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Immunological resilience of a freshwater Indian mollusc during aestivation and starvation

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ABSTRACT

Freshwater snail, *Pila globosa* is an edible mollusc which bears commercial and ethnomedicinal significance. During environmental aridity, *P. globosa* aestivates deep into the soil for four to five months. We report comparative analyses of immune associated parameters in the hemocytes and hemolymph of *P. globosa* during activity, experimental aestivation, arousal and starvation. Hemocyte is a major cellular component of molluscan immune system. They are functionally involved in phagocytosis, cytotoxicity, antioxidative defence and enzyme mediated lysosomal pathogen degradation. Determination of the total hemocyte count, phagocytosis, generation of superoxide anion, nitric oxide and activities of phenoloxidase, superoxide dismutase, catalase, glutathione-*S*-transferase, phosphatases and protein content in the hemocytes and hemolymph were carried out during the mentioned phases of life process of *P. globosa*. Total count of hemocyte was recorded to be higher during aestivation, arousal and starvation in comparison to the phase of activity. Phagocytic response of hemocytes of *P. globosa* was highest during phase of activation. *P. globosa* exhibited high level of generation of superoxide anion and nitric oxide in the hemocytes during aestivation. Activity of phenoloxidase was recorded to be highest in the hemocytes and hemolymph among the starved individuals. Activities of superoxide dismutase and catalase were highest in the hemocytes of starved *P. globosa.* Glutathione-*S*-transferase activity was estimated to be high in the hemolymph and hemocytes of active specimens. Aestivation and starvation exhibited modulation in the activities of phosphatases in the hemocytes and hemolymph. Total content of protein of hemocytes was recorded to be low in the aestivated, aroused and starved *P. globosa* in comparison to the active ones. Nonuniform status of the immune parameters of hemocytes and hemolymph during activity, aestivation, arousal and starvation was indicative to a status of immunological resilience of the species to ensure adaptation during different physiological and environmental conditions.

1. Introduction

Aestivation is a physiological adaptive response of organism under the challenge of environmental adversity. It is a special type of behavioural pattern of animals during scarcity of food resources (Chown and Storey, 2006) and is assumed to be a strategy of survival against environmental adversities like aridity and heat (Pinder et al., 1992; Storey, 2001). Aestivation is considered as an indispensable step in the life process of invertebrates like sea cucumber (Liu et al., 1996), land snail (Salway et al., 2010) and has been categorised into three distinct phases like preaestivated, aestivated and arousal (Wang et al., 2008). During unfavourable environmental conditions, many organisms including the molluscs undergo aestivation due to food and water deprivation. *Pila globosa* (*Mollusca: Gastropoda*) is a common variety of amphibian mollusc of Indian subcontinent. They evolved a highly developed gill and pulmonary sac which enabled them to perform respiration both in aquatic and terrestrial environments respectively. According to Haniffa (1978), aestivation of *P. globosa* depends on high environmental temperature. Monthly observation made on the aestivating *P. globosa* revealed that the environmental temperature above 35 °C was critical for induction of aestivation. This situation was reported to be associated with a relative loss of energy in the aestivating specimens. According to this report, the density of aestivating *P. globosa* was recorded to be the highest in the month of July in India. However, the duration of aestivation had been reported to be unpredictable in snails which may arouse within a few minutes with the rise in environmental humidity (Hermes-Lima et al., 1998).

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Being a poikilotherm, *P. globosa* experiences a wide range of thermal variation throughout the year. The status of physiological inactivity during aestivation has been considered as unique adaptive feature of this prosobranch distributed in the different region of India. It is reported that during aestivation metabolic rate of organism get depressed to several folds (Storey, 2002) to ensure survival. Lowering of metabolic rate enables the aestivator to survive for a longer period during environmental harshness (Ferreira-Cravo et al., 2010). Aestivation is considered as an adaptive feature which is characterised by water loss prevention and discontinuous breathing of organism distributed in dry environment (Guppy and Withers, 1999). During aestivation, *P. globosa* ceases feeding, closes its operculum and remains inactive inside the subsoil ecosystem with low moisture content. Hermes-Lima et al. (1998) reported the intermittent opening of pneumostome which allows gaseous exchange in the aestivating snail. The habitat of aestivated *P. globosa* appears to be dissimilar with that of active ones with reference to moisture content, temperature and nutrients status. Therefore, during active and aestivating phases of life, *P. globosa* are assumed to occupy two contrasting ecological niches and thus may be exposed to dissimilar immunological challenges.

P. globosa is an important bioresource of the freshwater ecosystem of India (Ray et al., 2015). Meat of the edible mollusc is in demand throughout the world (Baby et al., 2010). *P. globosa*, an edible gastropod, is widely consumed by the tribal and urban populations of India and Bangladesh (Baby et al., 2010). Flesh of *P. globosa* has been used as a supplementary food of prawn and poultry of different regions of India (Subba Rao and Dey, 1989; Baby et al., 2010). In Bangladesh, trading of *P. globosa* bears an influencing effect over the country's economy (Saha, 1998). According to Jahan et al. (2007), induced breeding of *P. globosa* during commercial farming appeared to be rational and profitable to utilize this aquatic bioresource in Bangladesh. Ethnomedicinal importance of *P. globosa* has been highlighted by several authors from different viewpoints (Prabhakar and Roy, 2009; Mahawar and Jaroli, 2007). According to them, diseases like arthritis, asthma, conjunctivitis, night blindness and cardiac diseases are reported to be curable by the pharmacological application of its different body parts and tissues. This indicates it's potentiality to act as a source of drug and other bioactive chemical substances (Mahawar and Jaroli, 2007).

Nutritional status of an organism is often associated with immunity of the organism (Butt et al., 2007; Xu et al., 2008; Al-Rawadeh, 2010) against the invasion of pathogen and parasite. Starvation induced changes in the hemocyte mediated parameters have been reported in the Zhikong scallop, *Chlamys farreri* (Xu et al., 2008). While examining the effect of starvation on cellular and biochemical parameters of crab, Matozzo et al. (2011) emphasized on two important aspects of the cellular immunity of crab, *Carcinus aestuarii*. Authors questioned whether starvation can influence immune related parameters and antioxidant status in *C. aestuarii* and the crab can modulate these immune parameters to overcome this starvation related stress.

Hemocytes, the chief immunoeffector cells of molluscs (Humphries and Yoshino, 2003), perform diverse immunological functions like phagocytosis (Hillyer et al., 2003; Cheng, 1977), cytotoxicity (Adema et al., 1991; Bogdan, 2001), aggregation (Chen and Bayne, 1995) and pathogen encapsulation (Humphries and Yoshino, 2003; Koutsogiannaki and Kaloyianni, 2010). On the other hand, hemolymph, the humoral component of molluscan immune system, is reported to exhibit the activities of superoxide dismutase (Monari et al., 2007), catalase (Liu et al., 2008), acid (Cheng and Butler, 1979) and alkaline phosphatases (Helal et al., 2003). Total hemocyte count in mollusc has been reported as an important immune parameter (Mello et al., 2010). Elevation of the total hemocyte count is an indication in the augmentation of immunity of invertebrates (Kacsoh and Schlenke, 2012). Phagocytosis is an established strategy of immune defence in invertebrates including mollusc. It is considered as principal immunological activity reported in many molluscan species (Goedken and De Guise, 2004; Garcia-Garcia et al., 2008). Major cytotoxic molecules like superoxide anion and nitric oxide generated by the circulatory hemocytes of mollusc are functionally associated with the destruction of pathogens (Manduzio et al., 2005; Rivero, 2006; Ray et al., 2013). Phenoloxidase is reported to be functionally associated with phagocytosis, self-nonself discrimination, cytotoxicity and melanisation response (Söderhäll and Cerenius, 1998; Cerenius and Söderhäll, 2004). Superoxide dismutase and catalase play a significant antioxidation role in the cellular physiology of mollusc. Both of these enzymes are involved in scavenging and deactivating the toxic oxidative radicals and protect the tissue from oxidative damage (Manduzio et al., 2005). Acid and alkaline phosphatases are functionally involved with pathogen destruction in phagolysosome and bear immunological significance (Murti and Shukla, 1984). Glutathione-*S*-transferase is functionally associated with general detoxification response of xenobiotics and antioxidation activity (Manduzio et al., 2004).

However, report of the effect of starvation and aestivation on the immune related parameters of Indian mollusc is absent in the current scientific literature. Nowakowska et al. (2009b) examined the extent of oxidative stress and activity of antioxidant enzymes in snail, *Helix pomatia* during aestivation. Physiological correlation of antioxidant defence and metabolic depression had been reviewed by Hermes-Lima et al. (1998). Starvation is reported to compromise with the immunological activity of a land snail, *Helix aspersa* (AL-Rawadeh, 2010). According to him, nutrition plays a major role in maintenance of selected immune parameters like total hemocyte count, phenoloxidase activity and phagocytosis in the same species. Present investigation is aimed to examine the modulation and relative plasticity of the innate immune parameters during experimental aestivation and starvation in *P. globosa*. Studied parameters included generation of cytotoxic molecules like superoxide anion, nitric oxide and phenoloxidase and the activities of superoxide dismutase, catalase, glutathione-*S*-transferase, acid phosphatase, alkaline phosphatase and total protein in hemocytes and hemolymph of *P. globosa* during activity, aestivation, arousal and starvation. Total count and phagocytic response of hemocytes were quantitated in the different phases of *P. globosa* too. Generated information is assumed to provide a better premise in estimating the immune status of *P. globosa* during the phases of activity, aestivation and starvation. Present data would provide an important information in the field of comparative immunity and physiology of this commercially important mollusc of India.

2. Materials and methods

2.1. Experimental design

Forty active specimens of *P. globosa* (shell length 4.2 ± 1.5 cm from apex to operculum) were manually collected from the selected natural habitats of the district of north twenty four parganas (22°86′N, 88°40′E) of the state of West Bengal, India. The collected specimens were transported to the laboratory and acclimated in aerated glass containers at an ambient temperature of 25–30 °C. A uniform light rationing of 12:12 dark–light cycle was maintained in the container throughout the acclimation period. After acclimation, each specimen was marked and divided into two groups of equal numbers and one group was induced to aestivate at 37 °C for 15 days. In laboratory, aestivating specimens were kept in plastic boxes without the physical contact of water and food. Whereas, the other group was further subdivided into two categories. One of the category was experimentally starved for 15 days, whereas, the