Inaccurate or disparate temperature cues? Seasonal acclimation of terrestrial and aquatic locomotor capacity in newts

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Summary

1. Many organisms respond to seasonal temperature fluctuations by the reversible modification of whole-animal performance. Semiaquatic ectotherms, which possess this acclimatory capacity in swimming speed, lack the plastic response in terrestrial locomotor performance and vice versa. Theory predicts that the presence of reversible (seasonal) thermal acclimation or fixed phenotypes depends on the predictability of future thermal conditions (i.e. accuracy of temperature cues) in a given environment. Alternatively, comparative data suggest that thermal acclimation is induced by disparate temperature cues in water and on land.

2. We tested both predictions by examining the seasonal acclimation response in thermal sensitivity of maximal swimming and running speed in adult alpine newts, *Ichthyosaura* (formerly *Triturus*) *alpestris*.

3. Following the seasonal variation in environmental temperatures, we exposed newts to 5 °C from November to March and, after a gradual temperature increase, to either a constant (15 °C) or fluctuating (10–20 °C) thermal regime from May to June. At the end of each treatment, we measured newt swimming and running capacity at five temperatures (range 5–25 °C). In the field, hourly temperatures were recorded in various aquatic and terrestrial microhabitats to obtain information about the predictability of thermal conditions in both environments.

4. Seasonal acclimation shaped the thermal sensitivity of swimming speed under both constant and fluctuating temperature treatments. Thermal sensitivity of running speed was markedly modified by a fluctuating thermal regime so that newts ran at the highest test temperature faster than cold-acclimated individuals. Natural thermal environment contained a similar proportion of predictable variation in water and on land.

5. Complex seasonal acclimation of locomotor capacity in newts was influenced by the disparate thermal cues, i.e. mean acclimation temperature or diel temperature fluctuations, rather than by the different accuracy of these cues in water and on land. Future confrontations of theory with empirical data will require more attention not only on the assumptions of adaptive thermal acclimation but also on the ecologically relevant thermal conditions during acclimation experiments.

Key-words: acclimation, amphibians, environmental predictability, running, swimming, thermal plasticity, thermal sensitivity, *Triturus*

Introduction

Many ectotherms possess the capacity to modify thermal sensitivity of functional traits in response to the seasonal shift in environmental temperatures. The significance of these plastic responses, termed as reversible thermal acclimation (Angilletta 2009), for adaptation to local conditions has intrigued evolutionary biologists since Darwin (Darwin 1859; Levins 1968; Kingsolver & Huey 1998; Gabriel 2005; Angilletta 2009). The recent theory predicts that reversible plastic phenotypes with short response delay should have a selective advantage over fixed strategies under predictable variation in thermal environment (Gabriel 2005). Unfortunately, though acclimatory ability is not ubiquitous among ectotherms (Else & Bennett 1987; Temple & Johnston 1998; Wilson & Franklin 2000), most empirical studies on thermal acclimation lack information about the reliability of thermal cues predicting future thermal conditions (but see...
Deere & Chown 2006 for an exception). To understand why the acclimatory capacity evolved in some thermal environments but not in others, clearly requires a stronger focus on assumptions underlying the occurrence and adaptive significance of thermal acclimation (Kingsolver & Huey 1998; Angilletta et al. 2006; Angilletta 2009).

Various proximate and ultimate factors other than the inaccuracy of thermal cues, can explain the absence of acclimatory capacity. Generally, both low additive genetic variation and the presence of genetic co-variation limit the evolution of phenotypic plasticity (Scheiner 1993). At the individual level, the plastic response involves costs associated with the loss of energy and time (Hoffmann 1995; DeWitt, Sih & Wilson 1998; Gabriel 2005), which diminish the benefit of thermal acclimation under certain conditions. An alternative explanation stems from the fact that most acclimation experiments were conducted under arbitrary chosen constant temperatures without respect to the natural temporal variation in environmental temperatures. The prolonged exposure to constant temperature or diel temperature fluctuations produces disparate acclimatory responses ranging from gene expression patterns (Podrabsky & Somero 2004) and membrane order (Williams & Somero 1996) to thermal sensitivity of swimming capacity (Měráková & Gvoždík 2009), and thus many results may represent methodological artefacts rather than biological reality. The extent to which results of acclimation studies are influenced by organismal/environmental properties as well as the design of acclimation experiments remains unclear.

In this present study, we examined seasonal thermal acclimation in adult alpine newts, Ichthyosaura (formerly Triturus) alpestris (Laurenti, 1768). Newts, like most amphibians, live intermitently in water and on land, which differently shapes their thermal performance curves for aquatic and terrestrial locomotor capacity (Gvoždík & Van Damme 2008). During their aquatic phase, newts have to frequently cross a vertical thermal gradient to replenish their oxygen stores (Šamajová & Gvoždík 2009), while they are both secretive and nocturnal on land. This fact together with low thermal inertia of newt bodies in water (Licht & Brown 1967) may influence both the total amount and the predictable proportion of variation in body temperatures, and consequently the adaptive significance of seasonal thermal acclimation in both environments (Angilletta et al. 2006). Indeed, the prolonged exposure of other newt species to warmer temperatures modifies their thermal sensitivity of maximal locomotor performance on land but not in water (Gvoždík, Puky & Šugerková 2007). On the other hand, some cold-exposed amphibians acclimated thermal sensitivities of aquatic locomotion but not those of terrestrial locomotion (Marvin 2003a,b). Such contradictory findings may reflect different thermal cues for the acclimation of aquatic and terrestrial performance, rather than disparate thermal properties in both environments (Gvoždík, Puky & Šugerková 2007). Finally, previous acclimation studies of locomotor performance in adult amphibians were carried out using constant-temperature treatments, which may further bias the general understanding of this phenomenon (see earlier references).

The aim of this study was to examine whether the different capacities to modify thermal sensitivities of aquatic and terrestrial locomotor performances stem from the disparate thermal properties in water and on land or from various temperature cues for the induction of acclimatory response in both environments. To fulfil this task, we quantified seasonal acclimation responses of the thermal sensitivity of swimming and running capacity in adult newts exposed to both constant and daily fluctuating temperatures. In addition, we simultaneously monitored temperatures in their aquatic and terrestrial habitats to provide information about temporal variation and the predictability of thermal conditions in water and on land.

Materials and methods

STUDY SPECIES AND MAINTENANCE

The alpine newt, I. alpestris (Fig. 1), is a medium-sized newt reaching a total length of up to 12 cm. Its distribution covers middle and high altitudes from western and central Europe to the Balkans (Griffiths 1996). Due to their biphasic life cycle, newts usually spend three to 4 months in water (April to July in the central Europe) and the rest of the season on land. Facultatively, they can stay in the aquatic phase throughout the whole season. Newts reproduce in a variety of standing waters, from lakes to temporary pools. Their diet consists of various invertebrates, mainly of chironomid larvae. The ecological significance of newt locomotor capacity is unknown. However, when disturbed or touched on the tip of their tail, newts respond with fast locomotion, which suggest that locomotor speed plays an important role in their escape from predators, as in other salamanders (Brodie, Dowdey & Anthony 1989; Dowdey & Brodie 1989).

For newt capture and maintenance, we followed the previously published approach by Dvořák & Gvoždík (2009). In short, we captured adult newts [males: mean snout-vent length (SVL) = 44.8 ± 0.7 mm, n = 12; females: mean SVL = 52.7 ± 0.8 mm, n = 12] from a population near Jihlava, Czech Republic, in April 2007. Pairs of newts (one male and one female) were placed in aquaria (50 × 30 × 18 cm high) filled with 15 L of tap water. Each cage was equipped with aquatic plants, Vesicularia dubyana and Egeria densa, and a piece of Styrofoam. Aquaria were placed in a room held at

Fig. 1. Alpine newt, Ichthyosaura alpestris.
16–20 °C with a natural light/dark regime for 6 months in order to reduce the influence of previous thermal history on measured traits. The temperature was then gradually decreased (by 2 °C per week) to 5 °C (cold treatment, CT). At this temperature, newts hibernated for 5 months (until April, 2008) under semiaquatic conditions, i.e. 5 L of water and a plethora of aquatic plants. With exception of the hibernation period, newts were fed with live chironomid larvae and *T. tabifex* worms twice per week. The water was changed on a weekly basis. After the experiment, newts were released at the site of their capture in July 2008.

**TEMPERATURE TREATMENTS**

After the first course of locomotion measurements (see below) in early April, we equipped all tanks with both aquarium heaters (50 W, Eheim Jäger, Wüstenrot, Germany) connected to precise control thermometers (Vertex 280, Exatherm, Jablonec n. N., Czech Republic) and aeration stones for water mixing to gradually increase the water temperature (by 2 °C per week) in the aquaria to 15 °C. Under natural conditions, the rate of weekly shift in water temperature broadly varies from −12 to 12 °C (J. Dvořák and L. Gvozdík, unpublished data), and thus chosen heating rates represent biologically realistic conditions. Afterwards, newts were randomly assigned to two groups (A and B) of equal size (*n* = 12; 1 : 1 sex ratio). Group A remained at a constant 15 °C (constant warm treatment, CWT), whereas group B experienced diel fluctuations between 10 °C and 20 °C (fluctuating warm treatment, FWT) for the following 6 weeks. Hence, newts in each group were subjected to two treatments, CT (November–April) and CWT or FWT (May–July). Temperature regimes were chosen according to modal bottom and surface temperatures (mean 144 ± 06 °C; Dvořák & Gvozdík 2010) and diel temperature fluctuations in sun-exposed pools (Méráková & Gvozdík 2009) measured during 2005 to 2008. Hourly water temperatures were recorded in four randomly selected aquaria (two per treatment) using thermocouple probes connected to temperature data loggers (resolution 0.1 °C; HOBO, Onset Computer, Bourne, MA, USA). Other conditions remained the same as the previous season (see above).

**LOCOMOTOR PERFORMANCE**

Before locomotor performance trials, each newt was transferred individually into a plastic (0.5 L) tub containing some water and left (30–60 min) in a walk-in climatic chamber until the water temperature equilibrated to experimental temperature (see below). To measure swimming speed, a randomly chosen newt was placed in the middle of a squared arena (60 × 60 × 10 cm³) filled with tap water to 2 cm. The arena was illuminated through the semi-transparent dish bottom. The newt was induced to move by tapping the tip of its tail with a soft paintbrush. Each newt was stimulated four times. Swimming bouts were recorded using a digital camera (frame frequency 50 Hz; Panasonic NV-GS500, Matsushita Electric Industrial, Osaka, Japan) mounted perpendicularly above the arena. The same arena and protocol was used to measure running speed, but water was replaced with moist filtration paper as the tracking surface. Locomotor trials were measured at 15, 25, 20, 10 and 5 °C in this temporal sequence. During the second run of measurements in July, newts were additionally retested at 15 °C to examine the possible influence of habituation, fatigue, etc. on locomotor performance over the course of experiments. Newts generally do not bask on land (Hutchison & Dupré 1992), therefore the range of experimental temperatures used encompass the regular temperatures experienced in the field (Fig. 2a,b). In each newt, we measured both locomotor performances at all experimental temperatures during both April and July measurements. No more than one trial per day was performed.

Video records were processed using motion analysis software (MaxTraq, Innovision Systems, Columbiaville, MI, USA). Each swimming trial was subjectively judged as good or bad. Bad trials (1%), e.g. swimming along the walls of arena, were discarded from further analyses. The maximal distance travelled by each newt during 0.02 s was used as an estimate of maximal swimming capacity at a

**Fig. 2.** Example of distribution and autocorrelations of environmental temperatures in aquatic (a, c) and terrestrial (b, d) microhabitats within and around a temporary pool [site (a) in Fig. 4] occupied by *I. alpestris* during April–July 2007.

given temperature. All locomotor capacity experiments (conducted in April and July) and their processing were carried out by one person (PS).

ENVIRONMENTAL TEMPERATURES

To obtain information about the availability of water temperatures in the wild, we followed a previously published protocol (Měráková & Gvoždík 2009). Briefly, we chose two pools (maximal depth 515 cm) occupied by newts from our study population. Four water-proofed (i.e. vacuum-sealed in plastic bags) dataloggers (resolution 0.5 °C; DS1921G-F5, Maxim Integrated Products, Sunnyvale, CA, USA) were used to characterize the range of available water temperatures in each pool during April–July, 2007. Two dataloggers were fixed to a piece of Styrofoam and anchored under the water surface approximately in the middle of the pool. The remaining two dataloggers were placed at maximum depth on the bottom. To obtain information about temperature variation on land we placed four dataloggers at two terrestrial microhabitats, i.e. full shade and 5 cm below ground in litter (see Jehle & Arntzen 2000), within the proximity of each pool. We deliberately omitted temperatures in sun-exposed microhabitats, because newts do not bask on land (Hutchison & Dupré 1992). Due to the non-detectable differences between temperatures recorded by dataloggers within a given microhabitat category, their pooled time series were used for further analyses. To evaluate the effect of evaporative cooling on datalogger temperatures, we compared the devices at 30 min intervals with those placed inside agar cylinders (6 × 15 cm; external temperature probe connected to HOBO datalogger, Onset Computer) during 24 h. Agar models have similar thermal properties as amphibian body (Navas & Araujo 2000), and thus they resembled newts placed in both terrestrial microhabitats. Datalogger temperatures were closely associated with the model temperatures recorded in both microhabitat categories (r = 0.88-0.98, mean difference 0.7-0.8 °C), which suggests a minor deviation of datalogger temperatures from newt operative temperatures in terrestrial environment. All dataloggers were calibrated against laboratory mercury thermometer before and after recording sessions.

STATISTICAL ANALYSIS

We used the general linear mixed-effect model to evaluate the effect of a seasonal temperature shift on thermal sensitivity of locomotor capacity in water and on land (Quinn & Keough 2002). To evaluate the seasonal shift in thermal sensitivity, two tests per locomotion mode were performed separately for the seasonal shift in acclimation temperature from CT to CWT and from CT to FWT. The comparison of data from CT and warm treatments within the same individual invalidates the assumption of the used model (i.e. the independence of data), therefore the CT always contained data from the other group (i.e. CT-A vs. FWT and CT-B vs. CWT). We considered experimental temperature as an ordinal categorical variable. Therefore, orthogonal polynomial contrasts were used for the analyses. This approach is advantageous over classical ANOVA, because it provides information not only about the effect of test temperature but also about the shape of the response. The model consisted of five fixed factors – acclimation temperature (Taccl), linear (L), quadratic (Q), cubic (C) and fourth order polynomial contrasts of test temperature (Ttest). The statistically significant L indicates the linear trend within the examined part of the thermal performance curve. Quadratic contrasts test whether performance at marginal Taccl is different from performance at central Taccl, whereas C test for the presence of a more complex pattern with two changes in direction, i.e. decreases and increases, of thermal performance curve. Results of the fourth order polynomial contrasts are difficult to interpret biologically, and were therefore not considered further. The estimation of thermal sensitivity required repeated measurements of locomotor performance on the same individuals, and thus individual identity was added as a random grouping factor. The statistical significance of random factors was tested using the likelihood ratio approach.

To evaluate the predictability of water temperatures, we used a recently published approach (Měráková & Gvoždík 2009) that divides the total variation in thermal time series between predictable (i.e. linear seasonal trend and diel fluctuations) and non-predictable components. We firstly examined autocorrelation patterns in time series during 168 h for the presence of trend, cyclic, and random variation (Chatfield 1996). After visual examination (Fig. 2c,d), we fitted temperature time series using the general linear model with an autoregressive error structure (Quinn & Keough 2002). The model consists of two parameters representing linear trend and diel cycles in time series data. The proportion of explained variance (PEV) by each factor was calculated as PEV = σ^2_factor/σ^2_total, where σ^2_factor is the variance explained by the factor, and σ^2_total is the total variance in a given time series. Due to large sample sizes, we considered the bias in the estimated variance of water temperatures due to time dependence as negligible (Brown & Rothery 1993). A significance level of α = 0.05 was used for all statistical tests. All means are reported ± 95% confidence intervals. Analyses were performed using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria).

Results

Thermal sensitivity of running speed was similar between CT-A and CT-B (P > 0.05 for all factors and their interactions), thus making them suitable controls for the seasonal acclimation testing. The running capacity showed the prominent relationship with Ttest [CT-A vs. FWT, Ttest(L): t88 = 3.42, P < 0.001; Ttest(Q): f88 = 2.16, P = 0.033; CT-B vs. CWT, Ttest(L): t88 = 7.64, P < 0.001]. The increase in acclimation temperature to a constant temperature of 15 °C showed no evidence of a shift in the thermal sensitivity of running speed (P > 0.05 for all interactions between Taccl and orthogonal contrasts of Ttest; Fig. 3a). In contrast, FWT induced an acclimatory response in thermal sensitivity by faster running speed at 25 °C, relative to the speed of newts exposed to 5 °C [Taccl × Ttest(L), f88 = 3.44, P < 0.001; post-hoc contrast; f88 = 2.17, P = 0.0033; Fig. 3b].

The swimming capacity of newts was strongly influenced by the Ttest in all treatments [CT-A vs. FWT, Ttest(L): t88 = 4.05, P < 0.001; Ttest(Q): f88 = 2.16, P = 0.033; CT-B vs. CWT, Ttest(L): f88 = 4.50, P < 0.001]. The thermal sensitivity of swimming speed showed a similar seasonal acclimation response in both constant and fluctuating temperatures (Fig. 3c,d). The interaction between Ttest(Q) and Taccl indicated that the plastic response affected the curvature of thermal performance curves (CT-B vs. CWT: t88 = 2.42, P = 0.018; CT-A vs. FWT: f88 = 2.28, P = 0.025). Specifically, CWT- and FWT-exposed newts demonstrated faster swimming speed at 15 °C at the expense of reduced speed at 5 °C (post-hoc contrasts; CT-A vs. FWT: t88 = 2.75,
Individual identity explained from 16 to 42% of total variance in both locomotor capacities ($P < 0.05$ in all cases). The association between the first and the last trial during the July course of experiments indicated good short term individual repeatability in both measured traits (swimming: $r = 0.51$, $P = 0.010$; running: $r = 0.56$, $P = 0.004$; $n = 24$).

To evaluate the predictability of environmental temperatures in water and on land, we analyzed 26,060 readings of hourly temperatures from two aquatic and two terrestrial microhabitats. Linear and cyclic factors (predictable components) explained from 76 to 86% of the total variation in the time series (Fig. 4; see Table S1 in Supporting Information). The difference in the proportion of predictable variation of temperature time series between water and land was statistically insignificant (80% in both environments; two sample $t$-test, $t_{6} = 0.125$, $P = 0.90$), suggesting that both environments similarly provide fairly predictable thermal conditions for newts.

**Discussion**

Theory predicts that the selective advantage of reversible acclimatory capacity hinges on the accuracy of cues predicting future thermal conditions (Gabriel 2005). In this study, environmental temperatures contained a similarly high amount of predictable variation in both aquatic and terrestrial microhabitats. Accordingly, the present results demonstrated that alpine newts possess the acclimatory capacity for both aquatic and terrestrial locomotor performance. However, newts modified thermal sensitivity of swimming speed in response to the shift in mean acclimation temperature, whereas the plastic response in running speed was more influenced by diel temperature fluctuations. These findings concur with
the hypothesis of diverse temperature cues inducing aquatic and terrestrial acclimation response (Gvoždík, Puky & Šugerková 2007).

In this present study, the shift in average acclimation temperature acted as a steady-state cue (Huey et al. 1999) for the induction of plastic response in swimming speed (i.e. environmental temperature has changed). Aquatic newts both frequently cross the vertical thermal gradient in still waters to replenish their oxygen stores during daytime (Šamajová & Gvoždík 2009) and are active in thermally-mixed water after sunset. Under these conditions, the acclimation response to intermediate rather than to marginal temperatures (daily minima and maxima) seems beneficial for them. In contrast, thermal acclimation of running speed was induced by the dynamic temperature cue, which ‘prepares’ organismal performance for the more extreme temperatures than current ones. A similar response has been reported in other ectotherms (Kaufmann & Bennett 1989; Huey et al. 1999; Deere & Chown 2006) including other newt species (Gvoždík, Puky & Šugerková 2007). Newts staying in sun-exposed pools consistently experience higher diel temperature fluctuations than newts breeding in shaded waters (Měráková & Gvoždík 2009). Accordingly, temperature fluctuations in water rather than average temperatures provide newts with reliable information about future thermal conditions on land, i.e. the presence of extreme temperatures, and thus enable newts to leave water with already adjusted running capacity for terrestrial thermal conditions. Hence, it seems that disparate thermal cues and acclimatory responses of swimming and running capacity are advantageous for newts during seasonal water-land transitions.

Why thermal acclimation improved running capacity only at extreme experimental temperatures remains unknown. Perhaps, because newts are relatively slow on land, they use escape only at body temperatures approaching thermal optimum for running capacity (26 °C; Gvoždík & Van Damme 2008), and rely on other antipredatory mechanisms (Kupfer & Teunis 2001) at lower temperatures. Another possibility is that warm-acclimated running capacity provides a higher chance of escape from overheated sites (Gvoždík, Puky & Šugerková 2007), which can be fatal for newts due to their low dehydration and thermal tolerance (Shoemaker et al. 1992; L. Gvoždík, unpublished data).

Acclimation temperature improved swimming capacity at a particular temperature but at the expense of lowered performance in other temperatures. If maximal swimming speed contributes proportionally to fitness, the acclimation response concurs with the beneficial acclimation hypothesis (Leroi, Bennett & Lenski 1994). However, this response modified the ascending part of the thermal performance curve without shifting its optimal temperature (26 °C; Gvoždík & Van Damme 2008), which is located about 10 °C above the local phenotypic optimum (i.e. daytime modal temperature; Dvořák & Gvoždík 2010). A recent review (Angilletta 2009) suggested that a similar pattern is quite common in diverse ectotherms (but see Glanville & Seebacher 2006). Whether selection favours acclimation of optimal temperature or thermal performance breadth depends on the relative response delay and the magnitude of seasonal variation in operative temperatures (Gabriel 1999). Unfortunately, information about acclimation rates of locomotor capacity or underlying enzymatic activity in amphibians remains scarce (Rogers, Seebacher & Thompson 2004), and thus future researchers should focus on the relative time lag of acclimation responses.

The almost symmetrically opposite shift of swimming speed between 5 and 15 °C suggests a minor contribution of the acclimation response to the estimation of parameters (i.e. breadth, height, thermal optimum) characterizing thermal performance curves (Huey & Stevenson 1979; Gilchrist 1996; Izem & Kingsolver 2005). Recent attention on thermal acclimation of the curve parameters (Deere & Chown 2006; Gvoždík, Puky & Šugerková 2007), missed this plastic response. Although the ‘parameter’ approach seems excellent for testing hypotheses about the magnitude and direction of plasticity in complete thermal performance curves, it is clearly inappropriate for the detection of acclimatory responses modifying only a part of performance curve.

In comparison with thermal acclimation of swimming speed in newt larvae (Měráková & Gvoždík 2009), the acclimation response in adults was induced not by diel temperature fluctuations, but by mean treatment temperature. This may result either from disparate proximate mechanisms of irreversible (developmental) and reversible (seasonal) thermal acclimation (Wilson & Franklin 2002; Gabriel 2005; Angilletta 2009) or from the ontogenetic shift in temperature-dependent selection gradient (Kingsolver & Gomulkiewicz 2003) modifying the induction of reversible acclimatory response. For example, hatched gill-breathing larvae need to stay in the surface water layer to maintain their preferred body temperatures (Smolinský & Gvoždík 2009), whereas body temperatures of bottom-walking adults are subjected to much higher variation during the daytime due to their air-breathing cycles (see above). In addition, one order of magnitude body size differences suggest that both stages are probably faced with various diversity, abundance, and size of predators. Hence, mechanisms and adaptive significance of the ontogenetic shift in acclimation-inducing temperature cues pose an interesting research agenda for future studies.

In conclusion, our study revealed the complexity of the thermal acclimation of terrestrial and aquatic locomotor capacity in newts. This shows that disparate thermal environments and lifestyles in water and on land shape not only evolutionary variation of thermal performance curves (Gvoždík & Van Damme 2008) but also its plasticity in newts. In addition, diverse acclimation responses in terrestrial and aquatic locomotor capacity resulted from disparate temperature cues (i.e. mean or fluctuating temperatures) rather than from different predictability of future thermal conditions in both environments. The accuracy of cues predicting future thermal conditions together with relatively high within-generation variation in ectotherms’ body temperatures and the short time lag for acclimation response, belong among the key assumptions for the evolution of reversible thermal acclima-
tion (Gabriel 1999, 2005; reviewed by Angilletta 2009). The natural thermal environment met both the heterogeneity and predictability assumptions in our study system (Dvořák & Gvozdík 2010; this study). In contrast, the use of merely constant temperature treatments would certainly have generated misleading conclusions about the occurrence of acclimatory responses in water and on land. Future investigations regarding evolutionary ecology of thermal acclimation will require more attention not only on the assumptions of evolutionary models predicting acclimatory capacities but also on the ecologically realistic design of acclimation experiments.

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References


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Supporting Information

Additional Supporting information may be found in the online version of this article.

**Table S1.** Parameters of GLM model.
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