*International Research Journal of Environment Sciences*_____ ISSN 2319–1414
Vol. **3(6),** 11-16, June **(2014)** *Int. Res. J. Environment Sci.*

Activities of selected Enzymes in various Tissues of *Pila Globosa* under Heat Shock

Dharanikota Malleswar, NairAbhinav and Rama Basavaraju
Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Prasanthi Nilayam Campus, Puttaparthi, Anantapur Dist., AP, INDIA

Available online at: www.isca.in, www.isca.me
Received 23rd April 2014, revised 31st May 2014, accepted 14th June 2014

Abstract

The physiology of Pila globosa (Indian apple snail) under heat shock was analyzed by measuring activities of two anti-oxidative enzymes and one oxidative enzyme in selected tissues. The temperature set points for the experiments were based on the climatic variations the snail gets exposed during an annual period in the specific geographical region. From the tissues of hepatopancreas, gills and foot, the enzyme activities were assessed to understand the compensatory mechanisms the snail utilizes by physiological adjustments. The results indicated that catalase (CAT), Glutathione-S-transferase (GST) and Lactate dehydrogenase (LDH) had tissue specific variations and play role in homeostasis. The study assumes significance with rising temperatures on earth due to global warming and also supports in understanding adaptive capabilities of these amphibious snails.

Keywords: *Pila globosa*, heat shock, Hepatopancreas, catalase (CAT), Glutathione-S-transferase (GST) and Lactate dehydrogenase (LDH).

Introduction

Animal adaptations for life in stressful environments typically include adjustments at multiple fronts like behavioral, biochemical, physiological etc. The phylum Mollusca is one of the largest invertebrate phyla within the animal kingdom which has evolved successfully and presenting a widespread distribution being able to survive in both aquatic and terrestrial environments. *Pila globosa* (Indian apple snails)are endemic to South India and are found in semi-arid regions of Andhra Pradesh state. The snail gets exposed to temperatures above 40^0 C during summer months and in some conditions enter into aestivation (summer sleep) when water bodies dry up. Being cold blooded animals they get exposed to range of temperatures during a day–night cycle. Fluctuations of temperatures in the habitat possibly create stress for the organisms leading to alterations in metabolism and further increases free radical formation[1]. Free radicals are reactive radicals and can trigger a chain reaction targeting cellular components such as nucleic acids, proteins and cell membrane[2]. To avoid free radical damage the body has a protection system of antioxidants which includes antioxidant enzymes.

Previous studies on snails showing the effect of environmental stress were, the physiology of *Pila globosa* under aestivation was analyzed by understanding the oxidation of glucose-U-^{14} c and glycogen synthesis in various tissues[3]. Catalase activity was reported in heart and foot of *Pila globosa*kept for 14 months in aestivation[4]. The effect of anoxia and aerobic recovery on the antioxidant defenses of the marine periwinkle, *Littorina littorea* L., was assessed in hepatopancreas and foot[5]. The enzymes glutathione reductase (GR), glutathione peroxidase (GPox), superoxide dismutase (SOD), catalase (CAT) were investigated. These analyses showed that the antioxidant defenses were important in maintaining cellular homeostasis among molluscs. The current research effort utilized heat stress as a model stress condition to derive the biochemical signatures to detect alterations in enzymatic components. The activities of two anti-oxidative enzymes catalase (CAT), Glutathione-S- transferase (GST) and one oxidative enzyme Lactate dehydrogenase (LDH) in selected tissues of hepatopancreas, gills and foot were examined.

Material and Methods

Specimen collection: The snail specimens of *Pila globosa* were collected from lakes, ponds, and rivers of Ananthapur district, Andhra Pradesh as shown in figure-1. These snails were maintained in specially made cement tanks with regular changes of water and provided with leafy vegetables.

Heat Shock Experimental Setup: A total of 28 specimen snails having a body weight of 50 +5 g. were selected. For heat stress experiment the snails were shifted to water baths as shown in figure-2 maintained at 30^0C, 36^0C and 42^0C for three days. The snails were continuously observed for three days at 36^0C and 30^0C and only one day for 42^0C as the samples did not survive for more than 24 hours. After every 12 hours four specimens were sacrificed at each temperature point. The hepatopancreas, gill and foot mass tissues were isolated and utilized for enzymatic studies.

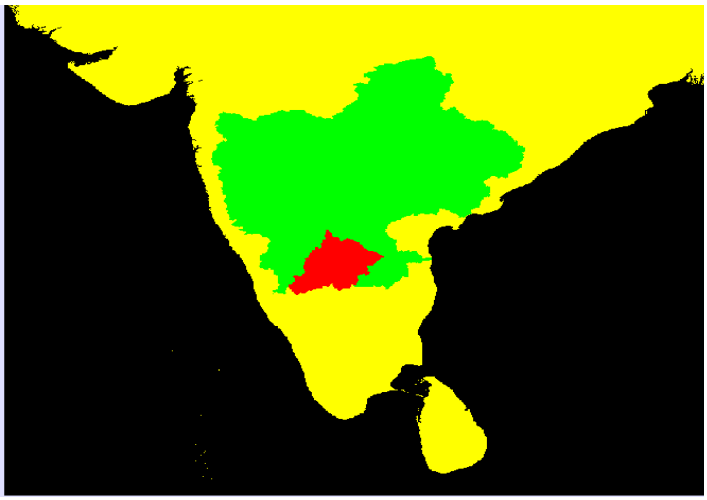

Figure-1
Sampling site (area in red) of Pila globosa collection. The area shown in green is the approximate known distribution of genera Pila

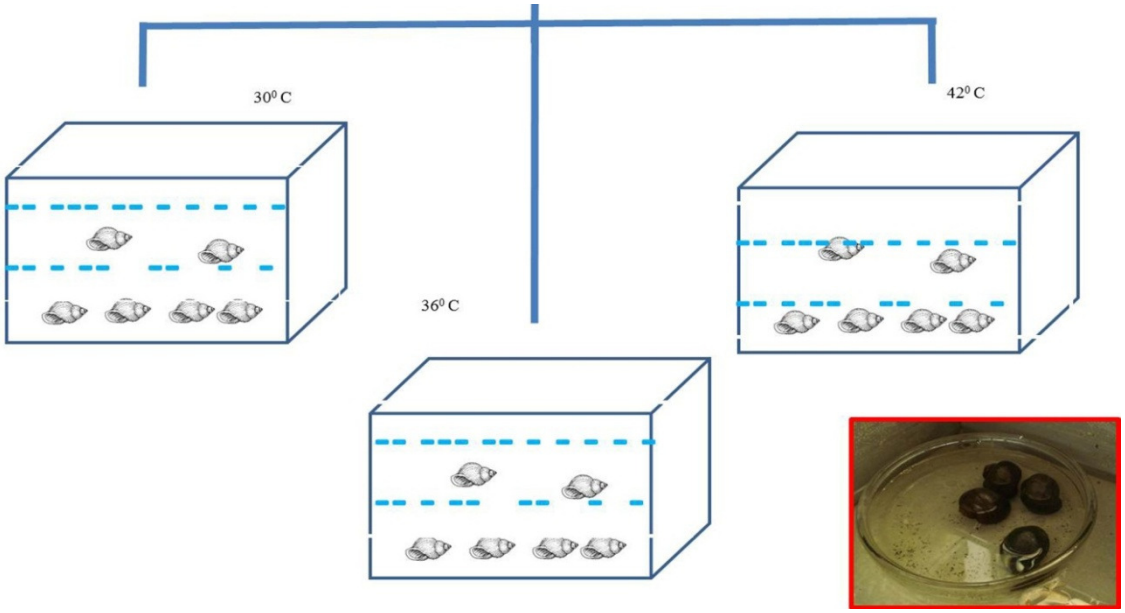

Figure-2
Schematic diagram showing the heat shock temperature set points used this for this study.Water baths as shown are maintained at 30 ^{0} C, 36 ^{0} C and 42^0. After every 12 hours four specimens were sacrificed at each temperature point. The hepatopancreas, gill and foot mass tissues were isolated and utilized for enzymatic studies

Tissues selected for the study: i. Hepatopancreas: The hepatopancreas occupies the coiled visceral mass of the shell. It is large, soft, triangular, brownish to dirty green in color. It`s function is similar to liver of mammals and is also involved in intracellular and extracellular digestive processes. ii. Gills: The gills or ctenidium is the organ of aquatic respiration situated on the extreme right side of the mantle cavity closely situated next to dorso- lateral wall of the brachial chamber. iii. Foot: The large, strong, muscular and ventral part of the body forms the foot. It is roughly triangular in shape in a fully expanded animal. The foot bears operculum on its dorsal side and it fits completely into the mouth of the shell.

Enzymes assays: Catalase (CAT): The activity of anti- oxidative enzyme catalase was measured by monitoring the disappearance of hydrogen peroxide spectrophotometrically at 240 nm[6]. CAT activity was expressed as decomposition of one micromole of H_2O_2 per minute at 25°C and pH 7.0 (Units/mg. wet tissue). The specific activity of the CAT was obtained by calculating protein concentration to express as Units per mg protein.

Glutathione-S-Transferase (GST): The activity of anti-oxidative enzyme glutathione-S-transferase activity was measured using 1-chloro-2, 4 dinitrobenzene (CDNB) as substrate[7]. One unit of GST activity is defined as the amount of enzyme producing one µmol of GSH-CDNB conjugate/min. The specific activity of GST was obtained by calculating with protein concentration to express as Units per mg protein.

Lactate Dehydrogenase (LDH): The activity of oxidative enzyme lactate dehydrogenase was determined by reaction velocity of decrease in absorbance at 340 nm resulting from the oxidation of NADH[8]. One unit causes the oxidation of one micromole of NADH per minute at 25°C and pH 7.3. The specific activity of the LDH was obtained by calculating protein concentration to express as Units per mg protein

Statistics: Results were analyzed by student t-test and a p value < 0.05 was considered statistically significant

Results and Discussion

Results: At 42^{0}C heat shock: The anti-oxidant enzymes catalase and GST showed steep rise in the hepatopancreas at 42 ^{0}C after 24 hours (figure-2). The rate of activities almost doubled in both cases. The foot and gills tissues had minimal changes with respect to catalase activity. GST activity was more in the foot after 24 hours of exposure at 42 ^{0}C. The oxidative enzyme LDH (figure-2) displayed a different trend with gills having highest activity (> 800 u.mol/mg/protein) after 24 hours of heat shock. The hepatopancreas exhibited lower activity than foot after 12 hours.

At 36^{0}C heat shock: The activity of the enzymes at 36 ^{0}C and 30^0 C had similar pattern for catalase and GST activities. For heat shock at 36^0 C the catalase levels (figures-3-4) displayed significant change ($p<0.05$) in hepatopancreas. Foot and gills followed identical activity levels till 60 hours after which the higher activity was observed in foot. The GST enzyme activity of hepatopancreas was two-fold higher than in gills and foot (figure-4). The foot presented enhanced activity of 4000 u.mol/mg/protein compared to gills at the end of 72 hours of heat shock. The Lactate Dehydrogenase (LDH activity) (figure-4) was highest in gills with significant changes ($p<0.05$) compared to hepatopancreas and foot (<500 u.mol/mg/ protein).

At 30^{0}C heat shock: Heat shock response at 30^0 C showed no significant changes compared to 42^0 C and 36^0 C. The hepatopancreas indicated slight variation of catalase (figure-5) and GST (figure- 5). In gills and foot changes were insignificant. The oxidative enzyme LDH in gills ($p<0.001$) revealed maximum changes upto 60 hrs of heat shock. Foot had higher LDH activity than hepatopancreas (figure-5).

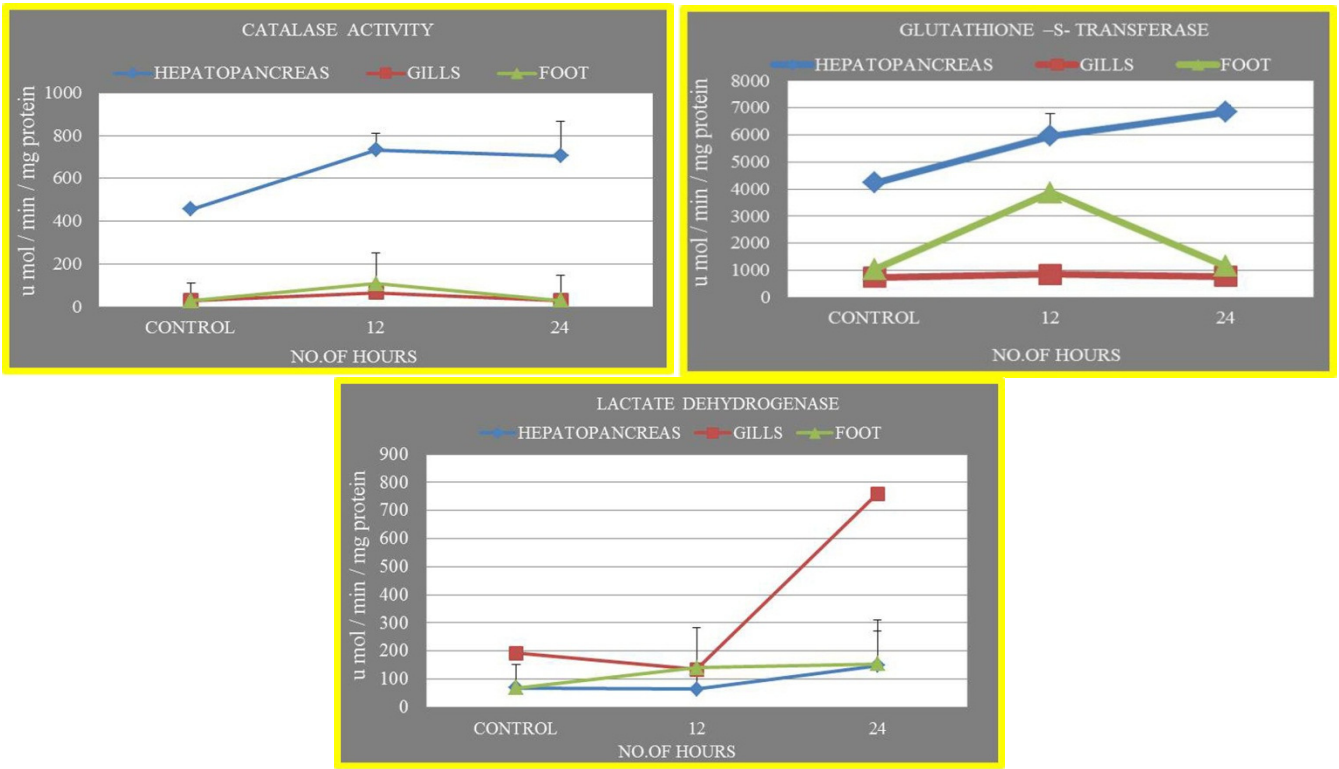

Figure-3

Enzyme activity levels in hepatopancreas, gills, foot of *Pila globosa* measured as µ.mol//min/mg.protein (Y-axis) vs. No. of hours of heat shock at 42^{0}C (X-axis). A total of 12 snails were utilized, four snails for each time point. A total of 12 snails were utilized, four snails for each time point.

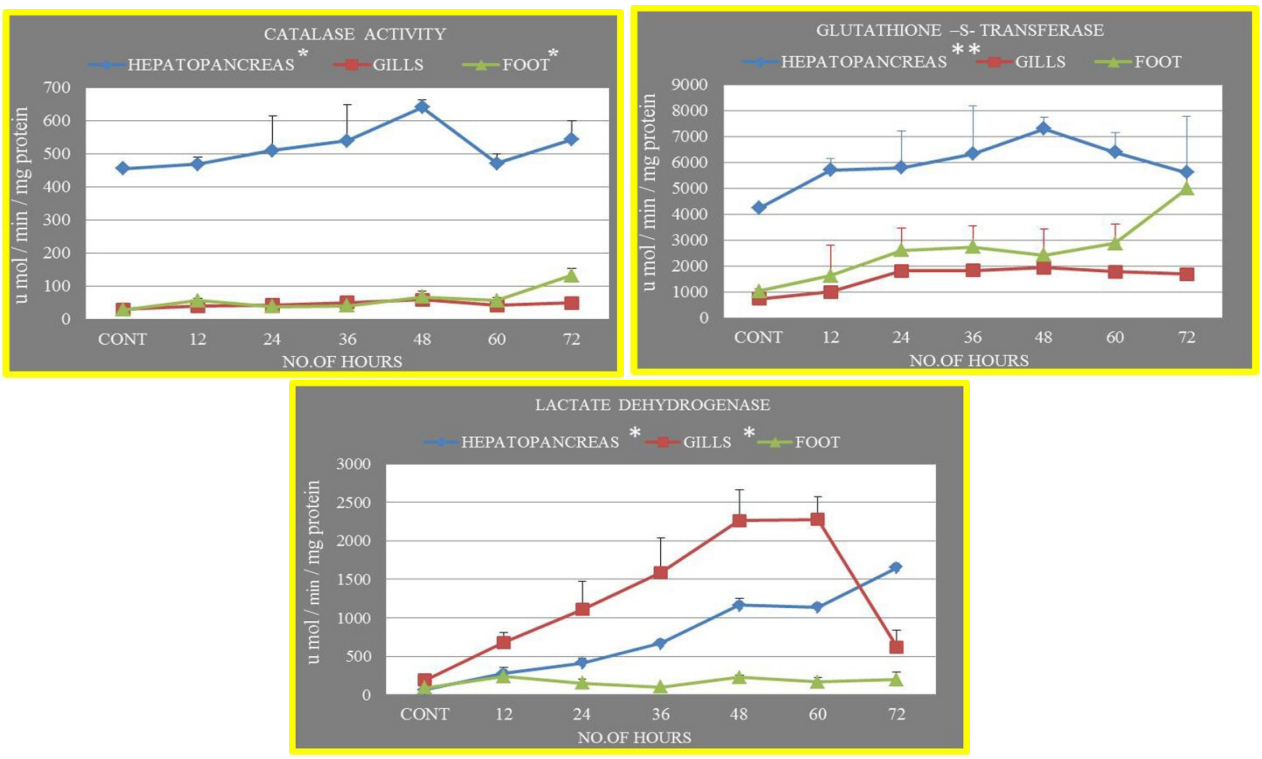

Figure-4

Enzyme activity levels in hepatopancreas, gills, foot of *Pila globosa* measured as µ.mol//min/mg.protein (Y-axis) vs. No.of hours of heat shock at 36^0C (X-axis). A total of 28 snails were utilized, four snails for each time point. $^{*}p < 0.05$, $^{**}p < 0.001$

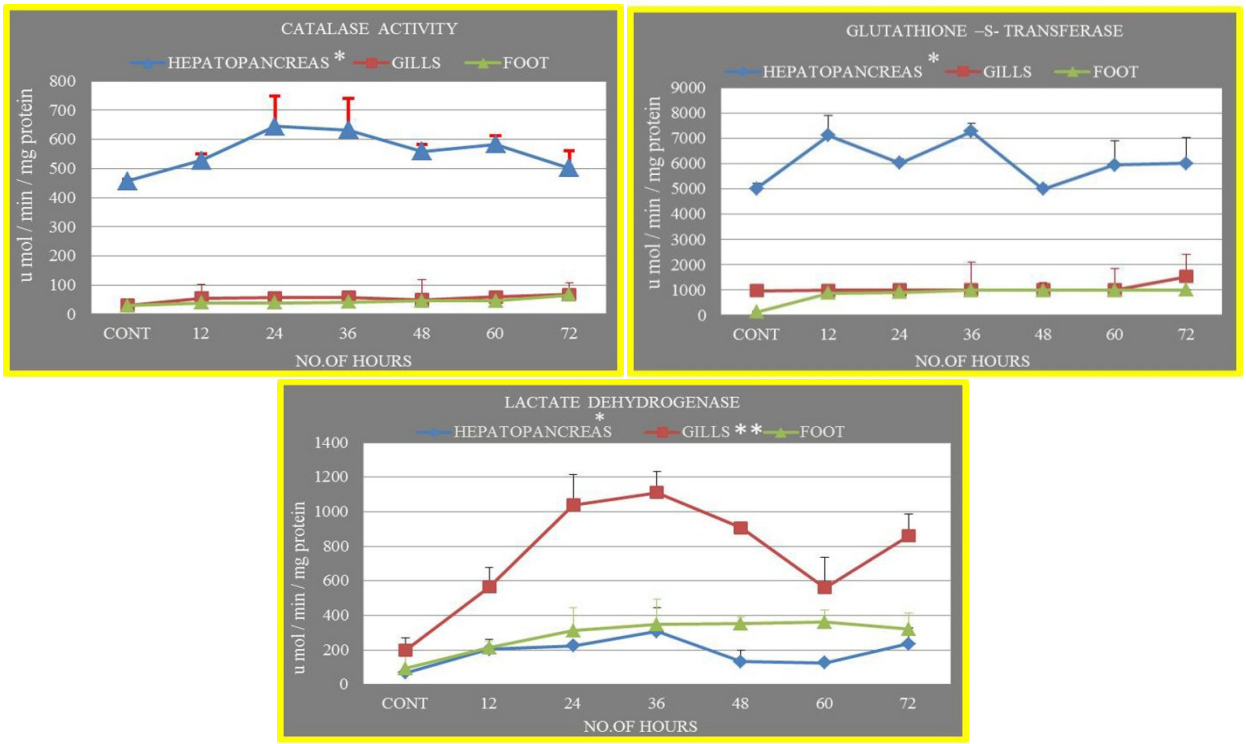

Figure-5

Enzyme activity levels in hepatopancreas, gills, foot of *Pila globosa* measured as µ.mol//min/mg.protein (Y-axis) vs. No.of hours of heat shock at 30^0C (X-axis). A total of 28 snails were utilized, four snails for each time point. $^{*}p < 0.05$, $^{**}p < 0.001$

Discussion: Global warming involves not only a rise of air temperature, but also more frequent heat waves in many regions on earth, and is predicted to intensify physiological stress especially in extremely changeable habitats[9]. *Pila globosa* in its habitat gets exposed to varied climatic changes especially with respect to summer temperatures ranging from 25^0 C to 45^0 C. To understand one important compensatory mechanism, the anti-oxidant enzyme response, the snails were subjected to three temperatures 30^0 C, 36^0 C, 42^0C. The considerably high activity of catalase (CAT) and Glutathione-S-transferase (GST)[10] in hepatopancreas highlighted tissue specific phenomena of the anti-oxidative enzymes[10-11]. The difference in induction of these enzyme activities responding to temperature changes indicated the differences concerning the inducibility of stress, as similar variations were observed for heat shock response in Antarctic intertidal limpet *Nacellaconcinna*[12]. *Pila globosa* which undergoes seasonal aestivation regularly might have developed this strategy of higher activity levels of catalase and GST to scavenge free radicals resulting from thermal stress. Likewise, *N.magellanica* experienced extreme fluctuations of temperature in its high shore habitat and thus expressed high levels of CAT and GST[13]. The increase in temperature stimulates all metabolic processes which also leads to enhanced oxygen consumption and therefore might increase free radicals as a consequence of intensified metabolism. Enhanced environmental temperature induces oxidative stress and was reported in marine snail, *Pernaviridis*[14], and fish *Carassius auratus*[15]. The activity of both CAT and GST were found to be elevated in hepatopancreas compared to gills and foot due to the fact that hepatopancreas is a metabolically very active organ because of its multiple roles in digestion, absorption, excretion etc.

Lactate dehydrogenase (LDH) being an oxidative enzyme presented a different trend in all these conditions. Gills had significantly higher activity at all the temperatures since it is in direct contact with the conditions and also mutilation to membranes was also observed[16]. Evidence was found that heat stress disrupting normal coupling of electron transport chain to oxidative phosphorylation and also drop of ATP levels[17]. Further, reduced oxygen levels due to high temperatures a possible scenario of anaerobic glycolysis leading to conversion of NADH and pyruvate to NAD+ and lactate by LDH was necessitated otherwise glycolysis cycle will end. A similar study in the land snail *Helix pomatia* regarding glycolysis cycle for anaerobic production of energy was reported[18].

Conclusion

In conclusion, due to rising temperatures and temperature fluctuations worldwide, fresh water organisms in semi-arid regions will make metabolic adjustments. In the short term before significant genetic changes occur, organisms such as *Pila globosa* whose physiological adaptation mechanisms are likely limited to a particular thermal tolerance window, will survive only within a particular geographical area. These results indicated the role of CAT, GST as protective enzymes during heat stress in the tissues of hepatopancreas, gills and foot of *Pila globosa*. On the other hand the significance of LDH activity was also understood during this analysis

Acknowledgements

Financial support from UGC SAP, DST FIST Govt. of India is gratefully acknowledged

References

1. Gordon M.R., McLeay D.J., Effect of photoperiod on seasonal variations in glycogen reserves of juvenile rainbow trout *Salmo gairdneri*, *Comp. Biochem. Physiol. A: Physiol.,* **60**, 349-351 **(1978)**

2. Halliwell B., Free radicals and antioxidants: updating a personal view, *Nutr. Rew.,***70(5)**, 257-65**(2012)**

3. Krupanidhi S., Raghavaiah K., B.P., N.; Ramamurthi R., Oxidation of glucose-U-14 c and synthesis of glycogen in different tissues of the garden snail, *Cryptozona ligulata* with reference to aestivation and starvation, *Proc Indian natn SciAcad.,* **87B**, 27-31 **(1978)**

4. Devi S.A., Effects of Aestivation and Rehabilitation on Tissue Catalase Activity of *Pila globosa*, *Proc. Ind. Natn. Sci.Acad.,* **B49,** 610-612 **(1983)**

5. Pannunzio, Antioxidant defenses and lipid peroxidation during anoxia stress and aerobic recovery in the marine gastropod *Littorina littorea*, *J. Exp. Mar. Biol. Ecol.,* **221**, 277–292**(1998)**

6. Beers R.F. and Sizer I.W., A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, *J. Biol. Chem*.**, 195(1)**, 133-40 **(1952)**

7. Pabst M.J., Habig W.H. and Jakoby W.B., Glutathione S-transferase A. A novel kinetic mechanism in which the major reaction pathway depends on substrate concentration, *J. Biol. Chem*., **249(22)**, 7131-7139 **(1974)**

8. Bishop M.J., Everse J. and Kaplan N.O., Identification of lactate dehydrogenase isoenzymes by rapid kinetics, *Proc. Natl. Acad. Sci. U S A.,* **69(7)**, 1761-5 **(1972)**

9. Bhandari G., Study on Climate Change Impacts and Adaptation Measures in Palpa District of Nepal, *Int. Res. J. Environmen Sci.,* **2(3)**, 15-23 **(2013)**

10. Warade N.V., Levels of Glutathione S - Transferase In Different Larval Tissues of *Papilio demoleus, Res. J. Recent Sci.,***1(ISC-2011)**, 313-316 **(2012)**

11. Malanga G., Estevez M.S., Calvo J. and Puntarulo S., Oxidative stress in limpets exposed to different environmental conditions in the Beagle Channel. *Aquat. Toxicol.,***69(4)**, 299-309 **(2004)**

12. Abele D., Burlando B., Viarengo A., Portne H.O., Exposure to elevated temperatures and hydrogen peroxide

elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna*, *Comp. Biochem. Physiol. B.,* **120**, 425–435 **(1998)**

13. Malanga G., Estevez M.S., Calvo J., Abele D. and Puntarulo S., Oxidative stress in gills of limpets from the Beagle Channel: comparison with limpets from the Antarctic, *Sci.Mar.,* **69(S2)**, 297-304 **(2005)**

14. Verlecar X.N., Jena K.B. and Chainy G.B., Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature, *Chem. Biol. Interact.,* **167 (3)**, 219-26 **(2007)**

15. Lushchak V.I. and Bagnyukova T.V., Effects of different environmental oxygen levels on free radical processes in fish, *Comp. Biochem. Physiol. B.,* **144(3)**, 283-9 **(2006)**

16. Malleswar D., *Pila globosa* (Indian Apple Snail) a Sentinel Organism to Monitor Climatic Stress in the Ecosystem: Mechanisms of Adaptation. Ph.D thesis., Sri Sathya Sai Institute of Higher learning, Prasanthi Nilayam, **(2013)**

17. Findley R.C., Gillies R.J. and Shulman R.G., In vivo phosphorous 31 NMR reveals lowered ATP during heat shock of tetrahymena, *Science***, 245(385)**, 39 **(1983)**

18. Wieser W., The initial stage of anaerobic metabolism in the snail, *Helix pomatia* L., *FEBS Lett.,***95(2)**, 375-8 (**1978)**