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Persistent sub-lethal chlorine exposure elicits the temperature induced stress responses in *Cyprinus carpio* early fingerlings

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Abstract

Thermal effluents discharged through cooling systems of nuclear power plants often contain chlorine (used to control bio-fouling), which may affect the metabolic status of fishes. In order to evaluate the hypothesis, we tested the effect of high temperature and a persistent sub-lethal chlorine exposure on stress responses in *Cyprinus carpio* advanced fingerlings. Fishes were acclimated to four different temperatures (26, 31, 33, and 36 °C) and maintained for 30 days in two different groups. Subsequently, one of the groups was exposed to persistent chlorine (0.1 mg L_{-1}) for another 28 days and was compared with their respective temperature controls (without chlorine exposure). Sub-lethal doses of pollutants and increasing temperatures with in the tolerance range may not always register any morphological changes Therefore, we studied organ specific biochemical pathways viz. aspartate amino transferase, alanine amino transferase (enzymes of protein metabolism) in liver and muscle; fructose 1,6 diphosphatase (gluconeogenic pathway), in liver; pyruvate kinase, malate dehydrogenase, and lactate dehydrogenase (glycolytic pathway) in muscle; glucose-6-phosphate dehydrogenase (pentose phosphate pathway) in liver; alkaline phosphatase (phosphorus metabolism) in intestine, liver, and muscle; acetylcholine esterase (neurotransmitting enzyme) in brain, and adenosine triphosphate (for membrane transport) in gills at two different acclimation periods (14 and 28 days). The results indicate that *C. carpio* fingerlings demonstrated metabolic readjustments with increasing temperatures, in order to cope with energy demand of the cell. However, exposure to chlorine at higher temperatures affected protein metabolism, gluconeogenic pathway and subsequently glycolytic pathway, leading to an energy-limited condition. In addition, alteration of membrane transport and neurotransmission might be an early indication of cellular damage. Overall results indicate that persistent sub-lethal chlorine exposure elicits temperature induced stress response in *C. carpio* early fingerlings.

Keywords: *Cyprinus carpio*; Acclimation temperatures; Chlorine; Metabolic enzymes; Stress

1. Introduction

In India, electricity is mainly derived from thermal, hydroelectric, and nuclear power stations. Only about 30% of thermal energy generated from fuels is converted into electricity and the remaining heat is released into the environment through water bodies. For the efficient operation of a nuclear power plant, uninterrupted supply of cooling water into the condensers is pre-requisite [1]. Nuclear power plants require, on an average, about 3 m^3 cooling water per minute per megawatt (MW) of electricity produced [2]. The dissipation of heat through the plant cooling water systems may affect growth, development, and

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distribution of flora and fauna in the surroundings, which is an important area of concern among the plant operators and environmentalists. Apart from increased temperature, the discharges often contain chemical stress factors in the form of biocides (e.g., chlorine) used for biofouling control [3]. Thus, condenser effluents from thermal power plants have the potential to impart thermal and chemical stress and, therefore, may pose environmental problems to the receiving water body [4]. In addition, increasing greenhouse gases in the atmosphere creates global warming and climatic changes over the years. The United States National Research Council proposed that the global mean temperature may increase by 1.5–4.5 °C in the next half century [5]. Thus, potential effect of thermal effluents discharge from power plants in addition to the increasing temperature due to global warming and climatic changes makes it vital to define the impact of temperature on fishes. In addition, the potential impact of chlorine in thermal effluents on fish physiology is not yet clearly understood.

Common carp (*Cyprinus carpio* L.) is the oldest cultured and the most domesticated fish species of the world [6]. *C. carpio* culture is well suited in temperate as well as tropical countries of the globe, as they can tolerate a wide range of environmental conditions and endure relatively poor water quality. Carps can survive extreme temperatures (4–39 °C) and low dissolved oxygen for several days [7]. In northern hemisphere, they are reported to tolerate 0–30 °C. However, the optimal temperature for carp growth from studies has been found to be around 25 °C [8]. Against this background, the present study was undertaken to assess the stress effect of increasing temperature combined with sublethal ($0.1\,mg\,L^{-1}$) level of chlorine, using *C. carpio* early fingerlings as a biomarker species. The acclimation temperatures chosen were 26, 31, 33, and 36 °C since it is in the range of preferred temperature of common carp, *C. carpio* [9] Indian Major Carps [10] Chlorine levels in the immediate vicinity of thermal power plants (near discharge canal) is about $0.1\,mg\,L^{-1}$ (personal communication from power plant operators).

Thermal acclimation process and their limits have been determined by enzyme activities at different temperature regimes [11]. We tested a few metabolic enzymes to describe the cellular physiology of the fish after exposure to high temperature and chlorine. Fishes utilize protein and lipid sources than carbohydrate for meeting their energy requirement [12]. Therefore, gluconeogenic pathway and protein metabolism, assumes importance in fishes under stress [13]. We analysed transaminases (alanine amino transferase, ALT and aspartate amino transferase, AST), enzymes of gluconeogenic pathway (fructose-1,6-diphosphatase, FDPase), glycolytic pathway (pyruvate kinase, PK malate dehydrogenase, MDH, and lactate dehydrogenase, LDH), pentose phosphate pathway (Glucose 6 phosphate dehydrogenase, G6PDH), phosphorus metabolism (alkaline phosphatase, ALP), neurotransmission (acetylcholine esterase, AchE), and membrane transport (adenosine triphosphatase, ATPase). ALT and AST were tested because it produces TCA cycle intermediates by transamination, which is used as the preferred substrate for gluconeogenesis [14,15]. PK is an allosteric enzyme, which act as a negative control of glycolysis, the main pathway for metabolic energy. The presence of FDPase in the liver, may act as a forward stimulator of PK. LDH, the terminal enzyme of glycolytic pathway, is responsible for reversible conversion of pyruvate to lactate and is present in most of the tissues. Under stressed condition, anaerobic pathway is activated and high levels of lactate are being produced by inter-conversion of pyruvate in the cell. Similarly, MDH (a sulfhydryl enzyme), which is involved in reversible conversion of L-malate and oxaloacetate. MDH activity is reported to increase under the influence of stress [16]. ALP, a zinc-containing metallo-enzyme, which plays an important role in metabolism of phosphorus in the body, which demonstrates stressor specific responses. Enzyme of neurotransmission, AchE is widely distributed in nervous tissue, stored and released from the synaptic vesicles, which helps in the depolarization of adjacent neuron and thus passes nerve signals. AchE is rapidly hydrolysed by choline esterase [17] under normal physiological conditions. AchE is said to be inhibited under the influence of xenobiotics, which affects the neurotransmission. ATPase is a membrane bound enzyme responsible for the transport of ions through the membrane and regulates Na^+/K^+ gradient along the cell membrane [18] and is considered affected under stress. From our earlier observations in experiments in fish (data unpublished), shellfish [19], these enzymes showed organ specific activity. Therefore, we tested ALT and AST in liver and muscle; FDPase in liver; PK, MDH, and LDH in muscle; G6PDH in liver; ALP in intestine, liver, and muscle; AchE in brain, and ATPase in gills. This organ specific metabolic profile may improve our current understanding on the impact of prolonged exposure to temperature and chlorine on the metabolic stress responses in *C. carpio* early fingerlings.

2. Materials and methods

2.1. Experimental fishes

Cyprinus carpio fingerlings (mean ± SE : 5.3 g ± 0.2) were brought in separate aerated open containers from Khopoli fish seed farm, Government of Maharashtra to wet laboratory, Central Institute of Fisheries Education, Mumbai and were acclimated for 30 days to laboratory conditions (30 °C). During this period, fishes were fed with supplementary feed (25% crude protein) before being subjected to acclimatory studies.

2.2. Acclimation of experimental fishes

A total of 144 fishes were distributed in eight different treatments in thermostatic aquaria (175 L water capacity) at the rate 1 °C/day from laboratory temperature (26 °C) to reach experimental acclimation temperatures (26, 31, 33,