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Histological Changes in the Vital Organs of *Labeo rohita* Acclimated to Increased Temperatures and Exposed to Critical Temperatures

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ABSTRACT

The present research study describes histological aberrations in the vital organs namely, kidney, liver and gill of *Labeo rohita* advanced fingerlings acclimated at 26 (Control), 31, 33 and 36^{0}C for 30 days, and those exposed to the Critical Thermal Maxima (CTMax) (40.63 ± 0.17^{0}C) and Critical Thermal Minima (CTMin) (13.73 ± 0.07 ^{0}C) (temperature increased or decreased at the rate of 0.3^{0}C/min from 26^{0}C). Fish acclimated at 26, 31 and 33^{o}C showed normal histoarchitecture. However, fish acclimated at 36^{o}C and those exposed to CTMax and CTMin showed damaged gill lamellae, hepatic hypertrophy and renal hyperplasia. Our report on histopathological aberrations in the important organs at higher acclimation temperatures (36^{0}C) and during acute thermal changes (CTMax and CTMin) will help to monitor health and proper management of *L. rohita* culture during climate change scenario.

Keywords *Labeo rohita,* Histology, Temperature acclimation, CTMax, CTMin.

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INTRODUCTION

Suitable environment is a prerequisite for maintaining physiological homeostasis essential for growth and reproduction in fishes. Temperature is an important environmental factor profoundly influencing growth, reproduction and health and survival of fish. Each fish species possesses distinct temperature tolerance thresholds as well as optimal conditions for growth, reproduction, and disease resistance (Borghetti and Canzi 1993). Within certain limits, increased water temperature may benefit fish culture leading to faster growth (Das *et al.* 2005, Besson *et al.* 2016). Conversely, extreme temperatures, whether high or low, can induce stress in fish, thereby disrupting their biochemical functions and cellular structures (Egginton and Sidell 1989, Das *et al*. 2009, Das *et al.* 2014, Dalvi *et al.* 2023). Histopathological investigations are widely used as biomarkers for evaluating fish health both in laboratory (Thophon *et al.* 2003) and in natural conditions (Teh *et al.* 1997). It is an imperative tool for monitoring organismal health compared to a single biochemical parameter (Segner and Branubeck 1988). Histological investigations are routinely used as indicators to evaluate stress levels and monitoring of fish health in aquaculture systems (Schwaiger *et al.* 1997).

Thermal tolerance research in fish has been conducted for over a century, highlighting its significant relevance (Beitinger *et al.* 2000). Indian major carps (IMCs), which include *Labeo rohita, Catla catla,* and *Cirrhinus mrigala*, are extensively farmed freshwater species across India due to their high demand and economic value. They thrive well between18.3^{0}C to 37.8^{0}C, however, temperatures below 16.7^{0}C and above 39.5^{0}C are fatal (Jhingran 1975). Our earlier investigations revealed temperature acclimation dependent adaptation in *L. rohita* (Das *et al.* 2004, 2005, Das *et al.* 2009). Surface water temperatures are anticipated to vary by as much as 40°C due to factors such as latitude, season, altitude, time of day, water depth, and other influences (Munro and Roberts 2001). In contrast to the open seas and oceans, shallow inland waters are expected to experience more frequent temperature changes (Kennedy *et al.* 2022). This situation underscores the importance of understanding how elevated water temperatures impact the histoarchitecture of essential organs in IMCs. Therefore, we investigated the effects of varying acclimation temperatures (26°C as control, 31, 33 and 36) as well as acute temperature change (critical thermal maximum (CTMax) and critical thermal minimum (CTMin)) on the histoarchitecture of key organs, specifically the kidney, liver, and gills of *L. rohita.*

MATERIALS AND METHODS

Maintenance of fish

L. rohita advanced fingerlings (mean weight ± SE: 31.5 ± 1.78 g) were bought from Pancham fish farm (Saphale, Maharashtra) transported with proper aeration to the wet laboratory at Central Institute of Fisheries Education, Mumbai. The fish underwent a 30-day acclimatization period in the laboratory at a temperature of 26 ± 1°C, during which they were provided with supplementary feed containing 35% protein.

Acclimation of fish and experimental setup

For acclimation studies, total 24 *L. rohita* were equally distributed (6 per aquaria) in four thermostatic aquaria (52 liters capacity, sensitivity ± 0.2^{0}C, Suan Scientific Instruments, West Bengal, India). Water temperature in the aquaria were increased at the rate of 1^{0}C per day over the ambient temperature (26^{0}C) to reach the test temperatures of 31,33 and 36^{0}C and maintained for another 30 days. During the acclimation period fish were fed with supplementary feed (protein 35%). Water quality parameters were assessed on a daily basis and remained within acceptable thresholds. The concentration of dissolved oxygen in the aquaria was sustained at 5.5 ± 0.5 mg /L through the use of an air blower. A 20% water exchange in the aquaria was performed every alternate day. For the critical temperature studies, 12 fish were evenly allocated between two aquaria and acclimatized at a temperature of 26°C for a duration of 30 days. Following this acclimatization period, the water temperature in the aquaria was systematically altered, either increased or decreased, at a consistent rate of 0.3°C per minute until the fish exhibited signs of loss of equilibrium, designated as CTMax (40.63 ± 0.17^{0}C) and CTMin (13.73 ± 0.07 ^{0}C), respectively (Beitinger *et al.* 2000, Das *et al.* 2004, 2005, Dalvi *et al*. 2009).

Tissue histology

Fish acclimated at 26, 31, 33 and 36^{0}C for 30 days, and those exposures to CTMax (40.63 ± 0.17^{0}C) and CTMin (13.73 ± 0.07^{0}C) were anaesthetized with clove oil (50µL.L^{-1}), organs (liver, gills and kidney) were carefully dissected, fixed in buffered neutral formalin, processed, embedded in paraffin wax, sectioned into thin slices of 6 µm, and stained with hematoxylin and eosin (The *et al.* 1997, Dalvi *et al.* 2023).

RESULTS AND DISCUSSION

Every fish species has specific temperature tolerance

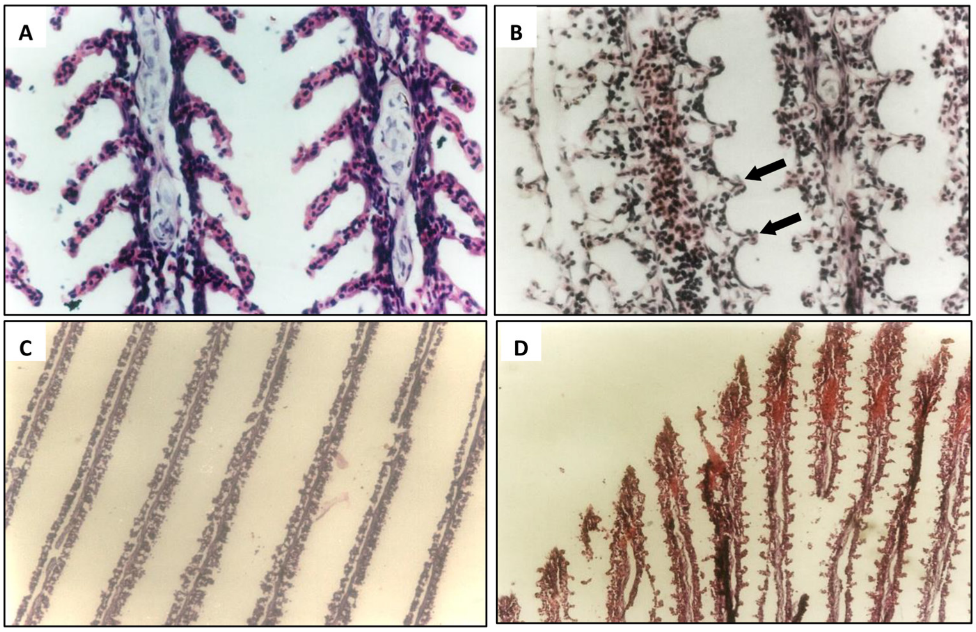

Fig. 1. Histology of the gill of *Labeo rohita* (H and E staining). A: Acclimated at 26, 31 and 33^{0}C showing normal histoarchitecture (magnification 320 X, H and E stain, representative figure), B: Gill of fish acclimated at 36^{0}C showing complete loss of 2/3rd of secondary gill lamellae (magnification 320 X, H and E stain), C: Gill of fish exposed to CTMax showing complete loss of normal structure and atrophy with congestion in the secondary gill lamellae (magnification 40 X, H and E stain); and D: Gill of fish exposed to CTMin showing marked congestion and atrophy in the secondary gill filament (magnification 80 X, H and E stain).

limit beyond which it experiences stress. In our study, *L. rohita* acclimated at 26, 31 and 33^{0}C showed normal histoarchitecture in the gill (Fig. 1A), kidney (Fig. 2A), and liver (Fig. 3A) tissues as these temperatures are within the optimum range of *L. rohita*, supporting our previous findings (Das *et al.* 2004). However, fish acclimated at 36^{0}C and those exposed to CTMax (40.63 ± 0.17^{0}C) and CTMin (13.73 ± 0.07 ^{0}C) showed severe changes in the tissue histology.

Fish acclimated at 36^{0}C showed significant changes, specifically complete loss of two-third of the secondary gill lamellae and mild atrophy observed in the upper third of the secondary gill lamellae from base of the gill arch (Fig. 1B). The gill in fish exposed to CTMax showed complete loss of normal structure and atrophy with congestion in the secondary gill lamellae (Fig. 1C), while the gill in fish exposed to CTMin exhibited marked congestion and atrophy in the secondary gill filament (Fig. 1D). Exposure to elevated temperatures can lead to respiratory distress and neurological impairments in fish. We observed that at 36^{0}C the effect on gill was profound than the other organs. The gills are most sensitive organs due to their close proximity with the external environment, functioning as heat exchangers that facilitate the transfer of heat between the surrounding water and the fish's body (Tattersall *et al.* 2012). Studies examining pollution effects in fish emphasized gill as an organ of paramount importance for assessing the degree of damage (Wang *et al.* 2022) and also serves as a significant marker for assessing thermal pollution (Alazemi *et al.* 1996). Elevated water temperatures have a dual impact on the oxygen demand and supply ratio in fish. While warmer water holds less dissolved oxygen, the metabolic rate of fish increases, leading to heightened oxygen uptake. Additionally, higher temperatures can disrupt the ability of fish to maintain osmotic balance, as changes in the lipids of gill cells can cause cell leakage, thereby diminishing the efficiency of salt excretion and regulation (Munro and Roberts 2001). Fish may experience respiratory distress and even coma as a result of lower temperatures. Although cold water contains a higher concentration

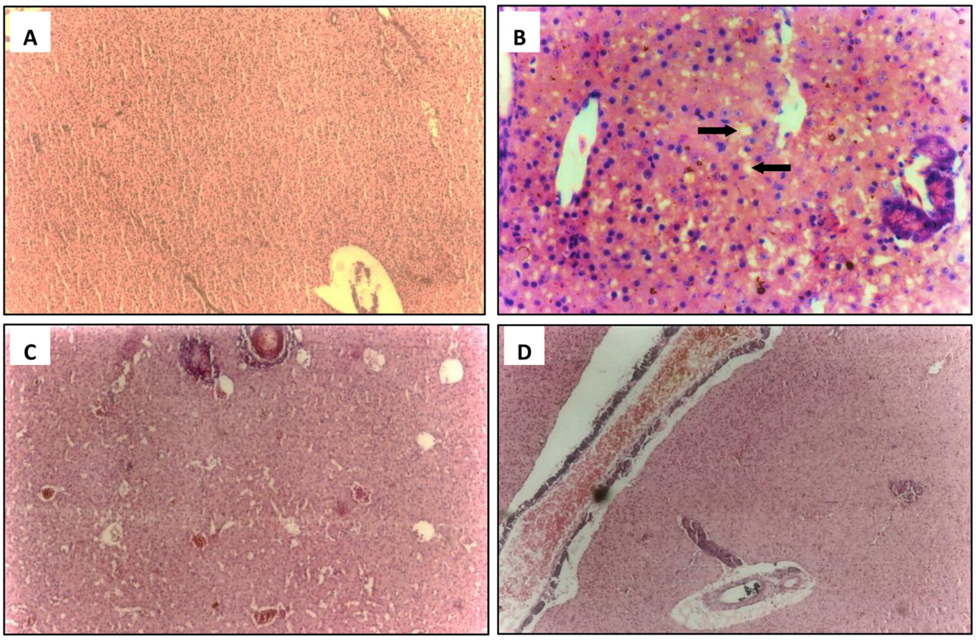

Fig. 2. Histology of the liver of *L. rohita* (H and E staining). A: Acclimated at 26, 31 and 33^{0}C showing normal histoarchitecture (magnification 80 X, H and E stain, representative figure), B: Liver of fish acclimated to 36^{0}C with hypertrophic hepatocytes and loss of cellular boundaries (magnification 320 X, H and E stain), C: Liver of fish exposed to CTMax showing focal areas of haemorrhages could be seen (magnification 80 X, H and E stain) and D: Liver of fish exposed to CTMin with showing marked swelling of the hepatocytes and constricted sinusoids (magnification 80 X, H and E stain).

of dissolved oxygen compared to warm water, the inhibitory effect on the respiratory rate of fish may stem from inadequate oxygen uptake, resulting in hypoxia (Holt and Jørgensen 2015). Our previous research has documented behavioral indicators linked to hypothermia, such as a reduced respiratory rate and impaired swimming coordination (Das *et al.* 2005, Das *et al*. 2014).

The liver in fish acclimated at 36^{0}C showed hypertrophy, loss of cellular boundaries and vacuolation of the hepatocytes (Fig. 2B), those exposed to CTMax showed focal areas of haemorrhages in the liver (Fig. 2C), while those exposed to CTMin showed mild swelling and sinusoids in the liver tissue (Fig. 2D). The liver is majorly associated with metabolism and detoxification of harmful metabolites. Therefore, liver histology is an important indicator of fish health and also useful for understanding its interaction with the environment (Aboka *et al.* 2017). In the present study, hepatocytes of *L. rohita* acclimated at 36^{0}C showed hypertrophy (due to proliferation of endoplasmic reticulum) which is similar with those reported in fish, exposed to aluminium (Hadi and Alwan 2012). Loss of cellular boundaries (total cellular degeneration) observed in *L. rohita* at 36^{0}C in our study, which is similar to those observed in *Labeo boga* exposed to higher temperature (Raina *et al.* 2015). We also observed hemorrhages in liver of fish exposed to CTMax, similar to those observed in perch exposed to metal contaminated water (Yancheva *et al.* 2016) and in sea-bass exposed to cadmium (Thophon *et al.* 2003), indicating total disarrangement and degeneration of the liver at near lethal temperature. We observed that fish exposed to CTMin showed marked swelling and constricted sinusoids, indicating total degeneration of liver tissue and cessation of metabolic process leading to cell death. Similar observations were made in *L. boga* exposed to lower temperature suggesting that lower temperature is more deleterious to physiological functioning of the liver compared to higher temperature (Raina *et al.* 2015).

The kidney in fish acclimated at 36^{0}C showed

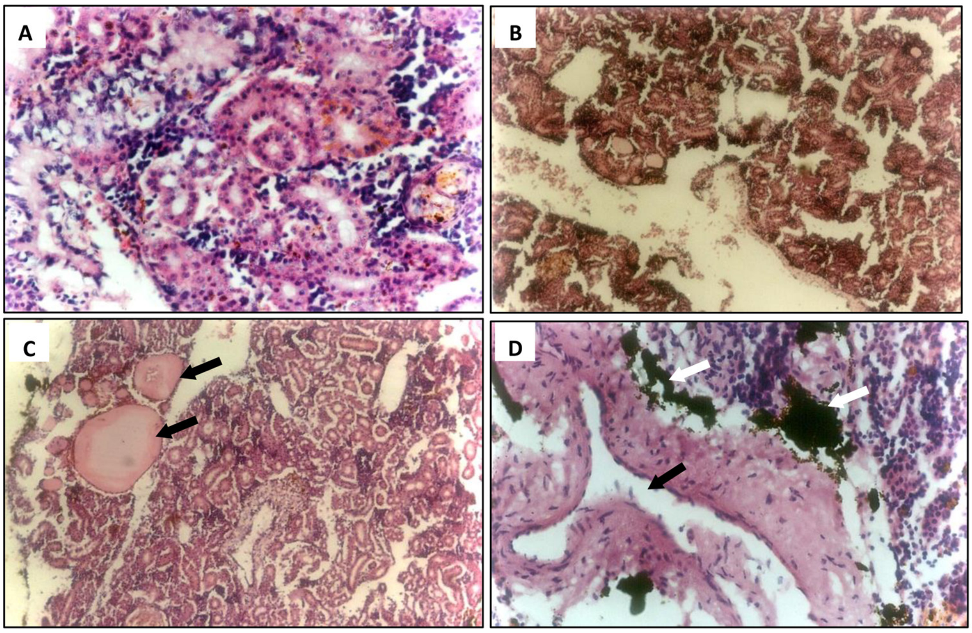

Fig. 3. Histology of the kidney of *L. rohita* (H and E staining). A: Acclimated at 26, 31 and 33^{0}C showing normal histoarchitecture (magnification 320 X, H and E stain, representative figure), B: Kidney of fish acclimated to 36^{0}C showing dilation of blood vessels with residual RBC's (magnification 80 X, H and E stain), C: Kidney of fish exposed to CTMax showing occasional accumulation of fibrinous masses in distal tubules (arrow) (magnification 80 X, H and E stain) and D: Kidney of fish exposed to CTMin showing hyperplasia and hyperactive melano-macrophage centers (white arrows) and vasoconstriction with deformed lumen of the blood vessels (black arrow) (magnification 320 X, H and E stain).

severe dilation of the blood vessels with residual red blood cells (Fig. 3B). Fish exposed to CTMax showed occasional accumulation of fibrinous masses in distal tubules of the kidney (Fig. 3C), while those exposed to CTMin showed hyperactive melanomacrophage centers and vasoconstriction with deformed lumen of the blood vessels in the kidney (Fig. 3D). In teleost, kidney is an important organ responsible for excretion. Drastic alteration in the water temperature creates stress in the renal tissues leading to complete degeneration and disarrangement of the cells (Dash *et al.* 2011, Aboka *et al.* 2017, Dalvi *et al.* 2023). In our study, kidney of *L. rohita* acclimated at 36^{0}C showed dilution of blood vessels with residual RBC similar to those reported earlier (Dash *et al.* 2011). We observed occasional accumulation of fibrinous masses in distal tubules of the kidney in fish exposed to CTMax, which is consistent to those reported earlier in different fish species exposed to various environmental stressors (Dash *et al.* 2011, Hadi and Alwan 2012, Velmurugan *et al.* 2007). Our observations on alterations in the kidney including hyperplasia and hyperactive melano-macrophage centers, vasoconstriction with de-shaping of the lumen of the blood vessels in fish exposed to CTMin, indicates severe degeneration and disarrangement, similar to those reported in liver of *L. boga* acclimated at lower temperature (Raina *et al.* 2015). Our observations on the histological changes in the gill, liver and kidney tissues corroborate with those reported earlier in *L. rohita* acclimated to higher temperatures (Dash *et al.* 2011) and in *Horabagrus brachysoma* acclimated at 36^{0}C and exposed to CTMax and CTMin (Dalvi *et al*. 2023).

CONCLUSION

Our study provides prima facie evidence elaborating extensive tissue damage in *L. rohita* acclimated at 36^{0}C and in those exposed to CTMax and CTMin. Our investigation confirms that *L. rohita* is most vulnerable when acclimated at 36^{0}C and also to extreme and acute change in water temperature. Results of the present study will help fish farmers and policy makers to monitor fish health and avoid temperature influenced tissue aberrations to ensure maximum production.

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REFERENCES

Aboka ER, Jian Z, Shengming S, Wuxiao Z, Han Y, Li SY, Jiahui Y, Jiabin Z (2017) Histopathological changes in gills, liver, and kidney tissues of bighead carp (*Aristichthys nobilis*) due to the effects of acute high-temperature stress. *Israeli Journal of Aquaculture – Bamidge*h 5(2) : 71-79. http://dx.doi.org/10.46989/001c.21062

Alazemi BM, Lewis JW, Andrews EB (1996) Gill damage in the freshwater fish *Gnthonemusptersii* (family: Mormyridae) exposed to selected pollutants: An ultrastructure study. *Environmental Technology* 17: 225-238. https://doi.org/10.1080/09593331708616381

Beitinger TL, Bennett WA, McCauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes* 58: 237-275. https://doi.org/10.1023/A:1007676325825

Besson M, Vandeputte M, Van Arendonk JAM, Aubin J, De Boer IJM, Quillet E, Komen H (2016) Influence of water temperature on the economic value of growth rate in fish farming: The case of sea bass (*Dicentrarchus labrax*) cage farming in the Mediterranean. *Aquaculture* 462: 47-55. https://doi.org/10.1016/j.aquaculture.2016.04.030

Borghetti JR, Canzi C (1993) The effect of water temperature and feeding rate on the growth rate of pacu (*Piaractus mesopotamicus*) raised in cages. *Aquaculture* 114 (1-2): 93-101. https://doi.org/10.1016/0044-8486(93)90253-U

Dalvi RS, Das T, Pal AK, Debnath D (2023) Histological changes in *Horabagrus brachysoma* acclimated to warm temperature and exposed to critical thermal maxima and minima. *Zoology and Ecology* 33(2): 97–109. http://dx.doi.org/10.35513/21658005.2023.2.1

Dalvi RS, Pal AK, Tiwari LR, Das T, Baruah K (2009) Thermal tolerance and oxygen consumption rates of the catfish *Horabagrus brachysoma* (Günther) acclimated to different temperatures. *Aquaculture* 295(1-2): 116-119. https://doi.org/10.1016/j.aquaculture.2009.06.034

Das T, Pal AK, Chakraborty SK, Manush SM, Chatterjee N, Mukherjee SC (2004) Thermal tolerance and oxygen consumption of Indian Major carps acclimated to four temperatures. *Journal of Thermal Biology* 29: 157-163. https://doi.org/10.1016/j.jtherbio.2004.02.001

Das T, Pal AK, Chakraborty SK, Manush SM, Sahu NP, Mukherjee SC (2005) Thermal tolerance, growth and oxygen consumption rate of *Labeo rohita* fry (Hamilton 1822) acclimated to four temperatures. *Journal of Thermal Biology* 30: 378-383. https://doi.org/10.1016/j.jtherbio.2005.03.001

Das T, Pal AK, Chakraborty SK, Manush SM, Dalvi RS, Apte SK, Sahu NP, Baruah K (2009) Biochemical and stress responses of *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) in relation to acclimation temperatures. *Journal of Fish Biology* 74: 1487-1498. https://doi.org/10.1111/j.1095-8649.2009.02216.x

Das T, Sahu NP, Chakraborty SK, Chatterjee N, Mohammed MS, Dalvi RS, Baruah K, Pal AK (2014) Ultrastructural alterations in the gills of *Labeo rohita* fingerlings exposed to thermal extremes. *Anatomia, Histologia, Embryologia* 43: 75-80. https://doi.org/10.1111/ahe.12039

Dash G, Yonzone P, Chanda M, Paul M (2011) Histopathological changes in *Labeo rohita* (Hamilton) fingerlings to various acclimation temperatures. *Chronicles of Young Scientists* 2(1): 29-36. https://doi.org/10.4103/2229-5186.79347

Egginton S, Sidell BD (1989) Thermal acclimation induces changes in subcellular structure of fish skeletal muscle. *American Journal of Physiology* 256: R1-R9. https://doi.org/10.1152/ajpregu.1989.256.1.R1

Hadi AA, Alwan SF (2012) Histopathological changes in gills, liver and kidney of freshwater fish, *Tilapia zilli,* exposed to aluminium. *International Journal of Pharmacy & Life Sciences* 3(11): 2071-2081.

Holt RE, Jørgensen C (2015) Climate change in fish: Effects of respiratory constraints on optimal life history and behavior. *Biology letters* 11(2): 20141032. https://doi.org/10.1098/rsbl.2014.1032

Jhingran VG (1975) Fish culture in freshwater ponds. In: Fish and Fisheries of India. Jhingran, VG ed, pp 276.

Kennedy IR, Runcie JW, Zhang S, Ritchie RJ (2022) A new look at physico-chemical causes of changing climate: Is the seasonal variation in seawater temperature a significant factor in establishing the partial pressure of carbon dioxide in the Earth's atmosphere?. *Thermo* 2(4): 401-434. https://doi.org/10.3390/thermo2040028

Munro ALS, Roberts RJ (2001) In: Fish Pathology (Harcourt Publishers Limited), pp 1 – 11.

Raina S, Sachar A, Gupta K (2015) Temperature fluctuations induced histopathological alterations in the liver of fish, *Labeo boga* inhabiting Jammu waters. *International Journal of Fisheries and Aquatic Studies* 2(4): 12-16.

Schwaiger J, Wanke R, Adam S, Pawert M, Honnen W,Triebskorn R (1997) The use of histopathological indicators to evaluate contaminant-related stress in fish. *Journal of Aquatic Ecosystem Stress and Recovery* 6(1): 75-86. https://doi.org/10.1023/A:1008212000208

Segner H, Braunbeck T (1988) Hepatocellular adaptation to extreme nutritional in ide, *Leuciscus idusmelanotus* L. (Cyprinidae). A morphofunctional analysis. *Fish Physiology and Biochemistry* 5: 79-97. https://doi.org/10.1007/bf01875645

Teh SJ, Adams SM, Hinton DE (1997) Histopathologic biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. *Aquatic toxicology* 37(1): 51-70. https://doi.org/10.1016/S0166-445X(96)00808-9

Tattersall GJ, Sinclair BJ, Withers PC, Fields PA, Seebacher F, Cooper CE, Maloney SK (2012) Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Comprehensive Physiology* 2(3): 2151-2202. https://doi.org/10.1002/cphy.c110055

Thophon S, Kruatrachue M, Upathan ES, Pokethitiyook P, Sahaphong S, Jarikhuan S (2003) Histopathological alterations of white sea bass *Lates calcarifer* in acute and subchronic cadmium exposure. *Environmental Pollution* 121: 307-320. https://doi.org/10.1016/s0269-7491(02)00270-1

Velmurugan B, Selvanayagam M, Cengiz E, Unlu E (2007) The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala. Journal of Environmental Science and Health-B* 42: 157-163. https://doi.org/10.1080/03601230601123292

Wang Z, Dong Z, Yang Y, Wang J, Yang T, Chen X, Liang L, Mu W (2022) Histology, physiology, and glucose and lipid metabolism of *Lateolabrax maculatus* under low temperature stress. *Journal of Thermal Biology* 104: 103161. https://doi.org/10.1016/j.jtherbio.2021.103161

Yancheva V, Velvheva I, Stoyanova S, Georgieva E (2016) Histological biomarkers in fish as a tool in ecological risk assessment and monitoring program : A review. *Applied Ecology and Environmental Research* 14(1): 47-75. http://dx.doi.org/10.15666/aeer/1401_047075