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 it has not been used to study thermal physiology of amphibians. *Hydromantes* terrestrial salamanders are of high conservation value for European fauna and strongly dependent 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 *H. italicus*) using infrared thermocamera. We photographed salamanders at the moment in which we found them and 2, 3, 4, 5 and 15 minutes after having kept them in the hands. Body temperature was equal to air temperature; salamanders attained the equilibrium with air temperature in about 8 minutes, 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.

Key words
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 could 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 Dupré, 1992; Seebacher and Alford, 2002; Spotila, 1972; Tracy et al., 2007).

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. Infrared thermocameras are an emerging approach for the study of thermal physiology of 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 to our knowledge they have never been applied to amphibians.

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 their 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 (Ficetola et al., 2012; Ficetola et al., 2013; Lunghi et al., 2014). 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 use 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 $T_b = T_a$, 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 measure the body temperature $T_b$ of salamanders. Overall, we analysed 31 *H. ambrosii* from two nearby caves (Cave A1: 44.18°N, 9.72°E and Cave A2, 44.12°N and 9.77°E) and 25 *H. italicus* from two nearby caves (Cave I1: 44.04°N, 10.25°E and Cave I2: 44.04°N and
10.26°E). 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 average temperature of pixels on head, trunk and tail (in average, 570 pixels per individual measured). Body temperature $T_b$ (i.e., 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); the size of sectors approximately corresponds to home range size (Ficetola et al., 2013; Lanza et al., 2006), covering the whole cave or until the position of the last salamander. In each sector, we used visual encounter surveys to detect the presence of active salamanders, and measured air temperature (°C) using a Lafayette TDP92 digital thermometer (accuracy: 0.1°C). 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 differences in temperature). Analyses were performed using the lme4 and lmerTest packages in R (Bates et al., 2014; Kuznetsova et al., 2015).
2.3 Differences between body parts

2.3. Test of equilibrium of body temperature.

To confirm that in our study system salamanders body temperature is at equilibrium, and to test the time required to reach thermal equilibrium, we manipulated 56 individuals. Individuals were captured within their habitat, weighed (accuracy: 0.1g), kept in the hands of an observer for 30 seconds, and then released at the collection point. Individuals were photographed using the infrared thermal imager to measure body temperature at the release and 1, 2, 3, 4, 5 and 15 min after release. Due to the difficult field conditions, some individuals were not photographed at all the time occasions (mean sample size: 38.4 individuals per time occasion).

We then used non-linear mixed models (nlmm) (Pinheiro et al., 2014) to evaluate how and how fast body temperature goes at equilibrium. We considered two potential models:

1) Exponential loss of temperature:
   \[ \Delta T^o = k + e^{a \times t + b} \]

2) Loss of temperature following an inverse power law:
   \[ \Delta T^o = k + a \times t^b \]

Where \( \Delta T^o \) is \( T_b - T_o \), \( t \) is the time after release, and \( k \), \( a \) and \( b \) are the parameters to be estimated by the models. The fit of the two models was compared using Akaike’s information criterion (AIC), and we then estimate model parameters, their significance, and the time required to achieve body equilibrium (defined as \( \Delta T^o \leq 0.1^oC \)).
We also tested whether the velocity at which body temperature goes at equilibrium was slower in large individuals. Unfortunately, if we put both time after release and body size as independent variables in the nlmm, the model showed convergence problems. We therefore used standard mixed models to analyse the relationship between $\Delta T^\circ$ and body mass at the six intervals after the release (1, 2, 3, 4, 5 and 15 min.).

3. RESULTS

3.1. Relation between $T_a$ and $T_b$

The infrared camera provided clear pictures of salamanders’ body, with a spatial resolution sufficiently fine to measure the temperature of different body regions (Fig. 1). Before any manipulation, body temperature ranged between 8.17 and 15.89°C. Salamanders were at thermal equilibrium with the air: the average difference between $T_a$ and $T_b$ was small (mean difference = -0.129°C; 95% CI = -0.541/0.282), and $T_b$ was strongly related to $T_a$ (mixed model: $F_{1,22.6} = 18.8$, $P = 0.0002$; $R^2_c = 0.98$). Nevertheless, we detected small but significant differences between head, body and tail ($F_{2,107.8} = 9.86$, $P = 0.0001$, Fig. 1, Fig. 2). Specifically, within individuals, head was slightly warmer than both the body (Tukey’s post hoc: mean difference ± SE: 0.05 ± 0.02°C, $P = 0.02$) and the tail (mean difference: 0.07 ± 0.02°C, $P < 0.0001$), while the difference between body and tail temperature was not significant (mean difference: 0.01 ± 0.02°C, $P = 0.70$).

3.2. Temperature differences among body parts

Nevertheless, we detected small but significant differences between head, body and tail ($F_{2,107.8} = 9.86$, $P = 0.0001$, Fig. 1, Fig. 2). Specifically, within individuals, head was slightly
warmer than both the body (Tukey’s post hoc: mean difference ± SE: 0.05 ± 0.02°C, \(P = 0.02\)) and the tail (mean difference: 0.07 ± 0.02°C, \(P < 0.0001\)), while the difference between body and tail temperature was not significant (mean difference: 0.01 ± 0.02°C, \(P = 0.70\)).

3.2. Equilibrium between \(T_b\)-\(T_a\) body temperature and air temperature

Keeping individuals in hand for 30 seconds determined an increase of \(T_b\) of 6-10°C. When animals were released, the difference between body temperature and air temperature (\(\Delta T^o\)) quickly decreased with time; after 15 minutes \(\Delta T^o\) was essentially zero (Fig. 3). The mixed model assuming exponential decrease showed much better fit than the one following an inverse power law (exponential model: AIC = 766.0; inverse power law model: AIC = 1044.9).

In the exponential model \(\Delta T^o = k + e^{ax+b}\) the parameter \(k\) was not significantly different from zero (Table 1), confirming that \(\Delta T^o\) quickly approaches zero. According to this model, \(\Delta T^o \leq 0.1°C\) after 8.2 minutes, i.e. body temperature reaches the equilibrium very quickly.

Salamanders with large body size required more time to reach thermal equilibrium. At the release, \(\Delta T^o\) was unrelated to weight of salamanders, but \(\Delta T^o\) decreased more quickly with time in small than in large salamanders. As a consequence, for a given time after release, \(\Delta T^o\) remained larger in the heaviest salamanders (Table 2, Fig. 4). For instance, 5 minutes after release small salamanders (weight < 1g) were essentially at the thermal equilibrium, while the largest salamanders showed a \(\Delta T^o\) of 2-3°C (Fig. 4b).

4. DISCUSSION

4.1. Thermoconformity of Hydromantes
Our results show that, in *Hydromantes* salamanders, body temperature is at equilibrium with environment temperature. Even though the thermal environment within caves may be heterogeneous, salamanders have extremely limited movements and may remain in the same cave sector for days (Lanza et al., 2006). If body temperature $T_b$ is at equilibrium, $T_b$ corresponds to the operative temperature $T_e$ of individuals (Bakken, 1992), which is the steady-state temperature organism would attain if placed indefinitely in a given environment (Kearney and Porter, 2009). As a consequence, in these salamanders important ecophysiological parameters, such as $T_b$ and $T_e$, can be easily estimated from air temperature, indicating that, at least for temperature, microhabitat features can be a good representation of operative conditions of individuals.

In several salamanders, it has been proposed that individuals can move within the thermal landscape to keep their body close to their preferred temperatures (Balogová and Gvoždík, 2015; Heath, 1975; Spotila, 1972). *Hydromantes* salamanders are not strictly cave-dwelling species, and can be active at the surface during cold, humid seasons (i.e. autumn and spring), but they have to move underground when outdoor conditions are too warm and dry, such as during Mediterranean summer. In summer, caves constitute a relatively continuous thermal gradient: cave sectors close to the surface have higher temperature, and temperature decreases in the deepest sectors (Lunghi et al., 2015). On the one hand, food is more abundant close to the surface (Ficetola et al., 2013; Lunghi et al., 2015), thus salamanders are restricted to a few tens of meters from the surface. On the other hand, given that salamanders are at thermal equilibrium (Fig. 3), they must remain in relatively deep sectors, where conditions are within the physiological tolerance limits of the species. The trade-off between these and other factors (e.g. humidity, not investigated by the present study) limits the distribution of salamanders to a narrow region of the underground space. The time needed to reach thermal equilibrium was slightly longer in salamanders with large body size (Fig. 4). These individuals might have slightly better opportunities to move toward unsuitable places for short time, and then coming back to more suitable areas. Large individuals can also have additional advantages, such as a better resistance to desiccation and to food shortage, and higher mobility. Actually, in the...
closely related *H. strinatii*, juvenile salamanders are restricted to a very narrow region (5-15 m from the surface), while adults can exploit a relatively broader region of the underground space, 5-30 m from the surface (Ficetola et al., 2013; Salvidio and Pastorino, 2002), suggesting that the improved tolerance of large individuals may allow them to exploit broader niches.

Body temperature showed regional differences along salamanders body, the head being slightly but significantly warmer (Figs 1 & 2). Until now, very limited information was available on regional differences of body temperature in salamanders. The high thermal resolution of thermocamera allowed to clearly detect the very small (about 0.05°C) temperature differences between the head and the rest of the body (Fig 1, Fig. 2). Plethodontid salamanders are sit-and-wait predators with a very low metabolism (Lanza et al., 2006). Even though they perform very limited movements, sensorial organs, such as eyes, Jacobson’s organ and buccal mucous are grouped in the head area (Lanza et al., 2006), and The activities of metabolism and sensory system probably adsorb most of the metabolism of these salamanders, which in turn likely produces a small amount of metabolic heat.

In conclusion, thermocamera offers a valuable tool for the study of thermal ecology of ectotherms. On the one hand, it allows to measure small temperature differences between body parts without manipulating individuals, thus enabling us to obtain new insights on the complexity of thermal properties of ectotherms. Furthermore, it is possible to quickly measure the major ecophysiological parameters of individuals in the wild, and to obtain data that can be used to better identify the thermal requirements of species, thus helping the characterization of species niches and improving our understanding of species responses to environmental variation.

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References


Vitae

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I'm a research scientist at the Alpine Ecology lab in France, with a multidisciplinary background in both modelling and genetics. I try to combine these two approaches to better understand how environmental systems change and which factors determine their modifications. My current research activities include: 1) Studying the factors that determine the distribution of amphibians and reptiles 2) Evaluating how evolutionary processes can be integrated into macroecological analyses; 3) Improving our understanding of biological invasions 4) Identifying strategies for amphibian conservation and 5) Using environmental DNA for the study of impact of human activities on biodiversity.
Table 1 Results of the exponential non-linear mixed effect model $\Delta T^\circ = k + e^{ax+b}$, evaluating the decrease of difference between body temperature and air temperature with time.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$B$</th>
<th>SE</th>
<th>$F$</th>
<th>d.f.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>-0.48</td>
<td>0.04</td>
<td>301.8</td>
<td>1.215</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$b$</td>
<td>2.11</td>
<td>0.02</td>
<td>19604.3</td>
<td>1.215</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$k$</td>
<td>-0.06</td>
<td>0.10</td>
<td>0.3</td>
<td>1.215</td>
<td>0.561</td>
</tr>
</tbody>
</table>
Table 2. Relationships between salamander weight and $\Delta T^\circ$, at specific moments after release. Sample size was not identical in the six test periods.

<table>
<thead>
<tr>
<th>Time</th>
<th>$B$</th>
<th>SE</th>
<th>$F$</th>
<th>d.f.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>At release</td>
<td>-0.05</td>
<td>0.20</td>
<td>0.1</td>
<td>1,23</td>
<td>0.810</td>
</tr>
<tr>
<td>1 min after release</td>
<td>0.52</td>
<td>0.17</td>
<td>9.4</td>
<td>1,47</td>
<td>0.004</td>
</tr>
<tr>
<td>2 min after release</td>
<td>0.81</td>
<td>0.12</td>
<td>42.4</td>
<td>1,44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3 min after release</td>
<td>0.77</td>
<td>0.12</td>
<td>40.0</td>
<td>1,33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4 min after release</td>
<td>0.55</td>
<td>0.12</td>
<td>22.8</td>
<td>1,33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5 min after release</td>
<td>0.44</td>
<td>0.11</td>
<td>15.7</td>
<td>1,41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15 min after release</td>
<td>0.04</td>
<td>0.02</td>
<td>5.7</td>
<td>1,36</td>
<td>0.022</td>
</tr>
</tbody>
</table>