Comparative Biochemistry and Physiology, Part A 203 (2017) 262–272

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Laboratory studies on the thermal tolerance and response of enzymes of intermediate metabolism in different land snail species

Alexandra Staikou [a], Evagelia Kesidou [b], Marina-Elena Garefalaki [a], Basile Michaelidis [b,*]

[a] Laboratory of Zoology, Department of Zoology, Faculty of Science, School of Biology, University of Thessaloniki, GR-54006 Thessaloniki, Greece
[b] Laboratory of Animal Physiology, Department of Zoology, Faculty of Science, School of Biology, University of Thessaloniki, GR-54006 Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form 7 October 2016
Accepted 10 October 2016
Available online 14 October 2016

Keywords:
Global warming
Temperature
Land snails
Mortality
Metabolism

ABSTRACT

Land snails species occur in a range of habitats from humid to semi-arid and arid ones and seasonal variations in their physiology and biochemical composition have been linked to annual cycles of photoperiod, temperature, humidity and water availability. In an effort to understand the thermal tolerance and the impact of temperature elevation on tissue metabolism of land snails we determined the mortality, heamolymph PO_2 and the activities of enzymes of intermediary metabolism in three land snail species (*Helix lucorum*, *Helix pomatia* and *Cornu aspersum*) differing in their geographical distribution and inhabiting areas with different climatic characteristics. No mortality was observed in both population of *Cornu aspersum*, while *Helix pomatia* exhibited higher mortality than *Helix lucorum*. PO_2 dropped within the first 10 days of exposure to elevated temperature in all species, although in *Cornu aspersum* this decrease was significantly lower. No significant reduction in the enzymatic activities of all glycolytic enzymes studied, as well as of citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (HOAD) was observed in the more thermal tolerant species *C. aspersum* from both populations studied. Significant reductions of enzymatic activity of the glycolytic enzymes phosphofructokinase (PFK), pyruvate kinase (PK) and D-Lactate dehydrogenase (D-LDH) was observed in *Helix lucorum* and *Helix pomatia*. The observed inter-specific differences seem to be in accordance with the life cycle characteristics of each species and may be attributed to climatic differences among habitats within their distribution range.

1. Introduction

According to the most recent climate change scenarios a significant temperature rise in European Mediterranean regions is expected to occur within the 21st century (IPCC, 2014). In view of the above mentioned climate change projections, it is anticipated that Europe's natural ecosystems and biodiversity will be substantially affected, and a great majority of organisms and ecosystems are likely to have difficulty in adapting (Alcamo et al., 2007).

It is well known that species, within their distribution range, experience stressful conditions of differing intensity and duration. Thus individuals become adapted to the range of environmental fluctuations they face and develop a rich repertoire of responses at all biological organization levels, including molecular and biochemical ones. Such responses determine in great extent an organism's tolerance to a stressful condition or its potential response to the scenery of changes in the environmental conditions due to climate change (Pörtner, 2002; Bozinovic et al., 2011). Animals' geographical boundaries are largely affected by their biochemical and physiological traits and under a global climate warming scenario these physiological and biochemical traits might determine their ability to survive. The physiological role of metabolic responses and energy (ATP) turnover to tolerance of stressful conditions has been studied extensively in several taxa and it seems that homeostasis of energy turnover play a crucial role to the ability of an organism to withstand stressful conditions (Hochachka and Somero, 2002; Sokolova et al., 2012). It has been reported that survival at high ambient temperature is closely related to animals' ability to keep their aerobic capacity and energy turnover (Pörtner, 2002; Bozinovic and Pörtner, 2015). However, most studies concern marine animals, while, according to our knowledge, there are few related studies on land invertebrates.

Land snails species occur in a range of habitats from humid to semi-arid and arid ones and seasonal variations in their physiology and biochemical composition have been linked to annual cycles of photoperiod, temperature, humidity and water availability (Machin, 1975; Riddle, 1983; Cook, 2001; Storey, 2002). Also, land snails exhibit a range of behavioural, molecular, biochemical and physiological adaptations that ensure their survival under adverse environmental conditions as high ambient temperature and aridity. Moreover, land snails respond to such stressful conditions (heat overload, extreme aridity) by entering into a quiescent stage characterized by a marked reduction of metabolic

* Corresponding author.
E-mail address: michaeli@bio.auth.gr (B. Michaelidis).

http://dx.doi.org/10.1016/j.cbpa.2016.10.002
1095-6433/

rate and ATP turnover (Guppy and Withers, 1999; Storey and Storey, 1990, 2010, 2012; Storey, 2015). Under high temperature regimes land snails often suffer from heat overload and desiccation, and respond to these conditions at the cellular level by induction of Hsps, as an important though energetically costly survival strategy (Arad et al., 2010). Additionally, such a physiological response may be well correlated with the estivation induced response of preparation for oxidative stress. This induction of antioxidant enzymes during hypoxia was viewed as a way to prepare animals for oxidative damage that may happen ultimately during reoxygenation (Nowakowska et al., 2010, 2011, 2014; Hermes-Lima et al., 2015; Moreira et al., 2016). On the other hand differences among species and populations in the intensity in which stress proteins are induced could be associated with differences among them in how temperature has affected their energy budgets (Mizrahi et al., 2010). Although it seems that land snails possess many common modes of responses to high temperature and dry environments there may be differences among species in their thermal limits and their ability to respond and adapt biochemically and physiologically to such stressful conditions. As shown recently, populations of land snail species from different latitudes or altitudes differ significantly in their growth and metabolic rates and in their sensitivity to respond to favorable conditions (Gaitán-Espitia et al., 2013a,b; Gaitán-Espitia and Nespolo, 2014; Staikou et al., 2016). Such differences in metabolic traits may contribute in shaping thermal limits and investigation on metabolic responses could be of great importance in understanding the impacts of global warming on land snail species.

The aim of the present work was to study the thermal tolerance and metabolic responses of three congeneric land snail species *Helix pomatia*, *Helix lucorum* and *Cornu aspersum* (formerly known as *Helix aspersa*) which differ in their geographical distribution and inhabit areas of different climatic characteristics. Also, we studied populations of *C. aspersum* from areas of different thermal regimes. *Helix pomatia* is a European species indigenous to central and southeast Europe but has been moved by humans all over Europe, Asia and the Americas. It lives up to 2100 m above sea level in the Alps, but usually below 2000 m. In the Balkans it occurs in open forests and shrubland and reaches the southern limits of its distribution in Europe, in the Rodope mountains along the Greek – Bulgarian border. Snails of the former populations hibernate during winter (November to March) and remain active throughout the rest of the year. Reproduction begins in mid-April, or July for populations in higher altitudes, and is extended throughout 137 summer until mid-September (Chatziioannou et al., 1989).

The distribution area of *H. lucorum* stretches from the Eastern Black Sea region through Asia Minor, the Balkan Peninsula as far as Albania until Italy west of the Apennine. The species has been introduced in regions of central Europe, in southern France and on the Iberian Peninsula. In Greece *H. lucorum* is found throughout the mainland and some islands especially on the northern part of the country, at altitudes ranging from sea level to 1200 m. In humid habitats in the north of the mainland and at high altitudes snails do not aestivate in summer and experience a continuous activity cycle from early spring when they exit hibernation until November. In these populations reproduction occurs from late spring to the beginning of autumn (Staikou et al., 1988).

Cornu aspersum is native to the Mediterranean region, probably originating from the north coast of Africa and subsequently spread to the north-west part of Europe and to the Eastern part of North Africa and eastern Mediterranean (Guiller and Madec, 2010). Today it is distributed from northwest Africa and Iberia, eastwards to Asia Minor and northwards to the British Isles. Also it is a typically ANTHROPOCHOROUS species and it has been spread to many geographical regions by humans, either deliberately or accidentally. In Greece *C. aspersum* is found throughout the southern part of the mainland along the coast and on almost all the islands of the Aegean and Ionian Sea. It is restricted to altitudes lower than 1000 m. Reproduction occurs during the autumnal months (September to November). In the southern part of its distribution (e.g. coastal populations in Crete and Cyprus) *C. aspersum* is active throughout the humid period from September to May, and aestivates during summer. In the northern parts (e.g. Corfu) snails aestivate during summer and hibernate during winter.

For the purpose of the present work we determined under laboratory conditions the mortality rate of the studied species and the changes in the activities of enzymes of intermediate metabolism during snails' exposure to high temperature as an indicator of their ability to keep energy turnover during warming.

2. Materials and methods

2.1. Animals and experimental design

Adult snails were collected from the field by the end of May. *Helix lucorum* was collected from an area near Axios river, Thessaloniki, Greece (40°43′44.6″N, 22°39′23.8″E), *H. pomatia* from an area near Echinos village, Xanthi, Greece (41°16′29.3″N, 24°58′05.6″E), *C. aspersum* from an area near Asini village, Argos, Greece (37°38′06″N, 22°43′44″E) and from an area near Nicosia, Cyprus (35°10′00″N, 33°21′00″E) (Fig. 1). Individuals of *C. aspersum* collected from Argos and Cyprus will be referred therein as *C. aspersum*-Argos and *C. aspersum*-Cyprus respectively.

Snails of all three species (*H. pomatia*: D ranged between 30 and 35 mm, *H. lucorum*: D ranged between 35 and 40 mm, *C. aspersum*: D ranged between 30 and 32 mm) were transferred to the laboratory where they were put in large glass boxes (40 cm × 40 cm × 60 cm) containing moist soil. Each box contained 30–35 individuals and for each species three replicate boxes were used. One box was used for mortality evaluation only, while snails used for PO_2 and enzymatic activity determination came from the other two boxes. All boxes were maintained in a temperature-controlled chamber at 20 ± 0.5 °C and a photoperiod of 14L:10D. Water was sprayed periodically to maintain soil moisture and high ambient humidity (above 80%). Snails were fed fresh lettuce leaves every day for 15 days. After this time period the chamber temperature was raised to 34 °C and snails were deprived from food and water. Snails were removed from the boxes at intervals of 5, 10, 20 and 30 days and their haemolymph was immediately sampled for the determination of PO_2. For the determination of enzymatic activities snails were removed as described above and the foot muscle along with mantle and hepatopancreas tissues were dissected, freeze-clamped between aluminum tongs cooled in liquid nitrogen and ground under liquid nitrogen. Tissue powders were stored at −80 °C until measurements of enzymatic activities. Snails kept under a temperature of 20 °C, daily food supply and high humidity were used as controls.

2.2. Collection of blood and haemolymph and determination of PO_2

Haemolymph collection from snails was performed as described by Pedler et al. (1996). In brief, after removing a small section of the shell to expose the pericardium, haemolymph was collected after puncturing the heart with a needle fitted to a syringe, previously equilibrated with pure nitrogen. After sampling the 205 snails were not used for further experimentation. The *PO2* was determined in snails' haemolymph using a Clarke type oxygen electrode (E5047) in the gas cuvette of the BMS3/MK2 Blood Micro System. All determinations were performed at the same temperature as the one corresponding to each sampling occasion.

2.3. Preparation of tissues homogenates for the determination of enzymatic activities

For the activities of enzymes of intermediary metabolism the assays were adapted from those described elsewhere (Storey and Storey, 1984; Brooks and Storey, 1991; Stuart et al., 1998; Michaelidis et al., 2008). CS and HOAD were adapted from Stuart and Ballantyne (1996).