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SHORT COMMUNICATION

Ultrastructural Alterations in the Gills of *Labeo rohita* Fingerlings Exposed to Thermal Extremes

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Summary

This study aimed to determine the cellular alterations in the gill of *Labeo rohita* exposed to lethal temperature maxima (LT_{Max}) and lethal temperature minima (LT_{Min}) by means of transmission electron microscopy (TEM). Acclimation of advanced fingerlings of *L. rohita* was carried out at 26°C for 30 days. Acclimated fish were subjected to a constant rate of increase or decrease in temperature (0.3°C/min) until the LT_{Max} and LT_{Min} values were reached. Dissected gills were processed for TEM, both at the end of acclimation period at ambient temperature (26°C) and at lethal temperatures. Results indicated that at ambient temperature, the gill tissues appeared normal. However, significant changes were observed at lethal temperatures. The gill tissues at lethal temperature maxima showed severely damaged lamellae, with more vacuolated space. At lethal temperature minima, gill tissues showed increased density of mitochondria. Our *prima-facie* report indicated that *L. rohita* exposed to lethal temperatures exhibited marked ultrastructural changes in the gills.

Introduction

Fishes are poikilotherms, and drastic change in the surrounding water temperature markedly influences their metabolic processes, behaviour, migration, growth, reproduction and survival. It has been shown in animals that extreme temperature changes induce stress in the organisms that is characterized by changes at the physiological (Tort, 2011) as well as at the cellular architectural levels (Egginnton and Sidell, 1989). However, moderate change (increase) in the water temperature has beneficial repercussions on the aquaculture animals; it increases the metabolism of the animals to a certain limit and facilitates faster growth. Fishes have temperature tolerance limits, as well as optimum temperature for growth (Das et al., 2005), reproduction (Das et al., 2006) and resistance to diseases.

It has been proposed by the United States National Research Council that the global mean temperature may increase by 1.5–4.5°C in the next half a century (Beitinger et al., 2000). The potential impact of global warming has compelled researchers to make continuous effort to define thermal adaptation in fishes and to elucidate their consequences on the health status. The various problems arising due to global warming have given greater urgency for understanding the biological responses due to temperature fluctuations, particularly when ectothermic organisms have limited independence from changes in their environmental temperature. Surface water temperature is likely to fluctuate up to 40°C depending on latitude, season, altitude, time of day, depth of water and other factors (Munro, 2001) and will be more frequent in tropical shallow freshwater bodies than in the sea. It is therefore essential to know the effect of thermal extremes (both lower and higher) on histo-architecture of vital organs in aquatic organisms. Among different organs, gills are vital because they are the main sites for gaseous

exchange (Hughes, 1982), osmoregulation (Verbost et al., 1994), acid-base balance (Lin and Randall, 1991; Goss et al., 1992) and excretion of nitrogenous compounds (Evans and Cameron, 1986; Sayer and Davenport, 1987) in fishes and shellfishes. Therefore, gills can be used as model organ for assessing the environmental impact in fishes (Nascimento et al., 2012). In India, freshwater aquaculture is dominated by Indian Major Carps (*Labeo rohita, Catla catla* and *Cirrhinus mrigala*), which contributes more than 80% of the total freshwater production (Kumar et al., 2010). *L. rohita* is widely cultured throughout India due to its high commercial value. However, little is known about the ultrastructural alterations in the gills of *L. rohita* exposed to thermal extremes. The present study employed transmission electron microscopy monitoring tool to investigate cellular changes in the gill architecture of *L. rohita* exposed to thermal extremes, lethal temperature maximum and lethal temperature minimum.

Materials and Methods

Experimental fish and acclimation

L. rohita advanced fingerlings (31.5 ± 1.78 g) were brought from Pancham fish farm, Saphale, Maharashtra, India, with proper oxygenation to the laboratory of the Central Institute of Fisheries Education, Mumbai, India, and held for 30 days at ambient water temperature (26 ± 1°C). During this period, they were fed at 2% body weight, twice daily with a formulated diet containing groundnut oil cake, fish meal, rice bran, binder and vitamin–mineral mixture. Manual water exchange (10%) was performed every day. Water quality parameters like temperature, dissolved oxygen, ammonia and pH were checked daily and were found within the optimum range. Feeding was stopped 24 h prior to the start of the experiment.

Exposure to lethal temperatures

For exposing to lethal temperatures, twelve fish were equally distributed in two thermostatic aquariums and were acclimated to the ambient temperature (26 ± 0.2° C) for 30 days. At the end of the acclimation period, fish were subjected to a constant rate of increase or decrease at the rate of 0.3°C/min until cessation of operculum movement was reached. This point was designated as the lethal temperature maximum (LT_{Max}) and lethal temperature minimum (LT_{Min}), respectively (Tushida, 1995; Das et al., 2004). This technique has been critically evaluated by numerous workers (Chatterjee et al., 2004; Debnath et al., 2006; Dalvi et al., 2009) and is well established as a powerful tool for studying the physiology of stress and adaptation in fish (Beitinger and McCauley, 1990; Paladino et al., 1980). Dissolved oxygen concentration was maintained at 5.5 ± 0.5 mg/L during the test by continuous aeration using a 2-HP centralized air blower. At the end of the test, the fish were immediately removed from the aquaria and anesthetized using an herbal anaesthetic formulation, CIFECALM (200 μL/L). Gills were dissected from both the control (acclimated to ambient temperature) and the treated fish (exposed to lethal temperatures) and fixed in 3% glutaraldehyde using 1 M sodium cacodylate buffer for 8 h at 4°C for electron microscopy study.

Ultrastructural analysis using electron microscopy

Transmission electron microscopic study was carried out using a transmembrane electron microscope (Model JEM-1010, Japan) at the Jaslok Hospital and Research Centre, Mumbai, India, following the protocol described by Ghadially (1986). Briefly, samples fixed in 3% glutaraldehyde using 1 M sodium cacodylate buffer were washed with sodium cacodylate buffer for 4 times, each time for 5 min and subsequently fixed in 1% osmium tetroxide and 1 M sodium cacodylate (v/v: 1:1) for 1–2 h at 4°C. The samples were washed with 1 M sodium cacodylate buffer for 10 min and dehydrated using a graded series of ethyl alcohol/acetone and finally embedded in SPURR's resin. Semi-thin sections were cut and stained with 20% toluidine blue and were observed under 40X. Suitable areas were selected and marked for ultra-thin sectioning. The ultra-thin sections were stained with lead citrate and uranyl acetate and subsequently scanned under TEM for observation and electron micrograph.

Results and Discussion

Thermal tolerance studies of poikilotherms have been carried out for more than a century, considering its multifaceted significance (Heath, 1884; Hazel and Prosser, 1974; Hernández et al., 1996; Beitinger et al., 2000; Díaz et al., 2000, 2002; Hernández and Bückle, 2002). Our previous investigations revealed that *L. rohita* demonstrates thermal acclimation-dependent adaptation (Das et al., 2004).

In the present study, we present the *prima-facie* evidence on cellular change in *L. rohita* in response to exposure to thermal extremes. Here, the gills were used as the model organ because they remain in direct contact with the environment, and therefore, they could act as an indicator of water quality. In addition, as thermal equilibrium between the environment and fish body mediates through gills, they are regarded as the heat exchangers

(Stevens and Sutterlin, 1976). The gills therefore are considered to be an appropiate organ for indicating thermal pollution (Alazemi et al., 1996) and also other related-pollution (Lichtenfels et al., 1996; Pawert et al., 1998).

The ultrastructural cellular architecture of the gill tissue in *L. rohita* acclimated to ambient temperature (26°C) and exposed to either LT_{Max} or LT_{Min} is presented in the Figs 1–3. In fish acclimated to ambient temperature, section of the secondary gill filament showed functional erythrocyte (E) and normal looking mitochondria (M) in the lining epithelial cell (Fig. 1a). Primary gill lamellae (P) and secondary lamellae (S) filled with erythrocytes, and cartilaginous cells (C) were evident at the centre of the primary lamella (Fig. 1b). Secondary gill lamellae showed endothelial lining cells (En) and erythrocyte (E) (Fig. 1c). This result indicated that fish acclimated to ambient temperature (26°C) had normal ultrastructures of the gills, suggesting healthy condition due to the preferred temperature of *L. rohita* (Jhingran, 1975).

Results also showed that the gill tissues of fish exposed to LT_{Max} showed endothelial cell (En) and more vacuolated space (V) (Fig. 2a). The tip of the primary lamella showed erythrocytes (E) and endothelial lining (En) (Fig. 2b and 2c). Fishes exposed to high temperatures may suffer respiratory stress and nerve disorders. Increasing water temperature exerts a dual effect on their oxygen demand/supply ratio. Warmer waters carry less dissolved oxygen, yet the oxygen uptake by the aquatic organisms is increased due to their high metabolic rate. Our earlier investigation indicated increased metabolic activity in the Indian major carps with increasing acclimation temperatures (Das et al., 2004). In this study, increased vacuolated space observed in the gill tissue of fish exposed to LT_{Max} could be attributed to osmotic imbalance as higher water temperature has been reported to alter the lipids in the gill cells, resulting in leakage of cells and reduction in the efficiency of salt excretion and balance (Munro, 2001). Our results are in line with the findings of Manush et al. (2007) who observed mitochondrial degeneration (devoid of cristae and severe destruction of the membrane) and vacuolated area in cytoplasm in the gill of *M. rosenbergii* acclimated to 35°C for 30 days.

In the present study, the gill tissue in fish exposed to LT_{Min} showed erythrocyte (E) in secondary gill lamella (Fig. 3a), desmosome (D), endoplasmic reticulum (Er) and increased density of mitochondria (Fig. 3b). Fishes encounter respiratory stress and coma as a direct effect of lower temperature. Even though cold water contains more dissolved oxygen than warm water, the suppressive effect on the respiratory rate of fish may be due to insufficient oxygen uptake and subsequent hypoxia. Behavioural signs associated with hypothermia include lower respiratory rate and loss of coordination during swimming (Donaldson et al., 2008) as was evident in

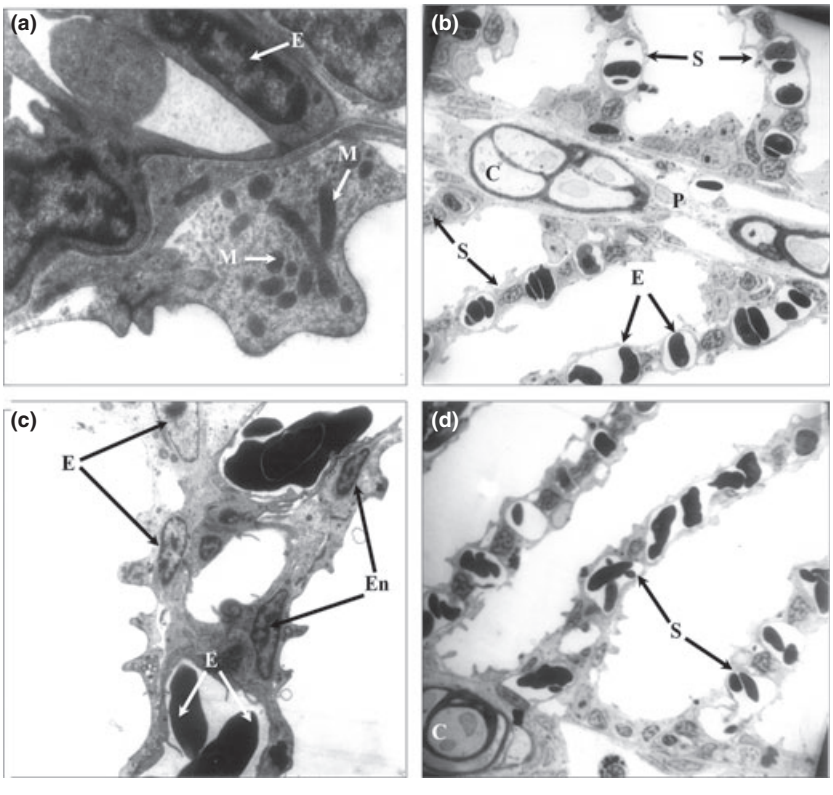

Fig. 1. Transmission electron microscopic (TEM) photographs of gill tissue in *L. rohita* acclimated to 26°C (Control). (Magnification: a: 12K; b: 1.5K; c: 5K; d: 1.5K) (C: Cartilaginous cells; E: Erythrocyte; E_n: Endothelial lining cells; M: Mitochondria; P: Primary gill lamella; S: Secondary gill lamellae)

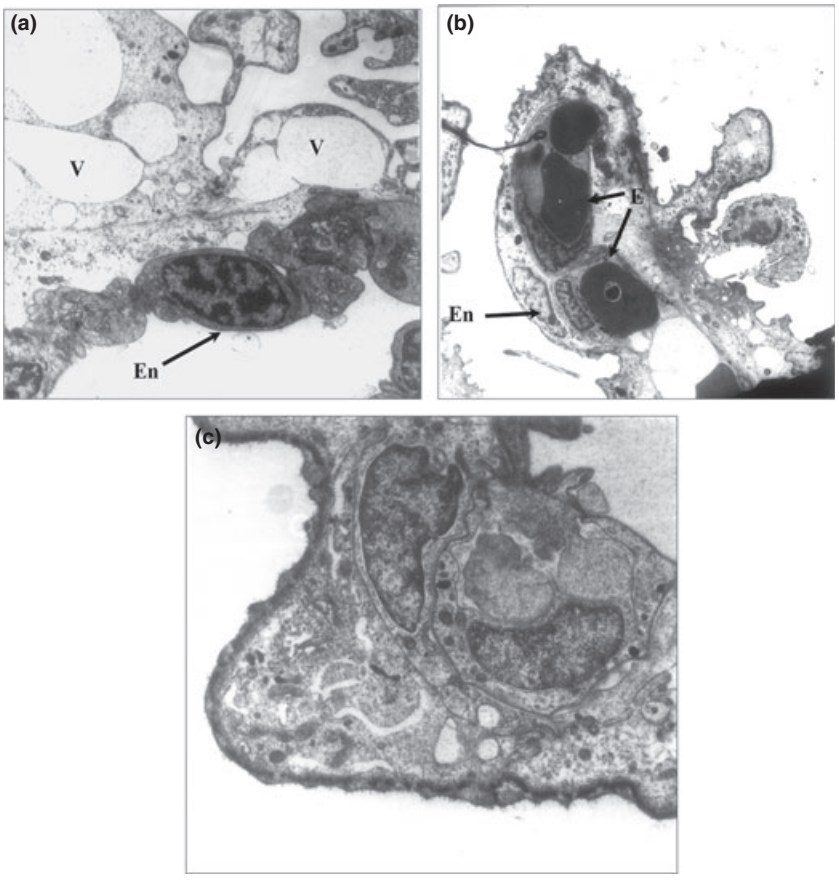

Fig. 2. Transmission electron microscopic (TEM) photographs of gill in *L. rohita* after exposure to lethal temperature maxima (LT Max) study. (Magnification: a: 6K; b: 4K; c:10K) (E: Erythrocytes; E_n: Endothelial cell; V: Vacuolated space)

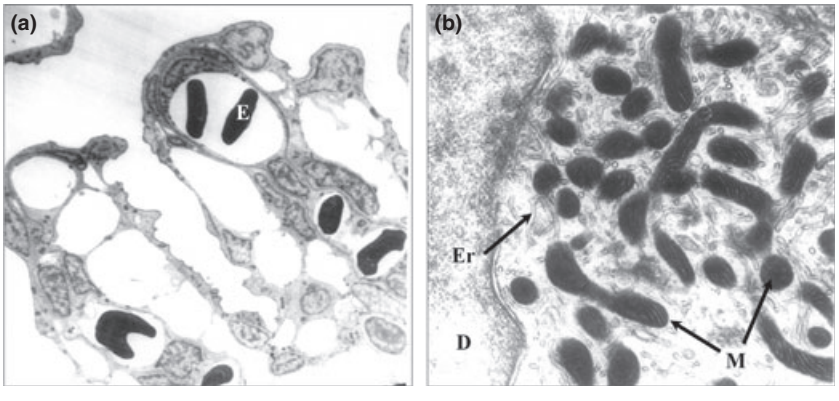

Fig. 3. Transmission electron microscopic (TEM) photographs of gill in *L. rohita* after exposure to lethal temperature minima (LT Min) study. (Magnification: a: 2.5K; b: 25K) (D: Desmosome; E: Erythrocyte; E_r: Endoplasmic reticulum; M: Mitochondria)

the present study. Das (1967) reported a relative hyperplasia of the hepatic cells in cold-adapted gold fish than those adapted at warm temperature. Hyperplasia in the langerhans tissue of *Anguilla anguilla* acclimated to 10°C than those acclimated to 20°C has been reported previously (Palayer, 1963). Hyperplasia is a phenomenon which appears to be much more frequent at low temperatures in all species of fishes (Roberts and Bullock, 1976). Our observations suggest that mitochondrial hypertrophy and hyperplasia may be a compensatory mechanism in *L. rohita* to derive energy for combating cold stress.

Results of our study showed that increased mitochondrial density in the gill tissue of *L. rohita* exposed to LT_{Min} could be an adaptive response to the thermal stress. It has been established that several species exhibit increased density of mitochondria for utilizing more energy to adapt or accommodate the cold stress. Overall, our results indicate that cellular integrity is altered in the gills in response to thermal extremes, which may cause

respiratory stress. Thus, this study may help in developing effective management strategies to overcome stress for aquaculture of *L. rohita* in field condition in the era of global warming.

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