Does developmental acclimatization reduce the susceptibility to predation in newt larvae?

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Many organisms respond to the heterogeneity of abiotic environmental conditions by plastic modifications of their phenotypes (acclimation or acclimatization). Despite considerable research efforts in this area, the beneficial (adaptive) effect of acclimation or acclimatization is still debated. We examined whether the development of newt larvae (Ichthyosaura alpestris) under different natural light and thermal conditions subsequently altered their susceptibility to predation in sun-exposed versus shaded tanks in nature. During predation trials in various light and temperature conditions, newt larvae that developed in sun-exposed warmer tanks consistently suffered from higher predation by dragonfly nymphs (Aeshna cyanea) compared to larvae from shaded or colder tanks. We conclude that higher sun exposure during embryonic and larval development negatively affects antipredator performance even in sun-exposed tanks: this result is inconsistent with the beneficial acclimation hypothesis.

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INTRODUCTION

The persistence of ectotherm populations depends on the intrinsic capacity to cope with the heterogeneity of an abiotic environment. This capacity involves not only genetic and demographic parameters, but also phenotypic plasticity (Williams et al., 2008). More than 150 years of research on this topic has demonstrated that developmental responses to laboratory-induced variation in one abiotic factor affect performance (developmental acclimation; Angilletta, 2009) in diverse ectotherm taxa (Johnston & Wilson, 2006). However, the adaptive significance of these plastic adjustments remains controversial. For a long time, all acclimation responses were considered to be beneficial. However, the number of empirical studies provided only weak support for this notion and falsified its generality (Leroy, Bennett & Lenski, 1994; Zamudio, Huey & Crill, 1995; Huey et al., 1999; Angilletta, 2009).

Aside from the various costs and limits of plasticity (DeWitt, Sih & Wilson, 1998; Auld, Agrawal & Relyea, 2010), methodological approaches used in studies of beneficial acclimation appear to be partly responsible for its ambiguous evidence. Among the studies detailing general methods of adaptive phenotypic plasticity (Gotthard & Nylin, 1995; Kingsolver & Huey, 1998), three have gained wider attention in acclimation studies. A recently adopted approach by Cooper, Czarnoleski & Angilletta (2010) carefully designed experiments to test predictions of optimality models. The second approach tracks the success of laboratory-acclimated individuals released in the field (Kingsolver, 1995; Loeschcke & Hoffmann, 2007). The final approach applies to rather elaborate reciprocal experiments that, in short, test the performance of individuals under various abiotic conditions to which they have previously acclimated or non-acclimated their phenotypes (Leroy et al., 1994; Zamudio et al., 1995; Huey et al., 1999). Given the above definition of acclimation, experiments were performed by manipulating only one factor, such as temperature, light or

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humidity, in the laboratory. However, under natural conditions, ectotherms are exposed to the heterogeneity of all abiotic factors at once, which may be necessary to provide the correct cues for the induction of beneficial plastic responses (Levins, 1968; Angilletta, 2009).

In the present study, we examined developmental acclimatization (i.e. developmental shift in performance to natural variation in abiotic conditions) in newt larvae. We chose larval susceptibility to predation (i.e. the proportion of exposed larvae that were killed per 24 h) by dragonfly nymphs as the fitness-relevant trait to investigate. Newt larvae are often exposed to intense predation by dragonfly nymphs (Van Buskirk & Schmidt, 2000), although varying thermal regimes during newt development induce plastic responses that affect swimming performance (Meráková & Gvoždík, 2009; Kurdišková, Smolinský & Gvoždík, 2011), which, in turn, may influence their success at escaping from predators (Watkins, 1996). In the field, newts breed in both shaded and sun exposed pools. Larvae developing in sun-exposed pools experience higher mean temperatures and greater diel temperature fluctuations than those developing in shadier pools (Dvorák & Gvoždík, 2010), and thus a study of potential developmental acclimatization to the light environment is ecologically relevant to this species. If beneficial acclimatization (Leroi et al., 1994) occurs, we expect that larvae from sun-exposed tanks have higher survival compared to larvae from shadier tanks when exposed to predators in sun-lit tanks, although a lower survival in shady ones.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

We captured adult alpine newts [Ichthyosaura alpestris (Laurenti, 1768)] and southern hawker (dragonfly) nymphs (Aeshna cyanea) in temporary pools close to Jihlava, Czech Republic, on 10 April 2010 and transported them 50 km to a research facility at the Institute of Vertebrate Biology. Pairs of newts (one male, one female) were placed in fiberglass tanks in the laboratory. In the present study, we examined developmental acclimatization (i.e. developmental shift in performance to natural variation in abiotic conditions) in newt larvae. We chose larval susceptibility to predation (i.e. the proportion of exposed larvae that were killed per 24 h) by dragonfly nymphs as the fitness-relevant trait to investigate. Newt larvae are often exposed to intense predation by dragonfly nymphs (Van Buskirk & Schmidt, 2000), although varying thermal regimes during newt development induce plastic responses that affect swimming performance (Meráková & Gvoždík, 2009; Kurdišková, Smolinský & Gvoždík, 2011), which, in turn, may influence their success at escaping from predators (Watkins, 1996).

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PREDATION TRIALS

Short-term survival trials were set up in plastic aquaria (18 L) filled with tap water and inoculated with plankton-containing pond water (0.5 L). To avoid the confounding influence of habitat complexity, each aquarium contained only a string (30 cm) of E. densa, provided with a perch for the dragonfly nymph, and a datalogger that recorded water temperatures and light intensity at hourly intervals. Aquaria were covered with a fine mesh. In total, we used forty aquaria arranged in a randomized block design: the treatment aquarium (i.e. with predator) was always...
paired with a control (i.e. no predator), and tanks were distributed outdoors under various light conditions. The light and temperature conditions during experiments were determined not only by the position of aquaria (sun or natural shade), but also by the substantial weather variation. This resulted in continuous light and temperature variation during the experiments. The presence of a control was necessary to identify causes of larval mortality other than predation during experimental trials.

We placed ten newt larvae in the final stage of development (i.e. the fifth toe clearly visible; Watson & Russell, 2000) into both treatment and control aquaria for 12 h before beginning of the experiment (20:00 h). Given the temporarily-limited availability of larvae in the required stage, only one treatment and control aquariums were simultaneously filled with larvae from one rearing tank. We then added one randomly chosen dragonfly nymph (total length $= 37.5 \pm 0.6$ mm) from rearing tanks into the treatment aquaria, and left it undisturbed for 24 h. To control for the different hunger levels of each predator, nymphs were starved for 3 days between successive trials. Nymphs were used repeatedly (two to four times) for predation trials. At the end of a trial, the number of surviving larvae was counted. A sample ($N = 10$) of randomly-selected larvae in the final developmental stage from each tank was photographed to measure the total length before predation trials. Given the time and sample size limitations, predation experiments were restricted to one developmental stage only. Predation trials were repeated depending on the availability of final-stage larvae in a given tank.

**STATISTICAL ANALYSIS**

The proportion of killed larvae was analyzed using a generalized linear mixed model for data with binomially distributed errors (Crawley, 2007). The numbers of killed and survived larvae were transformed to logits [i.e. log(killed/survived)] before further analyses. The full model contained fixed factors, the covariate, and random factors. Fixed factors consisted of treatment (i.e. predation and control trial), acclimatization regime (sun and shade), and their interaction. Mean light intensity, mean temperature and the diurnal range of temperatures during the predation trial were significantly intercorrelated, and so their first principal component (PC1) explaining 67% of total variance ($\chi^2 = 71.8$, d.f. = 5, $P < 0.001$) was used as the model covariate (factor correlations with PC1: 0.50, 0.58, and 0.65, respectively). Experimental abiotic conditions were considered continuous rather than categorical factors because light and temperature conditions seen between sun- and shade-exposed aquaria are highly overlapped during predation trials. Female, block, and predator identity were added as crossed random factors. To reduce overdispersion, female identity had both random intercepts and slopes. To test whether acclimatization regime affects larval body size at the same stage, a generalized linear mixed model, with one fixed (acclimatization regime) and two random factors (female and block identity), was applied. The best model selection was realized using the likelihood ratio approach. All analyzes were performed in R (R Development Core Team, 2009) using the ‘lme4’ library (Bates, Maechler & Bolker, 2012).

**RESULTS**

On average, shaded rearing tanks received 40% of the light intensity in sun-exposed tanks ($6624 \pm 339$ lux versus $16027 \pm 632$ lux; Fig. 1) and had cooler surface water temperatures than did sun-exposed tanks ($14.0 \pm 0.1$ °C versus $17.9 \pm 0.2$ °C, respectively). Thermal conditions in both light treatments were similar to those experienced by newt larvae in natural pools (see Supporting Information, Fig. S1). Although at a similar size ($30.1 \pm 0.3$ mm versus $29.4 \pm 0.4$ mm; $t_{18} = 0.85$, $P = 0.41$), the first larvae that developed in shaded tanks attained the larval climax stage $25 \pm 2$ days later than larvae from sun-exposed tanks. Accordingly, predation experiments were carried out five times between 11 and 26 June and 9 and 26 July for sun and shade treatments, respectively. Despite the temporal separation in predation experiments, larvae from both regimes experienced similar variations in light and thermal conditions during trials (Table 1).

![Figure 1. Hourly values of light intensity (mean ± SE) on the water surface in experimental tanks located in shaded and sun-exposed sites during the development of newt embryos and larvae.](image-url)
We performed 120 predator and control trials, respectively. Because all larvae survived in the control groups, the influence of the acclimatization regime, experimental light, and thermal conditions on their susceptibility to predation was further examined in predation trials alone. Because block, predator identity, and interaction between treatment and abiotic conditions contributed little to the model explanatory power (block: \(\chi^2 = 0, \text{d.f.} = 1, P = 0.99\); predators: \(\chi^2 = 1.57, \text{d.f.} = 1, P = 0.21\); interaction: \(\chi^2 = 0.38, \text{d.f.} = 1, P = 0.54\)), the minimum adequate model contained three factors: treatment, female identity, and PC1 of abiotic conditions as the covariate. Predation rates increased with the joint influence of light intensity, mean temperature, and the extent of diel fluctuations during these trials (\(z = 3.32, P < 0.001\); Fig. 2). Newt larvae that developed in sun-exposed tanks were killed by dragonfly nymphs more frequently than larvae from the shaded tanks, irrespective of whether experiments were run in sun or shade (sun: 0.27 ± 0.05 killed per survived larvae; shade: 0.16 ± 0.04 killed per survived larvae; \(z = 2.55, P = 0.01\)).

**DISCUSSION**

Although field experiments provide crucial information about the adaptive significance of plasticity (Gotthard & Nylin, 1995; Kingsolver & Huey, 1998; Loeschcke & Hoffmann, 2007), the beneficial effects of acclimatization have rarely been studied in animal models. Developmental acclimatization is considered beneficial if phenotypes that develop under a given set of abiotic conditions have a higher performance under these conditions than phenotypes reared in other conditions. Our results showed that newt larvae developed in sun-exposed tanks were more prone to predation than larvae from shaded tanks across the whole range of abiotic conditions. Clearly, this provides no support for the beneficial acclimatization hypothesis.

The consistent antipredatory failure of sun-acclimatized larvae is markedly similar to developmental acclimation patterns in other ectotherm taxa (Leroi et al., 1994; Zamudio et al., 1995; Stillwell & Fox, 2005). Overall, these patterns suggest the existence of optimal developmental temperatures (shaded conditions in the present study) rather than the beneficial acclimation response (Huey et al., 1999). The inferior performance of individuals that developed

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**Table 1.** Mean temperature, the extent of diel temperature fluctuations (temperature maximum – minimum), and mean light intensity during predation experiments, using newt larvae developed under shaded and sunny conditions

<table>
<thead>
<tr>
<th>Environmental variable</th>
<th>Treatment</th>
<th>Mean ± SE</th>
<th>Range</th>
<th>(t_{118})</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean temperature (°C)</td>
<td>Shade</td>
<td>19.5 ± 0.4</td>
<td>15.5–25.0</td>
<td>0.61</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Sun</td>
<td>19.1 ± 0.4</td>
<td>12.6–25.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature fluctuations (°C)</td>
<td>Shade</td>
<td>8.4 ± 0.5</td>
<td>3.0–14.0</td>
<td>0.11</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Sun</td>
<td>8.5 ± 0.5</td>
<td>3.0–14.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light intensity ((\times 10^3) lux)</td>
<td>Shade</td>
<td>11.4 ± 1.0</td>
<td>4.4–29.7</td>
<td>0.41</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Sun</td>
<td>11.9 ± 1.0</td>
<td>2.8–26.9</td>
<td></td>
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**Figure 2.** The influence of experimental abiotic conditions (the first principal component, PC1) on the numbers of killed newt larvae (logit transformation) in the presence of a predator, the dragonfly nymph. Newt larvae developed under disparate light and temperature conditions (sun and shade treatments). The PC1 represents 67% of total variance in mean light intensity, mean water temperature, and the extent of diel temperature fluctuations during predation trials (24 h). Upper right: predicted plastic response according to the beneficial acclimatization hypothesis.
under suboptimal temperatures was sometimes interpreted as a result of ecologically unrealistic stressful conditions during acclimation experiments (Huey et al., 1999; Wilson & Franklin, 2002; Woods & Harrison, 2002). Although ecological relevance of experimental conditions is an important issue in developmental acclimation studies (Měráková & Gvoždík, 2009), our results showed that a similar response was induced even under natural variation in an abiotic environment.

The nonbeneficial acclimatization response suggests that sun-exposed tanks represented stressful conditions that fell outside the limits historically experienced by individuals in such populations (Ghambor et al., 2007). The similarity between temperatures in sun-exposed tanks and a temporary pool occupied by newts in 2005 (see Supporting Information, Fig. S1) rejects this possibility or indicates that stressful conditions recently occurred even in natural habitat as a result of local climatic change (Dvořák & Gvoždík, 2010). Alternatively, some limits and costs of plasticity (DeWitt et al., 1998; Auld et al., 2010) prevented the evolution of a beneficial acclimatization response. We propose three likely scenarios: (1) the interaction with biotic (predator) cues moves the thermal/light reaction norm apart from a new adaptive optimum (Valladares, Gianoli & Gomez, 2007); (2) precocious muscle growth in high temperatures curtails antipredator performance (Johnston & Hall, 2004; Arendt & Hoang, 2005); and, finally, (3) because disparate light levels influence several acclimation-inducing factors in amphibian larvae, such as mean temperature (Wilson & Franklin, 1999), diel temperature fluctuations (Měráková & Gvoždík, 2009), or UV-B radiation (Alton, Wilson & Franklin, 2011), the nonbeneficial acclimatization represented a complex response to the correlated abiotic conditions.

Larval susceptibility to predation increased with light intensity and water temperature during experimental trials. In both predator and prey, higher temperatures accelerated their metabolic rates (Brey, 2010), and consequently their foraging activity, which, in turn, led to higher frequency of predation events (Skelly, 1994). In addition, dragonfly nymphs are visually-oriented predators, and so higher light intensity may increase their prey detection and attack rate (Luttbeg, Hammond & Sih, 2009). Hence, proximate causes responsible for this relationship are particularly complex and require further investigation.

Although higher susceptibility to predation negatively affected the fitness of individual larvae, the possible consequences on population dynamics are complex. Generally, the recruitment of amphibian juveniles is affected by a mortality/growth trade-off, rather than by predation alone (Werner, 1986; Benard, 2004). The somatic growth of newt larvae is accelerated by temperatures within the normal temperature range (L. Gvoždík, unpubl. data), meaning that faster growth may effectively buffer the nonbeneficial acclimatization of mortality-related performance trait. Hence, it follows that the overall effect of acclimatization on population dynamics is determined not only by the shape of reaction norms for mortality and growth, but also by the density of predators and the availability of resources in a given habitat (Bouskila et al., 1998; Skelly, Freudenburg & Kiesecker, 2002; Schiesari, 2006).

Although many studies have demonstrated a similar response after prolonged exposure to suboptimal values of one abiotic factor, temperature, the present study has provided the first evidence for nonbeneficial acclimatization in animals under natural variation of correlated abiotic factors. Our findings suggest that, after an abrupt shift in abiotic conditions such as windthrow or clearcutting, under some circumstances, acclimatization may decrease recruitment in newt populations. This has implications for the conservation management of forest amphibians. Because acclimatization represents an important component of a population’s adaptive capacity to climate change (Williams et al., 2008; Chown et al., 2010), the nonbeneficial response should be considered in future mechanistic models predicting the impacts of climate change on ectotherm populations.

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REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Distribution of the water surface temperatures in tanks located in sun-exposed (A) and shaded sites (B) in comparison with field temperatures from the warmest (A) and coolest (B) (2010) pools in the study population during 2005–2010 (Dvořák & Gvoždík, 2010). Both experimental and field temperatures were recorded at hourly intervals during the reproductive period of newts.