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Original Article

# High-resolution microscopic visualization of mitochondrial damage under oxidative stress in zebrafish, *Danio rerio*

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**Abstract:** Mitochondria is the major source of ROS which is neutralized by antioxidant enzymes. However, if there is an imbalance between the prooxidants and the antioxidants, then mitochondrial oxidative stress may occur. This is one of the main reasons for mitochondrial damage and dysfunction. The present study attempts to understand whether environmental stressors like hypoxia and acidic ambiances induce oxidative stress in zebrafish, *Danio rerio*. The enhanced production of MDA clearly states that the zebrafish skeletal muscle and liver tissues undergo oxidative stress when subjected to environmental stressors. Further, the study also aims to explore the mitochondrial biogenesis in skeletal muscle and liver tissues by confocal microscopy visualization through IraZolve-*mito* staining under oxidative stressed situations. Visualization of mitochondria from skeletal muscle and liver tissue through transmission electron microscopy (TEM) reveals that under oxidative stress, the structure of mitochondria is disorganized leading to reduced mitochondrial functioning and biogenesis. Through microscopic visualization, the study concludes that hypoxia and acidic ambiances can cause remarkable mitochondrial damage in tissues like skeletal muscle and liver.

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## Introduction

Mitochondria have an antioxidant system that can scavenge ROS through an energy homeostatic pathway. Therefore, mitochondrial proliferation promotes the removal of ROS. However, chronic ROS production causes oxidative stress in mitochondria leading to mitochondrial dysfunction (Pung et al., 2013). This oxidative damage of mitochondria by ROS is often called mitoptosis and autophagy (Venditti et al., 2013; Lushchak et al., 2021). As a counter mechanism of mitochondrial damage, the overall metabolic supplement in the body is regulated by the supplement of ATP, through mitochondrial biogenesis (MB). Indirectly, MB occurring through the division of pre-existing mitochondria plays a significant role in the growth of animals. Both the decrease and increase of MB and bioenergetics have been reported during oxidative stress conditions in mammals (Lee and Wei, 2005; Strobel et al., 2010; Kim et al., 2017). When ROS production occurs during normal metabolism in mitochondria, an increase in MB and bioenergetics occurs to prevent the cell from ROS. But, in conditions of severe oxidative stress, the MB and bioenergetics decrease as a result of dysfunction of the mitochondrial respiratory chain (Yoboue and Devin, 2012; Bhatti et al., 2017).

In fish, little is known about the consequences of oxidative stress on MB because most of these researches have been conducted using mammalian model organisms. A study on cold-acclimated fish revealed that MB increases due to long-term exposure to cold temperatures and acclimation (O'Brien and Mueller, 2010). Later on, Mueller et al. (2011) reported that due to an increase in temperature and changes in climatic conditions of Antarctic regions, the fish in those regions undergo a disrupted electron transport chain. Studies have also revealed that a high-fat diet and energetic stress in fish downregulate the proteins responsible for MB (Bremer et al., 2015; Lu et al., 2020; Dong et al., 2022). Except for this small

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piece of information, a detailed behaviour of mitochondria has hardly been reported in fish. Being the largest group of animals thriving in water and considering the changes in aquatic habitat, this knowledge gap in fish requires to be addressed not only as a general understanding of fish biology, but also to effectively act on management, conservation, or fisheries activities. In the present study, responses during oxidative stress caused by acidic and hypoxic ambiances were analysed through oxidative stress indicator and specified biochemical parameter, malondialdehyde (MDA) level in Zebrafish, *Danio rerio*. Being a by-product of lipid peroxidation, MDA has been widely used as a biochemical indicator of oxidative stress in mammals (Eze et al., 2008). Thereafter, the study aims at understanding the MB in skeletal muscle (as growth occurring tissue) and liver tissues through IraZolve-*mito* stain and transmission electron microscopy (TEM) studies which were used to detect the mitochondria under hypoxia (20-30% $O_2$ saturation) and acidic ambiance (pH = 4.5-5.4).

## Materials and Methods

**Fish collection and maintenance:** Zebrafish stock was collected from a local professional supplier in Howrah, West Bengal, India. After collection, the stock was brought to the laboratory and was kept in an aquarium under a laboratory environment (pH 6.5-7.5, temperature 25-28ºC, and Dissolved Oxygen (DO) 7-10 mg/l). The water of the aquarium was cleaned in a regular intervals with proper aeration. The stock of zebrafish was provided with fish food (Tetra bits complete) three times a day for their normal growth and development. The fish were acclimatized to the laboratory conditions for about one week.

**Stress exposure to zebrafish:** After one week of acclimatization, zebrafish (weight: 0.7±0.5 g, total length: 3.8±0.2 cm,) were used for further studies. The experiments were set under two different conditions *viz*. variation in DO and pH. Two different levels of pH were set (n = 10) to measure the responses of tissues against acidic stress. These two pH levels were 'acidic' (pH = 4.5-5.5, n = 10) and neutral (considering the control) (pH = 6.5-7.5, n = 10). The pH levels were maintained by the application of weak organic acid following the regression model $Y = 7.675 - 0.008X$ (Y = pH and X = Volume acetic acid, µl, $R^2 = 0.997$). A portable digital pocket-sized pH meter (HI98107P) was used to record the pH of the aquaria.

Similarly, experiments were set with two different levels of DO to measure the responses of tissues against saturated and low DO in water. These two DO concentrations were 20-30% of $O_2$ saturation (hypoxia), and 80% and above $O_2$ saturation (saturated, control). The DO levels were regulated using nitrogen gas (25 ml gas per sec) by the methods of Butler et al. (1994). A portable digital DO meter (Lutron DO-5510) was used to record the DO levels of the aquaria. Further analysis was done on each tissue (Skeletal muscle, Liver) against each DO level at four different time periods (4, 8, 12, and 16h). A similar analysis was also done on each tissue (skeletal muscle, and liver) against each pH level at four different time periods (1, 2, 3, and 4h). The time periods taken were determined by the analysis of the mortality rate of the fish for the lowest tolerable level of DO and pH. It was found that after 16h of exposure to the lowest tolerable level of DO, more than 50% mortality occurred. Similarly, after 4h, which is the lowest tolerable level of pH, more than 50% mortality occurred in the zebrafish population. Zebrafish (weight: 0.7±0.5 g, total length: 3.8±0.2 cm, n = 10 for each set of experiments) were further collected and malondialdehyde (MDA) levels were measured for both tissues to understand the most effective time period at which the MDA level was maximum indicating highest oxidative stress among all the time periods.

**Tissue collection and processing:** The pooled tissues (skeletal muscle, and liver) were collected at different time periods and at different levels of DO and pH, and were kept in lysis buffer (phosphate buffer) and then homogenized using a microtissue homogenizer. The tissues homogenized were then centrifuged (Genetix Biotech Asia Pvt. Ltd.) in 10000 g for 15 min. The supernatant was collected and used for further biochemical assessment.

**TBARS assay:** The biochemical assessment was

| | Liver (pH 4.5-5.5) | Liver (pH 6.5-7.5) | Muscle (pH 4.5-5.5) | Muscle (pH 6.5-7.5) |
|---|---|---|---|---|
| Hour-1 | 475.62 | 62.03 | 591.83 | 72.31 |
| Hour-2 | 1,540.95 | 97.55 | 4,702.43 | 99.32 |
| Hour-3 | 815.84 | 48.68 | 1,805.02 | 77.44 |
| Hour-4 | 521.06 | 39.37 | 1,653.93 | 65.99 |
| | Liver (DO 20-30%) | Liver (DO 80% and above) | Muscle (DO 20-30%) | Muscle (DO 80% and above) |
| Hour-4 | 312.5289 | 39.0412 | 515.3268 | 62.3990 |
| Hour-8 | 353.1451 | 79.1130 | 546.1905 | 75.3047 |
| Hour-12 | 582.3208 | 103.4363 | 889.2756 | 167.6918 |
| Hour-16 | 427.9446 | 77.8595 | 625.8060 | 106.7453 |

Figure 1. Heatmap for MDA analysis in liver and skeletal muscle tissues under pH=4.5-5.5(n=10), neutral pH (n=10, control) and pH=6.5-7.5 (n=10) and DO 20%-30% of $O_2$ saturation (hypoxia) and 80% and above $O_2$ saturation (saturated, considering the control).

performed for MDA following Aust (1985). MDA is a product of lipid peroxidation and reacts with TBA (thiobarbituric acid) to give a red species named TBARS (thiobarbituric acid reactive substance).

**Ira*Zolve-mito* staining:** IraZolve-*mito* is a fluorogenic dye that is used to stain mitochondria in live cells and tissues (Sorvina et al., 2018). This stain can easily cross membranes and accumulate in mitochondria. IraZolve-*mito* was procured from Cayman Chemical (Item no. 25910) and was used to stain skeletal muscle and liver tissues. Fresh tissue samples of 0.5-1 mm were collected and incubated in PBS containing IraZolve-*mito* at a concentration of 10-50 µM for 30 minutes at room temperature. Then the tissues were washed 3 times for 5 minutes each in PBS and were mounted in aqueous mounting media to observe under a confocal microscopy system (Leica DMi8).

**Transmission electron microscopy (TEM);** Tissues (skeletal muscle and liver) were trimmed into 1.0-1.5 mm cube size and then fixed in 2-3% glutaraldehyde in 0.1M phosphate buffer (pH 7.2). Fixation time is 4 hours at 4°C. Tissues were washed in 0.1M phosphate buffer (3 changes of 15 minutes each) and kept in the same buffer at 4°C until further processing for TEM studies (Dykstra and Reuss, 1992).

## Results

The present study examined two different tissues (skeletal muscle and liver) under hypoxic and acidic ambiances. The results are documented below-

**MDA analysis for skeletal muscle and liver tissues:** MDA was analysed for each tissue (skeletal muscle and liver) at two pH levels *viz*. 'acidic' (pH = 4.5-5.5, n = 10) and neutral (pH = 6.5-7.5, n = 10, (considered as control). Similarly, MDA was analysed for each tissue (skeletal muscle and liver) at two DO concentrations *viz*. 20-30% of $O_2$ saturation (hypoxia) and 80% and above $O_2$ saturation (saturated, considering the control). From the heatmap in Figure 1, compared to the control, MDA levels were found more in hypoxic and acidic ambiances. Among all the hours in pH and DO levels, the MDA level is maximum at 2h for acidic pH and 12h for hypoxic ambiance. Therefore, further analysis of mitochondrial dysfunction under hypoxic and acidic ambiances were performed at 12h and 2h, respectively.

**Ira*Zolve-mito* staining:** IraZolve-*mito* was used to stain live skeletal muscle and liver tissues of zebrafish under hypoxia and acidic conditions at 12h and 2h of exposure, respectively. Lesser mitochondrial stain was observed in both tissues of skeletal muscle and liver under hypoxia and acidic conditions in comparison to control (Fig. 2).

**Transmission electron microscopy (TEM):** Figures 3 and 4 show that mitochondria in both liver and skeletal muscle tissues are highly affected when exposed to hypoxic and acidic ambiances at 12h and 2h. Compared to the control, the mitochondria of the liver and skeletal muscle are highly degenerated. The cristae of mitochondria are disintegrated with a ruptured mitochondrial membrane in both the tissues under hypoxic and acidic ambiances at 12h and 2h,

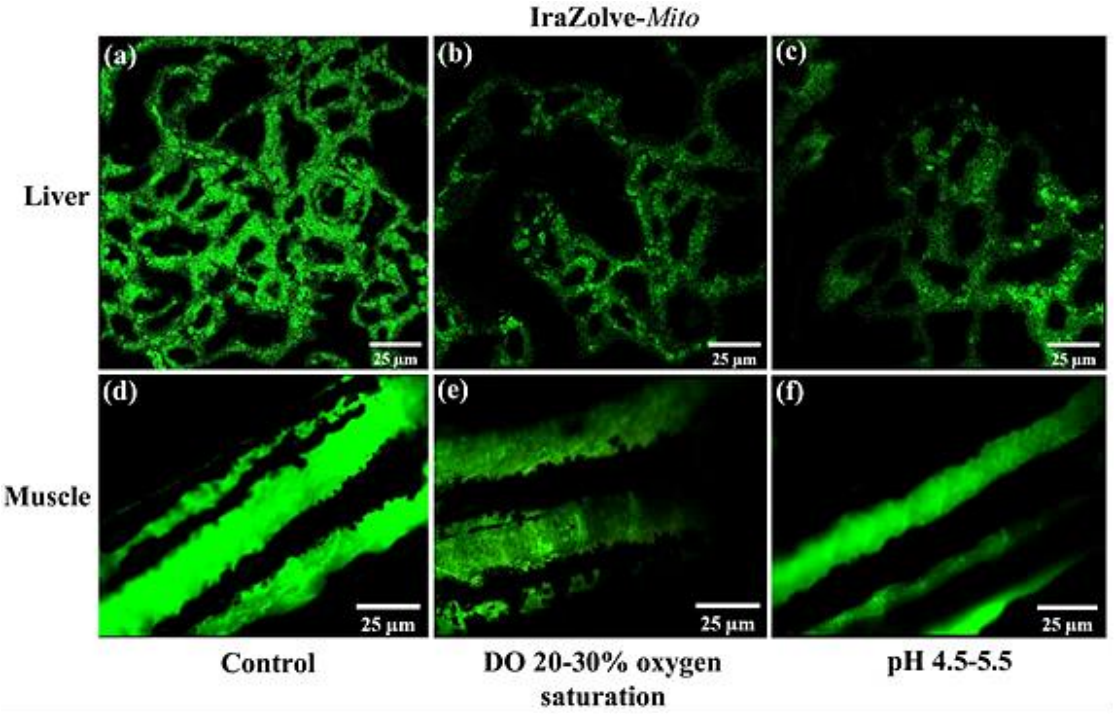


Figure 2. IraZolve-*mito* staining in skeletal muscle and liver at pH level of 4.5-5.5 at 2h and DO level of 20-30% oxygen saturation at 12h. (a) IraZolve-*mito* staining of liver tissue under control ambiance. (b) IraZolve-*mito* staining of liver tissue under DO 20-30% oxygen saturation. (c) IraZolve-*mito* staining of liver tissue under pH 4.5-5.5. (d) IraZolve-*mito* staining of skeletal muscle tissue under control ambiance. (e) IraZolve-*mito* staining of skeletal muscle tissue under DO 20-30% oxygen saturation. (f) IraZolve-*mito* staining of skeletal muscle tissue under pH 4.5-5.5.

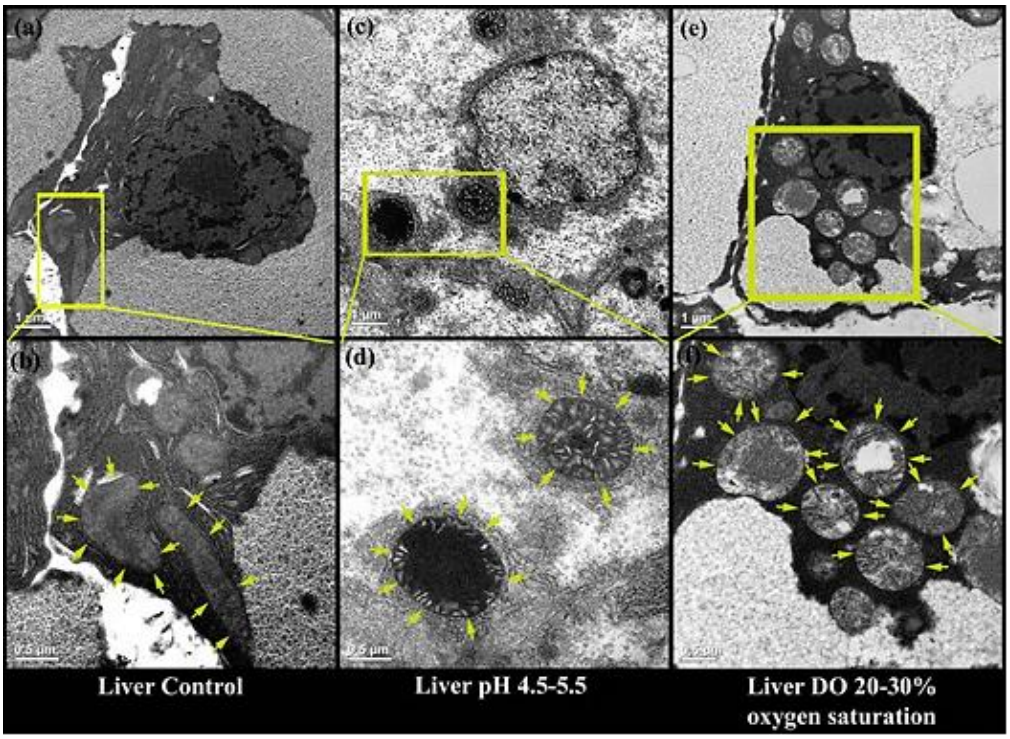


Figure 3. TEM images in liver at pH level of 4.5-5.5 at 2h and DO level of 20-30% oxygen saturation at 12h. (a) Mitochondrial imaging of control liver tissue at 2000x. (b) Mitochondrial imaging of control liver tissue at 5000x. (c) Mitochondrial imaging of liver tissue under pH 4.5-5.5 at 2000x. (d) Mitochondrial imaging of liver tissue under pH 4.5-5.5 at 5000x. (e) Mitochondrial imaging of liver tissue under DO 20-30% oxygen saturation at 2000x. (f) Mitochondrial imaging of liver tissue under DO 20-30% oxygen saturation at 5000x.

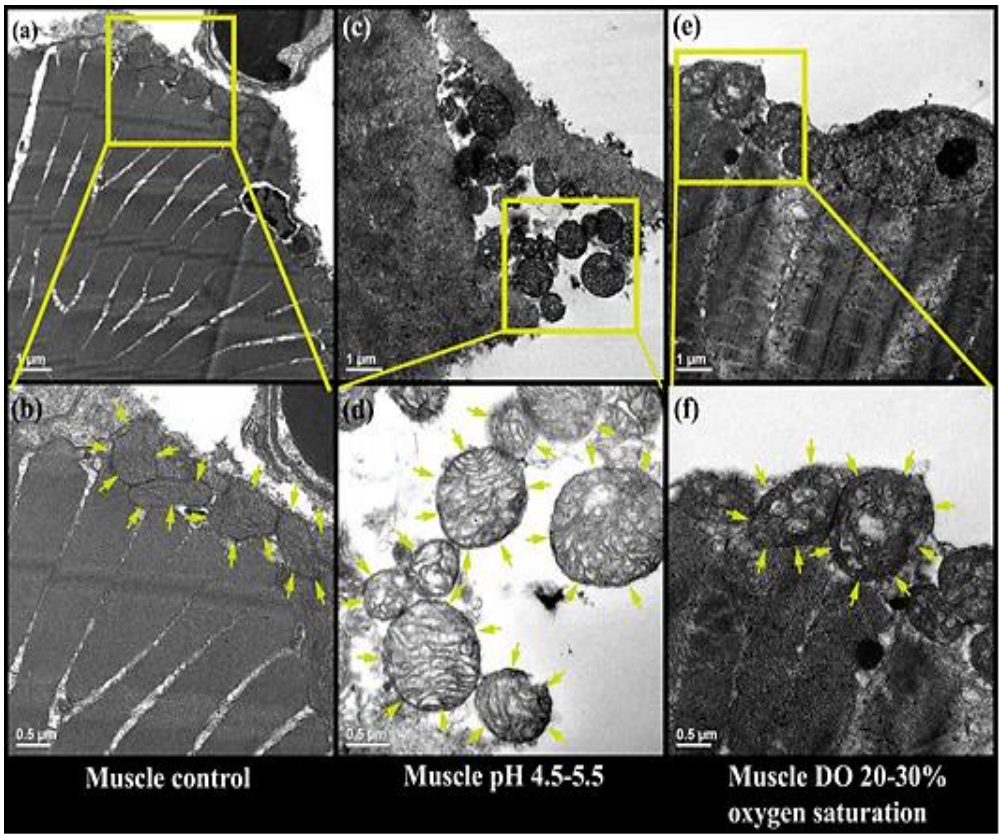


Figure 4. TEM images in skeletal muscle at pH level of 4.5-5.5 at 2h and DO level of 20-30% oxygen saturation at 12h. (a) Mitochondrial imaging of control skeletal muscle tissue at 2000x. (b) Mitochondrial imaging of control skeletal muscle tissue at 5000x. (c) Mitochondrial imaging of skeletal muscle tissue under pH 4.5-5.5 at 2000x. (d) Mitochondrial imaging of skeletal muscle tissue under pH 4.5-5.5 at 5000x. (e) Mitochondrial imaging of skeletal muscle tissue under DO 20-30% oxygen saturation at 2000x. (f) Mitochondrial imaging of skeletal muscle tissue under DO 20-30% oxygen saturation at 5000x.

respectively.

## Discussions

The aim of the present study was to understand the mitochondrial dysfunction caused in the skeletal muscle and liver tissues of zebrafish under hypoxic and acidic ambiance. Studies have shown that lipid peroxidation (resulting in the formation of MDA) can be considered a biomarker for understanding oxidative stress (Abdelkhalek et al., 2014; Srivastava and Reddy, 2017; Chowdhury et al., 2020). So, in the present study, MDA was analysed in both skeletal muscle and liver tissues under hypoxic and acidic ambiances to recognize the most effective time at which oxidative stress was maximum. It was already reported earlier that mitochondrial dysfunction resulting in reduced MB occurs in mammals with changes in environmental factors (McConnell and Petrie, 2004). Reports are also available that MB can be visualized by IraZolve-*mito* which is used to label mitochondria in live tissues in mammals and zebrafish (Grant et al., 2005; Sorvina et al., 2018).

In this study, live tissues of zebrafish were studied to label mitochondria and it was evident that compared to the control, reduced fluorescence was observed for the tissues exposed to hypoxia and acidic ambiance for 12h and 2h. TEM studies also allowed to understand the mitochondrial morphology under hypoxic and acidic ambiances. It was evident from the TEM studies that there is a drastic distortion in the shape and size of mitochondria with lot of abnormalities like diffusion and fragmentation of the population of mitochondria under hypoxic and acidic ambiances for 12h and 2h. Disruption in the mitochondrial cristae and large sized vacuoles were observed in the mitochondria under hypoxic and acidic ambiances compared to the control. It has already been studied in mammals that hypoxia can cause inefficient electron

transfer through the electron transfer chain leading to the accumulation of ROS resulting in mitochondrial damage and hence reduced mitochondrial biogenesis (Chiu et al., 2019). Such an outcome could be due to mitochondrial damage, which was evident from the present study, where, oxidative stress induced by stressors like reduced DO and pH have shown a disorganization in mitochondrial morphology. The altered mitochondrial structure is a common feature that is exhibited in many pathologies like obesity, a disease that can cause various metabolic syndromes, thereby, increasing the risk of muscular atrophy. A recent study in zebrafish has shown that a high-fat diet can induce mitochondrial damage and disorganization leading to the dysfunction of mitochondria (Zou et al., 2022). Therefore, IraZolve-*mito* stained mitochondria in the present study also exhibited disorganized mitochondria under the confocal microscopy under hypoxic and acidic ambiances induced live tissue samples. Not only IraZolve-*mito* staining, but transmission electron microscopy (TEM) also allows us to observe mitochondrial morphology and their overall organization under various circumstances (Dutta et al., 2021). The disintegrated cristae with a ruptured mitochondrial membrane in the present study affirmed that both the tissues under hypoxic and acidic ambiances at 12h and 2h, respectively were severely affected.

Mitochondrial dysfunction and oxidative stress in metabolic disorders have already been studied in mammals but in fish, such studies are still lacking (Bhatti et al., 2017). This is, indeed, a maiden ultramicroscopic observation on mitochondrial biogenesis in teleost. Furthermore, as already mentioned, very little is known in fish about the consequences of oxidative stress on MB because most of these researches have been conducted using mammalian model organisms. Studies of N-Nitrosodibutylamine treatment in the liver of mice result in altered mitochondrial morphology (Dutta et al., 2016, 2021). Mitochondrial alterations during oxidative stress may lead to chronic obstructive pulmonary disorders (COPD) and neurodegenerative diseases (Guo et al., 2013; Jiang et al., 2017). This study approves that Zebrafish too, undergo alteration of mitochondrial structures under ambient stressors, like hypoxia and acidic ambiance which can be linked to its dysfunction in these two tissues.

## Conclusion

The present study indicates that environmental stressors like reduced DO and pH can induce oxidative stress in zebrafish leading to abnormality in mitochondrial structure which reduces the mitochondrial biogenesis in skeletal muscle and liver tissues. Since, the effect of environmental stressors on fish mitochondrial structure and function is not researched yet, therefore, to some extent, this study can fill the knowledge gap in fish and can initiate a new direction of research.

**Ethics approval and consent to participate**: The work has been approved by the IAEC having IAEC approval No. IAEC/III-16/2020.

**Availability of data and materials**: The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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## References

Abdelkhalek N.K.M., Ghazy E.W., Abdel-Daim M.M. (2014). Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile

tilapia, *Oreochromis niloticus*: Impact on lipid peroxidation and oxidative stress. Environmental Science and Pollution Research, (22): 3023-3031.

Aust S.D. (1985). Lipid peroxidation. In: R.A. Greenwald (Ed.), Handbook methods for oxygen radical research. CRC Press Revivals, Florida. pp: 203-210.

Bhatti J.S., Bhatti G.K., Reddy P.H. (2017). Mitochondrial dysfunction and oxidative stress in metabolic disorders — A step towards mitochondria based therapeutic strategies. Biochimica et Biophysica Acta, (1863): 1066-1077.

Bremer K., Kocha K.M., Snider T., Moyes C.D. (2015). Sensing and responding to energetic stress: The role of the 2 AMPK-PGC1α-NRF1 axis in control of mitochondrial biogenesis in. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, (199): 4-12.

Butler I.B., Schoonen M.A.A., Rickard D.T. (1994). Removal of dissolved oxygen from water: A comparison of four common techniques. Talanta, (41): 211-215.

Chiu D.KC., Tse K.P.W., Law C.T., Xu IMJ., Lee D., Chen M., Lai R.K.H., Yuen V.W.H., Cheu J.W.S., Ho D.W.H., Wong C.M., Zhang H., Ng I.O.L., Wong C.C.L. (2019). Hypoxia regulates the mitochondrial activity of hepatocellular carcinoma cells through HIF/HEY1/PINK1 pathway. Cell Death and Disease, (10): 1-16.

Chowdhury S., Chatterjee S.K., Bhattacharya S., Maitra S., Saikia S.K. (2020). Acidic ambiance induced post-oxidative stress affects AMPK-PGC1a-SIRT1 axis in the skeletal muscles of zebrafish *Danio rerio* Hamilton, 1822. Acta Biologica Szegediensis, (64): 191-198.

Dong Y., Xia T., Yu M., Wang L., Song K., Zhang C., Lu K. (2022). Hydroxytyrosol attenuates high-fat-diet-induced oxidative stress, apoptosis and inflammation of blunt snout bream (*Megalobrama amblycephala*) through its regulation of mitochondrial homeostasis. Fishes, (7): 1-12.

Dutta S.P., Jamir R.P., Basaiawmoit S.P., Alam A. (2016). Effects of N-Nitrosodibutylamine on the liver mitochondria morphology and on the expression of liver mitochondrial membrane surface proteins in mice. International Journal of Pharmacy and Pharmaceutical Sciences, (8): 162-168.

Dutta S.P., Neog K., Alam A. (2021). Transmission electron microscopy of the liver mitochondria of N-Nitrosodibutylamine-treated mice. Microscopy Research and Technique (84): 2832-2836.

Dykstra M.J., Reuss L.E. (1992). Biological electron microscopy: theory, techniques, and troubleshooting. Springer, Berlin. 360 p.

Eze J.I., Anene B.M., Chukwu C.C. (2008). Determination of serum and organ malondialdehyde (MDA) concentration, a lipid peroxidation index, in *Trypanosoma brucei*-infected rats. Comparative Clinical Pathology, (17): 67-72.

Grant K.A., Raible D.W., Piotrowski T. (2005). Regulation of latent sensory hair cell precursors by glia in the zebrafish lateral line. Neuron, (45): 69-80.

Guo C., Sun L., Chen X., Zhang D. (2013). Oxidative stress, mitochondrial damage and neurodegenerative diseases. Neural Regeneration Research, (8): 2003-2014.

Jiang Y., Wang X., Hu D. (2017). Mitochondrial alterations during oxidative stress in chronic obstructive pulmonary disease. International Journal of Chronic Obstructive Pulmonary Disease, (13): 1153-1162.

Kim J.C., Park G.D., Kim S.H. (2017). Inhibition of oxidative stress by antioxidant supplementation does not limit muscle mitochondrial biogenesis or endurance capacity in rats. The Journal of Nutritional Science and Vitaminology, (63): 277-283.

Lee H.C., Wei Y.H. (2005). Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. International Journal of Biochemistry and Cell Biology, (37): 822-834.

Lu K., Policar T., Song X., Rahimnejad S. (2020). Molecular characterization of PGC-1β (PPAR Gamma Coactivator 1β) and its roles in mitochondrial biogenesis in blunt snout bream (*Megalobrama amblycephala*). International Journal of Molecular Sciences, (21): 1-13.

Lushchak V.I., Duszenko M., Gospodaryov D.V., Garaschuk O. (2021). Oxidative stress and energy metabolism in the brain: Midlife as a turning point. Antioxidants, (10): 1-19.

McConnell J.M., Petrie L. (2004). Mitochondrial DNA turnover occurs during preimplantation development and can be modulated by environmental factors. Reproductive Biomedicine Online, (9): 418-424.

Mueller I.A., Grim J.M., Beers J.M., Crockett E.L., O'Brien K.M. (2011). Inter-relationship between mitochondrial function and susceptibility to oxidative stress in red- and white-blooded Antarctic notothenioid fishes. Journal of Experiment Biology, (214): 3732-

3741.

O'Brien K.M., Mueller I.A. (2010). The unique mitochondrial form and function of Antarctic Channichthyid icefishes. Integrative and Comparative Biology, (50): 993-1008.

Pung Y.F., Sam W.J., Hardwick J.P., Yin L., Ohanyan V., Logan S., Vincenzo L.D., Chilian W.M. (2013). The role of mitochondrial bioenergetics and reactive oxygen species in coronary collateral growth. American Journal of Physiology-Heart and Circulatory Physiology, (305): 1275-1280.

Sorvina A., Bader C.A., Darby J.R.D., Lock M.C., Soo J.Y., Johnson I.R.D., Caporale C., Voelcker N.H., Stagni S., Massi M., Morrison J.L., Plush S.E., Brooks D.A. (2018). Mitochondrial imaging in live or fxed tissues using a luminescent iridium complex. Scientific Reports, (8): 1-8.

Srivastava B., Reddy P.B. (2017). Lipid peroxidation and DNA damage as biomarkers of pollution induced oxidative stress (OS) in fish. Life Science International Journal, (4): 194-198.

Strobel N.A., Peake J.M., Matsumoto A., Marsh S.A., Coombes J.S., Wadley G.D. (2010). Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. Medicine and Science in Sports and Exercise, (1): 1017-1024.

Venditti P., Stefano L.D., Meo S.D. (2013). Mitochondrial metabolism of reactive oxygen species. Mitochondrion, (13): 71-82.

Yoboue E.D., Devin A. (2012). Reactive oxygen species-mediated control of mitochondrial biogenesis. International Journal of Cell Biology, (1): 1-8.

Zou Y.Y., Chen Z.L., Sun C.C., Yang D., Zhou Z.Q., Xiao Q., Peng XY., Tang C.F. (2022). A High-Fat Diet Induces Muscle Mitochondrial Dysfunction and Impairs Swimming Capacity in Zebrafish: A New Model of Sarcopenic Obesity. Nutrients, (14): 1-11.