

## Behavioral and Physiological Changes in *Pila globosa* (Indian Apple Snail) During Aestivation



### Zoology

**KEYWORDS :** Aestivation, *Pila globosa*, Environmental stress

**VNS Malleswar. D**

Research Scholar, Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Prasanthi Nilayam Campus, Puttaparthi, Anantapur Dist., A.P., India.

**R. Basavaraju**

Professor & Head, Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Prasanthi Nilayam Campus, Puttaparthi, Anantapur Dist., A.P., India.

**S.Krupanidhi**

Professor, School of Biotechnology, Vignan University, Guntur, A.P., India

### ABSTRACT

Fresh water snails are widely distributed in terrestrial habitats, typically in moist environments that facilitate animals for gaseous exchange and reduce desiccation. Such habitats do undergo frequent seasonal changes that impact animal life. To survive dry, hot seasons and scarcity of food conditions, snails innately enter a dormant state by closing the operculum and this process is called aestivation (summer sleep). Factors leading to aestivation invariably display features of stress and hence it is of interest to understand and elucidate the behavioral changes during this stress in an aestivating amphibious Indian apple snail, *Pila globosa*.

*Pila globosa* whose distribution is mainly around equatorial & tropical regions of the world is subjected to seasonal variations and results in aestivation during summer. These factors are simulated in the laboratory conditions and results highlight the behavioral and physiological adjustments occurring in *Pila globosa* during aestivation which elucidate biological mechanisms involved.

### Introduction

Depression of metabolic rate is widespread survival strategy found across all kingdoms of life. When faced with environmental constraints that result in normal life being compromised and viability challenged many organisms retreat into a hypometabolic state (Storey, 2007).

Seasonal changes in humidity, water, temperature and vegetation create annual cycles of aestivation (summer sleep) in many amphibians (Hudson, Bennett, & Franklin, 2004), molluscs (Krishnamoorthy, 1968) and fishes (Sturla, 2002). Aestivation is a period of reduced metabolic activity which brings about changes in biochemical activities of an organism (Pakay, Withers, Hobbs, & Guppy, 2002). In arid and semi-arid regions of the world where conditions of high ambient temperature, low rainfall, low humidity and low vegetation exists, amphibious snails tend to undergo a period of torpor being characterized by a unique range of behavioral, biochemical and physiological adaptations to withstand thermal, ionic, water imbalances (Riddle, 1983; Tal Mizrahi, 2010).

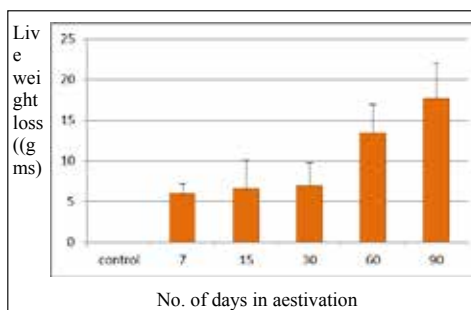
The endemic organism *Pila globosa*, Swainson, 1822 whose distribution is mainly around equatorial & tropical regions of the world, is subjected to seasonal variations and in turn undergoes self-induced stress condition of aestivation to evade long summer seasons. These factors are simulated in the laboratory to study the behavioral and physiological adjustments occurring in *Pila globosa* during aestivation.

### Materials and methods

Collection of snails was done from lakes, ponds, rice fields etc. around the regions of Ananthapuram town of Andhra Pradesh state. The snails were acclimatized to the laboratory conditions and regularly fed with leafy vegetables. The specimens were then exposed to simulated conditions viz. wooden containers (10 cms x 10 cms x 10 cms) with a bed of filter papers, a heat source was provided by keeping a 10 W bulb covered with silver foil to ensure only heat is emanated and not the light. The boxes were covered with lid fixed with a thermometer and maintained at a temperature of >30°C. After regular intervals the snails were collected and weighed.

### Results

The live-bodyweight of the snails decreased steadily with maximum loss of 18 grams at the end of 90 days of aestivation (Table.1). The operculum was closed by mucus epiphragum in the aestivating snails (Figure.2). Some of the snails before entering into aestivation were found to lay eggs in clusters (Figure.3). The shell texture during dissection was found to be brittle after 60 days of aestivation.



**Table.1. Gross Liveweight (gms.) loss in snails during aestivation**



**Figure.2. Aestivating snails have closed operculum. Active snails have their foot, tentacles protruding out.**



**Figure.3. Snails were found laying eggs before entering into aestivation. Mucus secretion was also seen.**

## Discussion

Phenotypic changes in relation to the survival and reproductive success of individuals contribute to their ability to cope with environmental stress conditions and adaptation to new niches (Prada, Schizas, & Yoshioka, 2008). Along with phenotypic adaptations, snails exhibit physiological and biochemical adaptations for survival under stress conditions (Storey, 2002). Some of the phenotypic changes observed in *Pila globosa* during aestivation were closure of operculum, mucus sections etc. which associate the organism's survival strategies in retention of water and preventing attacks from predators. Studies on aestivation of other snails like *Archachatina marginata*, *Achatina achatina* showed withdrawal into shells resulting in conservation of water by aperture closure with calcareous epiphragm. Water loss was further retarded by discontinuous gaseous exchange and intermittent opening of pneumostome (breathing organ). The snails were also found to reduce their live weight with increase in number of days of aestivation indicating probable consumption of energy reserves. The study also highlights the live weight changes as a good measure of aestivation tolerance formerly reported in *Archachatina marginata* during six week aestivation period (Onadeko, 2010).

Live weight changes as a measure of aestivation duration was observed in freshwater pulmonate snails *Helisoma trivolvis* with reduction of 50 % weight after 132 days of aestivation (Russell-Hunter & Eversole, 1976). Similarly *Achatina achatina* lost 44.6% drop in body weight after four months of aestivation (Lukong & Onwubiko, 2004), a loss of 52% weight loss in *Archachatina marginata* after six weeks of aestivation (Omoyakhi, 2007), *Littorina* sps a loss of 62% weight reduction (Emerson, 1967). These examples highlight the weight reduction as an inevitable phenomenon but a loss of only 17.2% after 90 days reported in this study on aestivation provides ample evidence that *Pila globosa* has phenotypically adequate adaptive features to survive extended periods of aestivation. Likewise results were also observed in *Pila virens* where aestivation was induced for as long as 14 months resulted in weight loss (Meenakshi, 1957).

It was also suggested that color of the shell plays an important role in survival during aestivation. Light color is beneficial for snails living in arid environments. A study conducted on *Sphincterochila* (*Albea*) *candidissima*, snails painted black lost more body weight than did light color ones presumably as a consequence of higher heat absorption (Morenorueda, 2008). *Pila globosa* being light greyish apparently absorbs less heat and hence less reduction in body weight.

Shell thinning was also observed in *Pila globosa* after 60 days of aestivation. Specific study on the role of shell during prolonged

aestivation highlighted that shell consists largest reserve of calcium carbonate and magnesium carbonate which play an important role in ion-balance as intracellular pH reduces rapidly (Scholnick, Snyder, & Spell, 1994). Further evidence was found in *Helix aspera* where shell thinning was observed after three months of starvation. The ratio of Mg/Ca in a related study indicated that the mineral salts (Magnesium carbonate and calcium carbonate) were transported from shell (ostracum and prostracum) via haemolymph to calcic cells and intestinal fluid (Porcel, Bueno, & Almendros, 1996). The study further identified that calcium released from calcic cells was used for growth and sexual maturation during aestivation in *Helix aspera* with little change in body weight and ion-balance enabling it to survive even after starvation upto three months.

According to investigators (Burton & Mathie, 1975) efflux of Mg and Ca ions from the shell may offset metabolic and respiratory acidosis by buffering extracellular fluid and haemolymph in *Helix pomatia*. Experimental acidification was also found to increase intracellular calcium in *Helix aspera* (Alvarez Leefmans, 1981). All these findings support that in *Pila globosa* that there exists a relation between shell thickness with shell and digestive gland's Mg/Ca levels suggesting that these organs are directly involved in mechanisms of ion-balance and maintaining body weight.

The mucus epiphragm formation during aestivation in *Pila globosa* reiterates an important behavioral adaptation to withstand stress. The secretion of mucus epiphragm is one of the several water preserving strategies accounting up to 20% saving during aestivation in *Theba psinia* (Arad, 2001), likewise function can be accepted in *Pila globosa*.

Epiphragm also serves as a deterrent to predators, pathogens, preventing mechanical damage to the inner soft tissue and also acts as holdfast organ. It also permits gaseous exchange and is sensitive to external environmental changes (Struthers, 2002). These findings support the role of mucus epiphragm and additional aspect of egg laying before aestivation makes *Pila globosa* one of the best model organism among snails to understand the behavior and physiology during environmental stress.

## Acknowledgements

Authors dedicate this study with gratitude to the founder Chancellor of Sri Sathya Sai Institute of Higher Learning, Bhagawan Sri Sathya Sai Baba. We thank the University authorities for providing facilities and financial support. The authors also thank UGC-SAP, DST-FIST, and DBT BIF.

## REFERENCE

- Alvarez Leefmans, F. J. R., T. J. Tsien, R. Y. (1981). Free calcium ions in neurones of *Helix aspersa* measured with ion-selective micro-electrodes. *Journal of Physiology*, 315, 531-548. | Arad, Z. (2001). Desiccation and rehydration in land snails – a test for distinct set points in *Theba pisana*. *Israel Journal of Zoology* 47, 41-53. | Burton, R. F., & Mathie, R. T. (1975). Calcium and pH homeostasis in the snail (*Helix pomatia*): effects of CO<sub>2</sub> and CaCl<sub>2</sub> infusion. *Experientia*, 31(5), 543-544. | Emerson, D. N. (1967). Some physiological effects of starvation in the intertidal prosobranch *Littorina planaxis* (Philippi, 1847). *Comparative Biochemistry And Physiology*, 20(1), 45-53. | Hudson, N. J., Bennett, M. B., & Franklin, C. E. (2004). Effect of aestivation on long bone mechanical properties in the green-striped burrowing frog, *Cyclorana alboguttata*. *Journal of Experimental Biology*, 207(3), 475-482. | Krishnamoorthy, R. V. (1968). Hepatopancreatic unsaturated fatty acids during aestivation of the snail, *Pila globosa*. *Comp Biochem Physiol*, 24(1), 279-282. | Lukong, H. A., & Onwubiko. (2004). A Role For Ca<sup>2+</sup> in the Thermal and Urea Denaturation of Haemocyanin from Aestivating Giant African Snails *Achatina achatina*. *Bio-Research*, 2(2). | Meenakshi, V. R. (1957). Anaerobiosis in the south indian apple snail *Pila virens* (Lamark) during aestivation *Journal of zoological society, India*, 9, 62-71. | Morenorueda, G. (2008). The colour white diminishes weight loss during aestivation in the arid-dwelling land snail *Sphincterochila* (*Albea*) *candidissima*. *Iberus*, 26(1), 47-51. | Omoyakhi, J. M. (2007). Chemical adjustments during aestivation by the giant African land snails, *Archachatina marginata* and *Achatina achatina*. | Onadeko, S. A. (2010). Liveweight changes and mortality rate in the giant african snail *Archachatina marginata* during six week aestivation period *World journal of zoology*, 5(2), 75- 81. | Pakay, J. L., Withers, P. C., Hobbs, A. A., & Guppy, M. (2002). In vivo downregulation of protein synthesis in the snail *Helix aspera* during estivation. *Am J Physiol Regul Integr Comp Physiol*, 283(1), R197-204. | Porcel, D., Bueno, J. D., & Almendros, A. (1996). Alterations in the digestive gland and shell of the snail *Helix aspersa* Müller (gastropoda, pulmonata) after prolonged starvation. *Comparative Biochemistry and Physiology Part A: Physiology*, 115(1), 11-17. | Prada, C., Schizas, N. V., & Yoshioka, P. M. (2008). Phenotypic plasticity or speciation? A case from a clonal marine organism. *BMC Evolutionary Biology*, 8, 47. | Riddle, W. A. (1983). Physiological ecology of land snails and slugs. (Vol. 6): Academic press, London. | Russell-Hunter, W. D., & Eversole, A. G. (1976). Evidence for tissue degrowth in starved freshwater pulmonate snails (*Helisoma trivolvis*) from tissue, carbon, and nitrogen analyses. *Comparative Biochemistry and Physiology Part A: Physiology*, 54(4), 447-453. | Scholnick, D. A., Snyder, G. K., & Spell, A. R. (1994). Acid-base status of a pulmonate land snail (*Helix aspersa*) and a prosobranch amphibious snail (*Pomacea bridgesi*) during dormancy. *Journal of Experimental Zoology*, 268(4), 293-298. | Storey, (2002). Life in the slow lane: molecular mechanisms of estivation. *Comparative Biochemistry and Physiology Part A: Physiology* 133(3), 733-754. | Storey. (2007). Tribute to P. L. Lutz: putting life on 'pause' -molecular regulation of hypometabolism. *Journal of Experimental Biology*, 210(10), 1700-1714. | Struthers, M. R. G.; Buckman, J.; Viney, C. (2002). The physical and chemical microstructure of the *Achatina fulica* epiphragm. *Journal of Molluscan Studies*, 68(2), 165-171. | Sturla, M. P., P. Carlo, G. Angela, M. M. Maria, U. B. (2002). Effects of induced aestivation in *Protopterus annectens*: a histomorphological study. *Journal of Experimental Zoology*, 292(1), 26-31. | Tal Mizrahi, J. H., Shoshana Goldenberg and Zeev Arad. (2010). Heat shock proteins and resistance to desiccation in congeneric land snails. *cell stress and chaperones*, 15, 351-363. |