ISSN: 2347-5129
IJFAS 2015; 2(4): 12-16

www.fisheriesjournal.com
Received: 01-01-2015
Accepted: 09-02-2015

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Temperature fluctuations induced histopathological alterations in the liver of fish, *Labeo boga* inhabiting Jammu waters

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Abstract
Presently an attempt has been made to elucidate the effect of temperature variance viz., 20 ºC, 26 ºC and 32 ºC on the liver of fish, *Labeo boga* for the experimental period of 60 days. The stress of different temperatures induced various histopathological alterations in the liver tissue that ultimately disrupt the entire metabolic functioning of the fish. Where higher temperature (32 ºC) induced only mild necrosis and increase in MMCS (to overcome the stress) in the liver while the stress of lower temperature (20 ºC) resulted in necrosis followed by vacuolation and total degeneration of the tissue. The results are the clear indicative of the fact that lower temperature (20 ºC) proved to be more deleterious to physiological functioning of the liver compared to higher temperature (32 ºC).

Keywords: Liver, *Labeo boga*, Temperature, Stress

1. Introduction
As ectotherms, fishes experience seasonal and daily fluctuations in water temperature in their aquatic ecosystem [1]. Since the fish's surrounding environment dictates the body temperature and hence may become vulnerable to any unseasonable thermal changes that can occur in their natural aquatic habitats [2]. Temperature fluctuations have been shown to affect histology of various organ systems of fish [3]. Histopathological biomarkers can be indicators of effects of various stressors (including temperature fluctuations) on organisms and are a reflection of the overall health of the entire population in the aquatic ecosystem including fishes [4] as these embodies even tissue lesions arising as a result of exposure of fishes to temperature changes. Among the haemopoietic organs (besides spleen and head kidney) liver have been found to show complete loss of cellular structure and organization at the extremely stressful temperatures [5]. Presently, the effect of varying temperature regimes (20 ºC, 26 ºC & 32 ºC) on the viz., liver of fish *L. boga* have been investigated for an experimental period of 60 days.

2. Materials and Methods
2.1.1 Sampling site
The fish *Labeo boga* for the experimental study were netted from the Nagrota stream of River Tawi, Jammu, J & K, India.

2.1.2. Setting up of the Experiment
To assess the effect of temperature, fish *L. boga* were divided into three groups. First group was exposed to lower temperature of 20 ºC, second group to optimum temperature of 26 ºC and third group to higher temperature of 32 ºC. These groups were kept in thermostat controlled water tubs and mercury bulb thermometer was used to monitor the temperature during the experimental period.

2.1.3 Tissue processing for histopathological observations:
After the termination of each set of experiments, the test fishes were promptly anaesthetized with a wild dose of benzocaine. The fishes were then dissected open in ringer saline solution and their liver was fixed in Bouin's fixative. After post fixation treatment and routine dehydration and clearing, tissues were embedded in histowax of 54-56 ºC. 5-7 µm thick sections were cut on microtome and were stained using haematoxylin eosin stain.

3. Results

Liver plays a key role in the metabolism and biochemical transformations of pollutants from the environment which inevitably reflects on its integrity by creating lesions and other histological alterations of the liver parenchyma [6, 7]. Temperature variations may disintegrate the structural composition of the liver tissue thereby affecting its functional efficiency.

3.1 Histology of fish liver at optimum temperature of 26 ºC

Microscopic examination of liver at optimum temperature (26 ºC) revealed the presence of polygonal hepatocytes (H) with centrally placed nuclei. Blood spaces or sinusoids (S) could be visibly seen in between the hepatocytes (Fig. 1). Melanomacrophage centres (MMCs) have also been observed in the liver tissue. At 26 ºC of optimum temperature, liver exhibited a mild increase in MMCs (Figs. 2-4) with the advancement of the experimental period.

3.2 Histology of fish liver at lower temperature of 20 ºC

At lower temperature (20 ºC), liver has been found to exhibit necrosis after the 12th day of experiment **(**Fig. 5) which extended upto 30th day (Fig. 6) and was followed by vacuolation of the liver cells. Both necrosis and vacuolation resulted in degenerative changes from 48th day (Fig. 7) and was ultimately observed to cause total degeneration of liver tissue (Fig. 8) by the end of the experimental exposure of low temperature.

3.3 Histology of fish liver at higher temperature of 32 ºC

Liver depicted increase in MMCs by the 12th day of experiment (Fig. 9) and its proliferation by 30th day of exposure (Fig. 10). Mild necrosis (N) was evident after 48th day of experiment (Fig. 11) and by the end of 60 days of high temperature exposure, there has been observed disintegration of normal cellular architecture (DCA) of liver tissue (Fig. 12).

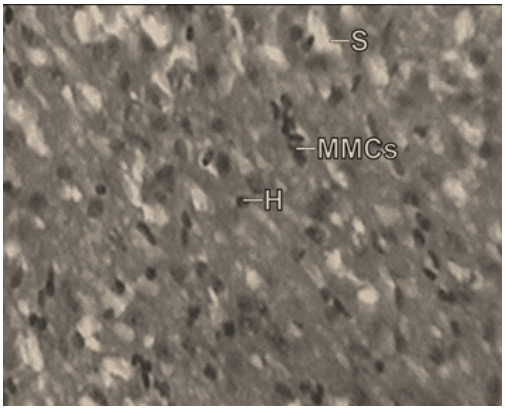

Fig 1: Microphotograph of liver from fish *Labeo boga* exposed to optimum temperature of 26 ºC showing Hepatocytes (H), Sinusoids (S) and Melanomacrophage Centres (MMCs) after 12th day of experiment H&E×1000.

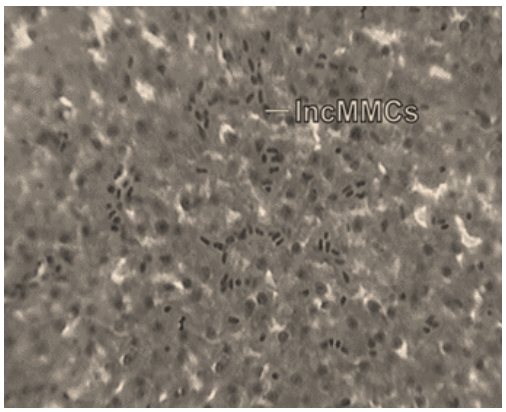

Fig 2: Microphotograph of liver from fish *Labeo boga* exposed to optimum temperature of 26 ºC showing increase in Melanomacrophage Centres (Inc MMCs) after 30th day of experiment H&E×1000.

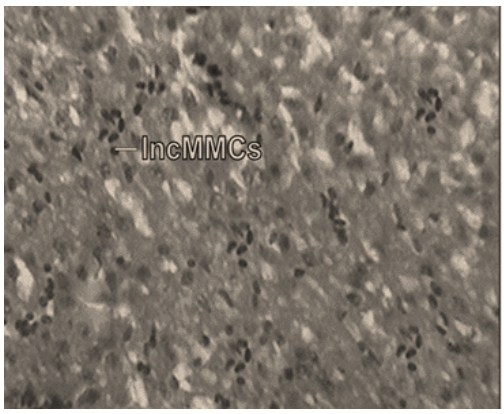

Fig 3: Microphotograph of liver from fish *Labeo boga* exposed to optimum temperature of 26 ºC showing increase in Melanomacrophage Centres (Inc MMCs) after 48days of experiment H&E×1000.

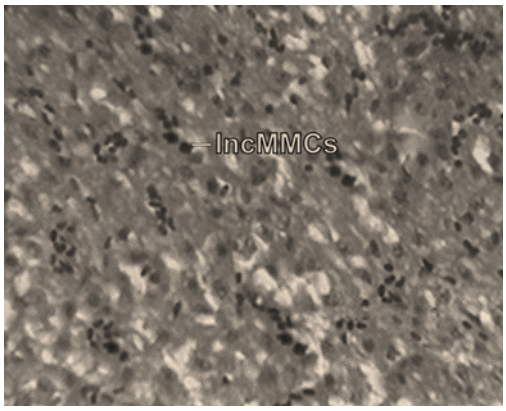

Fig 4: Microphotograph of liver from fish *Labeo boga* exposed to optimum temperature of 26 ºC showing increase in Melanomacrophage Centres (Inc MMCs) after 60th day of experiment H&E×1000.

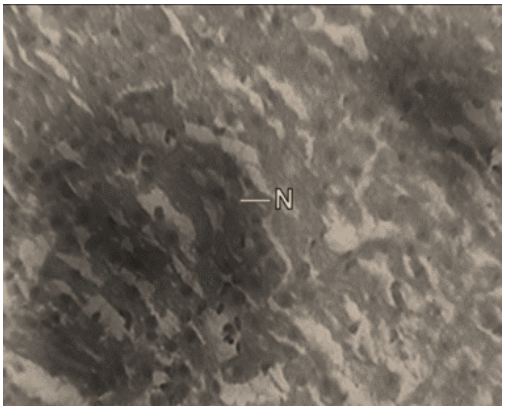

Fig 5: Microphotograph of liver from fish *Labeo boga* exposed to lower temperature of 20 ºC showing Necrosis of Hepatocytes (N) after 12th day of experiment H&E×1000.

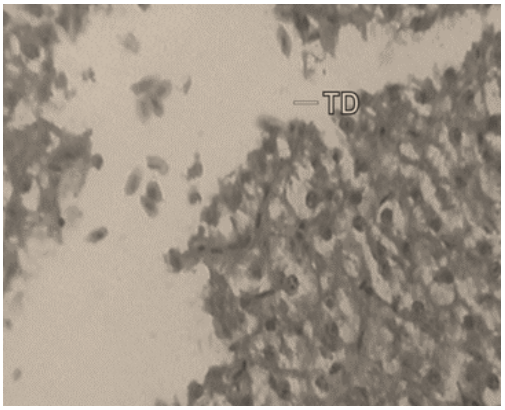

Fig 8: Microphotograph of liver from fish *Labeo boga* exposed to lower temperature of 20 ºC showing Total Degeneration after 60th day of experiment H&E×1000.

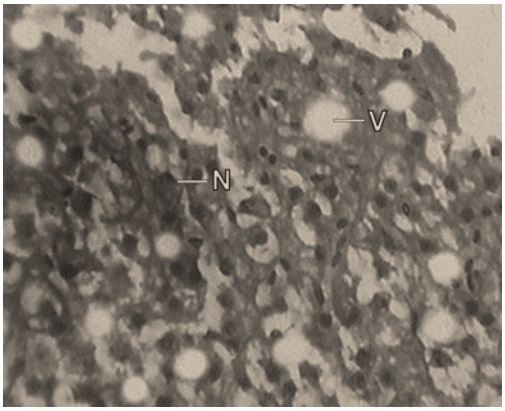

Fig 6: Microphotograph of liver from fish *Labeo boga* exposed to lower temperature of 20 ºC showing Necrosis of Hepatocytes (N) and Vacuolation (V) after 30th day of experiment H&E×1000.

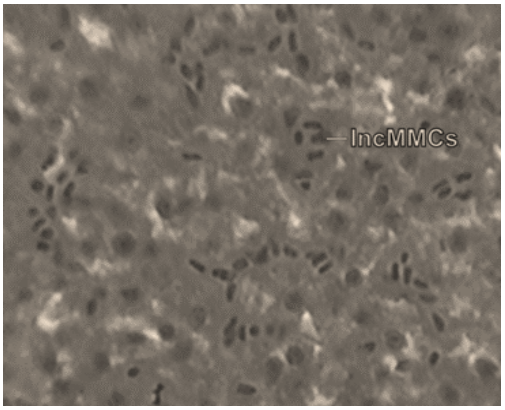

Fig 9: Microphotograph of liver from fish *Labeo boga* exposed to higher temperature of 32 ºC showing increase in Melanomacrophage Centres (Inc MMCs) after 12th day of experiment H&E×1000.

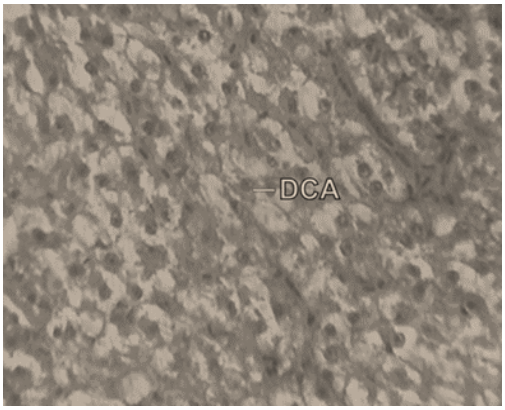

Fig 7: Microphotograph of liver from fish *Labeo boga* exposed to lower temperature of 20 ºC showing Degenerative Cellular Architecture (DCA) after 48th day of experiment H&E×1000.

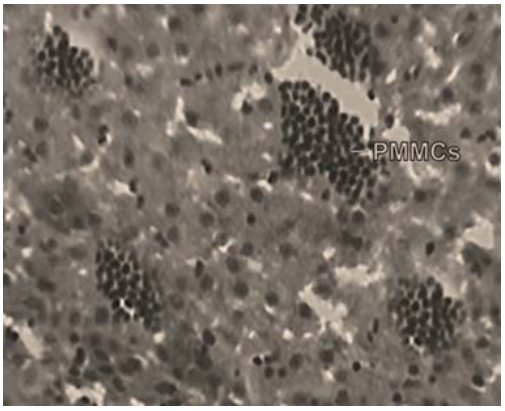

Fig 10: Microphotograph of liver from fish *Labeo boga* exposed to higher temperature of 32 ºC showing Proliferation of Melanomacrophage Centres (PMMCs) after 30th day of experiment H&E×1000.

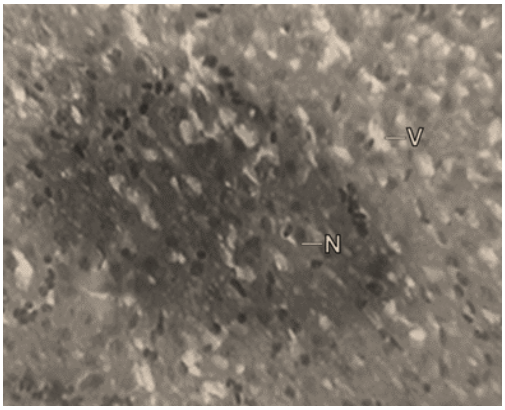

Fig 11: Microphotograph of liver from fish *Labeo boga* exposed to higher temperature of 32 ºC showing Necrosis (N) after 48th day of experiment H&E×1000.

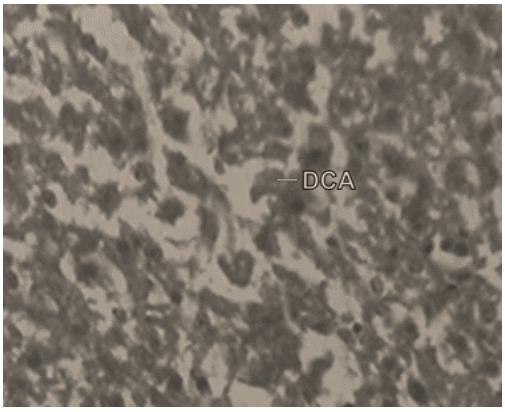

Fig 12: Microphotograph of liver from fish *Labeo boga* exposed to higher temperature of 32 ºC showing Total Degeneration (TD) after 60th day of experiment H&E×1000.

4. Discussion

Liver exhibited marked histological alterations viz., necrosis, vacuolation, commencement of degenerative changes leading ultimately to total degeneration when exposed to lower temperature of 20 ºC. Necrosis is an irreversible stage of degeneration and is characterized by dead hepatic cells [8]. Necrosis by causing loss of hepatocytes ultimately resulted in vacuolation of liver tissue. Vacuolation caused disruption in the synthesis of substances in hepatocytes as a result of which an imbalance may get created between their formation and release in the general circulation. Suja *et al*. [9] also reported lower temperature to be the causative of necrosis and vacuolation and they further added that these changes ultimately resulted in degenerative changes in the liver of fish *Colossoma macropomum*. Presently also necrosis and vacuolation have been found to result in degenerative changes in liver tissue. As exposure of low temperature, prolonged, liver tissue depicted total degeneration by the end of 60 days of the experimental period. Contrarily however, at higher temperature of 32 ºC, liver exhibited the increase in MMCs (mild increase at 26 ºC) which have been reported to be the chief indicators of stress induced by different kind of stressors (presently fluctuating temperature) [10]. MMCS are the aggregates of macrophages and are the key cells in the liver tissue dealing with foreign agents and cellular debris [11]. During high temperature periods, the pathogenic prevalence is more [12] and in order to combat it, MMCs increase i.e. proliferate and help fishes to get rid of such increased pathogenic attacks during high temperature exposure. Chinabut *et al*. [13] who reported an increase in MMCs in liver tissue as a response to counter the attack of pathogens during high temperature exposure lends a strong support for the present results. However, liver tissue revealed necrosis, which simply implies that as the exposure of high temperature, prolonged, liver becomes unable to withstand severity of high temperature resulting thereby in disintegration of normal cellular architecture of liver tissue. Dash *et al*. [5] in agreement with present findings also stated that higher temperatures are stressful to fishes. While studying the effect of higher temperature of 36 ºC on fish *Labeo rohita*, they also noted disarrangement of the hepatic cells and degeneration of the normal cellular architecture of fish liver.

5. Conclusion

From the above discussion on the effect of low as well as high temperatures on the histology of fish *L. boga*, it can be concluded that lower temperature has been found to be more deleterious to liver compared to higher temperature. On one side fishes at high temperature tried to combat the stress by increasing the melanomacrophage centres while on the other fishes at lower temperature exhibited degeneration of the tissue followed by necrosis and vacuolation of the tissue thereby affecting the entire metabolic functioning of the fish.

6. Acknowledgements

I am very thankful to Department of zoology, University of Jammu, Jammu for providing the necessary facilities regarding the smooth conduct of research work.

7. References

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