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Seasonal Acclimation of Preferred Body Temperatures Improves the Opportunity for Thermoregulation in Newts

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ABSTRACT

Seasonal acclimation and thermoregulation represent major components of complex thermal strategies by which ectotherms cope with the heterogeneity of their thermal environment. Some ectotherms possess the acclimatory capacity to shift seasonally their thermoregulatory behavior, but the frequent use of constant acclimation temperatures during experiments and the lack of information about thermal heterogeneity in the field obscures the ecological relevance of this plastic response. We examined the experimentally induced seasonal acclimation of preferred body temperatures ($T_p$) in alpine newts Ichthyosaura (formerly Triturus) alpestris subjected to a gradual increase in acclimation temperature from 5°C during the winter to a constant 15°C or diel fluctuations between 10°C and 20°C during the spring/summer. Both the mean and range of $T_p$ followed the increase in mean acclimation temperature without the influence of diel temperature fluctuations. The direction and magnitude of this acclimatory capacity has the potential to increase the time window available for thermoregulation. Although thermoregulation and thermal acclimation are often considered as separate but coadapted adjustments to thermal heterogeneity, their combined response is employed by newts to tackle seasonal variation in a thermoregulatory-challenging aquatic environment.

Introduction

Ectotherms cope with heterogeneity of the thermal environment by complex responses that involve thermoregulatory behavior, thermal acclimation, or thermal sensitivity of performance and life-history traits. The recent coadaptational theory of thermal biology (Angilletta et al. 2006) sheds more light on the puzzling combinations of behavioral, physiological, and life-history compensations. The center of this coadaptational thinking becomes the concept of thermoregulatory costs and benefits (Huey and Slatkin 1976). As costs of thermoregulation (i.e., energy, time, or predation risk) increase, this model predicts the relaxed thermoregulatory precision or thermoconformity that favors thermal generalization either through the fixed or plastic (acclimatory) change of thermal sensitivity. This response, in turn, alters the benefit received from maintaining a particular body temperature. Hence, studies on thermoregulatory behavior provide a key for understanding the evolution of thermal physiology in ectotherms (Bogert 1949; Huey et al. 2003; Angilletta et al. 2006; Marais and Chown 2008; Angilletta 2009).

Under the absence of thermoregulatory costs in laboratory thermal gradients, ectotherms often maintain their body temperatures within a narrower range than that of available operative temperatures ($T_e$). These preferred body temperatures ($T_p$) provide an estimate of target body temperatures that an ectotherm aims to achieve in the field and thus represent an important measure for the quantification of behavioral thermoregulation (Hertz et al. 1993). Nevertheless, many ectotherms, especially those living in thermoregulatory-challenging environments, possess the capacity to reversibly modify $T_p$ in response to seasonal change in body temperatures (reviewed by Hutchison andDupré 1992; Johnson and Kelsch 1998). Although the thermal acclimation of $T_p$ has received much attention, its contribution to thermoregulatory strategies in the field remains unclear for several reasons. Most studies focused on the modification of the mean value of $T_p$ rather than of the $T_p$ range, though the position of range limits, not of the mean (relative to a given distribution of operative temperatures [$T_e$]), affects the thermal habitat quality and accordingly ectotherm’s thermoregulatory strategy (Hertz et al. 1993). Acclimation studies on $T_p$ were often realized under constant temperature treatments (but see Lillywhite 1971; Podrabsky et al. 2008), though in ectotherms exposed to diel temperature fluctuations, the acclimatory response is sometimes triggered by diel cycles rather than by the shift in mean body temperature (Šámajová and Gvoždík 2010). In addition, stressful (i.e., constant and/or too high) acclimation temperatures may induce only the compensatory response of $T_p$ (Licht 1968), and thus miss the adaptive significance of $T_p$ acclimation under prevailing thermal conditions in the field. Finally, the significance of $T_p$ acclimation for thermoregulation in the field remains unexplored.

In this study, we examined the experimentally induced sea-
sonal (hereinafter “seasonal”) acclimation of $T_p$ in alpine newts Ichthyosaura (formerly Triturus) alpestris exposed to both constant and fluctuating acclimation temperatures. Newts seem especially suitable for this task, because at least three facts indicate the presence of reversible thermal acclimation of $T_p$ in this model system. First, field water temperatures vary considerably throughout the years (Dvůršák and Gvozďík 2010), and the relatively long-lived news (mean generation time 3–4 yr; Mlau et al. 2000) are presumably exposed to higher, rather than among-generation variation in $T_p$. Second, both seasonal trends in mean water temperatures and the magnitude of diel temperature fluctuations in their natural habitats are highly predictable (Měrák and Gvozďík 2009; Dvůršák and Gvozďík 2010). Finally, European newts generally show distinct thermoregulatory behavior in aquatic thermal gradients (Gvozďík 2003, 2005), though the opportunity for thermoregulation seems restricted in the field (Smolinský and Gvozďík 2009).

The available theory provides no testable predictions about the direction and magnitude of $T_p$ acclimation under seasonal change in body temperatures. However, assuming the parallel acclimation shift of both mean and range of $T_p$ with optimal temperature and the breadth of thermal performance curves, respectively (Glanville and Seebacher 2006), the seasonal shift in mean body temperature should favor the acclimation of mean $T_p$, and the plasticity of the $T_p$ range should be advantageous under variation in diel body temperature fluctuations (see also Gabriel 1999). Hence, we predict that the increase in mean acclimation temperature will induce the acclimatory response in mean $T_p$ and diel thermal fluctuations affecting the $T_p$ range. Because both $T_p$ characteristics are potentially sensitive to the variation in locomotor activity (Hutchison and Spriestersbach 1986), we provided detailed analysis of the association between thermoregulation and activity. In addition, we examined whether the acclimatory response has the potential to increase the proportion of daytime that allows thermoregulation (opportunity of thermoregulation) in the field.

Material and Methods

Study Species and Maintenance

The alpine newt Ichthyosaura alpestris is a medium-sized species (up to 12 cm total length) widespread at middle to high altitudes across most of western and central Europe (Griffiths 1996). It typically has a biphasic lifestyle with a reproductive aquatic period (April to June in the Czech Republic) and terrestrial periods during the rest of the year. In some populations, newts stay in water during the whole year. Newts reproduce in a variety of lentic waters, from shallow temporary pools to mountain lakes. The female oviposition period lasts several weeks. Newts feed on a variety of invertebrate prey, mostly crustaceans and chironomid larvae.

To enable comparison with a previous study conducted on thermal acclimation of locomotor performance (Šamajová and Gvozďík 2010), we strictly followed the same protocol for both general maintenance and temperature treatments. Adult newts (males: mean snout-vent length $[\text{SVL}] = 41.5 \pm 0.6$ mm, $n = 12$; females: mean SVL = 50.7 ± 0.7 mm, $n = 12$) were captured from a population near Jihlava, Czech Republic, in April 2008. Pairs of news (one male and one female) were placed in aquariums ($50 \times 30 \times 18$ cm high) filled with 15 L of tap water. Each aquarium was equipped with aquatic plants (Vesicularia dubayana and Egeria densa) and a piece of Styrofoam to enable newt emergence from the water. Aquariums were placed in an air-conditioned room kept at 20°–16°C with a natural light : dark regime for 6 mo in order to reduce the influence of previous thermal history on $T_p$. After a gradual decrease ($2\text{C}/\text{wk}$) of water temperature to $5\text{C}$ (cold treatment [CT]), newts wintered for 5 mo (until April 2009) under semiaquatic conditions, that is, 5 L of water and a plethora of aquatic plants. With the exception of the wintering period, newts were fed with live chironomid larvae and Tubifex worms twice per week. The water was changed (50% of volume) on a weekly basis. After completion of the experiment (August 2009), newts were released at the site of their capture.

Temperature Treatments

In April 2009, all tanks were provided with both aquarium heaters (50 W; Eheim/Jäger, Wüstenrot, Germany) connected to precise control thermometers ($\pm 0.2\text{C}$; Vertex 280, Exatherm, Jablonec, Czech Republic) and aerations stones for water mixing to gradually increase the water temperature ($2\text{C}/\text{wk}$) in the aquariums from CT ($5\text{C}$) to $15\text{C}$. Afterward, newts were randomly assigned to two groups ($n = 12; 1 : 1$ sex ratio). The first group remained at $15\text{C}$ (constant warm treatment [CWT]), whereas the second group was subjected to diel fluctuations in water temperatures between $10\text{C}$ and $20\text{C}$ (fluctuating warm treatment [FWT]; mean = 14.5° ± 0.07°C) for the following 6 wk (Fig. A1 in the online edition of Physiological and Biochemical Zoology). Hence, each group was subjected to unique treatment combinations, CT-CWT and CT-FWT. Because adult newts frequently move across a water column (Šamajová and Gvozďík 2009), temperature regimes were chosen according to modal bottom ($\text{mean} = 13.8° \pm 0.3\text{C}$) and surface ($\text{mean} = 15.5° \pm 0.6\text{C}$; Dvůršák and Gvozďík 2010) temperatures and diel temperature fluctuations ($\approx 10\text{C}$) in sun-exposed pools (Měráká and Gvozďík 2009) measured during 2005–2008. Hourly water temperatures were recorded in four randomly selected aquariums (two per treatment) using thermocouple probes connected to data loggers (resolution 0.1°C; HOBO, Onset Computer, Bourne, MA). During measurements of $T_p$ (2 wk), newts were fed at the same frequency but with a limited amount of food (≈0.5% of body mass) to minimize the postrandial shift of $T_p$ (Gvozďík 2003). Other conditions remained the same as the previous year.

Temperature Preferences

We recorded newt thermoregulatory behavior in a stainless steel tank ($240 \times 60 \times 60$ cm high; see detailed description in Gvozďík 2003). The tank was longitudinally divided into three lanes. Each lane consisted of 12 partially separated compart-
ments of equal size with different water temperatures (5°C–32.5°C) in steps of 2.5°C. The tank was filled with water to a depth of 4 cm. Water was intensively aerated in all compartments to prevent the establishment of an oxygen gradient. The tank was placed in a room at 18°C ± 2°C. Illumination was provided with fluorescent tubes (300 lx) during the day (0600–1800 hours) and with two red bulbs (<5 lx) during the night. The tank area was continuously monitored (12 frames/s) using a wide-angle camera (MTV-63S80H-A-ICR-R, Mintron, Taipei, Taiwan) connected to a PC surveillance system (V-Guard RT4, Chateau, Taipei, Taiwan).

To estimate temperature preferences, we placed a fasted newt in a separate lane of the tank and left it undisturbed for 12 h for habituation to the gradient conditions. After that time, newt behavior was recorded for another 24 h. All video files were later analyzed by one person (M.H.) to record the position of newts in the gradient at 10-min intervals. If the newt left the water during the interval (<2% of all observations), the record was omitted. The position values were transformed into temperatures using the calibrated values from each compartment. Because newt body temperatures closely approach those of the surrounding water (Licht and Brown 1967), we considered this method a reliable estimate of newt body temperatures. Both modal and mean \( T_p \) were closely related in this study \( (r = 0.76–0.90 \text{ in all treatments}) \), and thus we used the mean of individual \( T_p \) distribution \( (n_{\text{measurements}} = 168) \) as the measure of central tendency. We determined the \( T_p \) range by calculating the central 80% of body temperatures for each individual to eliminate possible temperature outliers due to occasional swimming into compartments with extreme high or low temperatures. Accordingly, the tenth and ninetieth percentiles of the individual \( T_p \) distribution were referred to as lower (LBT \( p \)) and upper (UBT \( p \)) bound of the \( T_p \) range, respectively. Based on another study where both newt spatial distribution in the thermal gradient clearly differed from the distribution under constant water temperatures (V. Marek and L. Gvozdik, unpublished data) and their locomotion at 5°C was not reduced significantly enough for them to be trapped at the cool end of the gradient (Anderson et al. 2007), we assumed that the method provided unbiased estimates of newt \( T_p \). To test for changes in locomotor activity between treatments, the index of locomotor activity was calculated as the mean number of crosses between compartments per 10 min (Gvozdik 2003). To examine the seasonal acclimation response in \( T_p \), each newt was measured twice, that is, in March and June. All experimental procedures were both approved by the Expert Committee for Animal Conservation of the Institute of Vertebrate Biology AS CR (research protocol 44/2005) and complied with the current laws of the Czech Republic.

**Opportunity for Thermoregulation**

We estimated the opportunity for thermoregulation as the proportion of total time that an individual could spend while keeping its body temperatures within the \( T_p \) range (Christian and Weavers 1996; but see Angilletta 2009 for different meaning). The minimum and maximum \( T_p \) values in temporary pools were obtained from a previous study (Dvořák and Gvoždík 2010). Because of low thermal inertia of newts in water (Licht and Brown 1967), we assumed that newt \( T_p \) matched temperatures of the surrounding medium. For the purpose of this study, we used time series of hourly bottom and surface temperatures from two pools (maximal depth 80 and 15 cm) measured during June 2005 and 2007. Selected pools represent the extreme range of thermal conditions experienced by the newts in this population (Fig. 1).

**Statistical Analysis**

First, we visually checked sample distributions and association between \( T_p \) characteristics (mean, 80% range, lower and upper bound) to verify assumptions (i.e., normality, homoscedasticity, and the relationships between variables) for further analyses. The possible association between time series of \( T_p \) and locomotor activity was examined using cross-correlation analysis.
Figure 2. Diel course of temperature preferences and locomotor activity (left) with their cross-correlation coefficients (right) in alpine newts from various treatment combination groups in March and June. Values are means ± SE. Temperature values were fitted (white lines) with the polynomial regression model (up to third degree) with an autocorrelated error structure. Gray areas refer to dark periods of time. Dashed lines in correlograms depict 95% of confidence limits. CT-CWT, cold treatment (5°C) in March and a constant warm treatment (15°C) in June; CT-FWT, cold treatment in March and a fluctuating warm treatment (10°C–20°C) in June.

(Chaffield 1996). The relationship between \( T_p \) (treatment mean) and time (10-min intervals) over a period of 24 h was tested using a polynomial regression up to third-order (cubic) polynomial. Because \( T_p \) values in time series violate the independence assumption, we used the regression model with an autocorrelated error structure. The best model was selected according to the lowest value of the Akaike Information Criterion. We used the general linear mixed-effect model (Quinn...
and Keough 2002) to evaluate the effect of a seasonal temperature shift (i.e., from 5°C to 15°C or from 5°C to 10°C–20°C) on locomotor activity, Tp characteristics, and the relative proportion of Tp within the Tp range. Because the last variable showed large departures from normality and homoscedasticity, we transformed raw data to ranks before analyses. This model consisted of two fixed factors (treatment combination and time of season) and their interaction. The time factor tests for the seasonal acclimation in Tp, whereas the treatment × time interaction tests for the effect of constant acclimation temperature versus diel fluctuations. For this model, we discriminated between two temperature treatment combinations, CT-CWT and CT-FWT (see details in “Temperature Treatments”). Temperature preferences were measured twice (March and June) in the same newt; therefore, time of season represented a repeated-measures factor within individual identity that was added as a random grouping factor. A significance level of α = 0.05 was used for all statistical tests. All means are reported ± SE unless stated otherwise. Analyses were performed using Statistica 6.0 (Statsoft, Tulsa, OK) and JMP 5.0 (SAS Institute, Cary, NC) statistical packages.

Results

We obtained 6,380 positions of the 24 newts in the aquatic thermal gradient. Newts maintained their Tp (group means/10 min) on average between 14°C and 19°C during a 24-h period (Fig. 2). The temporal patterns of Tp varied within and among treatment combinations. In CT-CWT newts, the time dependence of Tp during both March and June measurements was best explained by quadratic terms. In CT-FWT newts, time had a significant influence on Tp, in June, when temporal variation in Tp during 24 h showed a cubic trend, but not in March. In June, CT-CWT newts were more active toward the warmer end of the gradient, which resulted in the positive association between the index of locomotor activity and their mean Tp (Fig. 2h). In other months/treatments, cross-correlation coefficients between activity and Tp attained only negative significant values. The mean index of locomotor activity over 24 h showed both minor variations between treatments (treatment combination: F1,22 = 0.01, P = 0.92; time of season: F1,18 = 0.67, P = 0.42) and low association of the mean and range values of Tp (r = −0.40 to 0.08, P > 0.05 in all treatments), suggesting its negligible confounding effect on examined Tp characteristics.

The mean and range of Tp markedly differed between both groups. CT-FWT newts preferred higher mean body temperatures (18.3°C ± 0.5°C) than CT-CWT newts (16.3°C ± 0.6°C) during both March and June (F1,22 = 6.72, P = 0.02). Variation in the Tp range showed the opposite result; that is, CT-FWT newts maintained their body temperatures within the narrow range (4.0°C ± 0.5°C vs. 5.8°C ± 0.5°C) and vice versa (F1,22 = 4.85, P = 0.04), although this trait, unlike mean Tp, was also affected by the seasonal increase in treatment temperature (F1,18 = 8.15, P = 0.01). These peculiar results stem from the fact that individual mean Tp values were positively associated with the Tp range during both measurement periods (Fig. 3). Hence, we added mean or range variables to the respective statistical model as the covariate to correct for the variation in individual preference strategies (high mean–narrow range vs. low mean–wide range) between groups. After this correction, all measured traits clearly showed the seasonal acclimation response (Fig. 4). The shift in treatment temperature increased both mean Tp (F1,17 = 4.72, P = 0.04) and the Tp range (F1,17 = 11.57, P = 0.003). The seasonal shift of the Tp range was achieved by a decrease in LBTp (F1,17 = 9.97, P = 0.006) and an increase in UBTp (F1,17 = 5.44, P = 0.03). In all models, group × time interactions yielded statistically nonsignificant results, which provided no support for the influence of diel fluctuations on the acclimation of Tp. Individual identity explained 3%–20% of total variance in all measured traits.

The daily range of Tp in both temporary pools enabled newts to thermoregulate at least during a part of the day (Fig. 1). In both pools, seasonal acclimation of the Tp range improved the opportunity for thermoregulation in June (Fig. 5). Warm-
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Figure 4. Characteristics (adjusted mean ± SE) of preferred body temperatures ($T_p$) in alpine newts exposed to seasonal shifts in acclimation temperatures from 5°C in March to 15°C or 10°C–20°C in June. Because of the negative association between $T_p$ characteristics (Fig. 3), range measures were corrected for the variation in mean $T_p$ and vice versa. See Figure 2 for legend abbreviations.

acclimated newts would potentially spend an additional 3%–9% of total time within their $T_p$ range than newts exposed to a water temperature of 5°C (2005: $F_{1,17} = 9.82, P = 0.006$; 2007: $F_{1,17} = 12.09, P = 0.003$).

Discussion

Assuming a tight relationship between thermoregulatory behavior and thermal physiology (Huey and Bennett 1987), we predicted that (1) $T_p$ will show a similar direction and magnitude of acclimatory capacity to seasonal change in the thermal environment as the optimal acclimatory response for thermal performance curves (Gabriel 1999, 2005) and (2) that the response will increase the opportunity for thermoregulation. Our results provided partial support for these predictions. In alpine newts, the seasonal increase in mean water temperature induced the shift in both the mean and range of $T_p$ but without significant influence of diel temperature fluctuations on both characteristics. In the field, the seasonal acclimation of the $T_p$ range provided more time available for thermoregulation in acclimated newts than in nonacclimated individuals.

Both diel temperature fluctuations and constant temperatures had similar influence on the acclimation of $T_p$ characteristics. This result was unexpected given that the constant and fluctuating thermal regimens induce disparate acclimatory responses at different biological levels (Williams and Somero 1996; Podrabsky and Somero 2004; Podrabsky et al. 2008). However, detailed examination of both $T_p$ and activity time series revealed some notable variation between constant and fluctuating treatments. First, newts from constant temperature treatment increased their locomotor activity in warmer temperatures, whereas newts from other treatments showed the opposite pattern. Because various functions and processes occasionally have different thermal optima within an organism (Van Damme et al. 1991; Dorcas et al. 1997), the modified activity pattern may reflect compensation of prolonged exposure to suboptimal constant temperature. Second, the comparison of the diel course of $T_p$ variation with fluctuations in field $T_e$ (Figs. 1, 2) showed better correspondence in newts from fluctuating regimes than from constant treatments. The clear disadvantage of disassociated diel fluctuations between $T_p$ and $T_e$ imply that the constant temperature regime in fact neg-
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Figure 5. The relative frequency of operative temperatures (\(T_e\)) within the range of preferred body temperatures (\(T_p\)) in two temporary pools occupied by alpine newts during June 2005 (a) and 2007 (b). Values are means \(\pm\) SE. See Figure 2 for legend abbreviations.

The seasonal acclimation of \(T_p\) range in newts increased the opportunity for their thermoregulation in the field. Given the notable variation in thermal conditions between temporary pools (Dvořák and Gvozdík 2010), the relative advantage of \(T_p\) acclimation strongly depends on environmental context. Obviously, the “thermoregulatory advantage” prevails in deeper water bodies located in more open sites during sunny weather, whereas the “thermoconformer advantage” predominates in shallow and deeply shaded pools. This suggests that the combined acclimatory-thermoregulatory response constitutes the best solution to cope with various thermal conditions in aquatic habitats used by newts for reproduction.

The magnitude of acclimatory response in \(T_p\), and thus its potential time savings, was relatively small. Both favorable \(T_e\) in June (Smolinsky and Gvozdík 2009) and rather limited acclimatory change of the \(T_p\) range per degree increase in acclimation temperature (0.14°C–0.17°C; range: 0.2°C–0.3°C) contributed to this result. The extent of acclimatory change falls well within the scope reported for other taxa (Feder and Pough 1975; Hutchison and Spriestersbach 1986; Hutchison and Dupert 1992; Berner and Bessay 2006), which suggests generally limited plasticity of \(T_p\) in amphibians. However, costs of thermoregulatory behavior involve not only time but also other currencies, such as energy, predation risk, or missed opportunity (Huey and Slatkin 1976; Polo et al. 2005; Angilletta 2009). Therefore, it seems likely that the partial acclimation response reduces costs of thermoregulation more than our results suggest.

The comparison of our results with the acclimation of locomotor capacity (Šamajová and Gvozdík 2010) revealed a rather complicated pattern of seasonal acclimation in alpine newts. While mean \(T_p\) increased only 1.4°C–1.7°C in response to the 10°C upward shift in acclimation temperature, the same seasonal temperature change induced modification of thermal sensitivity not at the optimal temperature of 26°C (Gvozdík and Van Damme 2008) but at 15°C in the swimming speed of the newts. These findings support the notion that understanding the adaptive significance of acclimation requires focus on separate traits rather than on the whole organism (Woods and Harrison 2001, 2002). Given the importance of plastic responses in phenotypic evolution (Price et al. 2003; Ghalambor et al. 2007), it seems likely that diverse acclimatory capacities of thermoregulation and thermal sensitivity also affect the co-adaptation of thermal biology traits. Recently, increased attention has been devoted to evolutionary mismatch between thermal preferences and the optimal temperatures of thermal performance curves (Martin and Huey 2008; Angilletta et al. 2010; Ashbury and Angilletta 2010). The different capacity, magnitude, and direction of thermal acclimation in both traits provide another not-yet-considered factor responsible for this pattern.

Behavioral thermoregulation has been often considered as an evolutionary inhibitor rather than a driver of thermal sensitivity and thermal acclimation (Bogert 1949; Huey et al. 2003; Marais and Chown 2008). On the other hand, many ectotherms living in a thermally restricted habitat possess the acclimatory capacity of thermoregulatory behavior, which presumably reduces its buffering role in the evolution of thermal physiology. However, the diverse acclimatory responses of \(T_p\) have often been obtained using an ecologically unrealistic abrupt change in constant temperatures, which obscures the interpretation of these findings. Our study showed not only that the acclimatory response in \(T_p\) was induced under ecologically more realistic seasonal and diel variations of \(T_e\) but also that the acclimated newts were better tuned to local thermal conditions than nonacclimated individuals. This stresses the need to perceive thermoregulation and thermal acclimation as concerted rather than gradual responses to thermal heterogeneity in ectotherms subjected to thermoregulatory-challenging conditions.
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