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Thermal equilibrium and temperature differences among body regions in European plethodontid salamanders

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ABSTRACT

Information on species thermal physiology is extremely important to understand species responses to environmental heterogeneity and changes. Thermography is an emerging technology that allows high resolution and accurate measurement of body temperature, but until now it has not been used to study thermal physiology of amphibians in the wild. *Hydromantes* terrestrial salamanders are strongly depending on ambient temperature for their activity and gas exchanges, but information on their body temperature is extremely limited. In this study we tested if *Hydromantes* salamanders are thermoconform, we assessed whether there are temperature differences among body regions, and evaluated the time required to reach the thermal equilibrium. During summers of 2014 and 2015 we analysed 56 salamanders (*Hydromantes ambrosii* and *Hydromantes italicus*) using infrared thermocamera. We photographed salamanders at the moment in which we found them and 1, 2, 3, 4, 5 and 15 min after having kept them in the hands. Body temperature was equal to air temperature; salamanders attained the equilibrium with air temperature in about 8 min, the time required to reach equilibrium was longer in individuals with large body size. We detected small temperature differences between body parts, the head being slightly warmer than the body and the tail (mean difference: 0.05 °C). These salamanders quickly reach the equilibrium with the environment, thus microhabitat measurement allows obtaining accurate information on their tolerance limits.

1. Introduction

In animals, body temperature is a fundamental trait linked to the execution of all physiological activities, such as locomotion, immune resistance, foraging and growth (Angilletta Jr. et al., 2002). Each species has its own optimal temperature, which is the best temperature at which the organism could realize its functions (Raske et al., 2012). Endothermic species use their metabolism to regulate their own temperature and maintain optimal temperature during the time (Macdonald, 2010). On the other hand, ectotherms often use surrounding environments to maintain their body temperature into a specific range, that define conditions in which biological functions can be carried out (Angilletta Jr. et al., 2002; Gunderson and Leal, 2016; Navas, 1996; Navas et al., 2008). Due to the spatial and temporal heterogeneity of environments, many ectotherms adopt thermal behaviour to maintain body temperature close to their preferred one (Feder, 1982; Navas, 1997; Raske et al., 2012). Reptiles have a semi-impermeable skin that prevents water loss, so they quite easily use solar radiation for reach their favourite temperature (Kaufmann and Bennett, 1989). On the other hand, amphibians present some hurdles related to thermoregulation because they have to balance the intake of energy with evaporation of water through their skin (Hutchinson and Dupreé, 1992; Seebacher and Alford, 2002; Spotila, 1972; Tracy et al., 2007). In some cases, such as anurans living in ponds exposed to the sun, individuals can thermoregulate avoiding overheating and dehydration as they continuously absorb water through their ventral surface, thus also balancing the incoming heat (Lillywhite, 1970). Furthermore, some frogs living in arid

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environments with extreme temperature developed a specialized morphology to minimize heat gain and maximize heat loss while avoiding water loss (Kobelt and Linsenmair, 1995), and some newts can thermoregulate by selecting the portions of the water column which hold the optimal temperature (Balogová and Gvoždík, 2015).

Information on species thermal physiology is increasingly used to understand species responses to environmental changes. For instance, ecophysiological analyses can use information on thermal tolerance of species to identify areas where climatic conditions are suitable, and to estimate potential impacts of environmental changes (Kearney and Porter, 2009). Studies on species thermal tolerance often use air temperature (T_{a}) nearby active individuals as a proxy of the thermal environment: this information is easily available, but in some cases may be a poor measure of the conditions actually experienced by individuals (Sunday et al., 2014). Actually, body temperature (T_{b}) of ectotherms may be strongly different from T_{a} because it is affected by behaviour, solar radiation, metabolism, conduction and evaporation (e.g. Bakken, 1992; Kearney and Porter, 2009; Pough et al., 2013; Tracy, 1976). Understanding whether ectotherms are at thermal equilibrium with their environment (thermoconformity) is extremely important, as this information is needed to understand species responses to environmental variations, and to predict potential impacts of climate changes (Balogová and Gvoždík, 2015). In thermal physiology studies, body temperature is traditionally measured with thermometric probes, but this limits the number of body regions for which temperature may be recorded, and requires manipulation of individuals, with the risk of influencing behaviour and body temperature (Lillywhite, 2010). Infrared thermocameras are an emerging approach for the study of thermal physiology in ectotherms. They provide instantaneous, high resolution images of surface temperature without the need of handling individuals, allow to identify thermal heterogeneity within individuals and, for small animals, their results are consistent with more traditional techniques, such as cloacal thermometers (Luna and Font, 2013; Sannolo et al., 2014; Tattersall and Cadena, 2010). Thermocameras have been successfully used to study thermoregulation in reptiles, but as far as we know they have been applied to amphibians only once under controlled conditions (Đikić et al., 2011).

Within amphibians, Plethodontid salamanders are a very interesting taxon for studies on thermal ecology. Plethodontids represent about 66% of currently described caudate amphibians (AmphibiaWeb, 2016), and are among the tetrapods with the lowest metabolic rate (Chong and Mueller, 2012). This family is characterized by absence of lungs, so their respiration mainly occurs through the skin (Spotila, 1972). Gas exchanges require a constantly moist skin, thereby imposing limits on their habitat selection and thermoregulation (Feder, 1983; Huey, 1991; Peterman and Semlitsch, 2014). Several plethodontids are often associated with underground environments, in which humidity is very high but the heat sources are very limited (Camp et al., 2014). Early studies suggested that plethodontids generally are thermoconforms (Brattstrom, 1963), but it is possible that individuals regulate temperatures by selecting specific microhabitats (Spotila, 1972), or that the evaporative water loss reduces T_{b} at values significantly lower than ambient temperature (Bressin and Willmer, 2000).

European plethodontids (genus *Hydromantes*) often exploit underground environments, in which microclimatic features are suitable for their physiological needs (Lanza et al., 2006). Underground environments are dynamic systems in which few heat sources (e.g. rocks and external heat) seasonally interact with air flow and high moisture in determining complex thermal landscapes (De Freitas, 1982, 1987; Lunghi et al., 2015), and this affects the physiology and distribution of cave dwelling species (Sunday et al., 2014).

In this study we used thermocamera images to study the thermal ecology of *Hydromantes* salamanders. First, we assessed whether body temperature of salamanders is equal to air temperature (i.e. whether salamanders are at the thermal equilibrium with the environment). Air temperature is a quick and easy approach to the characterization of microhabitat for these salamanders and, if $T_{\mathrm{b}}=T_{\mathrm{a}}$, air temperature can be a good proxy of operative conditions actually experienced by individuals (Kearney and Hewitt, 2009; Sunday et al., 2014). Second, we evaluated whether there are temperature differences among body regions. Finally, we manipulated animals to evaluate the time required to reach the thermal equilibrium, and to assess whether body size confers a higher thermal inertia, thus increasing the time needed to reach equilibrium.

2. Methods

2.1. Study system

We used a Fluke Ti32 infrared thermal imager (thermal sensitivity <0.045 °C, spatial resolution 1.25 mRad) to photograph salamanders and measure their body temperature T_{b} (emissivity 0.98). Overall, we analysed 31 *H. ambrosii* from two nearby caves (Cave A1: 44.18°N, 9.72°E 190 sl.m., max. depth 200 m, external opening: 4 × 3.7 m; Cave A2, 44.12°N and 9.77°E 330 sl.m., max. depth 20 m, external opening: 1.8 × 2.5 m) and 25 *H. italicus* from two nearby caves (Cave I1: 44.04°N, 10.25°E 912 sl.m., max. depth 15 m, external opening: 1.2 × 2 m; and Cave I2: 44.04°N and 10.26°E 890 sl.m., max. depth 20 m, external opening: 2 × 4 m). Cavities (A1, A2 and I1) were natural, while the Cavity I2 was a test mine. Caves examined for *H. ambrosii* were located in a karstic area while caves for *H. italicus* were opened in schist rocks. Individuals were photographed, without manipulation, at a distance of 35 cm, on the cave wall where they have been observed to be naturally active. For each individual, we calculated the temperature of pixels on head, trunk and tail (average ± SE: 570 ± 1 pixels per individual measured) without counting limbs. Body temperature T_{b} was the mean temperature of pixels on head, trunk and tail.

2.2. Does air temperature represent body temperature of individuals?

Caves were divided in 3-m longitudinal intervals (hereafter: sectors), covering the whole cave or until the position of the last salamander; the size of sectors approximately corresponds to home range size (Lanza et al., 2006; Salvidio et al., 1994). Starting from cave entrance, every 3 m (e.g. at the end of each sector) we measured air temperature (°C) using a Lafayette TDP92 digital thermometer (accuracy: 0.1 °C). In each sector we used visual encounter surveys to detect the presence of active salamanders. We then photographed active salamanders using the infrared thermal imager to measure T_{b}, and calculated the average difference between the air temperature T_{a} of the sector and T_{b}. Subsequently, we used linear mixed models to assess the relationship between T_{a} and T_{b}. All mixed models considered sector, cave and species identity as random factors; this analysis was performed on 29 individuals for which data on body temperature and air temperature at the beginning of the experiment were available. Sample size was not homogeneous among groups, therefore in mixed models degrees of freedom were approximated and in some cases were not integer (Satterthwaite, 1946); the overall amount of variation explained by mixed models was assessed using conditional R^2 (Nakagawa and Schielzeth, 2013). Mixed models were also used to test whether temperature was significantly different between head, trunk and tail within the individuals (regional