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Persistent sub-lethal chlorine exposure augments temperature induced immunosuppression in *Cyprinus carpio* advanced fingerlings

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Abstract

Apart from increased temperature, thermal effluents discharged through cooling systems of nuclear power plants may often contain chlorine (used against bio-fouling), which may affect the immune status of fish. Therefore, a 28-day trial was undertaken to delineate the effect of high temperature and a persistent sub-lethal chlorine exposure on immunomodulation in *Cyprinus carpio* advanced fingerlings. Fish were acclimated to four different temperatures (26, 31, 33 and 36 °C) and maintained for 30 days in two different groups. One group was exposed to persistent chlorine (0.1 mg L^{-1}) and was compared with their respective temperature control groups (without chlorine exposure). Expression of heat shock proteins (hsp 70) was tested in muscle after 28 days using Western blotting. Haematological parameters (erythrocyte count, leucocyte count, haemoglobin), serum parameters (total protein, albumin, globulin, A/G ratio) and respiratory burst activity were tested to assess immuno-competence of *C. carpio* in response to temperature and chlorine exposure. Results indicated that hsp 70 was induced at 36 °C in temperature control groups but not in their respective temperatures in the presence of chlorine. Haematological parameters such as haemoglobin, erythrocyte and leucocyte counts appeared depressed in chlorine treated groups as compared to their respective temperature control groups. Serum protein and globulin were affected due to chlorine exposure at different acclimation temperatures. A decrease in NBT activity was recorded in chlorine treated groups as compared to their respective temperature control groups. Overall results indicate that increasing acclimation temperatures alters the immune status of *C. carpio* advanced fingerlings and persistent sub-lethal exposure to chlorine augments this temperature induced immunosuppression.

Keywords: *Cyprinus carpio*; Temperature; Chlorine; Hsp 70; Haematology; Phagocytic respiratory burst rate; Serum protein; Albumin; Globulin; Immunosuppression

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1. Introduction

In a healthy state, fish defend against potential invaders with a complex system of innate and adaptive immune mechanisms. In spite of limited pathogen recognition machinery, the strength of innate defence mechanisms against biotic and abiotic stressors is impressive [1]. Stressors may directly kill the fish or indirectly exacerbate diseased state by lowering the resistance and allowing the invasion of environmental pathogens [2]. Exposure to individual stressors may affect the immune system in a variety of ways; altering macrophage function [3,4] and circulating levels of immune cells. Ambient water temperature is critical in the development of both specific and non-specific immunity in fish [5]. Increasing temperature up to a certain limit favours fish growth by increasing the metabolic activities [6,7]. However, elevated water temperature (within the physiological range of fish) has been shown to alter the immune function [8]. For example, exposure of Catfish (*Heteropneustes fossilis*) to elevated temperature increases mitochondrial superoxide (O_2^-) production in the gills [9] and enhances antibody activity in Atlantic cod, *Gadus morhua* L. [10]. Further, beyond the range of preferred temperature zone, fish become more vulnerable to diseases due to metabolic injury, immunosuppression, carcinogens, etc. In addition to temperature, exposure to xenobiotics may act synergistically in causing immunosuppression in fish. However, the effect of multiple stressors on immunomodulation in fish still remains elusive.

Thermal discharges from nuclear power plants often contain chemical stress factors in the form of different biocides, in addition to high temperature. Amongst these, chlorine is widely used for bio-fouling control [11]. Chlorine is added to cooling effluent waters to neutralize mussel, algae and other marine fouling populations [12] in the immediate vicinity of the power plant, as growth of these aquatic organisms may hamper the flow of cooling waters to the condensers. For efficient operation of nuclear power plants, uninterrupted supply of cooling water to the condensers is a prerequisite [13]. One of our preliminary investigations indicated that the evaporation rate of chlorine increases with increasing temperatures (data unpublished). However, a steady level of chlorine is maintained in cooling condensers of nuclear power plants by continuous supplementation of chlorine at the intake point. Condenser effluents thus may have the potential to impart thermal and chemical stress on living organisms [14]. Continuous use of chlorine may thus affect non-target organisms by diffusing through their cell membrane, and inhibiting various metabolic activities.

There have been no reports available on the combined effect of persistent exposure to high temperature and chlorine on the immune status of fish. Therefore, in our study, *Cyprinus carpio* advanced fingerlings were selected to assess the effect of increasing temperature and a sub-lethal (0.1 mg L^{-1}) level of chlorine. *C. carpio* can tolerate a wide range of temperature (13–42 °C), as per our earlier investigations in early fingerlings [15], which may be the reason for their cosmopolitan distribution. The temperatures chosen (26, 31, 33 and 36 °C) in the present study were therefore well within the range of the test fish and a variety of carps, including Indian Major Carps [16]. A panel of assays considered pertinent in our study for assessing fish health includes RBC, haemoglobin, WBC and plasma protein values [36,37]. Respiratory burst activity has been considered as a critical effector mechanism in neutralising the biotic stressors [17]. Heat shock proteins (hsps), a class of acute phase proteins secreted in response to a variety of stresses, [18–20] were also tested.

2. Materials and methods

2.1. Experimental fish

C. carpio (mean ± SE: 11.13 ± 0.55 g) were brought in open aerated containers from Khopoli fish farm, Government of Maharashtra, to the wet laboratory, Central Institute of Fisheries Education, Mumbai, and were acclimatized for 30 days to laboratory conditions. Fish were fed with supplementary diet (25% crude protein) before being subjected to acclimation trials.

2.2. Chlorine dosage and analysis

As per earlier toxicity studies, LC_{50} of chlorine in *C. carpio* (average weight 8–10 g) was recorded as 0.4–0.5 mg L^{-1}. Therefore, a sub-lethal concentration (1/5th of LC_{50}) was selected for our study. A preliminary experiment was carried out to assess the evaporation rate of chlorine with respect to different temperatures and monitored