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Concerted Effect of Increasing Temperature and Persistent Sub-Lethal Chlorine Concentration on the Gills of *Labeo rohita* (Hamilton) Fingerlings

Rama R.[1*], Pal A.K.[2], Dalvi R.S.[2] and Usha Rani M.V.[1]
[1]Department of Environmental Sciences, Bharathiar University, Coimbatore 641046, Tamil Nadu, INDIA
[2]Division of Fish Nutrition and Biochemistry, Central Institute of Fisheries Education, Mumbai-400061, Maharashtra, INDIA

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Abstract

Thermal effluents discharged from nuclear power plants are the source of stress factors such as elevated temperature and chlorine to aquatic organisms. Therefore, the present investigation was carried out to assess morphological changes in the gill tissue of a freshwater fish Labeo rohita on exposure to increasing temperatures and sub-lethal concentration of chlorine. Fishes were segregated in to two different groups (control and experimental) and acclimated to four different temperatures (26, 31, 33 and 36°C) for 30 days. Then, the fishes in the experimental groups were subjected to 0.1 mg L^{-1} of chlorine, besides temperature treatments. At the end of 15 and 30 days of acclimation period gill tissue was examined for histopathological changes. Normal gill structure was observed in control group at 26, 31 and 33°C. However at 36°C marked histological alterations were noticed in the tissue. In the chlorine treated experimental groups discernible changes in the gills such as atrophic changes in primary and secondary gill lamellae, complete loss of secondary gill filaments, interlamellar infiltration of leucocytes, complete disintegration of secondary lamellae and clubbing of primary lamellae were observed. The results of the present study established that, elevated temperature affected the cellular integrity of the gills and the combined effect of increasing temperatures and chlorine further augmented the histological damage in the gill tissue of L.rohita.

Keywords: *Labeo rohita*, acclimation temperature, chlorine, histopathology, gill.

Introduction

Anthropogenic influence has been accountable for ecological damage in many ecosystems for decades. In aquatic ecosystems, industrial effluents containing a variety of physical and chemical stressors that are indiscriminately discharged into the water body causes potential deleterious effects on the aquatic organisms. Among the physicochemical factors, temperature is considered as the most striking example of a potent physical stressor in aquatic system. Alteration of natural water temperature regimes can create a wide variety of life history, behavioral and physiological responses in aquatic organisms[1-3] and small changes in water temperature can have considerable consequences for freshwater fish[4]. Anthropogenic influence has increased the frequency with which fish may face thermally extreme conditions[5]. One of the most extreme examples of thermally altered environments are thermal effluents associated with power plants[6]. Power plants require large amount of natural water for condenser tube cooling resulting in a rapid increase in the temperature of the entrained water. Heated effluents from such power stations are constantly released into these water bodies and consequently increase the temperature of these receiving water bodies. Besides increased temperatures, the heated effluent also contains chlorine used for control of biofouling. Chlorine has high acute toxicity for aquatic organisms[7] and therefore these discharges may be quite toxic to aquatic organisms[8]. Further, elevated temperatures increase the toxicity of chlorine to fish[9]. Many workers have studied the influence of thermal discharges on the physico-chemical parameters, fishes and other aquatic organisms of the receiving waters[10–14]. There are reports on the individual effects of temperature and chlorine in the different organs of fish species[15-22]. However, to our knowledge there have been no reports available on the combined effect of increasing temperatures and chlorine on the histology of Indian Major Carps. Therefore, based on these studies and considering the economic importance of *Labeo rohita,* the present study was undertaken to investigate the combined effect of increasing temperatures and sub-lethal level of chlorine on the gill tissue of *Labeo rohita,* a widely cultured Indian Major Carp. For the present work, the acclimation temperatures chosen were 26, 31, 33 and 36°C as they are in the range of preferred temperature of Indian Major Carps[23]. A sub-lethal concentration of 0.1 mg L^{-1} of chlorine was selected, as chlorine levels in the immediate vicinity of thermal power plants is about 0.1mg L^{-1} (personal communication from power plant operators).

Material and Methods

Experimental fish: *L. rohita* fingerlings (10±0.52 g) were procured from Aarey Fish Farm, Mumbai, Maharashtra, India and transported to the wet laboratory of Central Institute of Fisheries Education, Mumbai in polythene bags containing oxygenated water. They were acclimatized to laboratory

conditions for 30 days. The fish were fed with supplementary diet during the acclimatization and experimental periods. Water quality was maintained by the daily exchange of water (chlorine free freshwater).

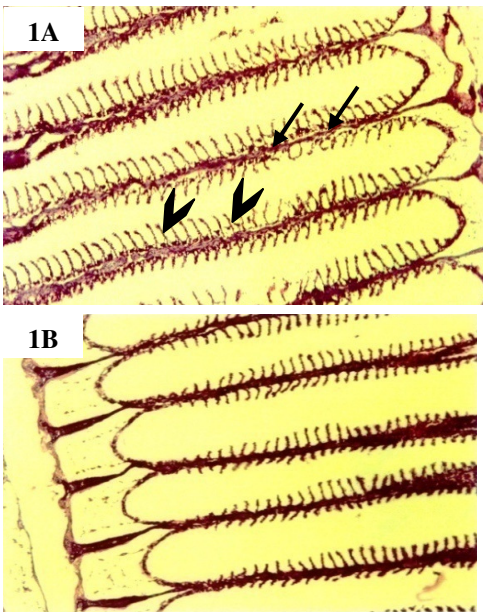
Experimental set-up: A total of 128 fishes were segregated in to two groups (control group and experimental group) of four treatments each. The fish of both the control and experimental group were acclimated to test temperatures (26, 31, 33, and 36°C) at the rate of 1°C per day from ambient temperature (26°C) to reach the test temperatures of 26, 31, 33 and 36°C. The fish were maintained at these temperatures for a period of 30 days. In the experimental group, once the test temperatures has been reached, a sub-lethal concentration (0.1 mg L^{-1}) of chlorine as sodium hypochlorite was added and maintained throughout the acclimation period. As chlorine evaporates rapidly due to aeration and temperature, its concentration was monitored at regular intervals and supplemented to maintain uniform level of chlorine during the experimental period. Spectroquant chlorine test kit (E- Merck, Germany; Accuracy - 0.01 mg L^{-1}) was used to monitor the chlorine concentration. Six fishes were sampled from each treatment of both the control and experimental group at two acclimation periods (15 and 30 days).

Chlorine dosage and analysis: The evaporation rate of chlorine at different temperatures (26, 31, 33 and 36°C) was assessed and chlorine levels were monitored and supplemented at every 8-h intervals to maintain a constant concentration of 0.1±0.03 mg L^{-1} in the experimental group. Sodium hypochlorite solution (Merck Ltd. Mumbai, India) was used as the chlorine source. Spectroquant chlorine test kit (E- Merck, Germany; Accuracy - 0.01 mg L^{-1}) was used to monitor the chlorine levels.

Preparation of tissue samples: At the end of 15 and 30 days of acclimation period, six fish from each of the treatments in the control and chlorine treated experimental group were anaesthetized using clove oil (50 µl L^{-1}). Gill tissue were removed and fixed in 10% neutral buffered formalin. After fixation, the tissues were dehydrated through graded series of alcohol, cleared in xylene and infiltrated in molten paraffin and then cast into paraffin blocks. Tissue sections of 7µm were prepared from paraffin blocks using a rotary microtome. The sections were then stained with haematoxylin-eosin. The slides were examined and the histopathological changes observed were photographed.

Results and Discussion

Control group at 15 days acclimation period: The gill tissue of *L. rohita* at 26, 31 and 33°C showed normal gill architecture with both primary and secondary lamellae in regular fashion (figures 1A and 1B). However, at 36°C marked histological changes like accumulation of erythrocytes at the tips was observed (figure 2).



Figures- 1A and 1B
Gill structure of control fish at 26, 31 & 33°C at 15 and 30 days period (H&E, 40X): Normal gill architecture with primary (arrow) and secondary lamellae (arrow heads) in regular fashion

Experimental group at 15 days acclimation period: Gill morphology at 26°C revealed atrophic changes in the primary and secondary lamellae, however the secondary lamellae at the tip were normal in structure (figures 3A and 3B). At 31°C tissue alterations like atrophy of the primary lamellae and complete disintegration of secondary lamellae at the base was observed (figure 3C), whereas the upper third of the gill lamellae showed normal arrangement (figure 3D). Complete loss of secondary gill filaments and atrophy of primary gill lamellae with interlamellar infiltration of leucocytes was noticed in the gills of the experimental fish at 33 and 36°C (figures 3E, 3F and 3G).

Control group at 30 days acclimation period: Normal gill architecture was observed at 26, 31 and 33°C (figures 1A and 1B). Histopathology of gills at 36°C revealed mild to moderate hemorrhages in the primary gill filaments and mild atrophy in the secondary filaments (figure 4).

Experimental group at 30 days acclimation period: Gill structure was characterized by extensive atrophic changes in the primary as well as secondary gill lamellae in the chlorine treated group at 26°C (figure 5A). However, normal histoarchitecture was noticed at the proximal one third of the secondary lamellae. Occasional thickening of cartilaginous tissue was observed in the primary gill lamellae. At 31°C the gills showed atrophy of primary gill lamellae with marked loss of secondary gill lamellae and clubbing of lamellae at some places (figures 5B and 5C). The gill tissue at 33 and 36°C exhibited discernible histological changes characterized by complete loss of secondary gill filaments, atrophy of primary lamellae with interlamellar infiltration of leucocytes and extensive clubbing of primary lamellae (figures 5D and 5E).

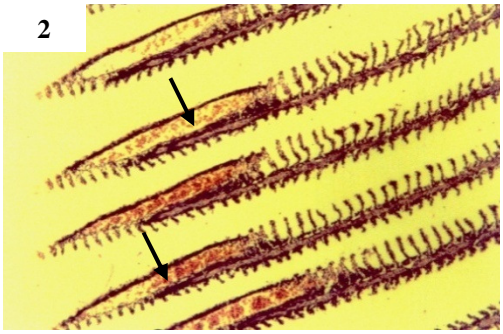
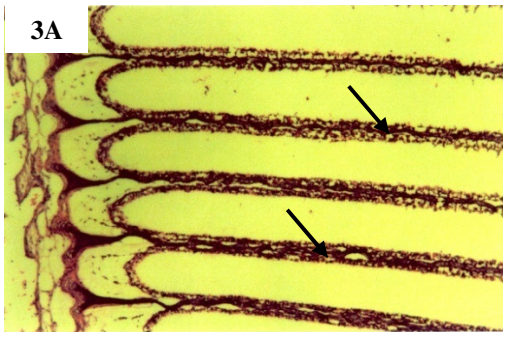
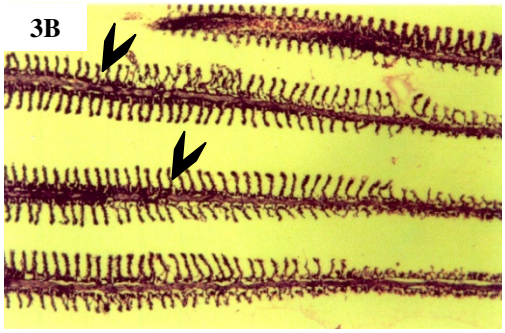
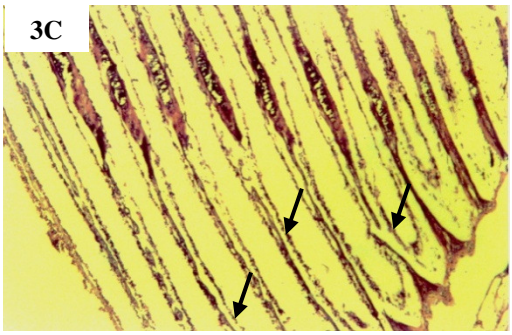


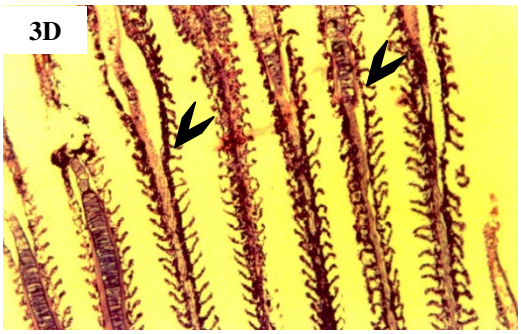
Figure-2
Gill tissue of control fish at 36°C at 15 days period (H&E, 40X): Tips were dilated with accumulation of erythrocytes (arrows)





Figures-3A and 3B
Gill tissue of experimental fish at 26°C at 15 days period (H&E, 40X): Atrophic changes (arrows) in the primary and secondary gill lamellae. Secondary lamellae at the tip appeared normal (arrow heads)





Figures - 3C and 3D
Gill tissue of experimental fish at 31°C at 15 days period (H&E, 40X): Atrophy of the primary lamellae and complete disintegration (arrows) of secondary lamellae at the base. Upper third of the gill lamellae appeared normal (arrow heads)

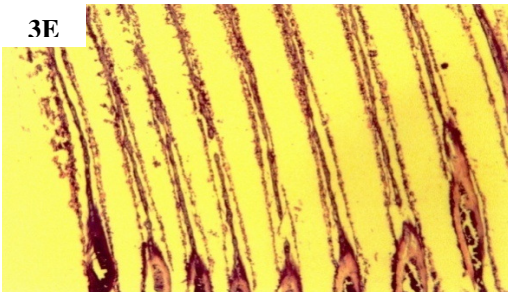
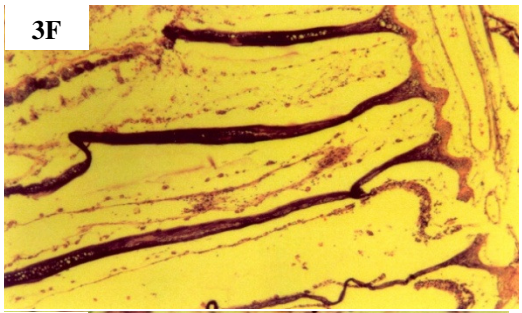
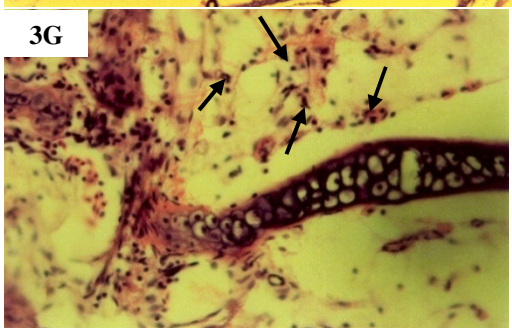


Figure-3E
Gill tissue of experimental fish at 33°C at 15 days period (H&E, 40X): Complete loss of secondary gill filaments and atrophy of primary gill lamellae with occasional interlamellar infiltration of leucocytes





Figures-3F and 3G
Gill tissue of experimental fish at 36°C at 15 days period (H&E, 40X & 160X): Complete loss of secondary gill filaments and atrophy of primary gill lamellae with interlamellar infiltration of leucocytes (arrows)

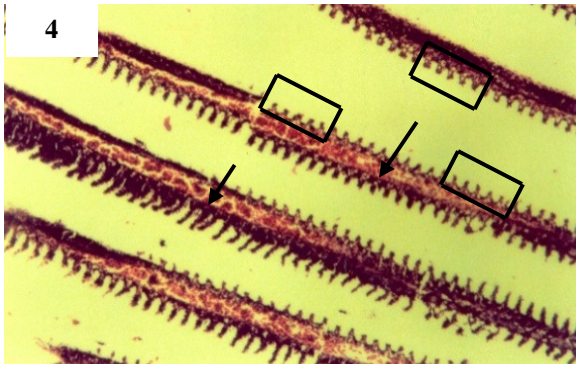


Figure-4
Gill tissue of control fish at 36°C at 30 days period (H&E, 40X): Slight to moderate hemorrhages (arrows) in the primary gill filaments and mild atrophy of secondary filaments (box)

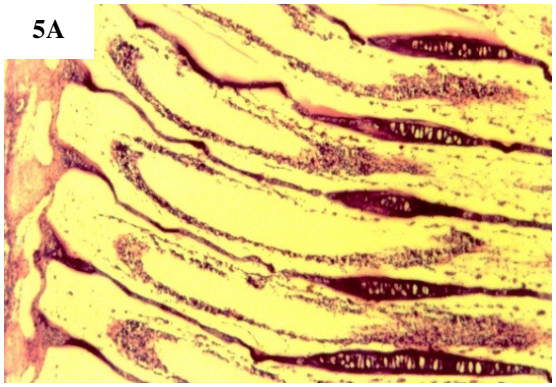
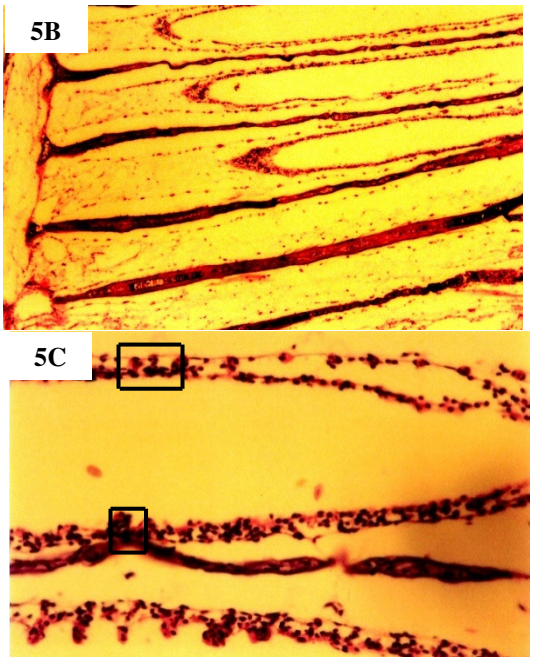


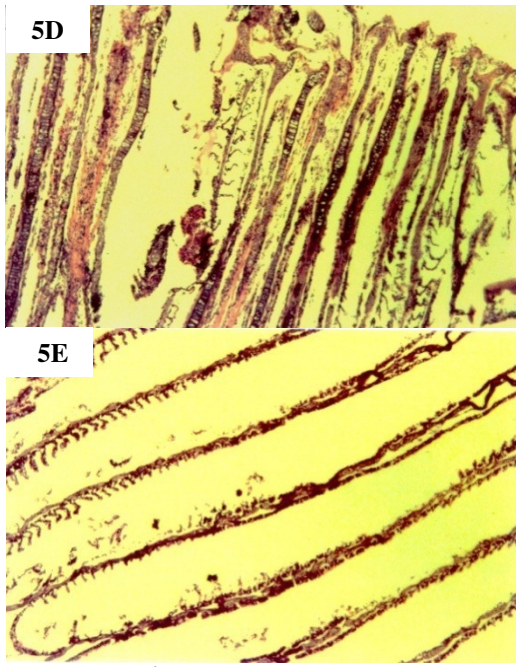
Figure-5A
Gill tissue of experimental fish at 26°C at 30 days period (H&E, 40X): Extensive atrophic changes in the primary and secondary gill lamellae. Secondary gill lamellae at the tip appeared normal with occasional thickening of the cartilaginous tissue in the primary gill lamellae

Discussion: The gill of teleost fish plays an important role in ion regulation, gas exchange, acid-base balance and nitrogenous waste excretion, which means, it has a key role at the interface of fish with its environment[24]. Besides these functions, gills also act as heat exchanger for conduction of heat between the environment and the fish's body. Therefore, gills are considered to be the most appropriate organ for indicating thermal pollution[25].

Normal histoarchitecture of the gill tissue at 26, 31 and 33°C in the control group revealed that these temperatures may not be detrimental to the fish. However, at a higher temperature of 36°C, profound changes observed in the gill structure viz., accumulation of erythrocytes and moderate hemorrhages in the primary gill filaments indicate that a temperature of 36°C and above may lead to cellular damage. Das[26] observed similar alterations in the gill structure of *Labeo rohita* and *Cirrhinus mrigala* acclimated to 36°C. Similarly, Manush et al.[27] reported normal gill structure in *Macrobranchium rosenbergii* acclimated to 25 and 30°C and tissue damage at 35°C.



Figures-5B and 5C
Gill tissue of experimental fish at 31°C at 30 days period (H&E, 40X & 160X): Atrophy of primary gill lamellae and marked loss of secondary gill lamellae. Lamellae were clubbed at some places (in box)



Figures-5D and 5E
Gill tissue of experimental fish at 33°C & 36°C at 30 days period (H&E, 40X): Complete loss of secondary gill filaments, atrophy of primary gill lamellae with interlamellar infiltration of leucocytes and extensive clubbing of primary lamellae

Marked histological changes observed in the gill of *L. rohita* in the chlorine treated experimental groups demonstrated the deleterious effect of increasing temperatures and chlorine on the fish. Laurent and Perry[28] consider the morphologic changes in gills, as a consequence of environmental changes, as adaptive attempts in conserving some physiological functions. Epithelial lifting and lamellar fusion were also suggested as a protective measure by decreasing the vulnerable surface area of the gills, to maintain its osmoregulatory function while sustaining a progressive loss of its basic functions[29]. However, such reactions that help slow down toxicant uptake could result in dysfunctional or even non-functional gills and eventually asphyxiate the fish[30].

Appearance of leukocyte infiltration in the gills supports the inflammation reaction indicated by hyperplasia and lifting of the respiratory epithelium[31]. Destruction of gill lamellae may be due to osmotic imbalance[27]. It is reported that higher water temperature affects the ability of fishes to maintain the osmotic balance by altering the lipids of gill cells, resulting in leakage of cells and reducing the efficiency of salt excretion and balance[32]. The observations of the above authors lend support to the findings of the present study. Therefore, histological changes like leukocyte infiltration, disintegration and complete loss of lamellae observed in the fish of experimental groups may correspond to inflammatory reaction and osmotic imbalance in the gills which reflect on the adverse effect of increasing temperatures and chlorine toxicity on the fish. Prolonged exposure of the fish to these experimental conditions may lead to respiratory distress.

Conclusion

Overall results in the present investigation clearly indicated that a temperature of 36°C and above were detrimental to fish health. Further, exposure to increasing temperatures and chlorine has caused various degrees of discernible cellular alterations in the gill of fish reflecting the synergistic effect of increasing temperatures and chlorine on *L. rohita*. Additionally, the progressive degenerative histopathological changes observed in the chlorine treated fishes with increasing temperatures clearly delineated the effect of temperature augmented chlorine toxicity in the fish. Prolonged exposure of the fish to these experimental conditions may result in severe physiological problems like respiratory distress which may ultimately lead to the death of the fish. From the findings of the present investigation, it can be concluded that there is direct correlation between the increasing temperature induced augmented chlorine toxicity and the histological aberrations observed in the gill tissue of *L. rohita*. The present work also emphasizes the need for further studies on the mechanism of interactive action of temperature (a potent physical stressor) and chlorine (a highly toxic chemical stressor) on freshwater fish species and other aquatic organisms.

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