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Original research

The influence of global warming on oxidative stress and antioxidant defense system in blue tilapia (O. aureus)

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Abstract:

Global warming is shifting the population of animals around the world, due to the frequent extreme heatwaves. Scientists anticipate that the ocean temperature will rise by 1-4°C by the year 2100. Fishes as an ectotherm are particularly vulnerable to global warming. However, there is limited information about the possible impact of global warming on fish physiology. This study was designed to investigate and compare the effects of long-term thermal acclimation on antioxidant enzymes activities and the potential stress in blue tilapia (O. aureus) (20±1.7 g). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and glutathione reductase (GR), as well as γ -glutamyl cysteine synthetase (γ -GCS) and γ-Glutamyl transferase (γ-GT). In comparison to fish acclimated to winter ambient temperature, the activity of the liver and white muscle enzymes increased dramatically in fish adapted to 30°C and 40°C. While the levels of lipid peroxidation (LPO) were significantly increased in tissues of fish acclimated to 40°C, as compared to fish at both ambient and 30°C.

Keywords: Enzymatic antioxidant, Global warming, Temperature, Blue tilapia (*O. aureus*), Oxidative stress.

1- INTRODUCTION

Global warming is drifting the population of animals around the world, due to the frequent extreme heatwave events. Scientists anticipate that the ocean temperature will rise by 1-4°C by 2100 worldwide. The majority of fish species are ectothermic, which means they don't and can't regulate their internal body temperature and have a body temperature that matches their surroundings. However, there are limited information about the possible impact of global warming on fish physiology.

Furthermore, fish use a range of metabolic methods to cope with temperature variations in their surroundings. Considering the importance of the optimum water temperature for fish to maintain normal metabolism, feeding behaviour and normal growth rate, and variation of water temperature can disturb the feed efficiency, growth rate, physiological status, stress resistance and immune response (Liu *et al*., 2019; Wu *et al*., 2019).

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Tilapia was classified as eurythermal, it can tolerate a quite wide range of temperatures (8 – 42°C) (Dan and Little, 2000). Temperature is a limiting factor for the chemical reactions and the thermodynamic activation of enzymes on which biological system acts are based. Temperature affects the antioxidant system of the freshwater fish (Kaur *et al*., 2005; Bagnyukova *et al*., 2007).

Acclimation of an ectothermic animal to different temperature for a long period can result in a series of physiological alterations, that improve the animal's performance at the new temperature, this process known as thermal acclimation (Johnston and Temple, 2002; Tattersall *et al*., 2012). Fish thermal acclimation is a complex process that involves changes in enzyme activity, protein synthesis as well as gene expression (Ju *et al*., 2002; Zhou *et al*., 2019).

In Egypt, the mean temperatures of water during summer season in 2018 were about 30.2°C, which is ideal for fish (Ali *et al*., 2020) among different governorates. The optimal growth and feed utilization for Tilapia were recorded at temperatures between 26 and 30°C (Azaza *et al.,* 2008). While, water temperature at Nasser Lake recorded 39°C in July 2020, upper Egypt is hotter than Delta region (Abd-Elhamid *et al.,* 2021).

Though, water temperature increases about 0.6 to 0.8 degrees for every one degree increase in air temperature (Morrill *et al*., 2001). The combined temperature of land and ocean-surface in 2021 has increased 0.93°C above the 20th century, according to the 2020 Global Climate Report from NOAA National Centers for Environmental Information. Regarding the impacts of the climate change on water temperature, many scientists anticipate higher temperature in the future.

High temperature induced a physiological stress through three steps; 1) spurring mitochondria to generate more oxygen radicals, 2) suppressing antioxidant enzymes activity, 3) initiates oxidative stress in aquatic animals (McKenzie *et al*., 1996; Coppola *et al*., 2018).

Normally, reactive oxygen species (ROS) is generated in the mitochondria during aerobic respiration (Fridovich, 2004; Abele and Puntarulo, 2004).

The antioxidant defense systems regulate excessive ROS generation such as; superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$). Ectothermic fish have a lesser antioxidant defense capacity than endothermic creatures like birds and mammals (Wilhelm-Filho, 2007). High quantities of ROS can cause damage to cell structures, nucleic acids, and lipoproteins (Valko *et al.,* 2006). ROS generation, and antioxidants activity are directly related to temperature (Wilhelm *et al*., 2000).

The antioxidant system initiate action with superoxide dismutase (SOD), which is an enzymatic antioxidant, that catalysis the transformation of the superoxide anion to molecular oxygen and hydrogen peroxide. Catalase (CAT) catalysis the conversion of hydrogen peroxide to molecular oxygen and water, glutathione peroxidase (GPx) catalysis the transformation of hydrogen peroxide into water, and glutathione S-transferase (GST) detoxifies a variety of reactive intermediates and products of oxidative stress, and glutathione reductase (GR) which catalyzes the reduction of oxidized glutathione (GSSG) into reduced glutathione (GSH) (Halliwell, and Gutteridge, 2007).

GSH is a GPx and GST substrate that is thought to be the first line of defense against ROS in the cell. GSH is the most abundant non-protein thiol in the cell, with levels in the millimolar range in most cells (Voronkova *et al*., 2018). Superoxide dismutase (SOD) activity was affected by temperature fluctuations in fish (Kammer *et al*., 2011). Moreover, the levels of oxidized

glutathione in the liver of warm-acclimated fish were higher than in the liver of cold-acclimated fish (Leggatt *et al*., 2007).

The purpose of this study was to study the possible impact on oxidative stress and antioxidant enzymes activities in fish exposed to global warming. Through, investigating the long-term thermal stress of global warming on fish in laboratory, providing insight into what might be anticipated in the wild.

2- MATERIALS AND METHODS

2.1. Fish husbandry

Blue tilapia juveniles (35±1.7 g) were taken from fish hatchery pond in January and transported to the laboratory. In the laboratory, fish were kept in 48 glass aquaria of 33 L with a constant air flow system, five fish per aquarium, and a typical photoperiod. Commercial fish dry pellets (25.2 percent protein, carbohydrate, lipids, and fibers, with total energy of 2505 Kcal/kg were used during the whole experiment. Fish were fed 3 % of their body weight once a day at 9 a.m. Fish were acclimated to the laboratory conditions for two weeks before experiment.

2.2 Thermal acclimation

fish were sub-grouped to control (Egypt's ambient water temperature in winter 15°C), 30°C (the optimal temperature for tilapia) and 40°C (represents the possible climate change thermal stress) fish were divided into 16 aquaria (33 L capacity) in each group, at 1.5 g L^{-1} rearing density (APHA, 1998). The temperature was raised by 1°C every 2 days to obtain the experiment temperature (30 and 40°C). Thermal acclimation extended to four weeks (Guderley, and Gawlicka, 1992). Half of the aquaria water was daily replaced by de-chlorinated water which pre-adjusted to each experiment temperature.

2.3. Tissue sampling and protein extraction

At the end of the feeding trial, fish were starved for 24 h, then removed from the holding tank and immersed into anesthetic solution (Simoes *et al.*, 2011), and dissected on ice. Tissues (Liver and white muscle) were excised, rinsed in isotonic NaCl saline, dried and weighed.

Tissues were immediately homogenized in 10% w/v ice-cold phosphate buffer using a homogenizer at 10,000 xg. The homogenate was centrifuged at 2,000 xg for 15 minutes at 4 °C, and the supernatant was preserved. Once again, the supernatant was centrifuged at 6,000 xg at 4°C for 15 minutes, yielding a supernatant containing total antioxidant enzymes (both cytosolic and mitochondrial enzymes).

2.4. Enzyme activity assays

Superoxide dismutase (SOD) activity was assayed according to the method of Paoletti and Mocali (1990). Catalase (CAT) activity was assayed according to the method of Cohen *et al.* (1970). Glutathione S-transferase (GST) activity was assayed according to the method of Habig *et al.* (1974). Glutathione peroxidase (GPx) activity was assayed according to the method of Paglia and Valentine (1967). GR activity was measured by the method described by Cribb *et al.* (1989). γ-glutamyl cysteine synthetase (γ-GCS) activity was assayed using the method of Huseby and Stromme (1974). γ-Glutamyl transferase (γ-GT) activity was assayed according to the method of, Silber *et al.* (1986). Lipid peroxidation activity (LPO) was assayed by the thiobarbituric acid (TBA) reaction using the method of Ohkawa *et al*. (1979).

2.5. Statistical analyses

Results are presented as mean ± S. D of 8 fish. The mean differences of enzyme activity were assessed using a one-way ANOVA followed by Tukey test. Differences were considered significant when $P \leq 0.05$.

3.Results and Discussion

In table 1, the activity of antioxidants enzymes showed a significant increase in both liver and white muscles of fish acclimated to 30°C and 40°C as compared to ambient fish. Moreover, enzymes activity recorded a significant reduction in liver and white muscles of fish acclimated to 40°C, as compared to 30°C fish, except for GPx and GR.

The total SOD activity enhanced by 4.2 and 3.6 folds in liver and white muscle of fish acclimated to 30°C, while increased by 2.7 and 2.3 folds in liver and white muscle of fish acclimated to 40°C, as compared to ambient temperature. CAT activity increased by 2.3 and 2.9 folds in both liver and white muscle of 30°C acclimated fish, and enhanced by 1.6 and 2.1 folds in liver and white muscle of fish acclimated to 40°C as compared to ambient fish. GST activity in were increased by 1.4 and 2 folds in liver and white muscle at 30°C, although increased by 1.2 and 1.7 in prementioned tissues in fish acclimated to 40°C, when compared to ambient fish. In comparison to ambient fish, GPx activity in fish acclimated to 30°C were enhanced by 2.3 and 4.9 folds in liver and white muscle, while increased by 2 and 3.2 folds in 40°C acclimated fish in comparison with ambient fish. The enzymatic activity of GR was increased by 1.4 and 1.5 folds in liver and white muscle of 30°C fish, although improved by 1.2 and 1.2 in 40°C fish as compared to ambient fish. γ-GCS activity showed an increase in fish acclimated to 30°C by 1.8 and 1.6 folds in liver and white muscle, meanwhile recorded a significate increase by 1.4 and 1.4 folds in comparison with ambient fish. γ-GT activity was significantly increased by 3.2 and 3.8 folds in liver and white muscle of fish acclimated to 30°C, while increased by 2 and 1.6 folds in fish acclimated to 40°C in comparison with ambient fish.

Table 1. Antioxidant activity in liver and white muscles of blue tilapia fish acclimated to ambient, 30°C and 40°C (mean ± S.D 8 fish).

		15°C (Ambient)	30°C (Optimal)	40°C (Thermal stress)
SOD	Liver	36.95 ± 3.55^{c}	157.2 ± 6.53^{a}	100.27 ± 6.73^{b}
(U/mg)	white muscles	21.1 ± 2.94^{c}	76.6 ± 4.57^{a}	48.54 ± 2.62^{b}
CAT	Liver	86.57 ± 1.5^{c}	201.18 ± 0.88^{a}	141.40 ± 1.2^{b}
(µM/min/gram)	white muscles	31.33 ± 1.12^{c}	90.77 ± 1.36^{a}	65.47 ± 1.26^{b}
GST	Liver	1.41 ± 0.10^{c}	2.05 ± 0.16^{a}	1.77 ± 0.11^{b}
(µM/min/gram)	white muscles	0.13 ± 0.01^{c}	0.26 ± 0.01^{ab}	0.22 ± 0.03^{b}
GPx	Liver	7.55 ± 1.5^{c}	17.92 ± 2.04^{ab}	15.47 ± 2.25^{b}
(µM/min/gram)	white muscles	1.19 ± 1.02^{c}	5.88 ± 1.4^{ab}	3.91 ± 1.6^{b}
GR	Liver	2.38 ± 0.45^{c}	3.46 ± 0.61^{ab}	2.94 ± 0.63^{bc}
(µM/min/gram)	white muscles	1.84 ± 0.25^{c}	2.86 ± 0.22^{ab}	2.34 ± 0.25^{b}
γ-GCs	Liver	1.84 ± 0.26^{c}	3.36 ± 0.25^{a}	2.54 ± 0.19^{b}
(µM/min/gram)	white muscles	1.44 ± 0.17^{c}	2.43 ± 0.15^{a}	2.12 ± 0.18^{b}
γ-GT	Liver	0.35 ± 0.03^{c}	1.12 ± 0.07^{a}	0.70 ± 0.05^{b}
(µM/min/gram)	white muscles	0.18 ± 0.07^{c}	0.70 ± 0.1^{a}	0.30 ± 0.1^{b}

Data were presented as mean ± SD (n=8 fish). Values within a row with different superscripts differ significantly (tukey Multiple Range Test, $P<0.05$).

Table 2. LPO levels in liver and white muscles of blue tilapia fish acclimated to ambient, 30°C and 40°C (mean ± S.D 8 fish).

		15°C (Ambient)	30°C (Optimal)	40°C (Thermal stress)
TBA (nmol/mL)	Liver	1.33 ±0.23 [c]	2.27 ±0.25 [b]	4.74 ±0.34 [a]
	white muscles	0.44 ±0.08 [c]	0.57 ±0.10 [bc]	1.2 ±0.11 [a]

Data were presented as mean ± SD (n=8 fish). Values within a row with different superscripts differ significantly (tukey Multiple Range Test, P<0.05).

LPO levels in liver of fish acclimated to 30°C and °40C were significantly enhanced by 1.7 and 3.5 folds as compared to ambient fish. White muscles recorded insignificant LPO increase in fish acclimated to 30°C, while significantly increased in 40°C fish as compared to ambient fish in Table 2.

The levels of antioxidant enzyme activity in the liver and white muscle of Blue tilapia acclimated to three different temperatures exhibited substantial variances. In compared to fish acclimated at 30°C, the enzyme activity of fish acclimated at 40°C were reduced. In contrast, the activity of enzymes in the liver and white muscle of fish acclimated to 30°C were increased in comparison with ambient fish.

Fish under laboratory circumstances, usually experience extensive exercised (Hyndman *et al*. 2003), this energy is mainly powered by white muscle and supported by anaerobic metabolism, which consumes a large amount of the adenosine triphosphate (ATP), and glycogen (Wood 1991). Later, during the recovery the oxygen consumption rate increases to compensate the consumption of ATP (Gaesser and Brooks 1984; McKenzie *et al*. 1996; Lowe and Davison 2006). Moreover, higher water temperature associated with metabolic activation and increased oxygen consumption rate.

High temperatures represent physiological stress which enhance ROS production, suppresses antioxidant enzymes and initiates oxidative stress in aquatic animals (McKenzie *et al*. 1996; Coppola *et al*., 2018). Therefore, ROS generation, and antioxidant status are directly related to temperature (Wilhelm Filho *et al*., 2000). Higher oxygen consumption rate may enhance the risk of LPO and exacerbate lipid radical chain propagation by over production of ROS in mitochondria (Fridovich, 2004). Wilhelm-Filho and Boveris (1993) found antioxidant enzymes activity in liver are directly related to levels of oxygen consumption in fish.

Few studies have shown the effects of long-term temperature acclimatization on fish antioxidant enzyme systems (Yang *et al*., 2018).

The present enzymatic activity increasement could be attributed to the temperature coefficient Arrhenius plot (Q10), which links the changes of a process rate and the temperature fluctuation by 10°C. Temperature can significantly affect most of the physiological activities through enzymatic production rate for every 10 °C change (Tattersall *et al*., 2012).

Moreover, antioxidant enzymes are affected by a variety of ecological factors, such as seasonal variations of temperature (Aras *et al*., 2009, Pavlovi *et al*., 2010), which increased the metabolism rate in fish (Wilhelm Filho *et al*., 2000).

On the other hand, warm water holds lesser oxygen than cold water, lesser oxygen induce hypoxia in fish and expose fish to radical's overproduction and eventually oxidative stress (Dawood *et al*., 2021). Moreover, Tolerance of higher temperature has declined the growth rate, which is an evidence of oxygen uptake alteration in tilapia (Blasco *et al*., 2020). Tilapia displayed

a higher oxygen uptake and gill ventilation when the temperature increased (Maricondi-Massaria *et al*., 1998). Hyperactivity of tilapia acclimated to high temperature provoked an increase in both oxygen consumption and ammonia excretion (Zeng *et al*., 2010).

This may illustrate the enzymatic reduction, along with LPO enhancement in fish acclimated at 40°C. While fish needed more oxygen as exposed to thermal stress and showed hyperactivity, water failed to hold enough oxygen and fish experienced hypoxia, which induce oxygen free radical production via mitochondria. This anticipation verified by higher LPO levels, which suppress the production of antioxidant enzymes.

Although, the present pattern of antioxidant enzymes activity did not exhibit inhibition. This may reflect a high ability of tilapia to cope with ROS over production induced by thermal stress as an adaptive response

Conclusion

Water temperatures affect the antioxidant enzymes levels in both liver and white muscle of blue tilapia juveniles. This increase seems to be related to the increased oxygen consumption as temperature increased. Therefore, we can anticipate that higher ambient temperature will increase the oxygen consumption rate, which consequently enhance the production of ROS. The present pattern of antioxidant enzymes activity at high temperature did not exhibit inhibition. This may reflect the adaptive responses of blue tilapia at high ambient temperatures and stimulate protective mechanisms against ROS.

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