

Impact of heat stress on the immune response of fishes

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Received: May 2018

Accepted: August 2018

Abstract

Changes in the environmental temperature mediate oxidative stress state that affects the survival rate of fishes. In general, stressors could affect directly as fish death or indirectly through inhibiting immunity thus allowing pathogen invasion and disease incidence. Rapid temperature fluctuations cause severe physiological stress on fish so any temperature change affects the immune system. Fishes elicit generalized physiological and immunological stress response against heat stress. As in other vertebrates, this generalized stress response comprises physiological responses that are common to a wide range of environmental, physical and biological stressors. This mini review provides insight into the effect of heat stress on the fishes and the immune response against heat stress.

Keywords: Apoptosis, Heat stress, Immune response, Oxidative stress.

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Introduction

The aquatic ecosystem mainly depends on water quality, as the number and diversity of biota is affected by the physicochemical parameters of water. Measuring physiological parameters of fish could evaluate the stress caused by elevated temperature (Diaz *et al.*, 2007; Vanlandeghem *et al.*, 2010). Fish are ectothermic which means that external temperature could affect their immune system directly; also bony fish have a complete vertebrate immune system resembling the mammalian immune system. These reasons suggest that fish is the ideal example used to study the effect of temperature on their immune system.

Heat stress (HS) and its effect on fishes

Stress occurs when any environmental change (physical or physiological) could alter the normal animal homeostasis (Bly *et al.*, 1997). In general, stressors could affect directly as fish death or indirectly through inhibiting immunity thus allowing pathogen invasion and disease incidence (Zelikoff *et al.*, 1991). Rapid temperature fluctuations cause severe physiological stress on fish (Crawshaw, 1979) so any temperature change affects the immune system badly (Lillehaug *et al.*, 1993).

Water temperature is considered one of the most important environmental factors which affect animal survival and growth aquaculture farming. In the teleost fish, any change in the culture water temperature could affect the fish survival, physiological conditions and

immune responses (Bowden, 2008; Lee *et al.*, 2014). The need to study fish reaction to sublethal temperature becomes an urge due to world climatic changes and heat stress (HS) (Hari *et al.*, 2006). Many factors effect on water temperature fluctuation such as daily changes of air temperature, water body size, flow rate volume, degree of mixing and solar radiation exposure. Ambient temperature fluctuations could effect on the animal physiological state causing animal stress and resulting in the “general adaptation syndrome” (Selye, 1950; Iwama *et al.*, 2004). It was found that, the sea surface temperature has risen 0.8 (Godbold and Calosi, 2013) leading to a disruption in species abundance and distribution and affecting the community and ecosystem (Vinagre *et al.*, 2009; Peck *et al.*, 2012; Madeira *et al.*, 2016). Animals usually respond to high temperatures through many behaviors such as: feeding, shoaling, body color, breath function (operculum movements), swimming activity (equilibrium and swimming speed) and escaping (Ohlberger *et al.*, 2007; Lopez-Olmeda and Sanchez-Vazquez, 2011). These behaviors are difficult to notice in marine animals leading to poor understanding of a species response to ocean warming (Healy and Schulte, 2012; Cardoso *et al.*, 2015).

HS mediates oxidative stress and generation of reactive oxygen species (ROS)

In normal conditions, ROS production and elimination are well managed in regular cell metabolism. Although this balance between ROS production and antioxidant defense system is disturbed due to environmental stress leading to ROS overproduction which has toxic effects on cells (Achard-Joris *et al.*, 2006; Qiu *et al.*, 2011). In normal conditions, ROS plays many important roles in cellular metabolism such as: cell growth, apoptosis and signaling (Luo *et al.*, 2014). Normal cellular defense systems such as enzymatic defense system and naturally antioxidant system counteract the negative effects of oxidative stress and balance the intracellular redox status. These antioxidant enzymes are considered to be the first intracellular defense against ROS and regulate redox-dependent signaling, which are essential for the innate immunity (Selvaraj *et al.*, 2012).

HS could induce ROS overproduction leading to damage of cellular biomolecules such as proteins, lipids and DNA, which eventually cause impaired cellular functions (Liu *et al.*, 2014; Luo *et al.*, 2015).

HS induces apoptosis

Extensive oxidative stress leads to disruption of cell signaling, severe DNA damage and cellular apoptosis (Chandra *et al.*, 2000). Apoptosis (programmed cell death) could be defined as a regular physiological process that is involved in the development, cellular homeostasis and immune responses. Apoptosis could be

triggered through two major mechanisms: the first is the extrinsic pathway (death receptor pathway) which is induced by binding of tumor necrosis factor (TNF) family receptors (Gao *et al.*, 2013). The second pathway is the intrinsic apoptotic pathway (mitochondrial pathway) which involves cell death signals cascade initiated from mitochondria (Luzio *et al.*, 2013). Apoptosis plays an important role in the immune system as it is involved in enhancing the phagocytic removal of dying or infected cells (Gao *et al.*, 2013; Luo *et al.*, 2017), preventing autoimmune disorder (Chen *et al.*, 2006) also inhibiting pro-inflammatory cytokines (Savill, 1997).

Immune system of fishes

Fish possess a complex immune system of innate and acquired immune defense mechanisms. The innate immune system which is considered to be the first line of defense for fish is sensitive to environmental changes that lead to disruption of immune function (Fevolden and Roed, 1993).

One of the essential components of fish immune system is the complement system. It plays an important role in the immune defense against the bacterial invasion and the inflammation. C3 is the key modulator of both classical and lectin pathways involved in various immune effector functions (Holland and Lambris, 2002). C3 mRNA expression level was found to increase due to environmental stressors (Qi *et al.*, 2011). The complement system activation is a good indicator of fish

immuno-competence under stressors (Carroll, 2004). It was suggested that HS could activate the complement system (Cheng *et al.*, 2018).

Effect of HS on the immune system of fishes

High temperature that is within the physiological range of the fish species could affect the immune function (Bly and Clem, 1992). For example, exposure of Catfish (*Heteropneustes fossilis*) to high temperature leads to mitochondrial superoxide (O_2) production in the gills (Prakash *et al.*, 1998) and induced antibody activity in Atlantic cod, *Gadus morhua* L. (Magnadottir *et al.*, 1999). Exposure of Atlantic halibut to high water temperature had no effect on phagocytosis, but inhibited the immune response against bacteria (Avtalion, 1981).

In teleost, blood cell plays a major role in immune defense so measuring the parameters of these blood cells could determine the physiological changes induced by stressors (Wu *et al.*, 2015). It was demonstrated that elevated temperature fluctuation lead to reduction in blood cell count in teleost (Qi *et al.*, 2013; Cheng *et al.*, 2017). Ellis (1981) found that, white blood cell count could be used to evaluate some diseases and injuries in fish body; such as rearing environment affecting the number of circulating leukocytes. It was found that there was increase in the white blood cell count in rainbow trout (*Oncorhynchus mykiss*) (Houston *et al.*, 1996) and carp (Engelsma *et al.*, 2003)

at elevated temperature and after cold shock, respectively. Ndong *et al.* (2007) found that, when Mozambique tilapias (*O. mossambicus*) were transferred from 27°C to 19°C or 35°C, white blood cell counts decreased significantly. The phenomenon of strong disease resistances in tilapia at optimal temperature could be explained by (Qiang *et al.*, 2013). It was found that, under temperature stress of 19°C and 35°C from 27°C, tilapia decreased its resistance against *S. iniae*. WBC counts of sea bass *Dicentrarchus labrax* and tench were significantly elevated at suitable water temperature in summer than in winter (Pascoli *et al.*, 2011).

Immune response of fishes against HS

Heat shock proteins (HSPs) are a group of highly conserved proteins which exist widely in all living organisms. HSPs families play a critical role in protein folding, intracellular transport, protein degradation, and cell signaling (Geething and Sambrook, 1992), which are responsible for the maintenance of cellular viability by preventing the irreversible loss of vital proteins and facilitating their subsequent regeneration due to stresses (Pörtner, 2002). It is also believed that HSPs act as biomarkers for assessing the organism's response to environmental stressors (Dalvi *et al.*, 2017). Among HSP family, HSP70 and HSP90 play a major role in folding newly synthesized proteins and refolding denatured proteins, also they participate in the stress immune response (Fu *et al.*, 2011). HS on fish cause over-

expression of HSP70 and HSP90, leading cells to recover from stress and protect them from further insults (Wu *et al.*, 2012). Overexpression of inducible HSP70 could be due to elevated protein damage and increased stress tolerance (Feder and Hofmann, 1999). HSPs were found to be heat inducible in many species, such as zebrafish (*Danio rerio*) (Lele *et al.*, 1997), medaka (*Oryzias latipes*) (Werner *et al.*, 2003), and sea urchins (Wu *et al.*, 2012). It was noticed that elevated levels of HSP70 and HSP90 lasted only for some hours, may be due to energy consumption for other heat shock responses (Somero, 2002) and the available last energy was limited.

Fish could adapt to the elevated water temperature of its habitat till a specific limit, although HS occurs when the temperature exceeds the threshold extent. The temperature at which stress occurs to fish depends on a fish's acclimation or previous experience. Additionally, there is a sub-stressful temperature at which stress occur regardless fish acclimation (Barton, 2002).

After the elevation of environmental temperature, the water oxygen concentration decreases and the metabolic rate increases thus inducing tissue oxygen demand. Fish adapt to fluctuation in environmental temperature and hypoxia by inducing their total hemoglobin content (Brix *et al.*, 2004). As a result to the elevated metabolic rate due to HS (Campbell *et al.*, 2008; Otto and Zahn, 2008); fish increase their fat consumption due to

increased energy demand, and finally lead to reduced body mass. As fishes are poikilothermic aquatic animals, elevated water temperature could change their body temperature. This ectothermic character could affect the innate and adaptive immunity, leading to increase susceptibility to infection or death (Dittmar *et al.*, 2013). Generally, fish could modulate their metabolism to overcome the effects of temperature fluctuations (Ibarz *et al.*, 2010). Aquatic ectotherms usually increase the anaerobic component so as to control the damage caused by different stressors (Devi, 1996).

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