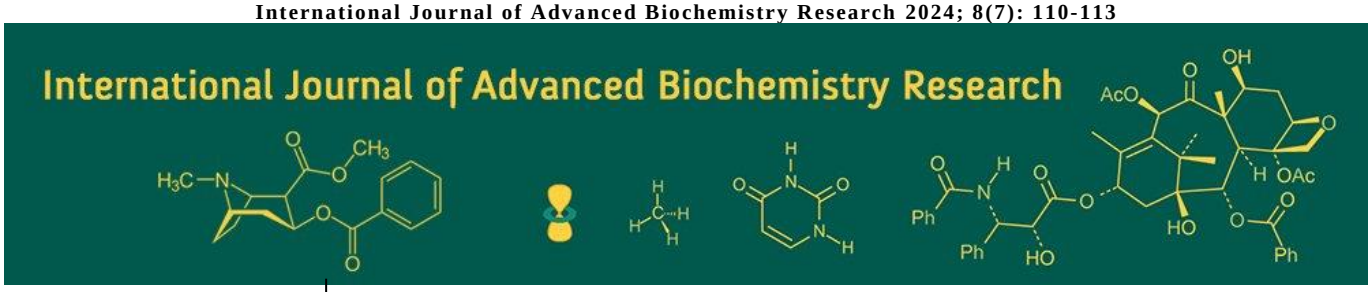

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Evaluating lipid peroxidation in the liver, belly and caudal muscles of *Anabas testudineus* (Bloch) in response to water temperature gradient

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Abstract
In the last few decades, there has been an overall increase in average global temperatures and other severe climatic changes affecting every aspect of life on earth. One such aspect is the effect of temperature variation due to climatic change mostly rising temperature affecting the aquaculture of many edible and commercial fish species. *Anabas testudineus*, a mostly freshwater-dwelling fish, is known for its good nutritional value, being a rich source of proteins, vitamins, minerals, etc. This is a much easily available fish as a source of food for many people across South Asia. The experiment performed studies the effect of different temperatures on the stress levels of *Anabas testudineus*, measured by the levels of lipid peroxidation (LPX) in the liver, belly, and caudal muscle. In doing so, the tolerance of the fish to different temperatures was assessed. From the study it was found that the liver showed an initial rise of 79.65% in LPX levels after 24 hours of exposure at the low temperature (20 °C) with respect to control but soon declined (9.20%) after 48 hours of exposure with respect to control, indicating supposed acclimatization of the fish to the cold temperature. The high temperature (30 °C) caused a steady rise in LPX values in the liver, initially decreasing (44.13%) compared to the control, for 24 hours of exposure, but rising with increasing time i.e., after 48 hours the levels have gone up from the initial value significantly showing about 38.03% rise compared to control in the same condition. In belly muscle, there was an increase in LPX values at both the lower and higher temperatures compared to control for both exposure periods, with a very high percentage increase in LPX at 30 °C after 48 hours (with respect to initial LPX levels for 24 hours exposure period) supporting previous research stating a rise in temperature leads to a prominent increase in LPX values with increasing exposure period. While the lower temperature leads to an initial rise (469.23% compared to control) but lowers after 48 hours of exposure to 91.42% compared to the control, giving a perception of adjustment. But the changes in the level of LPX for caudal muscles show a distinct pattern different from earlier trends for muscle tissues seen on exposure to a different temperature. The patterns of LPX changes for different exposure periods at different temperatures require more future analysis. These insights at the considered temperatures, about the stress condition of *Anabas testudineus* can be used to have better temperature management for aquaculture purpose for commercial purposes, since any stress on the liver or muscle may cause low productivity due to oxidative damage to these organs causing many physiological changes or in extreme situations death of the fish.

Keywords: Lipid peroxidation, liver, belly, caudal muscles, *Anabas testudineus* (Bloch)

Introduction
Anabas testudineus is commonly known as climbing perch or koi in the local language. It is widely distributed in the tropical waters of South Asia (more specifically in Southeast Asia) as well as in China (Jamsari, Muchlisin, Musri, Siti Azizah, & Research, 2010) [2]. A hardy fish as it is tolerant to extreme environmental conditions, unlike other normal fish species that cannot withstand these conditions. They are potamodromous (fishes that complete their entire life-cycle in freshwater) in nature.
Anabas testudineus is well known for its tolerance toward very extreme environmental conditions, like low oxygen availability (hypoxia), the acidity of the water (lowering of pH), high level of salinity, and excess ammonia in the surrounding environment (Paul *et al*., 2017) [5]. This fish is also adaptable to very high contamination in field or laboratory conditions (Li *et al*., 2019) [3].

Thus it proves that the climbing perch can sustain highly contaminated medium or extreme situations (Prakash & Prakash, 2021) [6]. But the mechanisms behind these types of tolerance are still unknown, for organic pollutants. Because of its survival in such adverse conditions, *Anabas testudineus* can be used to analyze the toxic effects of various harmful chemicals and pollutants as well as the adaptation system developed for longer sustenance in such conditions (Zhang *et al*., 2019) [11].

Hence it is a perfect test model organism for laboratory or even field experiments regarding various subjects, mainly the hostile conditions – high or low temperature, pH, salinity, or levels of applied chemicals like enzymes, toxins, etc.

Global warming has resulted in a high frequency of heat waves; such events have led to prominent population changes among fishes in comparison to the average temperature rising regularly. Most times fish try to search for a temperature favorable to them in changing temperatures but it is not possible in all kinds of geographic locations or habitats that the fish may be residing. A warm water situation because of rising temperature can directly cause changes in the biochemical activity, hence affecting the physiology of fish, as these biochemical reactions are under the control of temperature only (Sarthoria *et al*., 2022) [8] These physiological changes help in the adjustment of fish to the high temperature, by intensifying stress hormone release in the common blood vascular system at the basal level and more neurotransmitter release in the neural pathways. As a result of high temperature, the energy expenditure also rises in the form of more metabolic reactions that lead to a rise in development activities, faster growth, and increased motility as the speed of swimming increases (Yanik *et al*., 2018) [10]. Another damaging effect of rising temperature includes a mutation in DNA, denaturation of protein structure, and oxidative stress that finally leads to the death of a cell. High temperature may also lead to more occurrence and graveness of many diseases both parasitic and non-parasitic.

Objective of the Study

Since there has been no earlier research regarding the effect of temperature variations on oxidative damage in *Anabas testudineus*, this experiment was planned to assess the oxidative damage in the form of lipid peroxidation (LPX) level in the liver, belly, and caudal muscle of *Anabas testudineus* on exposure to temperatures – 20 °C, 25 °C and 30 °C, for 24 hrs and 48hrs in case of each temperature.

Methodology

Anabas testudineus belonging to the age group of around 7±2 weeks, and approximate weight and height of about 26±4gm and 15±3cm respectively were collected. The fish were given commercial fish feed as food at around 5% of their body mass, throughout this acclimatization phase.

One control (25 °C) and two treatment groups were (20 °C and 30 °C) were in three tanks each. Each tank contained five fish, thus totaling to 15 fish for each treatment. The control sample temperature of about 25 °C is maintained in BOD Incubator. Samples were collected after sacrificing the fish at 24 hours and 48 hours of temperature maintenance. In similar fashion samples for test temperatures of 20°C and 30 °C at exposure periods of about 24 and 48 hours were extracted. The tissue from liver, muscle and belly were collected and homogenized with phosphate buffer. The supernatant was collected after centrifugation at 10000 rpm at 4 °C for 10 minutes.

Lipid peroxidation level was examined in the liver, belly and caudal muscles of *Anabas testudineus* after different temperature exposure for two varied periods by the method given by Ohkawa *et al*., (1979) [12].

Two-way ANOVA (Anova: Two-Factor Without Replication) was performed to evaluate the significance of the difference in the mean values of the data at 0.05 for various parameters between control and treatment. All statistical analyses and graphs were done using XL-STAT.

Results and Discussion

Three tissues – liver, belly muscle, and caudal muscle, extracted from the fish *Anabas testudineus were* exposed at different temperatures which were 20 °C, 25 °C, and 30 °C. The tissue samples were categorized based on their exposed temperature. Tissue samples exposed to 25 °C were taken as control hence called C. While tissue samples with the treatment of 20 °C and 30 °C were given the symbol T_1 and T_2 respectively.

In Caudal Muscle

Table 1: Percentage change in the mean of LPX level in caudal muscle (nmol/mg protein) for 24 hours and 48 hours with respect to control (25 °C).

Exposure Temperature	24 Hours	48 Hours	Percentage Change At 24 Hours	Percentage Change At 48 Hours
20 °C	0.079±0.000	0.076±0.004	-68.89%	-35.59%
25 °C	0.254±0.003	0.118±0.008	0.0%	0.0%
30 °C	0.739±0.011	0.106±0.000	190.94%	-10.16%

In Belly Muscle

Table 2: Percentage change in the mean of LPX level in belly muscle (nmol/mg protein) for 24 hours and 48 hours with respect to control (25 °C).

Exposure Temperature	24 Hours	48 Hours	Percentage Change At 24 Hours	Percentage Change At 48 Hours
20 °C	0.296±0.001	0.201±0.000	469.23%	91.42%
25 °C	0.052±0.001	0.105±0.015	0.0%	0.0%
30 °C	0.194±0.007	0.330±0.020	273.07%	214.28%

In liver

Table 3: Percentage change in the mean of LPX level in liver (nmol/mg protein) for 24 hours and 48 hours with respect to control (25 °C).

Exposure Temperature	24 Hours	48 Hours	Percentage Change At 24 Hours	Percentage Change At 48 Hours
20 °C	0.521±0.001	0.296±0.002	79.65%	-9.2%
25 °C	0.290±0.002	0.326±0.002	0.0%	0.0%
30 °C	0.162±0.002	0.450±0.009	-44.13%	38.03%

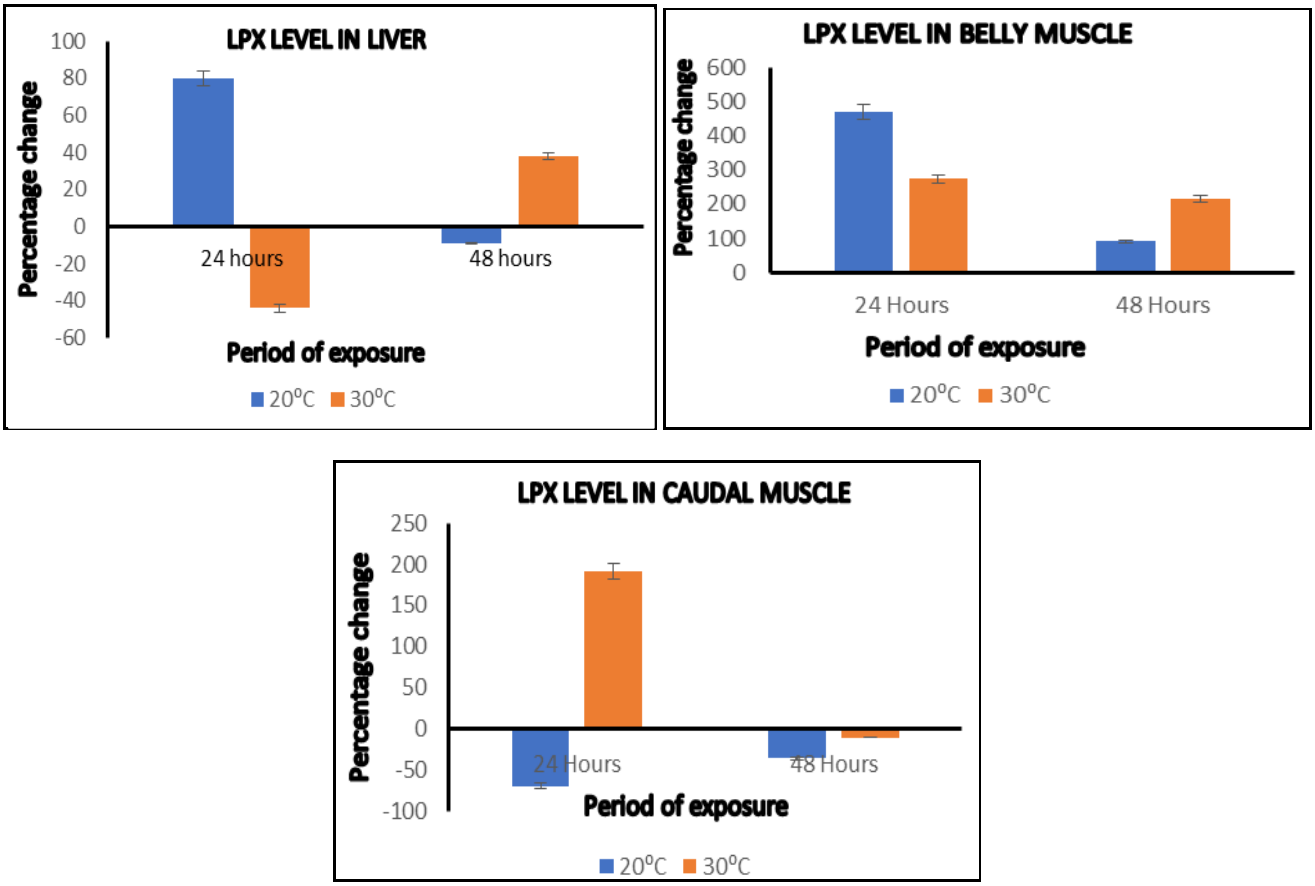

Fig 1: Graphical representation for change in percentage of LPX level (nmol/mg protein) at 20 °C and 30 °C temperatures in comparison to control temperature (25 °C) in liver, belly muscle and caudal muscle tissue.

From the experiment, it was found that there is a varied pattern of lipid peroxidation level according to the type of tissue into consideration as well as the temperature it was exposed to for two different exposure periods in the fish *Anabas testudineus*. The temperature treatments of 20 °C and 30 °C were given in comparison to the control temperature taken of 25 °C, one treatment is lower than the control while the other is higher in comparison to the control, this plays a role in an irregular pattern of changes in LPX level and MDA levels as a result.

LPX level (also declined from initial absolute values at 24 hours exposure) in comparison to liver tissue at control after the same period of treatment (that had also shown a slight rise in LPX level from initial 24 hours value) suggesting the acclimation of the fish to the lower temperature in with respect to control. Chien and Hwang, (2001) [1] found that fish raised in lower water temperature had more PUFA (polyunsaturated fatty acids) in their liver, which gets oxidized as a result of lipid peroxidation (Li *et al*., 2019) [3]. Hence suggesting low lipid peroxidation occurs in fish acclimatized to comparatively lower temperature in comparison to other test temperatures taken, as the scientists duo saw in the thorn-fish (*Teraponjabua)* exposed to 28 °C, 32 °C, and 36 °C had higher PUFA levels at 28 °C and hence suggesting lower LPX level compared to other test temperatures (Nwani *et al*., 2010) [4].

Since the liver tissue in *Anabas testudineus* presents an initial rise in LPX levels at 20 °C after 24 hours but later for 48 hours there is a decrease in LPX levels in comparison to control at the same temperature for both periods, it can be assumed that this fish acclimatizes to 20 °C hence a cooler temperature for the fish. But the opposite happens at 30 °C, the initial low LPX levels at 24 hours of exposure rise with time and increase after 48 hours, with respect to the control for the same hours of treatment suggesting 30 °C as a heat stress-inducing temperature for the liver.

Hence the pattern of LPX level due to temperature variation responses in caudal muscle in the performed experiment is very different from those discovered earlier in muscle tissue, while the belly muscle almost follows the general path of muscle level LPX changes due to temperature variation (Vinagre *et al*., 2012) [9].

Conclusion

The experimental work carried out for this thesis gives an idea of the optimum temperature for adaptation to oxidative stress occurring at high levels in the liver of *Anabas testudineus* and also the temperature causing higher stress

with increasing time. The trend of lipid peroxidation in belly muscle for the same fish is quite similar to other kinds of fish but exposure periods associated with changes require more work to address the mechanism behind these changes. Further, the unique pattern of caudal muscle LPX level needs further exploration.

All these studies concerning thermal-induced oxidative stress in *Anabas testudineus* were to understand the tolerance capacity of this fish which has a crucial role as a source of food across the world and as a medicine in many tribal cultures across India. This research holds importance due to the rise in global temperature as a result of climate change and global warming which are at the forefront of the serious risks faced by all living beings, including food shortage. A reduction in the population of commercial fishes, like *Anabas testudineus*, a good source of proteins and other nutrients can cause a gap in the nutritional index of the populations depending on them for food.

Hence future assessments to further the study on oxidative stress due to temperature variation are required to find the mechanisms behind the adaptation process and the resistance to it in the various organs mentioned at different temperatures, to help in maintaining an optimal environment for proper growth and cultivation of this fish despite a temperature change.

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