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Seasonal variation in thermal tolerance, oxygen consumption, antioxidative enzymes and non-specific immune indices of Indian hill trout, *Barilius bendelisis* (Hamilton, 1807) from central Himalaya, India

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ABSTRACT

We studied the season dependent thermal tolerance, oxygen consumption, respiratory burst response and antioxidative enzyme activities in juveniles of *Barilius bendelisis*. The critical thermal maximum (CTmax), lethal thermal maximum (LTmax), critical thermal minimum (CTmin) and lethal thermal minimum (LTmin) were significantly different at five different seasons viz. winter (10.64 °C), spring (16.25 °C), summer (22.11 °C), rainy (20.87 °C) and autumn (17.77 °C). The highest CTmax was registered in summer (36.02 °C), and lowest CTmin was recorded during winter (2.77 °C). Water temperature, dissolved oxygen and pH were strongly related to CTmax, LTmax, CTmin and LTmin suggesting seasonal acclimatization of *B. bendelisis*. The thermal tolerance polygon area of the *B. bendelisis* juveniles within the range of seasonal temperature (10.64–22.11 °C) was calculated as 470.92 °C^2. Oxygen consumption rate was significantly different ($p<0.05$) between seasons with maximum value during summer (57.66 mg O_2/kg/h) and lowest in winter (32.60 mg O_2/kg/h). Total white blood cell count including neutrophil and monocytes also showed significant difference ($p<0.05$) between seasons with maximum value during summer and minimum number in winter and were found correlated to temperature, dissolved oxygen, pH and respiratory burst activity. Respiratory burst activity of blood phagocytes significantly differed ($p<0.05$) among seasons with higher value during summer (0.163 $OD_{540\ nm}$) and minimum in winter season (0.054 $OD_{540\ nm}$). The activity of superoxide dismutase, catalase and glutathione-s-transferase both in liver and gill, also varied significantly ($p<0.05$) during different seasons. Overall results of this study suggest that multiple environmental factors play a role in seasonal acclimation in *B. bendelisis*, which modulate the thermal tolerance, oxygen consumption, respiratory burst activity and status of anti-oxidative potential in wild environment.

1. Introduction

Thermal tolerance plays a significant role in any organism's life history (Ficke et al., 2007). In fishes, thermal tolerance is influenced by numerous factors like species (Das et al., 2004), prior thermal experience history (Beitinger et al., 2000, Das et al., 2004; Akhtar et al., 2013), acclimation temperature as well as acclimation duration (Diaz et al., 2007; Akhtar et al., 2014), salinity (Jian et al., 2003), photoperiod and seasonal effects (Hutchinson, 1976; Bulger and Tremaine, 1985, Layne et al., 1987). Daily and seasonal fluctuations in environmental temperature also affect variation in thermal tolerance of many species of ectotherms including fishes (Rome et al., 2002). Until today, most of the thermal tolerance studies concentrated on laboratory acclimated fish (Das et al., 2004; Debnath et al., 2006; Wang, 2009; He et al., 2014) than from wild habitat where fish is predisposed to multiple biotic and abiotic environmental factors such as temperature, dissolved oxygen, pH, salinity, photoperiod, reproduction, pollutants, etc. The thermal sensitivity of organism needs investigation at both the high and the low end of the temperature spectrum. The crucial mechanism of thermal limitation and adaptation should link low and high tolerance limits since they shift unidirectionally during thermal acclimation (Pörtner, 2002). Fish being poikilothermic/ectothermic species are adapted to and depend upon the maintenance of the characteristic temperature window of their natural

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environment. Hence, severe shift in the characteristics of natural environment beyond the optimum limits of a particular fish species influences metabolic processes, growth, reproduction, behavior and migration which in turn may cause several physiological disturbances in the organism (Portner, 2001; Pörtner, 2002; Akhtar et al., 2011; Wilson et al., 2014).

The critical thermal temperature is vital for the extensive understanding of adaptation of aquatic organisms to diverse thermal regimes (Herrera et al., 1998). The critical and lethal thermal limits (CTmax and LTmax & CTmin and LTmin) are the most common physiological indices used to measure fish tolerance to extreme low or extreme high temperatures and to determine fish resistance to different thermal phenomena (Mora and Ospina, 2001; Madeira et al., 2012a). Hence, thermal tolerance studies have gained importance in apprehending the effect of global warming on aquatic organisms including fish.

In fishes, the metabolic rate is indirectly measured as their rate of oxygen consumption (Brett, 1979; Kutty, 1981). Numerous factors affect rate of oxygen consumption in fish, including acclimation temperature, acclimation period, salinity (Das et al., 2004; Manush et al., 2004; Ern et al., 2014) and hematological parameters, gill surface area, ventilation volume and organ to body size ratio (Jobling, 1994; Benfey, 1999). Fish oxygen consumption rate is widely used indicator of metabolic rate (Jobling, 1994) mainly because knowing the metabolic cost of maintenance enables dietary energy needs to be calculated, and it is an important economic factor in aquaculture sector (Ibarz et al., 2003).

White blood cells (leukocytes) and other hematological parameters are repeatedly used as indicators of health status in fish because these are essential components of non-specific or innate immune defense and provide protection against commonly encountered pathogens through phagocytic functions (Ballarin et al., 2004; Zarejabad et al., 2010). Leukocyte numbers in fish are highly influenced by a number of extrinsic and intrinsic factors including environmental (e.g. photoperiod, salinity, temperature and season) (Valenzuela et al., 2007; Zarejabad et al., 2010; Thomas et al., 1999; Pradhan et al., 2014a) and biological (e.g. age, sex, maturity, etc.) (Hrubec et al., 2001; Pradhan et al., 2014a). Fish show a wide diversity of leukocytes, with different cell types and structural heterogeneity even between closely related species (Shigdar et al., 2009). In differential count in fishes, leukocytes in blood smears are commonly characterized by lymphocytes, neutrophils, monocytes, and eosinophils (Blaxhall and Daisley, 1973). Neutrophils and monocytes, which are long-lived phagocytic cells, are associated with the defense of infections and bacteria (Davis et al., 2008). The differential leukocyte count, like other hematological parameter, is dependent on sex, season, feeding regime and nutritional status (Francesco et al., 2012; Pradhan et al., 2014a) of the species. Therefore, these indices have been employed in effectively monitoring the responses of fishes to different environmental factors/stressors to know their health status in wild as well in captive conditions.

The immune system (both specific and nonspecific) of poikilothermic vertebrates including fish also exhibits seasonal changes (Zapata et al., 1992; Collazos et al., 1998; Collazos et al., 1995; Kaattari and Ottinger, 2000; Leu et al., 2001; Magnadóttir, 2006; Bowden et al., 2007; Tolarova et al., 2014). These changes are probably due to abiotic factors i.e. temperature, photoperiod, environmental pollution, etc. (Nikoskelainen et al., 2004; Magnadóttir, 2006; Lamkova et al., 2007; Morgan et al., 2008; Valenzuela et al., 2007; Tolarova et al., 2014) as well as biotic factors i.e. pathogens or parasites (Zapata et al., 1992; Bowden et al., 2007). The nonspecific immune response, particularly phagocytosis, is one of the fundamental mechanisms involved in the host protective response against microbial infections (Secombes, 1996; Alvarez-Pellitero, 2008; Grayfer et al., 2014). Phagocytosis is mediated by phagocytic cells such as neutrophils, monocytes, and macrophages. During the phagocytosis of parasite/pathogens, leukocytes upsurge their consumption of molecular oxygen through the NADPH oxidase enzyme complex and produce numerous reactive oxygen species (ROSs) in a process called the respiratory burst (Abbas et al., 2012; Takahashi-Biller et al., 2013). Several methods to measure the respiratory burst activity of neutrophils and monocytes have been used in fish, such as nitroblue tetrazolium reduction (NBT), chemiluminescence (CL) and flow cytometry (FC) assays. Leukocyte respiratory burst test using the reduction of nitroblue tetrazolium (NBT) to formazan as a measure of superoxide anion production is a potential tool to measure the reactive oxygen species produced during phagocytosis and can be applied to evaluate the immune response (Moritomo et al., 2003).

Production of reactive oxygen species (ROS) by fishes is very common in aquatic environments (Lesser, 2006). ROS comprise of both free radical forms (superoxide radicals and hydroxyl radical) and non-radical forms (hydrogen peroxide and singlet oxygen) which could be produced naturally during cell metabolism (Liu et al., 2015). Under stressful environments, such as high temperature, low dissolved oxygen, pathogen load, the balance between production and scavenging of ROS could be disturbed, causing oxidative stress in fish (Tovar-Ramírez et al., 2010). This result in tissue injury in many cellular components like proteins, lipids, carbohydrates, free amino acids and DNA (Toyokuni, 1999). In order to cope with these injuries, cells have antioxidant defence which consists mainly of antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-dependent enzymes and non-enzymatic defence such as amino acids and vitamins E, K and C (Martínez-Álvarez et al., 2005). Superoxide generated as the primary ROS is converted into hydrogen peroxide (H_2O_2) by SOD, which is then detoxified by catalase (Guemouri et al., 1991). Glutathione-S-transferases (GST) are a family of dimeric multifunctional enzymes involved in numerous processes including (1) detoxification of xenobiotics; (2) defense from oxidative damage; and (3) intracellular transport of hormones, endogenous metabolites and exogenous chemicals in diverse organisms (Goto et al., 2009). Further, the activity of antioxidative enzymes is affected by various environmental factors i.e. food availability (Pascual et al., 2003), season (Parihar et al., 1997; Aras et al., 2009; Pavlović et al., 2010), dissolved oxygen concentration (Cooper et al., 2002), feeding behavior, temperature, salinity and the influence of xenobiotics (Lushchak, 2011; Li et al., 2003; Martínez-Álvarez et al., 2005). Nowadays antioxidative enzymes are used as potential biomarkers of oxidative stress in fishes in both captive and wild conditions.

Barilius bendelisis is a benthopelagic fish belongs to family cyprinidae with a potential of a candidate species for aquaculture. In the wild, *B. bendelisis* is distributed along the Indian Himalayas (mainly in Ganga, Brahmaputra and Indus basins) including Pakistan, Nepal, Bangladesh, Sri Lanka, Bhutan and Myanmar (Talwar and Jhingran, 1991; Oo, 2002). *B. bendelisis* is potential food fish consumed by Himalayan population achieved a market price of Rs 160–200 per kg. This species is also demanding in the aquarium trade in India under the name of 'Indian hill trout' or 'Hamilton's baril and reported to be exported from India as ornamental fish (Mir et al., 2015; Jayalal and Ramachandran, 2012). Despite being a popular ornamental species among aquarists as well as a food fish (in terms of protein and minerals) for populace of Himalayan region, is poorly studied and as a result, this species has undergone a drastic reduction in its availability in natural water in the recent past (Mir et al. 2015). Further, there is no published data available on seasonal variation in thermal tolerance, oxygen consumption, respiratory burst activity and antioxidative enzymes of this species. Considering the threat of global warming and to ensure sustainable availability as well as management of this species, it is