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EFFECT OF THERMAL STRESS ON TOTAL PROTEIN CONTENTS OF LAND SNAIL, ACHATINA FULICA

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ABSTRACT:

Climate change due to increasing temperature has brought about severe alterations in ecosystem and biodiversity all over the world. Thermal pollution affects the aquatic as well as terrestrial flora and fauna, but physiological and biochemical changes in terrestrial molluscs with reference to thermal stress are poorly known. The present study is therefore undertaken to find out the effect of thermal stress on total protein content of the gastropod, Achatina fulica. After acclimation to laboratory conditions for seven days, upper and lower temperature tolerance limits were determined by the dynamic method. Snails were exposed to temperatures of 10°C, 15°C, 35°C and 40°C in the laboratory for 24 hours as well as 48 hours. After exposure to these temperatures, quantitative estimation of total proteins in hepatopancreas was conducted by the Bradford method and compared with that of control protein content. The average protein content of control molluscs was found to be 67.9 mg/gm of hepatopancreas tissue. After thermal exposure for 24 hours at 10°C and 15°C, the protein content decreased whereas at 35°C and at 40°C it increased. After exposure for 48 hours to 10°C, 15°C, and 35°C, protein content consistently increased, whereas exposure to 40°C was lethal for the snail.

KEYWORDS: *Achatina fulica, thermal stress, proteins, temperature*

INTRODUCTION

Increasing temperature of our planet has become an issue of immense importance. Climate change is one of the major problems of the environment all over the world in recent years. Various causes and impacts of the climate change have been observed. Increasing atmospheric carbon dioxide is the main cause of changes in global temperatures, climate, and even surface seawater composition. (Hofmann G.E., 2010) The effects of climate warming have been attributed to increased concentration of greenhouse gases. (Sauter R., 2013) Oscillations in the earth's climate leads to associated fluctuations in the temperature regimes of many marine and terrestrial ecosystems. (Pörtner, 2001) Climate change is the greatest threat to biodiversity due to changes in species through shifting habitat, changing the life cycles and the adaptive evolution of new physical and sexually selected traits. In 1950, in one of the studies of 1700 plants and animal species it was found that due to increased global temperature the species have shifted average 3.8 miles towards poles. Moreover species move three times faster. Even marine species moved towards the colder water. (Schwartz, 2014) Gustafsson (2017) studied eco-evolutionary dynamics of ornamental expressions of secondary sexual traits in Collared Flycatchers and found

that the forehead patchsize has declined and has influenced male reproductive success. These traits might evolve rapidly due to environmental changes. After cold breeding season more ornamented males were favoured but were not favoured after warm breeding seasons. Since late 1970s, despite annual variability, clear trend of elevated global temperature can be observed.

In the present study, thermal pollution as thermal stress is studied in *Achatina fulica*. *A. fulica* belongs to phylum Mollusca and class Gastropoda. The Achatinidae family is native to East Africa. *A. fulica* was attributed the pest status due to its nature to damage crop plants. This snail was introduced in India from its native place in Kenya (East Africa) through Mauritius as early as 1847 when a few specimens were brought to Botanical Garden, Calcutta by the conchologist W. H. Benson. Later, the snails multiplied and spread. Now the snails are noted as a menace to agro-horticultural economy in several states of India and neighbouring countries. (Vinci G. K., 1988; Avhad S.B., 2013). Snail causes the loss of agricultural productivity due to herbivory on crop plants, either through damage to crop itself or to other plants that provide shade or soil enrichment of key elements such as nitrogen. Damage may also take the form of transmission of plant pigments (Raut, 2002).

There has been very little work on terrestrial molluscs in this field. The present study is undertaken to find out how thermal stress affects the protein content of *Achatina fulica*. Proteins play a vital role in the physiology of *A. fulica*. Proteins are long chains of amino acids forming three dimensional structures. Proteins play both structural and functional roles at the cellular level. Being an integral part of the cell membrane, intracellular and extracellular passages are linked through proteins. (Anilkumar, 2012) Any cellular metabolism occurring in the body involves one or many different proteins. Proteins are among the most abundant biological macromolecules and are extremely versatile in their function and interaction during its metabolism. (Harper, 1978)

MATERIALS AND METHODS:

Achatina fulica were collected from the gardens of Chhatrapati Shivaji Maharaj Vastu Sangrahalaya, Fort Mumbai and were carried to the M. D. college Zoology laboratory in cloth bag consisting of moist garden soil. In the laboratory, the animals were kept in plastic boxes of dimensions of 37cm x 25cm x 17cm. Three fourth part of the plastic container was filled with four kg garden soil and covered by a mesh. About ten snails were kept at room temperature in each container for acclimation. Snails with shell length between 6-8 cm and weight ranging between 20-40gms were selected for experiments and were acclimated to laboratory conditions for one week. During acclimation, the snails were provided with 100gms of cabbage leaves and about 250ml water per box was sprinkled on soil on alternate days. Temperature tolerance was carried out by the dynamic method (Maya, 2006) in B.O.D. incubator using the same plastic containers which were used for acclimation of the animals. Six snails were exposed to temperature of 30°C in incubator. Upper lethal temperature was obtained by gradually raising the temperature of incubator at a rate of approximately 0.3°C/min. Temperature was raised by 5°C per day. To check mortality, the mobility of the foot of every specimen was recorded after poking with a blunt spatula. Temperature at which 100% mortality was obtained, was considered as upper lethal temperature. An equivalent method was used to obtain lower lethal temperature where temperature was reduced by 0.3°C/min. from30°C and 5°C per day.

After obtaining the temperature tolerance range, sub-lethal temperatures were selected for the experiments. Ten sets of snails were prepared. Each set, containing six snails, was exposed to temperatures of 10°C, 15°C, 35°C and 40°C for 24 hours as well as 48 hours along with the sets of snails at control temperatures (room temperature 27°C ±3°C). Each experiment was repeated thrice. At every temperature, animals were sacrificed. Hepatopancreas was removed, homogenized and used for further analysis. Protein concentration of hepatopancreas homogenate exposed to different temperatures was measured with the Bradford assay method. (Bradford, 1976)

For the said study of snails exposed to four temperatures of 10°C, 15°C, 35°C and 40°C, the protein content was studied and analysed for two temperature periods of 24 and 48 hours respectively. The average protein level and corresponding standard deviation (SD) has been evaluated. The Shaipiro Wilks test was carried out to test the Normality of the data. The analysis of variance was further

executed to study the significance of effect of different temperatures on the protein level of the snails by using R software version 3.6.1.

OBSERVATIONS AND RESULTS:

The total protein content in the hepatopancreas of *Achatina fulica* at the control temperature (27°C ±3°C) was found to be 66.94 ± 6.62 mg/g and 66.90 ± 8.34 mg/g for 24 hours and 48 hours respectively. It was observed that after exposure to 10°C and 15°C for 24 hours, the protein content decreased to 56.61 ± 11.13 mg/g and 59.94 ± 24.78 mg/g respectively. As the duration of exposure for lower temperatures i.e. 10°C and 15°C increased to 48 hours, protein contents increased to 75.22 ± 17.57 mg/g and 87.00 ± 25.19 mg/g respectively.

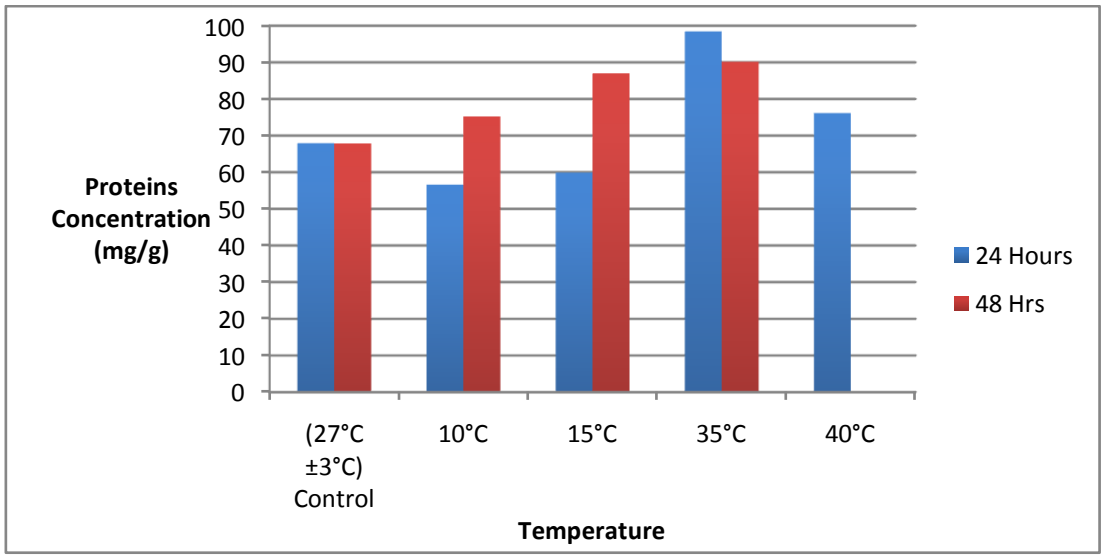
At higher temperature of 35°C, the protein contents increased to 98.44 ± 15.95 mg/g at 24 hours and 90.16 ± 24.55 mg/g at 48hours of exposure. At 40°C protein contents increased to 76.16 ± 28.36 mg/g at 24 hours of exposure, whereas after exposure to 40°C for 48 hours all the animals died.

After the data for protein content was obtained in above experiments, the statistical tests were carried out. The data was tested for normality using the Shaipiro Wilks test and p value obtained was 0.003. After executing the ANOVA test the p value was zero.

Table1: Changes in the protein contents in the hepatopancreas of *A. Fulica*, expressed as mg of proteins/gm of tissue, exposed to different temperatures, in degree celsius, for 24 and 48 hours. n = Number of snails. X ± Sd= Mean ± Standard Deviation.

Temperature (°C)	n	Proteins Content (mg of proteins/gm of tissue)	
		24 Hours X ± SD	48 Hrs X ± SD
Control (27°C ±3°C)	18	67.94±6.62	67.90±8.34
10°C	18	56.61± 11.13	75.22± 17.57
15°C	18	59.94± 24.78	87.00± 25.19
35°C	18	98.44± 15.95	90.16± 24.55
40°C	18	76.16± 28.36	Lethal temperature

Graph1: the variation in the total concentration of proteins In hepatopancreas of *Achatina Fulica* after exposure to different temperatures for 24hrs & 48hrs



DISCUSSION:

The hepatopancreas acts as a depot to store extra proteins. Exposure to lower temperatures develops stress in the animal. To counteract this stress, energy is required which might be derived from degradation of the stored proteins. Hence decrease in the total protein content was observed after exposure of *A. fulica* to lower temperatures of 10°C and 15°C for 24hrs. The same trend was observed in some studies on molluscs and other organisms after stress period. Patil (2011) studied protein changes in the fresh water bivalve *Parreysia cylindrica* after exposure to toxic indoxacarb and inferred that digestive gland is the main site of metabolism of pollutants in the body of bivalves. The digestive gland seems to be the main site of degradation and detoxification of pesticides and hence has the largest demand of energy for the metabolic processes resulting in increased utilization of protein to meet the energy demand. The higher degradation of protein in the digestive gland provided better indication of the extent of toxicity. A marked fall in the protein level in all the tissues indicates a rapid initiation of breakdown of protein. To meet energy demand during toxic stress increased utilization of protein might have taken place. Decrease in protein content was possibly due to stress conditions caused by toxicity of indoxacarb on protein metabolism or due to enhanced proteolytic activity as a consequence of increased metabolic demands.

In *A. fulica*, we observed that after increasing the duration of exposure from 24 hours to 48 hours for lower temperatures of 10°C and 15°C, the protein content showed increasing values. This might be due to increase in the stress due to exposure to longer duration at lower temperature. Exposure to lower temperatures might have resulted in the production of antifreeze proteins for tolerance and adaptation to lower temperatures to avoid freezing. Freeze-tolerant invertebrates at low temperatures produce antifreeze proteins (AFPs) or antifreeze glycolipids (AFGLs) for survival. AFPs and AFGLs encourage the growth of many small crystals and cease the expansion of large crystals in a process of ice recrystallization inhibition. (Walters K. R., 2011). Even with the help of AFPs and AFGLs, ice formation able to alter proteins, membranes and other structures. Freeze tolerant invertebrates thus accumulate polyhydric alcohols and sugars, such as glycerol, sorbitol. At Intracellular level, these cryoprotectants stabilise proteins and membranes, and prevent freezing, while extracellularly their function is to limit the osmotic imbalance that occurs during freezing, by maintaining water content above the critical minimum cell volume. (Everatta M. J., 2014)

At higher temperatures of 35°C and 40°C for 24 as well as 48 hours of exposure, the protein contents increased by almost 30%. This increase can be attributed to the rise of protective proteins at higher temperatures, called as molecular chaperons or Heat shock proteins (HSPs) (Whitley D., 1999; Santer, 2010) The overexpression of HSPs indicates universal molecular mechanism to manage the stress. The cDNA of HSPs has cloned from some molluscs and found that formation of HSPs can be induced by heat stress, hypoxia, heavy metal contamination, etc. (Dongwu Liu, 2013) Venketesh studied the effect of higher temperature on the crab *Scylla serrata* and stated that a decrease in protein content was observed, this may be due to the fact that during heat stress many proteins are denatured as a consequence of which these proteins are degraded leading to a fall in total protein content. (Venketesh, 1999)

It is observed that there is an average increase in the protein level of snail with an increase in the temperature exposure period i.e. from 24 to 48 hours for all temperature levels 10°C, 15°C and 40°C except for 35 °C. At 35 °C the average protein level shows a decreasing trend from 98.4 mg/g to 90.17mg/g. The p value happens to be significant for both the Normality test ($p = 0.003$) as well as for ANOVA ($p = 0$). Thereby revealing the acceptance of the alternative hypothesis. We accept and conclude that there is a significant effect of temperature on the protein content of snail.

Organisms have evolved a greatly conserved cellular response called the heat-shock or stress proteins response (SPR) that proliferates their tolerance to extreme environmental conditions. The tissues of two molluscs, *Mytilus edulis* and *Collisell apelta*, respond to stressful situations by eliciting this response. It was found that both heat-shock and Cadmium can induce the SPR in both species. (M.Sanders, 1988) Thermal stress might affect number and distribution of *A. fulica* in the future. According to the present literature, various terrestrial animals such as amphibians, birds, butterflies,

insects, lizards, mammals and worms are studied for climate driven extinctions and projected impacts on biodiversity. (S. Fred Singer, 2011)

CONCLUSION/ INTERPRETATION:

In conclusion, the results obtained in the present study reveal that total protein levels of *A. fulica* showed direct relationship with temperature. After exposure to thermal stress, *Achatina fulica* exhibits two different trends, either decrease in protein concentration or increase in protein concentration as compared to that of controlled temperatures.

1. In particular at lower temperatures of 10°C and 15°C initially at 24 hours there is decrease in the protein contents but as exposure duration is increased to 48 hours protein contents increase.
2. Whereas at higher temperatures 35°C and 40°C exposure, it shows increase in the protein content uniformly.
3. Longer exposure of 48 hours at 40°C temperature is found to be lethal.
4. When animals were exposed to the temperatures of 10°C, 15°C, 35°C and 40°C, it was noticed that the exposure produced significant effect on protein contents, which means that fluctuation in control temperature sultimately affects the physiology of *A. fulica*.

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