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Influence of Water Temperature on Physiological Functions and Histology of Crucian Carp, *Carassius carassius*

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Abstract

The present study was conducted for forty-five days to check the effect of water temperatures on hemato-biochemical parameters and histopathology of gills and liver of Crucian carp, *Carassius carassius.* Two hundred fishes were randomly stocked in five treatments each with four replicates in 72 litre tanks. Five treatments namely T_1, T_2, T_3, T_4 and T_5 were maintained with water temperature of 16°C, 20°C, 24°C, 28°C and 32°C respectively. The hemoglobin, hematocrit and Total Red blood cells increased with the increase in acclimation temperature. Whereas, total leukocyte count showed an inverse trend. Differential leukocyte count showed significant ($P<0.05$) increase in neutrophil and drop in lymphocyte count. Minimum glucose level was observed at T_3. Total-protein, albumin and globulin increased with the increase in temperature. whereas, Triglycerides and cholesterol showed inverse trend. The histological examination revealed that gills and liver were adversely affected below T_3 and above T_4, with most marked changes noticed at 32°C (T_5). This fish displayed adaptive physiological changes to counteract the effect of temperature variations. The findings of this study indicate that *C. carassius* can be reared between 24°C to 28°C and is important for understanding how fish adapt to temperature changes, especially in context of climate change.

Introduction

Temperature is a crucial environmental factor with a substantial impact on the physiology of fish. The rising water temperatures associated with global warming are causing significant apprehension among aquaculturists and fishery biologists. Increase in temperature due to climate change is already influencing physiological processes in fish, leading to a reduction in fish populations and in some cases, the potential extinction of certain species (Cheng et al., 2013). Fish are greatly sensitive to changes in the physical and chemical properties of water, which can manifest in changes to their blood constituents (Pinna et al., 2023). Fish blood can be readily obtained, and examining blood parameters can offer valuable insights into how fish react physiologically to environmental variations that disrupt their internal equilibrium (Salam et al., 2015; Sharmin et al., 2016). Blood indices parameters are immediately impacted when fish encounter temperature stress (Costa et al., 2016). Therefore, haematological research can shed light on fish's

physiological condition and level of tolerance (Witeska et al., 2023). It is recommended to use hematological parameters to evaluate the general health state of animals and to provide early warning of potentially harmful changes in stressed organisms (Fazio, 2019). These parameters, in addition to serving as stress biomarkers, are intricately influenced by a variety of variables, including the quantity and quality of dietary protein (Ahmed & Ahmad, 2020), which has a significant impact on energy metabolism and causes significant fluctuations in the fish's various serum biochemical parameters. Understanding the hemato-biochemical changes that occur in fish raised in intense environments helps enhance production, fish welfare and the standard of culture procedures (Fazio, 2019; Uiuiu et al., 2021).

For a long time, histological studies have been acknowledged as trustworthy biomarkers of stress in fish (Van et al., 2003). Variations in temperature have been demonstrated to impact the histology of several fish organ systems. The histological examination of target organs, tissues that respond to a particular stimulus, either internal or external is a sensitive and accurate method for understanding changes in tissues. Abiotic factors like temperature make some organs more vulnerable to alterations than others. The gills and liver are among the organs to be taken into account for histological analyses, both in vertebrates and aquatic invertebrates. These organs are in charge of critical processes like respiration, excretion, and the accumulation and biotransformation in fish (Gernhofer et al., 2001). Understanding histopathological changes in fish due to thermal stress is crucial for assessing fish health and environmental quality. Studies have shown that elevated temperatures can cause significant alterations in vital organs such as gills, liver, kidney, and heart (Dash et al., 2011; Aboka et al., 2017). These changes include epithelial lifting, edema, necrosis, and hyperemia, which can impair respiratory and other physiological functions (Aboka et al., 2017). While fish can adapt to some temperature changes, chronic exposure to high temperatures can lead to degenerative changes and reduced survival (Dash et al., 2011). It also serves as an important tool for evaluating fish health, water quality and the presence of pollutants in aquatic environments (Lopes, 2021). This understanding is particularly valuable for aquaculture management, helping to reduce disease impact and improve productivity (Aisyah & Andriani, 2024). Overall, histopathological studies provide crucial insights into fish adaptation mechanisms and environmental health assessment. The aim of the present study was to investigate the effects of changing water temperature on hemato-biochemical parameters and histopathology of gills and liver of *C. carassius*, a warm-water fish species which has ornamental value and significant commercial value that is found throughout Northeastern Asia in nations including Taiwan, Japan, China and Korea (Everard, 2025).

Materials and Methods

Procurement and Acclimatization of Fish

Fish samples were procured from commercial catches of Dal-lake, Kashmir from the local fishermen. Before acclimatization, fishes were given a prophylactic treatment of 0.5 ppm potassium permanganate ($KMnO_4$). Dead fishes were removed and the remaining fishes were acclimatized for one week to laboratory conditions. Fishes were then transferred into glass tanks (60 cm × 30 cm × 40 cm) containing 72 litres of water, wherein gradual acclimatization was done as per the treatments and this procedure took around a week.

Experimental Procedure

The experiment was conducted in Fishery Engineering laboratory of Faculty of Fisheries, SKUAST-K, Rangil for a period of 45 days. Completely randomized design (CRD) was used with five treatments, namely T_1, T_2, T_3, T_4 and T_5 maintained at water temperatures of 16°C, 20°C, 24°C, 28°C and 32°C respectively, each with four replicates. Temperature was adjusted using thermostats "Rs- Electrical" (Rs-200 W) and was monitored using digital thermometer. Fish of average initial weight 34.88± 0.15 g were randomly assigned ten fish per tank. The fish were fed twice daily 2-3% of body weight with a diet containing 35% crude protein. Tanks were cleaned once a day by siphoning and 25% of water was replaced everyday with filtered water to assure water quality. Each day dead fish (if occured) was removed with the help of hand net. Water quality parameters like dissolved oxygen (DO), pH, carbon dioxide (CO_2), total alkalinity and hardness were recorded once in a week as per the standard methods given by Adoni et al. (1985); APHA (2017).

Sample Collection and Analyses

After a period of forty-five days rearing experimental fishes at different treatments, four fish from each replicate were anaesthetized before sampling with clove oil (150 µl/L) in water. Once the fish lost equilibrium, the blood samples were drawn by caudal vein puncture using heparinised syringe having 25-gage needle. Collected blood was stored in EDTA tubes at 4°C until the period of hematological analyses and the blood in non-heparinized eppendorf tubes was centrifuged at 5000 rpm for 5 min at 4°C to undergo biochemical analysis. The collected serum was stored at − 20°C for further analyses. Also, the gills and liver tissues dissected from fish were first fixed in 10% formalin. Tissue processing was done by using haematoxylin and eosin staining techniques for histopathological changes (Hussain et al., 2019). The slides were examined by using (OLYMPUS-CX31) binocular microscope and the changes in the organs were identified using as per the characterization given by Dash et al. (2011). Hemoglobin

was estimated using cynmethemoglobin method, hematocrit using wintrobes tube method (Mondal & Budh, 2019). Total leukocyte count (TLC) and total red blood cell count (RBC's) were estimated by the microdilution method using hemocytometer (Stevens,1997), Differential Leucocyte Count (DLC) was performed using the standard protocol (Hudson & Hay, 1991). Biochemical parameters were estimated by using standard kit from Medsource Ozone Biomedicals Pvt. Ltd. Erythrocyte indices *viz*. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were evaluated as per formula of Dacie and Lewis, (1991).

Statistical Analysis

Data was analysed by using one way analysis of variance (ANOVA) and Tukey's multiple range test was carried out for post-hoc comparison of means ($P<0.05$) to check if they were significantly different. All statistical analysis of the data was carried out by using SPSS (version 20) for windows and Microsoft excel.

Results

Effects of Temperature on Hemato - biochemical Parameters

The hemoglobin (Hb), hematocrit (Hct) and total RBC's increased with the increase in acclimation temperature. Highest mean Hb (g/dl) level was encountered at T_3 (24°C) and the lowest value of Hb was encountered at T_2 (20°C). The Hb level in T_3 and T_4 differed significantly ($P<0.05$) from T_1 and T_2. However, no significant difference ($P>0.05$) was observed in T_3, T_4 and T_5 treatments. Maximum Hct % was observed at T_3 (24°C) whereas, lowest Hct% was observed in T_1 (16°C). Hct% in T_3, T_4 and T_5 differed significantly ($P<0.05$) from T_1 andT_2. Highest average of RBC's count was observed at 24°C. whereas, lowest mean of total red blood cell count was observed at T_1 (16°C). Total leukocyte count decreased with the increase in acclimation temperature with the highest count observed at 16°C and lowest count observed at T_5 (32°C). Differential leukocyte count (DLC) showed significant ($P<0.05$) increases in neutrophil (neutrophilia) with the highest count observed at T_3 (24°C) and T_4 (28°C) and decreases in lymphocyte count (lymphocytopenia) with the highest count observed at T_1 (16°C). The other hematological indices such as Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) did not show any significant difference ($P>0.05$) among treatment groups.

The result based on serum biochemical parameters showed that with the increase in temperature the blood glucose level increased upto T_2 (20°C), declined in T_3 (24°C) and T_4 (28°C) and again increased in T_5 (32°C) treatment group. The total protein, albumin and globulin showed the same trend to increase with increasing temperatures and then decreased at high temperature. However, at T_4 (28°C) the concentration of mean albumin showed a decrease which differed significantly ($P<0.05$) from T_3 (24°C) and T_5 (32°C). Triglycerides decreased with the increase in temperature and cholesterol varied in the same fashion as triglycerides with the highest value observed at T_1 (16°C) and lowest value observed at T_5 (32°C). Data pertaining to hematological and serum biochemical parameters of *C. carassius* at 16°C, 20°C, 24°C, 28°C and 32 are illustrated in Figure 1 and Table 1 respectively.

Effects of Water Temperature on Histology of Gills and Liver

No specific histological alterations were observed in the gills and liver of the fishes exposed to 24°C (T_3) and 28°C (T_4). Most of the histopathological alterations were observed after the prolonged exposure of 32°C. In the current investigation, exposure of *C.carassius* to 16°C (T_1) resulted in lamellar fusion, clubbing of secondary lamellae, primary lamellar hyperplasia, hyperplasia of epithelial cells at the bases of branchial secondary lamellae and lamellar aneurysm (Figure 2). Lamellar degeneration, epithelial hypertrophy, epithelial necrosis, primary lamellar hyperplasia, secondary lamellar hyperplasia was observed at 20°C (T_2). The acclimation temperature of 32°C (T_5) displayed severe lamellar degeneration, epithelial lifting, telangiectasis, cellular hyperplasia, necrosis, chloride cell proliferation and epithelial hyperplasia. The liver of the fish at 16°C (T_1) showed various histopathological changes (Figure 3) such as mild melano-macrophage centres and their prevalence increased as the temperature increased, except for T_4 (28°C). Necrosis of hepatocytes, smaller vacuoles were seen and blood spaces or sinusoids were evident at 20°C (T_2). The severity of liver tissue damage at 32°C (T_5) was more. Complete degenerative cellular architecture, severe necrosis of hepatocytes, increased vacuolation, total degeneration, melanin production and pyknotic nuclei were observed.

Discussion

Temperature exerts a pronounced influence on diverse physiological processes in fish, standing out among various environmental factors. The present study aimed to comprehend the implications of temperature on the hemato-biochemical parameters and histology of gills and liver of *C. carassius,* thereby delving into its impact on the overall physiological well-being. Changes in Hct%, Hb concentration and RBC's are indicative of stress, and have been used to determine the acute thermal stress response in fish (Smit et al., 1981; Wendelaar, 1997). In the current study, the RBC's, Hb and Hct showed a common trend of increase with respect to increase in acclimation temperature. However, significant decrease was observed at higher

temperature in RBC's. In previous studies conducted by Das et al. (2002) in *Labeo rohita* and *Rita rita*; Ahmad et al. (2011) in *Cyprinus carpio;* Stewart et al. (2019) in *Ictalurus punctatus;* Pinto et al. (2019) in *Piaractus mesopotamicus* reported a significant increase in Hct% and Hb when temperature was increased. Significant reduction in Hemoglobin at 32°C evident in the current study might be because of compromised function of hematopoietic system under stressful condition caused by elevated temperature (Islam et al., 2019). Some situations may increase metabolic rates (e.g, increase in temperature) and consequently, increase the energy demand can trigger an increase in haemoglobin concentration for oxygen transport, altering the percentage of hematocrit (Brauner & Harter, 2017; Wells, 2009). Decrease in Red blood cells number at low temperature should be observed in conjunction with the hemoglobin values. Usually, this decrease is associated with cases of erythrocytopaenia, possibly occurring due to increased activity of the haem- oxygenase enzymes in the liver when the organism is exposed to low temperatures, resulting in the degradation of the haem group in hemoglobin (Maekawa & Kato, 2015). Total Leukocyte Count (TLC) decreased with the increasing acclimation temperature which defines a weakened defence (immunosuppression) at higher temperature. Generally, at higher temperatures, the TLC counts decrease (Verma et al., 2007). Same was evident in the current study wherein, significant decrease ($P<0.05$) in leukocyte counts at the acclimation temperature of 32°C was observed and this is in alignment with the previous studies conducted by Verma et al. (2007); Ahmad et al. (2011) in *C. carpio* and Akhtar et al. (2012) in *L. rohita* who found reduction in TLC on exposure to elevated temperatures. Differential Leucocyte Count (DLC) showed significant rise in neutrophil and decline in lymphocyte count as the acclimation temperature increased. These results indicate an altered immune status of fish at 32°C. Neutrophilia and Lymphopaenia in fish are hematological responses to stress (Davis et al., 2008). Also, the ratio of neutrophils to lymphocytes can be used as an index of a secondary stress response. Our

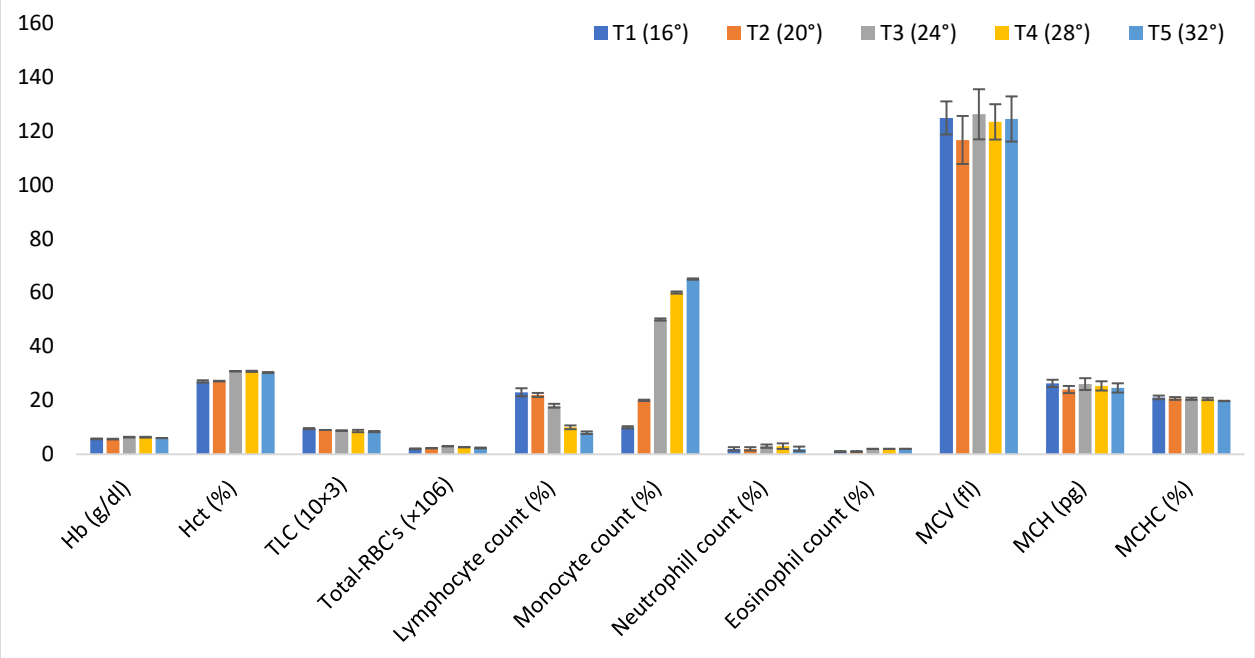
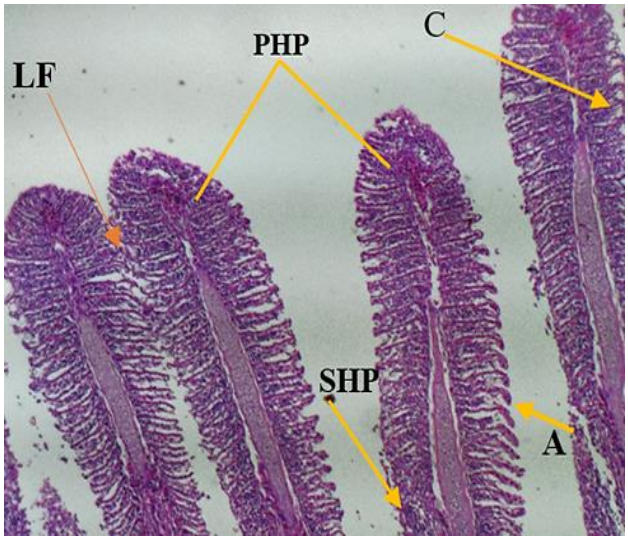


Figure 1. Hematological parameters of Crucian carp, *C. carassius* in response to different levels of water temperatures. Hb(g/dl)= Hemoglobin, Hct (%)= Hematocrit percentage, RBC's= Red Blood Cells, TLC= Total Leukocyte Count, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration.

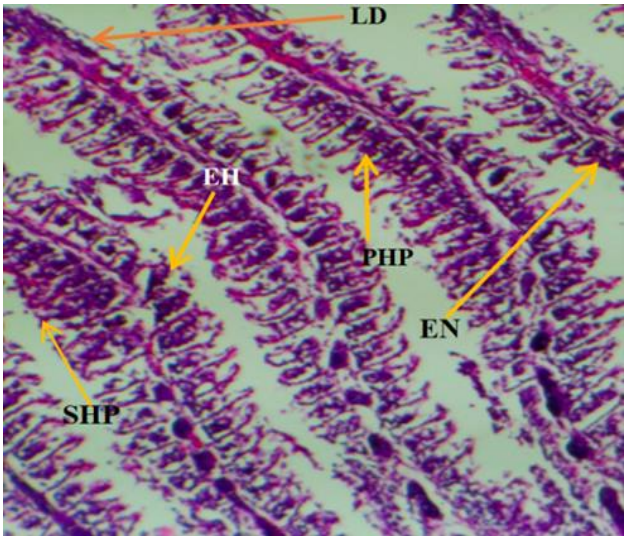
Table 1. Serum biochemical parameters of Crucian carp, *C. carassius* in response to different levels of water temperatures

Parameters	T_1 (16°C)	T_2 (20°C)	T_3 (24°C)	T_4 (28°C)	T_5 (32°C)
Glucose (mg/dl)	80.15 ± 0.09^{c}	84.61 ± 0.26^{d}	68.78 ± 0.88^{a}	70.35 ± 0.17^{b}	80.38 ± 0.24^{c}
Total-Protein (gm%)	4.01 ± 0.01^{a}	4.69 ± 0.07^{b}	4.97 ± 0.02^{c}	5.00 ± 0.01^{c}	4.96 ± 0.02^{c}
Albumin (g/dl)	1.50 ± 0.01^{a}	1.71 ± 0.01^{b}	1.77 ± 0.01^{c}	1.67 ± 0.01^{b}	1.79 ± 0.01^{c}
Globulin (g/dl)	2.51 ± 0.01^{a}	2.99 ± 0.06^{b}	3.20 ± 0.03^{cd}	3.33 ± 0.01^{d}	3.18 ± 0.02^{c}
Albumin-globulinratio	0.60 ± 0.01^{c}	0.57 ± 0.01^{bc}	0.56 ± 0.01^{b}	0.50 ± 0.01^{a}	0.56 ± 0.02^{bc}
Cholestrol (mg/dl)	80.15 ± 0.10^{d}	84.61 ± 0.26^{c}	68.79 ± 0.44^{b}	70.36 ± 0.17^{b}	80.38 ± 0.24^{a}
Triglycerides (mg/dl)	120.45 ± 0.21^{d}	110.88 ± 0.36^{c}	107.96 ± 0.66^{b}	110.01 ± 0.01^{c}	99.84 ± 0.03^{a}

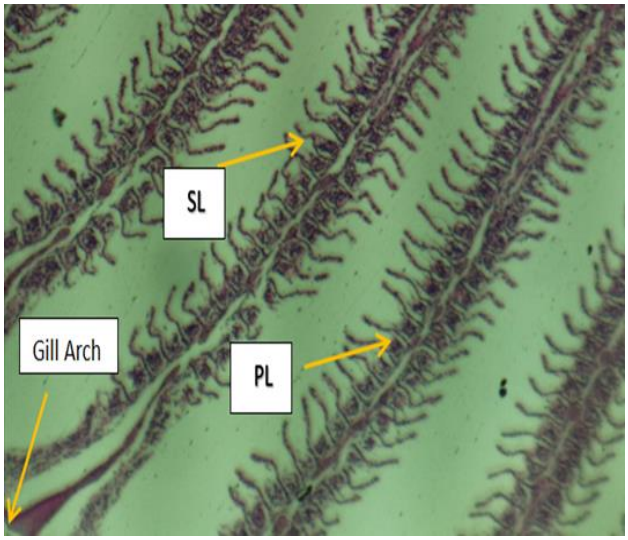
Values with different alphabets as superscripts in the same row differ significantly ($P<0.05$) among different temperatures. All values expressed as mean±SE.



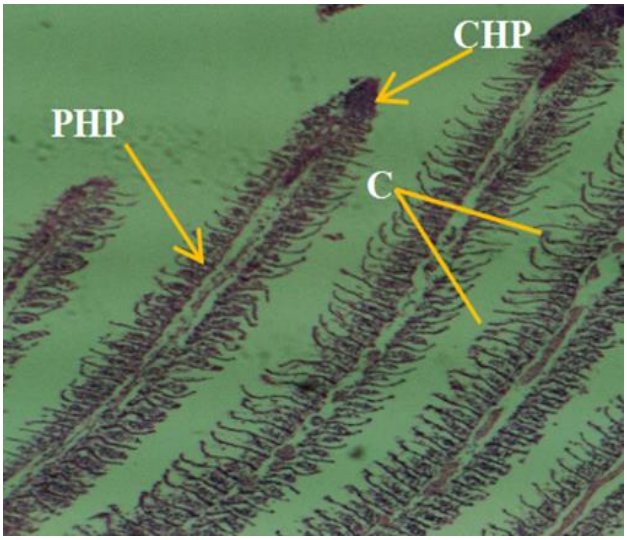
T_1 (16°C)



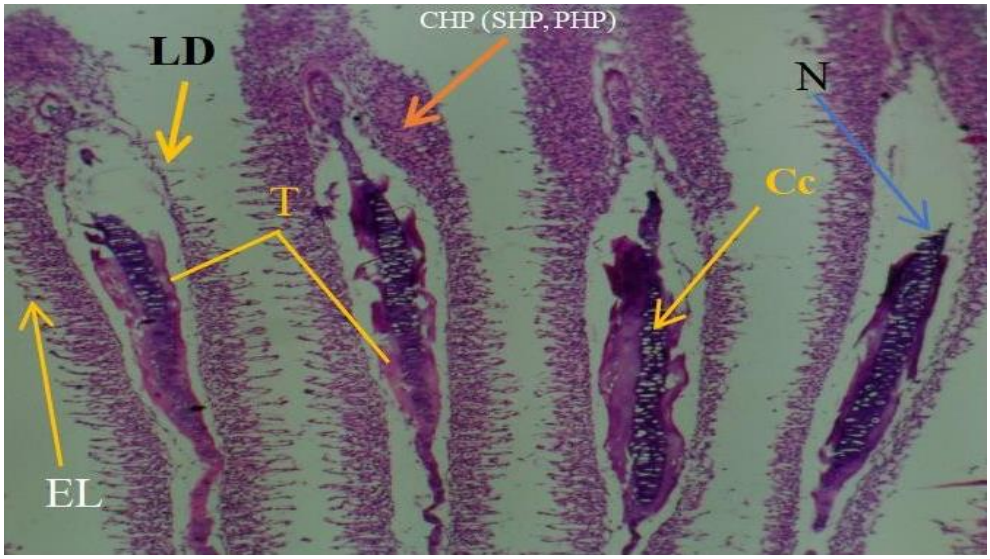
T_2 (20°C)



T_3 (24°C)

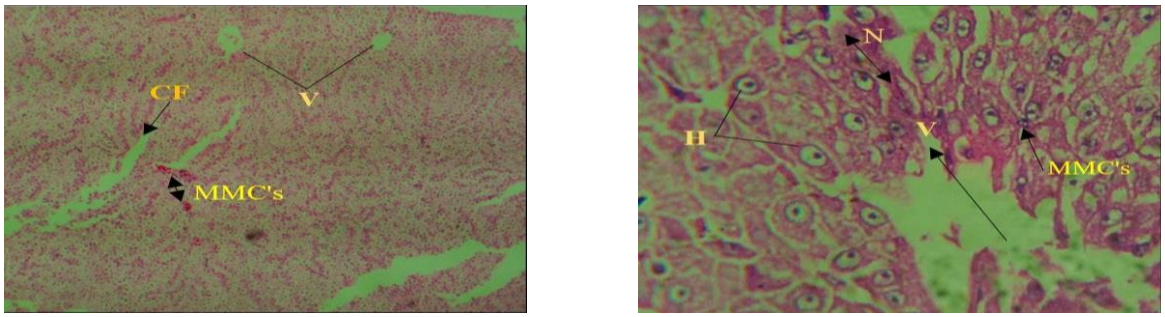


T_4 (28°C)

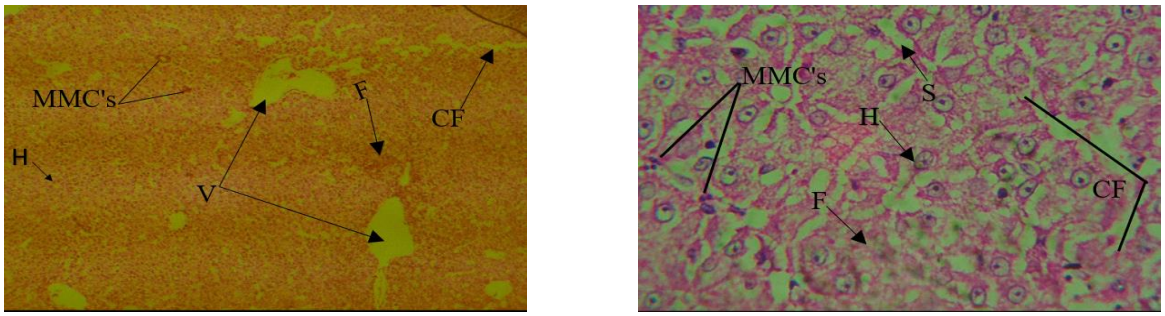


T_5(32°C)

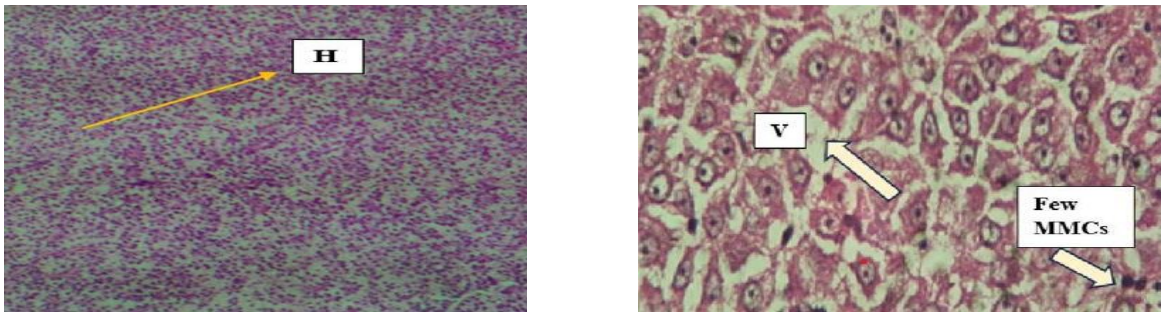
Figure 2. Histomorphological examinations of gills of *C. carassius* in different treatments after exposure for 45 days. (Stain: H& E; magnification: 10X; scale bar: 30 µm) Abbreviations: LF: Lamellar fusion, C: clubbing of secondary lamellae; PHP: Primary lamellar hyperplasia, SHP: Hyperplasia of epithelial cells at the bases of branchial secondary lamellae, A: Lamellar aneurysm, LD: Lamellar Degeneration, EH: Epithelial Hypertrophy, EN: Epithelial Necrosis, CHP: Cellular Hyperplasia, EL: Epithelial Lifting



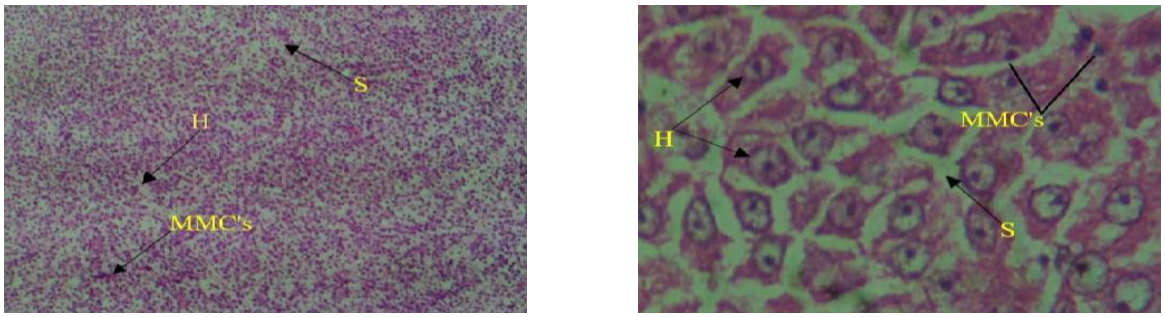
T_1 (16˚C; 10X and 40X)



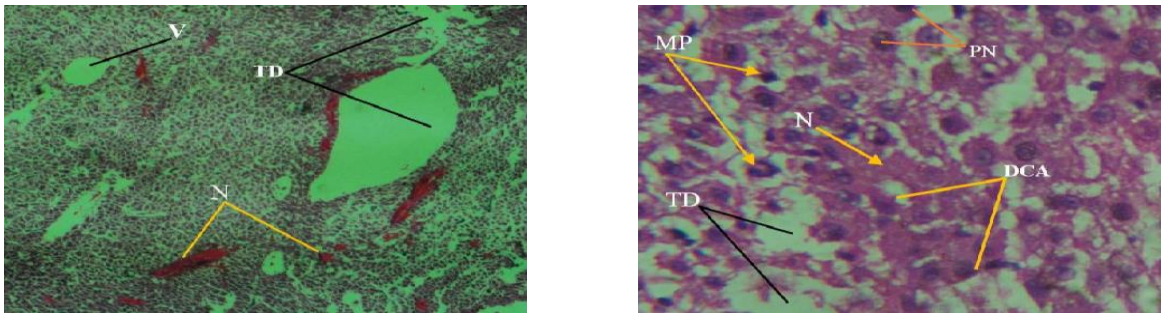
T_2 (20˚C; 10X and 40X)



T_3 (24˚C; 10X and 40X)



T_4 (28˚C; 10X and 40X)



T_4 (28˚C; 10X and 40X)

Figure 3. Histomorphological examinations of liver of *C. carassius* in different temperature treatments after exposure for 45 days. (Stain: H& E; magnification: 10X and 40X respectively; scale bar: 30 µm). Abbreviations: MMC's: Melano-macrophage centres, CF: Canaliculi formation, V: Vacuolation, H: Hepatocytes, S: Blood spaces or sinusoids, F: Fibrosis, DCA: Degenerative cellular architecture, N: Necrosis of hepatocytes, TD: Total degeneration, MP: Increased melanin production, PN: Pyknotic nuclei, MP: Increased melanin production.

result was compatible with the findings of Shahjahan et al. (2018) in *Pangasianodon hypophthalmus,* when subjected to the highest temperature (36°C). The current study indicates that prolonged exposure to elevated temperatures may potentially induce stress in fish. The MCV, MCH and MCHC did not change with the temperature. Previous study done by Adeyemo et al. (2003) in *C. gariepinus* also observed that MCV, MCH and MCHC were temperature independent.

The blood glucose level increased with the increase in acclimation temperature upto a certain limit then showed a slight decrease and then again increased at higher temperature (32°C). Our findings are compatible with the study of Adeyemo et al. (2003) in *C. gariepinus;* Ahmad et al. (2011) in *C. carpio;* Islam et al. (2019) in *P. hypophthalmus*. Blood glucose has been shown to be a sensitive indicator of environmental stress (Beyea et al. 2005). Elevated glucose levels in fish subjected to high temperatures could stem from the conversion of glycogen into glucose, addressing the heightened energy requirements associated with combating temperature-induced stress. The release of glucocorticoids and catecholamine hormones, known to induce hyperglycemia in animals, is triggered rapidly by stress stimuli, originating from the adrenal tissue of the fish (Pickering, 1981). This rise might result from stressed fish increasing gluconeogenesis as they try to meet their heightened energy requirements (Winkaler et al., 2007). Total protein, albumin and globulin also increased with the increase in temperature. Previous study done by Costa et al. (2016) in juveniles of *Lophiosilurus alexandri*; De et al. (2019) in hybrid grouper (*Epinephalus fuscoguttatus*♀ × *Epinephalus lanceolatus*♂) and Prabu et al. (2022) in *Trachinotus blochii* juveniles reported that the serum protein, albumin and globulin levels showed increasing values as the temperature rose and dip at higher temperatures. The elevated total protein, albumin and globulin contents in the serum indicate a better immune function in fish until the fish is alive (Liang et al., 2018). Also, these proteins confer protection against subsequent and lethal hyperthermia, a phenomenon referred to as thermo tolerance (Li & Werb, 1982). Triglycerides and cholesterol levels decreased with the increase in water temperature. Similar results were found by Roychowdhury et al. (2020) in *L. rohita* who found that serum cholesterol and triglyceride level decreased in the elevated water temperatures (37-38°C) than the optimal water temperature (28-30°C). This could be because cholesterol is an essential biomolecule of biological membranes and maintains membranes fluidity at higher temperatures to protect the cells and tissues of animals (Malekar et al., 2018).

Gills maintain osmotic and ionic balance, acid-base regulation, elimination of nitrogenous waste and modulate neurotransmission (Evans et al., 2005). Gill lamellae serve as the main location for gas exchange, constituting the majority of the respiratory surface area within gills. The effect of water temperature was noticeable in T_1 (16°C), T_2 (20°C) and T_5 (32°C), exhibiting significant histological changes. In contrast, T_3 (24°C) and T_4 (28°C) did not manifest any substantial alterations in gill morphology; nonetheless, mild changes were noted in the T_4 treatment. The microscopic observations in the gills subjected to temperatures outside the certain range leads to the alterations in morphology of gill structure as the water temperature beyond optimal range of the fish increases the oxygen demand and subsequently increase biological respiration which exerts pressure on the oxygen- carrying system, and ultimately morphological changes in the gill lamellae. Under high temperature, cellular hyperplasia and hypertrophy were an important feature and the rupture of the gill epithelium (epithelial degeneration) could cause haemorrhage, this lesion can be interpreted as a reflection of the direct action of the thermal agent on the tissue (Temmink et al., 1983). Under low temperature, the gills showed close by similarity to the lesions brought about by high temperature especially lamellar cell hyperplasia and haemorrhage between the branchial secondary lamellae, as well as the appearance of partial fusion of some secondary lamellae, which is example of defence mechanism (Camargo & Martinez, 2007).

The fish liver plays a crucial role in essential functions such as fundamental metabolism, storage and transformation of substances, and the removal of toxins from their bodies. Additionally, it serves as a reliable indicator of the overall well-being of fish (Capkin & Altinok, 2013; Nunes et al., 2014). The impact of water temperature in the current study was noticeable across all treatment groups mainly the presence of melano-macrophage centres (MMC's) and T_1 (16°C), T_2 (20°C) and T_5 (32°C) displaying more pronounced changes. However, it's worth noting that at T_1 (16°C) mild MMC's were detected, and their prevalence increased as the temperature increased, except for T_3 (24°C) in which fewer MMC's were observed, hinting at the possibility that this temperature might be suitable for the fish, as it exhibited fewer alterations compared to the other treatment groups. Other alterations *viz.* mild necrosis and vacuolation, when exposed to (T_1) 16°C were observed and present result is in allignment with the previous study of Dash et al. (2011) in *L. rohita* and Raina et al. (2015) in *Labeo boga*) who observed that the higher temperature and lower temperature, respectively, resulted in necrosis followed by vacuolation and total degeneration of the tissue. If the lower temperature persists for an extended period, it could lead to the development of oxidative damage in the liver. Necrosis represents an irreversible stage of degeneration and is characterized by the death of hepatic cells (Pal, 2006). Necrosis, through the loss of hepatocytes, ultimately leads to the presence of vacuoles within the liver tissue. Additionally, presence of pyknotic nuclei, which are highly condensed forms of chromatin material in the nucleus were seen. These pyknotic nuclei, as explained by (Pal et al., 2012) are

indicative of severe liver tissue damage and can lead to cell death through apoptosis. The observed tissue damage at high temperature (32°C) in the current study appears to be a consequence of both apoptotic and necrotic processes that surpass the cellular antioxidative defence. This is supported by an increase in Thiobarbituric Acid Reactive Substances (TBARS) and a significantly oxidized redox potential, coupled with a reduction in the activity of the enzyme superoxide dismutase, as found in the *Zoraces viviparus,* eel species when exposed to the extreme temperature, as reported by Heise et al., (2006). Many poikilothermic organisms respond to temperature fluctuations by adapting the physical properties of their cell membranes. This adaptation, referred to as "home viscous adaptation" by Sinensky et al. (1974), aims to maintain the functional and structural integrity of these membranes. The effectiveness of homeviscous adaptation, which measures how well cells compensate for temperature changes, can vary among different tissues and membranes (Lee & Cossins, 1990). Adjusting the physicochemical properties of membranes in response to temperature changes is expected to be a rapid and reversible process, ensuring proper functionality under fluctuating thermal conditions in fish.

Conclusion

The ideal thermal range for the *C. carassius* is between 24°C and 28°C, as this range has been found to optimize the fish's metabolic processes and physiological functions. The outcome of this research may help us understand the significance of thermal tolerance limit in *C. carassius* and provide valuable insights into their culture under controlled conditions especially in context of climate change wherein we cannot rely completely on natural water bodies and this could be the alternative and this knowledge may contribute to enhancing production of *C. carassius* and advancing sustainable efforts in aquaculture.

Ethical Statement

Experimental protocol followed the guidelines of the Institutional Animal Ethics Committee of SKUAST-K, J&K, India. The approval for study was granted number: SKUAST/IAEC-17/2023/09.

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Author Contribution

Asra Imtiyaz Mattoo: Performed the experiments; manuscript writing; **Darve Sabina Iqbal:** Concept of manuscript; literature analysis; manuscript writing; **Farooz Ahmad Bhat:** Microscopy; literature analysis**; Gohar Bilal Wani:** Provided laboratory facilities for trials; revision of manuscript**; Tasaduq Hussain Shah:** literature analysis**; Bilal Ahmad Bhat:** Data Analysis; **Mansoor Ahmad Rather:** literature analysis**; Hakim Mudasir Maqsood:** Data Analysis.

Conflict of Interest

The authors have declared that no conflict of interests exist.

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