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Thermoregulatory strategies in an aquatic ectotherm from thermally-constrained habitats: An evaluation of current approaches

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ABSTRACT

Many ectotherms employ diverse behavioral adjustments to effectively buffer the spatio-temporal variation in environmental temperatures, whereas others remain passive to thermal heterogeneity. Thermoregulatory studies are frequently performed on species living in thermally benign habitats, which complicate understanding of the thermoregulation–thermoconformity continuum. The need for new empirical data from ectotherms exposed to thermally challenging conditions requires the evaluation of available methods for quantifying thermoregulatory strategies. We evaluated the applicability of various thermoregulatory indices using fire salamander larvae, *Salamandra salamandra*, in two aquatic habitats, a forest pool and well, as examples of disparate thermally-constrained environments. Water temperatures in the well were lower and less variable than in the pool. Thermal conditions prevented larvae from reaching their preferred body temperature range in both water bodies. In contrast to their thermoregulatory abilities examined in a laboratory thermal gradient, field body temperatures only matched the mean and range of operative temperatures, showing thermal passivity of larvae in both habitats. Despite apparent thermoconformity, thermoregulatory indices indicated various strategies from active thermoregulation, to thermoconformity, and even thermal evasion, which revealed their limited applicability under thermally-constrained conditions. Salamander larvae abandoned behavioral thermoregulation despite varying opportunities to increase their body temperature above average water temperatures. Thermoconformity represents a favored strategy in these ectotherms living in more thermally-constrained environments than those examined in previous thermoregulatory studies. To understand thermal ecology and its impact on population dynamics, the quantification of thermoregulatory strategies of ectotherms in thermally-constrained habitats requires the careful choice of an appropriate method to avoid misleading results.

1. Introduction

The body temperature of ectothermic organisms is primarily determined by the heat obtained from the surrounding habitat, and thus many ectotherms are not passive to the thermal heterogeneity of environment. They maintain their body temperatures (T_b) higher than the mean and within a narrower range than operative temperatures (T_e) by behavioral means (Adolph, 1990; Hertz, 1992; Bauwens et al., 1996), providing them with a substantial fitness advantage (Gilchrist, 1995). Recently, behavioral thermoregulation is receiving renewed attention, because it allows ectotherms to cope with the pitfalls of climate change (Kearney et al., 2009; Huey et al., 2012; Sunday et al., 2014). The thermoregulatory effort varies substantially among species however, within a continuum between thermoconformity and effective thermoregulation (Hertz et al., 1993; Christian and Weavers, 1996), which complicates predictions about the impact of climate change on their populations (Sunday et al., 2014). Despite its obvious ecological relevance, determinants of thermoregulatory strategies are not fully understood.

The source of variation in thermoregulatory strategies among species may stem not only from ecological factors but also from methods of their quantification. Since 1993, thermoregulatory strategies of ectotherms have become quantified using thermoregulatory indices (Hertz et al., 1993). However, if the thermal conditions of the habitat prevent the attainment of the

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ectotherms' target temperatures, the index values reflect not only thermoregulatory effort but also the presence of thermal constraints. Following this reasoning, Herczeg et al. (2006) proposed calculations of thermoregulatory indices as deviations from the maximum attainable T_e rather than from the T_p range. Despite sound rationale for its use (Angilletta, 2009), the field evaluation of this approach is missing.

In this study, we examined the applicability of various thermoregulatory indices using behavioral thermoregulation in salamander larvae as a suitable model system. We found a population of fire salamanders with their larvae developing in two water bodies with contrasting thermal conditions, i.e. a forest pool and well, located only 20 m apart. Because water is mixed by the bottom spring in the well, its temperatures are lower and less heterogeneous than in the pool (K. Piasečná and L. Gvoždík, unpublished data). Both habitats lacked amphibian competitors and predaceous insects, which suggests that their potential thermoregulatory behavior was little affected by biotic interactions (Gvoždík et al., 2013). Accordingly, we predicted that if thermal conditions permit larvae to attain their T_p, the larvae would thermoregulate. However, if thermal conditions prevent them attaining their target range (i.e., if there are thermal constraints) then the cost(s) of thermoregulation could be too high relative to the benefit(s) for growing larvae, and thus they would become thermoconformers. Hence, this system provides an excellent opportunity to evaluate the applicability of thermoregulatory indices in ectotherms exposed to thermally-constrained conditions.

2. Materials and methods

2.1. Study organisms

Fire salamanders, *Salamandra salamandra*, are approximately 20 cm long tailed amphibians distributed across Western, Central, and Southern Europe. In Central Europe, larviparous females lay up to 40 larvae into water in May (Kopp and Baur, 2000). They usually prefer clean flowing waters such as creeks and springs for laying, but occasionally use still water bodies (Thiesmeier and Grossenbacher, 2004). Bottom-dwelling larvae prey on various aquatic invertebrates. Larval development lasts 6–12 months, which largely depends on thermal conditions in a given habitat (Zakrzewski, 1987). In Central Europe, larvae often overwinter in water and finish their metamorphosis during the next spring.

2.2. Study site

The study was carried out in two water bodies located on a birch-forested slope near Hukvaldy, Czech Republic (49°37′44″N, 18°13′25″E; 350 m). The forest pool (7.4×1.7 m^2) and the well (1.2×0.8 m^2) were oval-shaped and had the same maximum depth of 20 cm. Both water bodies were equally shaded by surrounding trees. In addition to the salamander larvae, two invertebrate taxa were abundant in both water bodies, amphipod crustaceans (*Gammarus* sp.) and caddisfly larvae (*Chaetopteryx* sp.).

2.3. Thermal preferences

To test thermal preferences and to estimate preferred body temperatures, 36 salamander larvae (total length [mean ± SD] = 43.9 ± 2.7 mm from pool and 40.4 ± 5.2 mm from well) from each habitat were captured and transported to the laboratory in May 2011. Larvae were transported in plastic bags (two larvae per 1 l water) inside Styrofoam boxes (10–15 °C) for a five hour drive. In the laboratory, larvae were placed individually in aquaria ($40 \times 26 \times 18$ cm^3) with 5 l of well (non-chlorinated) water at 9–11 °C, which were the most frequent water temperatures in their natural habitat in May 2010 (K. Piasečná and L. Gvoždík, unpublished data). Aquaria were equally equipped with five dry birch leaves, some water vegetation (*Egeria densa*), and an aeration stone. Larvae were fed with live *Chironomus* larvae and *Tubifex* worms at three day intervals. Water (50% of total volume) was regularly changed at three-day intervals. Larvae were subjected to laboratory conditions at least one week prior to the beginning of thermal preference trials.

Table 1
Definitions of acronyms used in this paper.

Acronym	Definition
T_b	Field body temperatures.
T_e	Operative temperatures; temperatures of a physical model with the same thermal characteristics as a study organism. For small aquatic organisms, operative temperatures equal water temperatures.
T_p	Preferred body temperatures; body temperatures that an ectotherm maintains in the laboratory thermal gradient under the absence of abiotic and biotic limitations.
d_e	Index of thermal quality of habitat; the mean absolute deviation of T_e from the T_p range.
$d_{e'}$	Thermally-constrained version of d_e; the mean deviation of T_e from the maximum T_e at a given time.
d_b	Index of accuracy of thermoregulation (originally the accuracy of T_b); the mean absolute deviation of T_b from the T_p range.
d_b'	Thermally-constrained version of d_b; the mean deviation of T_b from the maximum T_e at a given time.
E	Index of effectiveness of thermoregulation; $E=1-(d_b/d_e)$.
E'	Thermally-constrained version of E; $E'=1-(d_b'/d_e')$.
E_{diff}	Alternative measure of E; $E_{diff}=d_e-d_b$.
E_{diff}'	Thermally-constrained version of E_{diff}; $E_{diff}'=d_e'-d_b'$.

Thermal preferences were tested in a stainless steel tank ($240 \times 60 \times 60$ cm^3 high) divided into nine longitudinal lanes (7×240 cm^2) using solid partitions. The tank bottom was equipped with computer-controlled Peltier modules and a heat-exchange unit to maintain various thermal profiles along the tank (see Gvoždík (2003) for further details). The tank was filled with water to a depth of 2 cm. To test thermal preferences, either a thermal gradient (7–28 °C) or constant temperature (10 °C) was maintained in the tank. The tank was located in a room at 18 ± 2 °C. Illumination was provided with fluorescent tubes (300 lx) during the day (0600–1800). The water was completely changed after each trial.

Because wild-caught larvae contained food in their stomachs, examined larvae were fed 24 h prior to trials. For a thermal preference trial of the experimental group in a thermal gradient (7–28 °C), we randomly selected (without replacement) nine salamander larvae and placed them individually into the 10 °C section of the lane 14 h before the trial (1800). To estimate thermal preferences and preferred body temperatures, every two hours, we carefully (to not disturb the animals) measured the larval horizontal position along the length of the tank (to 5 cm) and water temperature (to 0.1 °C) exactly where it was positioned using a thermocouple probe connected to a digital thermometer (HH 22; Omega Engineering, Stamford, USA). Given the small size of experimental animals and high thermal conductivity and heat capacity of water, we assumed that body temperatures of larvae matched the temperature of the surrounding water (Lutterschmidt and Hutchison, 1997). According to preliminary observations, the presence of experimenter during temperature measurements has a negligible influence on larval motor activity patterns. For each individual, we used the mean and boundaries (minimum and maximum) of preferred body temperatures (T_p) measured over 10 h (0800–1800), i.e. the same time period as in the field (see below). Previous analyses (Smolinský and Gvoždík, 2009)