependent manner. The value in the bar diagram denoted mean ± SE. ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant

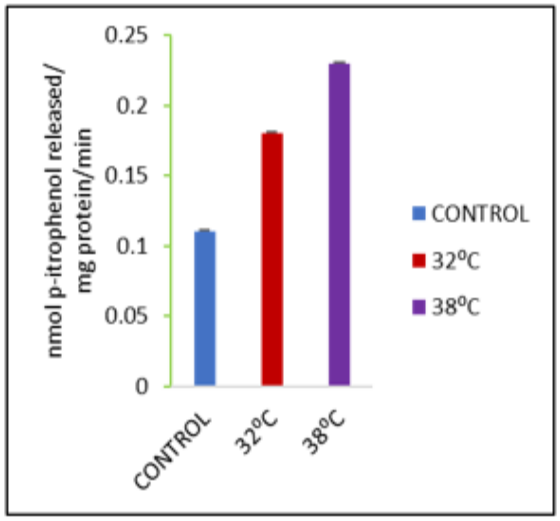

Fig. 7. Activity of Alkaline Phosphatase (ALP) of *Danio rerio* in response to temperature stress time-dependent manner. The value in the bar diagram denoted mean ± SE. ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant

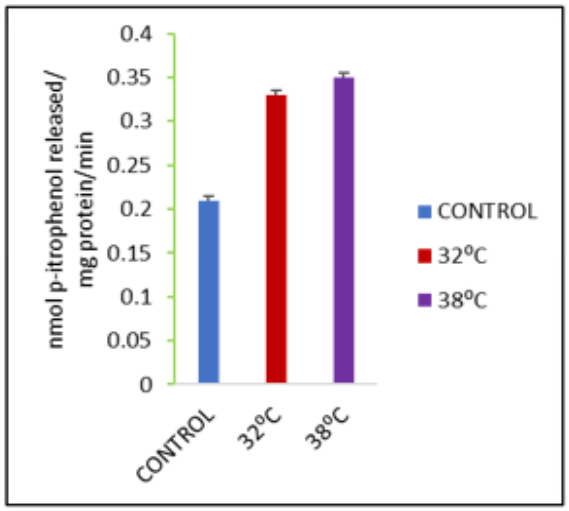

Fig. 8. Activity of Acid Phosphatase (ACP) of *Danio rerio* in response to temperature stress time-dependent manner. The value in the bar diagram denoted mean ± SE. ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant

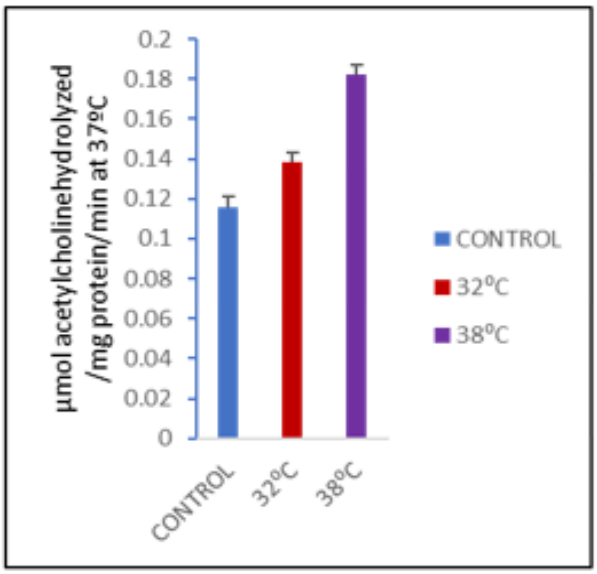

Fig. 9. Activity of Acetylcholinesterase (AChE) of *Danio rerio* in response to temperature stress time-dependent manner. The value in the bar diagram denoted mean ± SE. ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant

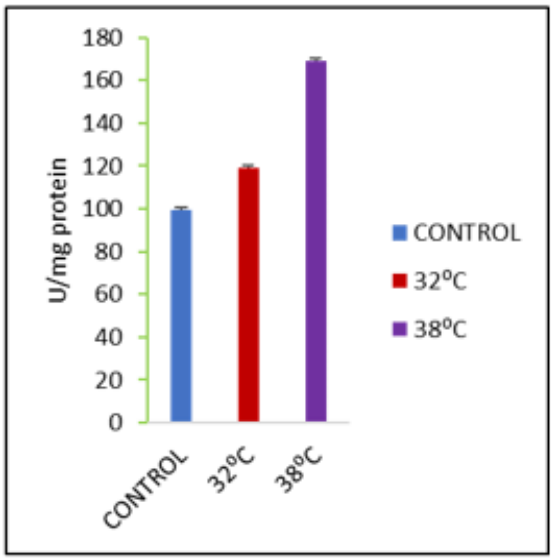

Fig. 10. Activity of Beta amylase of *Danio rerio* in response temperature stress time-dependent manner. The value in the bar diagram denoted mean ± SE. ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant

Acetylcholinesterase (AChE) activity exhibited a moderate but significant increase in Zebra fish exposed to elevated temperatures. At 32°C, AChE activity rose by approximately 1.18 folds, and at 38°C, it increased to about 1.56 folds compared to the control group. This elevation suggests enhanced cholinergic neurotransmission and neural excitability in response to thermal stress. The increase in AChE activity may

reflect adaptive neurophysiological adjustments or early signs of stress-related neural stimulation in *Danio rerio* (Fig. 9). ANOVA followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

3.1 Interpretation of Correlation Results (Table 1)

Correlation analysis provides insight into the interrelationships between different biochemical markers in response to thermal stress. In this study, the analysis reveals both positive and negative correlations among antioxidant enzymes (SOD, CAT, GSH), oxidative damage marker (MDA), metabolic enzymes (ALP, ACP, β-amylase), and the neuro-enzyme AChE. A strong positive correlation was observed among SOD, CAT, and GSH. This suggests a coordinated antioxidant defense system, where activation of one enzyme supports or induces the upregulation of others. Such relationships are expected under oxidative stress, as superoxide radicals (handled by SOD) are converted to hydrogen peroxide (handled by CAT), while GSH contributes to detoxification and redox balance. This alignment reflects the synergistic action of enzymatic and non-enzymatic antioxidants to maintain redox homeostasis. The positive correlation of MDA with ALP and ACP indicates that increased oxidative stress is linked with elevated phosphatase activity. As MDA levels rise due to lipid peroxidation, ALP and ACP likely respond to membrane damage and cellular stress, possibly as part of a tissue repair or remodelling mechanism. These results suggest that ALP and ACP may serve as secondary stress markers triggered by ROS-induced membrane instability. The significant positive correlation between AChE and SOD/CAT indicates a neuroprotective relationship, where the nervous system increases AChE activity alongside antioxidant defenses to maintain neurotransmission under stress. This suggests that AChE is not only a neurotoxicity marker but also may reflect adaptive neural responses in early or moderate thermal stress. β-Amylase showed a moderate to strong positive correlation with both GSH and MDA, indicating a link between energy metabolism and oxidative status. This may reflect increased glycogen breakdown to meet energy demands, which in turn could exacerbate ROS generation through elevated metabolic activity.

Table 1. Correlation table between different enzymes and antioxidants following Pearson Correlation method

Enzymes	Correlation values	Comments
MDA/SOD	0.938547	Very high positive correlation
MDA/CAT	0.817918	High positive correlation
MDA/GSH	1	perfect correlation
MDA/ALP	0.927729	Very high positive correlation
MDA/AChE	0.784708	High positive correlation
MDA/β-amylase	0.75114	High positive correlation
MDA/ACP	0.996192	Very high positive correlation
SOD/ALP	0.999547	Very high positive correlation
SOD/ACP	0.965067	Very high positive correlation
SOD/β-amylase	0.932829	Very high positive correlation
SOD/AChE	0.950433	Very high positive correlation
SOD/CAT	0.966232	Very high positive correlation
SOD/GSH	0.938281	Very high positive correlation
CAT/AChE	0.998457	Very high positive correlation
CAT/ALP	0.973553	Very high positive correlation
CAT/ACP	0.864966	High positive correlation
CAT/β-amylase	0.994174	Very high positive correlation
GSH/ALP	0.927441	Very high positive correlation
GSH/ACP	0.996124	Very high positive correlation
GSH/β-amylase	0.750631	High positive correlation
GSH/AChE	0.78423	High positive correlation

4. Discussion

Thermal stress is a significant environmental factor affecting the physiological and biochemical balance in ectothermic aquatic organisms such as *Danio rerio*. The present study explored the effects of elevated temperature (32°C and 38°C) on various biochemical markers, including antioxidant enzymes (SOD, CAT, GSH), lipid peroxidation (MDA), metabolic enzymes (ALP, ACP, β-amylase), and the neurotoxicological marker acetylcholinesterase (AChE). The findings indicate a coordinated but variable stress response among different biochemical systems in Zebra fish, reflective of their adaptive physiological mechanisms. The activity of superoxide dismutase (SOD) showed a significant increase under both temperature conditions, indicating an upregulation of the primary antioxidant defense mechanism (Yu et al., 2017). SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently neutralized by catalase (CAT). In the current study, CAT activity also exhibited a strong positive response, with a 1.97-fold increase at 32°C and a 2.41-fold increase at 38°C, demonstrating an enhanced enzymatic ability to detoxify hydrogen peroxide generated during oxidative stress. This synergistic rise in SOD and CAT highlights a well-coordinated antioxidant response to thermal challenge (Okogwu et al., 2014). Similarly, reduced glutathione (GSH) levels were elevated (1.97-fold at 32°C and 2.02-fold at 38°C), suggesting reinforcement of the non-enzymatic antioxidant defense system (Li et al., 2020). GSH plays a critical role in ROS scavenging and maintaining redox balance, and its upregulation is consistent with heightened oxidative load. Together, the increased activities of SOD, CAT, and GSH underline the organism's effort to maintain redox homeostasis under thermal stress. Despite the activation of antioxidant defences, malondialdehyde (MDA) levels an index of lipid peroxidation were significantly elevated in all heat-exposed groups (Forgati et al., 2017). This indicates that while antioxidant enzymes were upregulated, they were not fully able to neutralize all ROS, leading to oxidative damage of membrane lipids (Hoseinifer et al., 2020). The elevated MDA content suggests that cell membrane integrity was compromised, especially at higher temperatures, reinforcing the notion of oxidative damage despite the antioxidant response (Chowdhury & Saikia, 2020) (Fig. 11). Thermal stress also altered the activity of key metabolic enzymes (Yang et al., 2020). ALP activity increased significantly 1.63-fold at 32°C and 2.09-fold at 38°C suggesting enhanced cellular membrane activity, tissue remodelling, or stress-induced changes in phosphate metabolism. Similarly, ACP activity was elevated 1.57-fold and 1.66-fold, respectively which may reflect increased lysosomal activity and autophagic processes triggered by heat-induced cellular stress. β-Amylase activity showed a rising trend with temperature 1.20-fold at 32°C and 1.70-fold at 38°C, indicating enhanced carbohydrate metabolism. This likely reflects increased energy demands for coping with thermal stress. The upregulation of β-amylase suggests that Zebra fish may mobilize stored glycogen more rapidly under elevated temperatures to support heightened metabolic activity (Webb et al., 2019). AChE activity also increased in a temperature-dependent manner 1.18-fold at 32°C and 1.56-fold at 38°C, which may represent an adaptive response to increased neural activity under stress. AChE regulates synaptic acetylcholine levels and thus controls neurotransmission; its elevation may be a compensatory mechanism for enhanced cholinergic signalling or mild neurotoxic stress (Yuan et al., 2025). Although often associated with inhibition under toxicant exposure, a moderate increase in AChE under thermal stress may also suggest increased neural excitation or metabolic compensation. Overall, the results point to a complex interplay between stress perception, metabolic compensation, and antioxidant activation. Thermal stress in *Danio rerio* activates both enzymatic and non-enzymatic antioxidant systems, elevates metabolic enzyme activities, and induces lipid peroxidation, highlighting cellular strain despite protective responses. The tissue-specific elevations of ALP, ACP, and β-amylase further suggest active involvement of liver and muscle tissues in coping with thermal changes. The coordinated increase in SOD, CAT, GSH, and metabolic enzymes indicates an adaptive physiological adjustment, while elevated MDA and AChE levels serve as early markers of oxidative and neuro-metabolic strain. These findings are consistent with previous studies reporting thermal stress-induced ROS generation, antioxidant modulation, and enzyme fluctuation in fish species (Liu et al., 2024; Zheng et al., 2019). The correlation data support the notion that thermal stress in *Danio rerio* induces a system-wide biochemical response, involving tightly linked pathways of oxidative defense, membrane integrity, metabolic adjustment, and neural activity. The strong positive associations among antioxidant enzymes confirm the existence of a well-orchestrated defense mechanism, while the relationship of MDA with phosphatases emphasizes their roles as indirect oxidative stress indicators. Furthermore, the correlations involving AChE and β-amylase reflect the neuro-metabolic integration in stress adaptation, providing a holistic picture of how Zebra fish cope with environmental heat challenges. These correlations not only validate the individual biochemical findings but also demonstrate that these markers can be used together to form a multi-biomarker index for evaluating thermal stress in aquatic organisms.

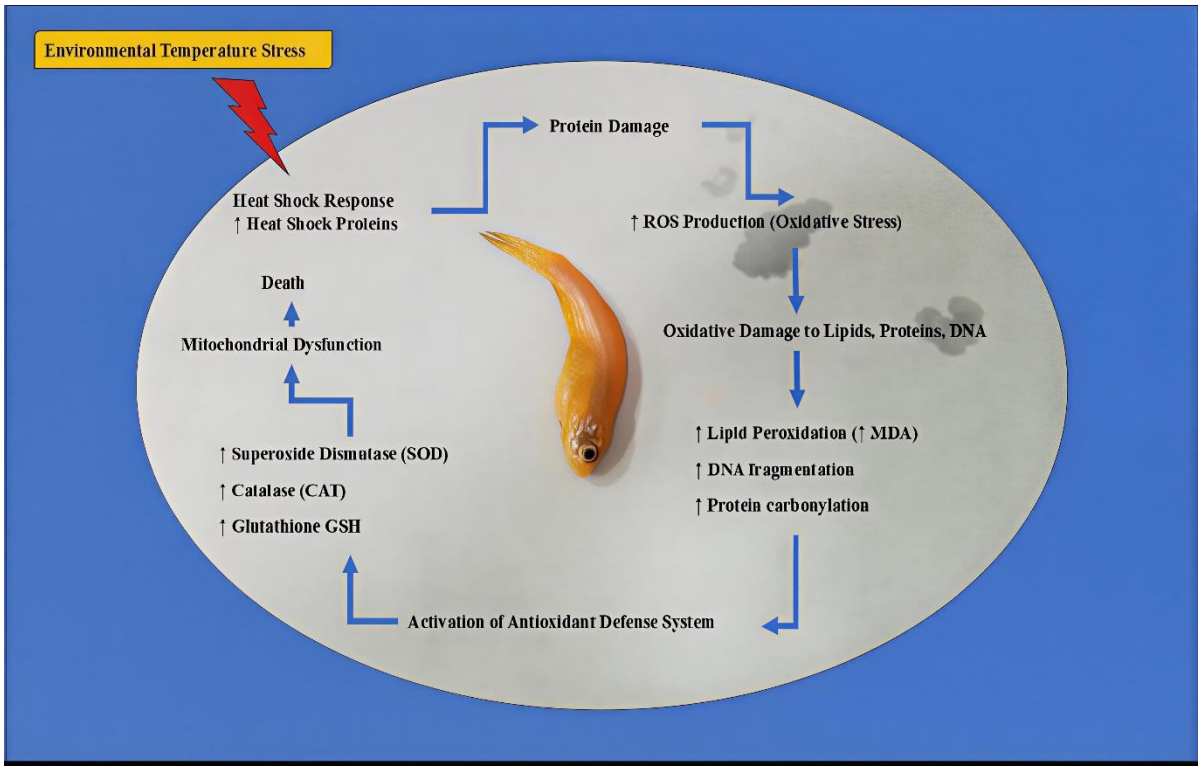

Fig. 11. Thermal stress mechanism in *Danio rerio*.

5. Conclusion

The study investigates the physiological and biochemical responses of *Danio rerio* (Zebra fish) to thermal stress, focusing on antioxidant defense mechanisms, oxidative damage, metabolic enzyme activity, and neurophysiological adaptation. It found that thermal stress increases oxidative stress, leading to upregulation of antioxidant enzymes like SOD, catalase, and GSH. Metabolic enzymes like ALP, ACP, and β-amylase also show increased activity, suggesting increased tissue metabolism and energy mobilization. The study employs a gradual temperature increase (1°C every 3 days), but intermediate physiological responses during this progression are not reported. This limits the ability to distinguish between acute and chronic thermal stress effects. The study suggests that thermal stress induces a systemic biochemical shift in Zebra fish, providing valuable tools for ecotoxicological assessment and environmental monitoring.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s).

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Acknowledgement

The writers are grateful to Dr. Pradip Ghosh, Honourable director, Midnapore City College, and all the Higher Authorities of Biodiversity and Environmental Studies Research Centre, Midnapore City College, for providing all the necessary assistance in order to complete this work.

Competing Interests

Authors have declared that no competing interests exist.

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