**Some like it hot? Developmental differences in yellow-bellied toad tadpoles (Bombina variegata) from geographically close but different habitats**

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Some like it hot? Developmental differences in yellow-bellied toad tadpoles

*(Bombina variegata)* from geographically close but different habitats

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Some like it hot? Developmental differences in yellow-bellied toad tadpoles 
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Abstract

The key for the long-term survival of species is their potential to respond to changing conditions. These reactions are usually species-specific and may vary between populations. The yellow-bellied toad, *Bombina variegata* (L., 1758), occurs in forested and open areas. We wanted to know whether tadpoles react plastically to different environmental conditions – and if so, whether reaction norms are species-, population- or season-specific. In a common garden experiment we compared developmental traits (developmental time, size, body-condition) of metamorphs from different habitats (forest *versus* quarry) in close geographic proximity. Tadpoles from both habitats grew up under shaded and sunny conditions. The experiments were run during early and late breeding season. We detected different developmental strategies between populations, concerning treatments and season on a microgeographic scale. Tadpoles with quarry origin developed faster and reached larger body sizes, at the expense of lower body-condition. Major risks affecting tadpole’s survival in the open habitat are high temperatures and high desiccation. Forest tadpoles were comparatively smaller in size, but showed higher plasticity and higher body-condition. Under changing climatic conditions quarry population may reach temperatures above their thermal limits. In contrast forest conditions may mitigate increasing temperatures. Forest populations could be better adapted to future climate change.
Keywords

Adaptation, plasticity, developmental traits, seasonality, climate change

Introduction

Species have three principal options to survive changing environmental conditions: i) react plastically to the change; ii) evolve adjusted new characters (e.g. concerning physiology or behavior); or iii) migrate to more favourable habitats (Parmesan 2006; Bellard et al. 2012). The respective importance and availability of these options for the long-term survival are species specific. Local adaptation should favour traits which are specialized to a specific environmental situation, and therefore are crucial for individual fitness and the short-term survival of populations (Kawecki and Ebert 2004; Bellard et al. 2012). Ideally individuals should exhibit local habitat specific adaptations and still be able to react plastically to changing conditions (or migrate into other habitats).

An animal group known to react particularly sensitive to environmental change, and thus being impacted by climate change, are amphibians (Blaustein et al. 2010). Due to a comparatively low migration potential (Sinsch 1990), many species have a limited ability to leave unfavorable habitats (Wells 2007). Consequently, the species-specific potential to react locally to changing environments might be a key for the survival of populations and species. This is particularly true for species that breed in ephemeral ponds, as the water holding capacity of breeding sites directly determines the survival chances of tadpoles (Blaustein et al. 2010), and pond duration is impacted by temperature (desiccation rates) and precipitation pattern (amount and timing of rainfall).

The breeding of Bombina variegata (L., 1758) occurs opportunistically after spring and summer rains, in small, shallow, temporary puddles and ponds (Barandun et al. 1997;
Abbühl and Durrer 1998). The development and survival of the tadpoles highly depend on water temperature (Barandun and Reyer 1997a), where temperatures above 31°C cause high pre- and post-hatching mortality (Pawlowska-Indyk 1980) and temperatures below 12°C seem to be unsuitable for growth and development (Rühmekorf 1958a). The upper thermal limit for the survival of tadpoles is stated to be 36°C (Böll 2002). In addition, high temperatures increase the evaporation rate and desiccation of the breeding waters, which is the main factor of larval mortality (Barandun and Reyer 1997b). Increasing temperatures and changing precipitation patterns, as predicted for Central Europe (IPPC 2013), may directly impact the species future survival. In Central Europe, *B. variegata* occurs in quarries or other open, secondary habitats, as well as in forested areas (Gollmann and Gollmann 2012; Sztatecsny 2014). Thus, geographically close populations can occur in habitats with remarkably different environmental temperature regimes. Given the vast distribution of *B. variegata*, and the wide range of different and usually unpredictable dynamics of breeding waters (Buschmann et al. 2013; Canessa et al. 2013), it can be expected that the species *per se* can cope well with a broad array of ecological conditions. However, the reaction potential of *B. variegata* populations to changing environments on small geographic scale is unknown.

Developmental traits in ectothermic organisms highly depend on environmental temperature; i.e. higher temperatures leading to faster development and shorter time to metamorphosis (Smith-Gill and Berven 1979). However, a fast development will usually go with the costs of smaller size at metamorphosis, and therefore may affect future survival probabilities (Newman 1988; Berven 1990). Populations of *B. variegata* in quarry habitats face high temperatures and evaporation rates, due to low vegetation cover. Tadpoles should exhibit a fast development to leave the puddles before they desiccate, but potentially at the expense of an appropriate metamorphic size, needed to e.g. minimize desiccation and predation risk (Thorson 1955). In contrast, forest populations face lower temperatures and
lower desiccation risk, thus, the tadpoles should have a lower developmental rate, but reach larger metamorphic size (Angiletta et al. 2004).

Timing and size of metamorphosis in temperate regions are crucial factors for the survival during proceeding hibernation; therefore prolonged breeding species should show seasonal differences in their developmental patterns. In particular, tadpoles need to finish metamorphosis before temperatures get too low; otherwise they will freeze in their shallow breeding waters. Further, it should be beneficial to have a higher mass at metamorphosis in order to have stored enough energy to survive hibernation (Edge et al. 2013).

We herein test the developmental responses of *B. variegata* tadpoles from geographically close but different habitat types (forest versus quarries) to different environmental conditions (sun versus shade) and seasons (early versus late). Our questions were: i) do *B. variegata* larvae from a particular population react phenotypically plastic to different environmental conditions (temperatures); ii) do populations from different habitats exhibit local environmental adaptations, and thus differ in their reaction to different developmental temperature; and iii) do these reactions depend on season. To address these questions, we raised tadpoles from different habitat types under shaded and sunny conditions, in the early and late breeding season and compared developmental traits (developmental time, size and body-condition at metamorphosis) in a common garden experiment.

**Materials and methods**

We declare that animals were cared for in accordance with guidelines on the care and use of wildlife (CCAC 2003). According to the German Protection of Animal Act ("Tierschutzgesetz", latest adapted on 9 December 2010; http://www.gesetze-im-internet.de/tierschg/BJNR012770972.html; assessed on 12 June 2013) painless experiments with and observations of vertebrates neither require permission nor disclosure (§ 1/§ 7
TierSchG). The vertebrates involved, *Bombina variegata* tadpoles and metamorphs, experienced no pain, suffering, complaints or harm. Thus, no Institutional Animal Care and Use Committee (IACUC) or ethics committee approved this study as this was not required by German law. Peter Krämer (higher nature conservation authority of Lower Franconia - “Regierung von Unterfranken”) approved the research in accordance with the Federal Conservation of Nature and Landscape Act (“Bundesnaturschutzgesetz”).

**Study area and period**

The study area was located in the Steigerwald (northern Bavaria; WSG84: N 49°55', E 10°33'). The landscape is characterised by vast continuous blocs of deciduous mixed forest with many puddles, ponds and small streams (Habbe 2003). Several active sandstone quarries are scattered within the forest.

We collected *B. variegata* eggs from small puddles and road ditches after rain. Quarry eggs originated from Gleußner (N50°1.9', E10°40.2'; 4 puddles), Schleifsteinwerke (N50°0.9', E10°40.9'; 5 puddles) and Klaubholz (N50°2.2', E10°39.7'; 5 puddles). The depth of breeding sites in the quarries varied from 4 to 16 cm, their surfaces ranged from 0.4 to 20 m². The water temperature in 9 quarry puddles ranged from 11 to 40°C (mean = 21 ± 5°C) during the study period (May–September 2012) and was measured every 3 h with a thermologger (Theromochron® iButtons© DS 1921G; accuracy ± 0.5°C, Maxim Integrated, San Jose, USA). Forest eggs originated from Böhlberg (N49°56.2', E10°30.8'; 2 puddles), Hundelshausen (N49°56.1’, E10°29.6’; 2 puddles), Wotansborn (N49°55.5’, E10°31.0’; 2 puddles), Geusfeld (N49°53.9’, E10°30.1’; 1 puddle) and Obersteinbach (49°54.2’, E10°31.3’; 2 puddles). The depth of forest breeding sites varied from 3 to 40 cm, their surfaces ranged from 0.3 to 20 m². Water temperatures in the forest were lower and varied less than in the quarries, ranging from 8 to 32°C (mean = 19 ± 3°C, 7 puddles with iButtons©, temperature
measured every 3 h).

The reproductive period of *B. variegata* starts in early May and ends in late July. Our first experiment started on May 9 and ended (last metamorph) on August 19, 2012; in the following termed: “early season”. The second experiment started on July 9, the last metamorph being recorded on September 19; in the following termed: “late season”. We stopped the second experiment on September 19. Rühmekorf (1958a) reported that development stops when tadpoles were kept at 12°C. In our experimental tanks mean temperatures did not exceed 12.5°C in mid-September. Therefore, we concluded that thermal conditions restrict development of the tadpoles and they will not reach metamorphosis until the end of the season. Thus, we released all remaining tadpoles in their respective original habitats at September 20, and treated them as having failed metamorphosis in our analyses.

**Common garden experiments**

All experiments were conducted as a common garden setup at the ecological field station in Fabrikschleichach (N49°55.4’, E10°33.3’). We raised tadpoles from quarry and forest habitats under shaded and sunny conditions. All eggs were first kept in polystyrene tanks (37 x 30 x 22 cm, water volume 11 L), until tadpoles hatched and started active swimming (stage 25 after Gosner 1960). One tank contained several (5-20) egg masses from one location and date. All eggs and tadpoles faced equal conditions until experiments started. The hatching tanks were filled with water from a nearby spring (pH = 6.75-8.20, conductivity µS = 60-210; Combo Tester HI 98129/130, HANNA® Instruments, Kehl am Rhein, Germany) and tadpoles were not fed.

To focus exclusively on the factor temperature, we always kept 15 randomly selected tadpoles from one location and sample date per experimental tank (same tank size as above; filled with 4 cm of sediment and 10 cm water). All tadpoles not needed for an experiment
were instantly released at their original sites. If tadpoles died in the experiments, they were
not replaced. In comparison to natural densities, 15 tadpoles per 11 L of water compares to
low density-condition (Hartel 2007). In addition, the species’ larval development seems to be
little affected by high densities (Heusser 1972). Nevertheless, we tested the effect of tadpole
density on developmental traits by comparing developmental parameters with the number of
metamorphs per tank. We monitored water chemistry (pH and conductivity with ComboTester
(HI 98129/130, HANNA® Instruments, Kehl am Rhein, Germany) and replaced 3 L of water
in each tank once per week (total water volume kept constant at 11 L). Tanks were covered
with plastic gauze to exclude predators. The tadpoles were fed every second day with the
same amount of commercial fish food (one tablet of Tetra TabiMin®, Tetra GmbH, Melle,
Germany, per tank). Each tank was equipped with an iButton, measuring temperature every
three hours. During the early season we had a total of 40 tanks, 10 tanks per habitat type and
treatment; during the late season we had 20 tanks, 5 tanks per habitat type and treatment. The
lower sample size in the late season was due to lower breeding activity.

Larvae in stage 25 (Gosner 1960) from one sample location (forest or quarry) and
sample date were split in two treatments; one treatment with sunny conditions, the other one
with shaded conditions. Sunny condition tanks were exposed to sun for 12 hours per day.
Shaded condition tanks, were placed below trees and received a maximum of 6 sun hours per
day (10 am to 4 pm). At the metamorphic climax (Gosner stage 42, breakthrough of
forelimbs, fully developed tail) we recorded the developmental time, the size (without tail;
snout-vent-length (SVL) in mm, accuracy ± 0.5 mm) and the weight (PCB 350-3 balance,
accuracy ± 0.05 g; Kern & Sohn GmbH, Balingen, Germany) of the froglets. After taking all
measurements metamorphs were released to their native habitat.

We were not only interested in the developmental time, size and mass at
metamorphosis, but also in the condition of metamorphosing froglets. Size and mass of
froglets are parameters commonly assessed, but as single parameters not necessarily informative concerning the body condition of an individual. The (relative) amount of energy reserves at metamorphosis may better indicate the individual’s future survival chances after metamorphosis (Scott et al. 2007), during hibernation (Reading 2007), or its future fecundity (Reading and Clarke 1995). A nonlethal method to get information about stored energy is calculating body-condition-indices (BCI; Peig and Green 2009, 2010). Such BCI indicates not only the condition of an individual, but also the environmental conditions, including stress during the individual’s development (Reading 2007). Thus, BCI is a useful proxy for habitat quality in situ (Sztatecsny and Schabetsberger 2005; Janin et al. 2011; Scheele et al. 2014), e.g. availability of food and favourable micro-climate.

Many different BCIs have been introduced into literature. Some of them have been successfully tested with B. variegata (Băncilă et al. 2010; Scheele et al. 2014). We chose the scaled mass index (Peig and Green 2009; MacCracken and Stebbings 2012), because it is independent of life stage and size; it is comparable between populations and studies (if the exponent is similar); and it has a reasonable unit (g) for comparison. The BCI was calculated with the formula: BCI_i = weight_i [g]*\left(\frac{\bar{x}_{\text{size}} [\text{mm}]}{\text{size}_i [\text{mm}]}\right)^{b_{\text{SMA}}} The exponent (b_{\text{SMA}}) derives from the slope of a standard major axis regression of log_{10} weight on log_{10} size. The mean size was calculated from all metamorphs of all treatments and experimental runs (n = 783). Therefore, the final formula to calculate the BCI was: BCI_i = weight_i [g]*(13.79 [mm]/size_i [mm])^{4.11}.

The calculation of the b_{\text{SMA}} value was performed with the R package lmodel2 (Legendre 2013). Since individual tadpoles in a tank cannot be regarded as independent samples, we calculated mean values of each tank. Thus, means of developmental time, BCI and size of all 15 larvae in one tank represent one sample. Data were tested for normality (Shapiro-test) and homogene variances (Bartlett-test). We checked for linear dependency of
temperature (mean) with developmental parameters (Pearson correlation coefficient). We used Pearson correlation to test for density effects in the tanks where mortality occurred. Where it seemed biologically reasonable, we performed a linear regression model – mean temperature (independent variable) with the developmental time (dependent variable).

We compared mean size at metamorphosis (SVL in mm), body condition index (BCI in g) and developmental time (time from laid egg to metamorphosis in days) of the two habitat types, two treatments and two seasons with a three factorial analyses of variance (ANOVA). Because season was a significant factor, and the main effects between seasons varied (see results), we conducted a two-factorial ANOVA for each parameter and used interaction plots for visualization. All analyses were conducted with the statistical software R 3.1.1 (R Core Team 2013). For data visualization we used the R package ggplot2 (Wickham 2009).

Results

Metamorphic success was high (87%; 783 of 900 tadpoles) but varied between original habitat type, treatment and season (Table 1 and 2). During the early season more tadpoles from forest than from quarry populations died, independent of treatment (Fig. 1, Table 2). The high mortality in the shade treatments during the late season was due to counting non-metamorphosed tadpoles as dead (see Materials and methods). The overall mortality (13%) was considerably lower than under natural conditions (87-93%; Barandun and Reyer 1997a). On average 13 individuals per tank (standard deviation = 3) reached metamorphosis. There was no correlation between final number of tadpoles per tank and metamorphic size (Pearson $r = -0.11; p = 0.49; n = 60$); BCI (Pearson $r = 0.12; p = 0.45; n = 60$); or developmental time (Pearson $r = 0.17; p = 0.29; n = 60$). Thus, within our experimental setup (maximum of 1 tadpole per 0.72 L), we observed no density effects on developmental traits.
As a 3-factorial ANOVA revealed a strong effect and interactions of season with developmental traits (Table 3), we conducted 2-factorial ANOVAs and separated our analyses by season (Table 4).

The mean temperature per treatment during the two experimental seasons was similar; although the minimum and maximum temperatures in the late season were slightly lower (Table 5). In both periods we detected significant differences between mean temperatures of sun treatments ($\bar{X} = 19^\circ$C) and shade treatments ($\bar{X} = 16.5^\circ$C) (ANOVA $p < 0.001$, early season: $n = 40$, late season: $n = 20$) (Table 5).

Temperature and developmental time were correlated in the early (Pearson, $r = -0.69$, $p < 0.001$, $n = 40$), and late season (Pearson, $r = -0.95$, $p < 0.001$, $n = 20$). Thus, higher temperatures lead to faster development. A linear model, conducted for both seasons, revealed that a $1^\circ$C mean temperature increase decreased the time to metamorphosis by 4-5 days in our mean temperature regime between 15 and 20$^\circ$C. In both seasons, the origin and the treatment significantly impacted developmental time (Fig. 2a, b, Table 4). Metamorphs from the quarry developed faster in both seasons; independent of treatment (genetic component). The metamorphs from both habitat types raised in the sun showed an accelerated development compared to the ones raised in the shade (environmental component).

The SVL at metamorphosis varied significantly between populations from the quarry and the forest during the early season (Table 4). There was a clear effect of origin (genetic component), but no treatment effect (environmental component; Figure 2c). Metamorphs originating from the quarries reached larger sizes than those from the forest, independent of sun or shade treatment. Although temperatures differed between treatments, we revealed only a weak negative correlation between mean temperature and mean SVL (mm) at metamorphosis (Pearson $r = -0.31$, $p = 0.05$, $n = 40$). Froglets which developed in sunny conditions were smaller than froglets from shaded conditions, even though there was no
statistical difference, for example the ones originating from quarry and kept in the shade were the largest; the ones originating from forest and kept in the sun the smallest (Table 5, Fig. 2c).

In the late season the SVL at metamorphosis varied significantly between treatments, but not between the origin of eggs (Fig. 2d, Table 4). Thus, tadpoles raised in the sun reached larger sizes than those in the shade, independent of their original habitat type. However, SVL was generally smaller than in the early season, independent of tadpole origin and treatment (Fig. 2d). The correlation of temperature and size was strongly positive (Pearson $r = 0.68; p = 0.001, n = 20$) and froglets raised in the sun were the largest; i.e. the warmer the temperature was, the larger were the froglets (Table 5, Fig. 2d).

The BCI comparison in the early season revealed differences between metamorphs of different origin (genetic component), but not between treatments (environmental component) (Table 4); i.e. froglets originating from the forest reached higher BCI values in both treatments than those from the quarries, even if they were smaller (Fig. 2e). There was no correlation between developmental temperature and BCI (Pearson, $r = 0.2, p = 0.21, n = 40$).

The variation of BCI values in metamorphs was greater in the late season (Table 5) and we detected significant differences between metamorphs of different origin and between treatments (Fig. 2f, Table 4). The metamorphs from the forest had higher BCI values than those from the quarries in both treatments (genetic component). We likewise revealed a treatment (environmental) effect, i.e. higher BCI values in the shade, even if those metamorphs were smaller in SVL. During the late season the BCI correlated negatively with temperature (Pearson $r = -0.7, p < 0.001, n = 20$), this means that metamorphs which experienced higher temperatures had the lowest BCI values. Developmental time correlated positively with BCI (Pearson $r = 0.8, p < 0.001, n = 20$), i.e. metamorphs with longer developmental time had the highest BCI values (Fig 2f).
Discussion

Our study species, *Bombina variegata*, occurs in a variety of habitats with often unpredictable breeding site availability and pond persistence (Barandun and Reyer 1997b). Thus, it is expected that the species has a high ability to cope with different environmental conditions. However, it was unknown to which extent populations can perform plastically, and if and how this plasticity depends on original environmental parameters, such as e.g. the stability of their environment. Local plasticity might be crucial for the long-term survival of species under changing climatic conditions (Bellard et al. 2012). In our study area, *B. variegata* reproduce in two habitat types which are geographically close, but differ fundamentally with respect to their microclimate: open, sunny, and warm quarries, and closed, shady and cooler forests.

Our study revealed that variability in developmental traits between *B. variegata* populations from open versus closed habitats was influenced by origin (genetic component), temperature (environmental component), and season. The detected differences between populations imply that *B. variegata* is able to plastically react to differing environmental conditions, but likewise shows population specific adaptations to specific habitats on small geographic scale. In particular, tadpoles originating from quarry habitats were adapted to higher temperatures and higher desiccation risk, but on the cost of comparatively low body condition. Tadpoles from forest populations needed longer for their development, and often did not reach the sizes of quarry metamorphs, but still gained a better BCI.

Numerous studies on ectotherms, including anuran tadpoles, showed the effect of temperature on developmental time (Smith-Gill and Berven 1979; Kingsolver and Huey 2008; Zuo et al. 2012), as well as population specific responses to temperature variation (Laugen et al. 2003; Edge et al. 2013). First of all, this has a physiological base, meaning higher temperatures leading to higher metabolism (Gillooly et al. 2001; Angiletta et al 2002; Zuo et
Therefore, we expected that tadpoles from both habitat types would develop faster in the sun treatment, compared to the shade treatment. Indeed, our results showed that a mean increase of water temperature by 1°C shortens the time to metamorphosis by 4-5 days. Nevertheless, tadpoles from quarry origin generally displayed a faster growth and higher developmental rate, regardless of treatment. That can be seen as an adaptation to a higher risk of desiccation in an unpredictable and comparatively hot environment (Newman 1988; Böll 2002). The breeding ponds in the quarry habitat regularly dry up and tadpoles face a high mortality risk. In contrast puddles in the forest habitat dry up less often (CD et al., unpubl. data).

In unpredictable environments, such as small ephemeral waters, the timing of and size at metamorphosis should be particularly plastic, taking into account the trade-off between developmental time, size at metamorphosis and survival probabilities (Wilbur and Collins 1973; Rudolf and Rödel 2007). Increased developmental rates, and thus a shorter larval period, decrease mortality risk due to desiccation, but are usually connected to a smaller size and lower weight at metamorphosis (Denver et al. 1998). Based on studies on anuran species with similar breeding habitats (e.g. *Epidalea calamita*: Tejedo and Reques 1994; *Spea hammondii*: Denver et al. 1998), we expected that the snout-vent length at metamorphosis in sun treatments would be smaller, due to a higher developmental rate. This expectation was met in the early season with metamorphs being smaller in the sun treatment, independent of their origin, but without statistical significance. Nonetheless, the interesting effect was that metamorphs from the quarry were larger than those from the forest, regardless of treatment. We interpret these results as a selection for fast development and larger size at metamorphosis in an open and hot habitat. This can be seen as an example of co-gradient variation, where the environmental influence (higher temperature) and the genetic influence (developmental rate) act in the same direction (Conover and Schultz 1995). The quarry genotype shows a fast
developmental rate under both treatments (local adaptation), which should lead to a small size at metamorphosis. Interestingly this was not the case in our experiment, and our results thus are in contrast to counter-gradient variation often found in amphibians (Berven 1982; Laugen et al. 2003; Skelly 2004). However, the trait under selective environmental force in the quarry is size, because temperature can rise up to 40°C and exceed the thermal tolerance of the species (larvae 36°C, Böll 2002; adults 38°C, Sos 2007). Larger size at metamorphosis could provide a better protection against desiccation (Thorson 1955); and reduce the range of potential predators (Caldwell et al. 1980). Juvenile size is often associated with adult size and larger individuals show higher fecundity and survival rates (Semlitsch et al. 1988; Berven 1990; Morey and Reznick 2001; Altwegg and Reyer 2003). Furthermore, jumping performance increases with larger size (Zug 1978), and better locomotor ability reduces the risk of predation in an open habitat with few hiding places (Wassersug and Sperry 1977).

Remarkably, we found a different pattern in the late season, where metamorphs reached larger SVL in the sun treatment, independent of origin, although the sizes at metamorphosis in all treatments remained small compared to the early season. The small sizes could be explained by an increasing mortality risk in the aquatic habitat. A model from Werner (1986) predicts an optimal size at metamorphosis in order to reach maximal growth rate in the juvenile stage. The optimal size to leave the aquatic habitat mainly depends on the ratio of mortality risk in the aquatic to mortality risk in the terrestrial habitat. This ratio will change towards the end of the season, because ponds are drying and temperatures are decreasing. Thus, at the end of the season, the mortality risk increases in the aquatic stage and it is potentially beneficial to leave the pond earlier at a smaller size. This was not possible for the tadpoles in the shade treatment, because the environmental conditions (low temperatures) restrict development to a degree that cannot be overcome. Similarly Miesler and Gollmann (2002) detected a reduction in body size of metamorphs (13.1 mm) at the end of the
reproductive season, and explained this by desiccating ponds and low temperatures. Furthermore, they stated that the late metamorphs seemed to be “underdeveloped”, which can be linked to unfavourable temperatures late in season. Not only were preferred temperatures for *B. variegata* tadpoles (24.6–29.7°C, Rühmekorf 1958b) no longer reached in the shade treatment, the temperature often dropped under a limit where growth was no longer possible (Rühmekorf 1958a).

In addition to timing and size at metamorphosis, the body condition (energy reserves) is crucial for the future survival and performance of froglets. For instance, animals with higher body condition may have better chances to escape predation or competition e.g. by moving further distances (Lowe et al. 2006), or have higher fecundity as adults (Reading and Clarke 1995; Castellano et al. 2004).

We observed a slight difference of BCI between the populations during the early season. Metamorphs from the forest displayed higher body condition, with a higher variability than the population from the quarries, regardless of the treatment. Forest tadpoles needed more time to develop and had the opportunity to gain more weight i.e. accumulate more reserves. These population differences became more pronounced during the late season, and were accompanied by an additional treatment effect. The tadpoles in the shade treatment had the slowest development, but the highest BCI. We assume that the low temperatures support the accumulation of fat reserves, caused by a slowdown of the growth rate and a prolonged developmental time. Another explanation could be that frogs which reach metamorphosis later, due to a colder environment in the shade treatment, have less time to grow in the terrestrial habitat to establish fat deposits for hibernation. Hence, they should gather some deposits during their larval development (Edge et al. 2013).

Contrary, the tadpoles raised in the sun developed faster and had the lowest BCI. Thus, it seems that they used energy for a faster development and bigger size; rather than
accumulating reserves for hibernation. Their strategy is probably to metamorphose and leave the pond before desiccation occurs, and then gain weight as juveniles. Here, the food availability in the aquatic habitat potentially plays an important role. The lower temperatures towards the end of the season are leading to reduced growth of algae in the aquatic environment (Butterwick et al. 2005). Thus, instead of staying in an unfavourable aquatic (herbivorous) environment, it is useful to leave the pond and to search for suitable terrestrial microhabitats where food is abundant. The chance to gain a bigger amount of fat reserves is higher with a carnivorous diet (Álvarez and Nicieza 2002), and a higher amount of fat reserves increases the probability to survive the first terrestrial months (Pfennig 1992; Scott et al. 2007) and hibernation (Reading 2007).

We also found a seasonal influence on developmental traits and believe that season per se, most likely via photoperiod length and temperature affected developmental traits, as found in other studies (Wright et al. 1990; Laurila et al. 2001). Seasonality can play an important role in the variation of life-history traits between populations with differing growth seasons (Hjernquist et al. 2012), and therefore, it could affect developmental traits in differing habitats. Several studies show that larval and adult amphibians react to differing photoperiods and that they can estimate time to hibernation or aestivation by different physiological mechanisms (Schmuck et al. 1994; Lampert and Linsenmair 2001; Bradshaw and Holzapfel 2007). A shorter photoperiod can lead to faster growth (Gutierrez et al. 1984); what we observed as well. Time to metamorphosis in the sun treatment decreased towards the end of the reproductive season for tadpoles of both origins, even when minimum temperatures were lower and rates of development and growth should decrease. The lack of respective developmental responses in the shade treatments, most likely was due to temperatures that were too low to allow for continued growth.

Our results indicate that quarry populations may already reach their limits of adapting
to warming environments, and might face temperatures above their thermal limits in the future. Even if adults are long-lived and skip reproduction under unfavourable conditions (Abbühl and Durrer 1998), a loss of offspring over several years will negatively impact population dynamics, and may ultimately lead to local extinctions.

On the other side, our results support the study from Scheele et al. (2014), who demonstrated that adults from forest habitat showed a better body condition than those from open habitats, and thus concluded that forest ponds provide a high quality habitat for *B. variegata*. We likewise think that forest habitats could potentially mitigate the negative effects of mean environmental temperature increase, induced by climate change for *B. variegata* in the future. Thus, forest management supporting *B. variegata* breeding sites may play a significant role in the long-term survival of this species in Central Europe.

Acknowledgements

We thank Peter Krämer (Regierung von Unterfranken), Ulrich Mergner (Bayerische Staatsforsten) and the owners of the quarries Klaubholz, Schleifsteinwerke and Gleußner for permits and permissions. Hans J. Poethke and the staff of the ecological field station supported the study by providing access to local infrastructure. Christopher Edge and two anonymous reviewers improved our manuscript with their constructive criticism. The study was part of the project „Adaptation capacity of selected species to climate change (2011 FKZ 3511 86 0200)“ funded by the German “Federal Agency for Nature Conservation” (BfN).

Reference


**Table 1** Summary of three-way ANOVA (type III ss) examining the main and interaction effects of origin (quarry/shade), treatment (shade/sun) and season (independent variables) on mortality (non-metamorphosed individuals) of *Bombina variegata* at metamorphic climax (Gosner 42).

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Sum of squares</th>
<th>df</th>
<th>F-ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>origin</td>
<td>1.63</td>
<td>1</td>
<td>0.46</td>
<td>0.50</td>
</tr>
<tr>
<td>treatment</td>
<td>28.03</td>
<td>1</td>
<td>7.81</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>season</td>
<td>67.50</td>
<td>1</td>
<td>18.81</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>origin x treatment</td>
<td>0.07</td>
<td>1</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>origin x season</td>
<td>0.02</td>
<td>1</td>
<td>0.01</td>
<td>0.65</td>
</tr>
<tr>
<td>treatment x season</td>
<td>64.07</td>
<td>1</td>
<td>17.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>origin x treatment x season</td>
<td>0.83</td>
<td>1</td>
<td>0.23</td>
<td>0.63</td>
</tr>
<tr>
<td>error</td>
<td>186.6</td>
<td>52</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Note:** Analyses were based on the mean of each tank (*n* = 60). df= degrees of freedom, p-values in bold show significant differences.
Table 2 Summary of two-way ANOVA (type II ss) examining the main and interaction effects of origin (quarry/forest) and treatment (shade/sun) (independent variables) on mortality, separated by two seasons (early season: May-August; late season: July-September).

<table>
<thead>
<tr>
<th>season</th>
<th>Source of variance</th>
<th>Mortality</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum of squares</td>
<td>df</td>
<td>F-ratio</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>early</td>
<td>origin</td>
<td>14.40</td>
<td>1</td>
<td>5.57</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>0.10</td>
<td>1</td>
<td>0.04</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>origin x treatment</td>
<td>0.90</td>
<td>1</td>
<td>0.35</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>error</td>
<td>93.00</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>late</td>
<td>origin</td>
<td>3.20</td>
<td>1</td>
<td>0.55</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>168.20</td>
<td>1</td>
<td>28.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>origin x treatment</td>
<td>3.20</td>
<td>1</td>
<td>0.55</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>error</td>
<td>93.60</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Analyses were based on the mean of each tank (early n = 40, late n = 20), df= degrees of freedom, p-values in bold show significant differences.
Table 3 Summary of three-way ANOVAs (type III ss) examining the main and interaction effects of origin (quarry/forest), treatment (shade/sun) and season (early/late) (independent variables) on developmental traits (dependent variable); body size (SVL in mm), body condition index (BCI in g) and developmental time in days of *Bombina variegata* at metamorphic climax (Gosner 42).

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>SVL in mm</th>
<th></th>
<th></th>
<th>BCI in g</th>
<th></th>
<th></th>
<th>Developmental time in days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum of squares</td>
<td>df</td>
<td>F-ratio</td>
<td>p-value</td>
<td>Sum of squares</td>
<td>df</td>
<td>F-ratio</td>
<td>p-value</td>
</tr>
<tr>
<td>origin</td>
<td>5.21</td>
<td>1</td>
<td>23.34</td>
<td>&lt; 0.001</td>
<td>&lt;0.01</td>
<td>1</td>
<td>0.02</td>
<td>0.91</td>
</tr>
<tr>
<td>treatment</td>
<td>1.93</td>
<td>1</td>
<td>8.63</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>1</td>
<td>6.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>season</td>
<td>1.83</td>
<td>1</td>
<td>8.18</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>1</td>
<td>1.44</td>
<td>0.24</td>
</tr>
<tr>
<td>origin x treatment</td>
<td>0.13</td>
<td>1</td>
<td>0.59</td>
<td>0.45</td>
<td>&lt; 0.01</td>
<td>1</td>
<td>0.52</td>
<td>0.47</td>
</tr>
<tr>
<td>origin x season</td>
<td>2.36</td>
<td>1</td>
<td>10.58</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>1</td>
<td>1.73</td>
<td>0.19</td>
</tr>
<tr>
<td>treatment x season</td>
<td>1.94</td>
<td>1</td>
<td>8.71</td>
<td>&lt; 0.01</td>
<td>0.03</td>
<td>1</td>
<td>12.70</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>origin x treatment x season</td>
<td>0.07</td>
<td>1</td>
<td>0.29</td>
<td>0.59</td>
<td>&lt; 0.01</td>
<td>1</td>
<td>0.11</td>
<td>0.74</td>
</tr>
<tr>
<td>error</td>
<td>11.60</td>
<td>52</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>52</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Note:** Analyses were based on the mean of each tank (*n* = 60). df = degrees of freedom, p-values in bold show significant differences.
Table 4 Summary of two-way ANOVAs (type II ss) examining the main and interaction effects of origin (quarry/forest) and treatment (shade/sun) (independent variables) on developmental traits (dependent variable); body size (SVL in mm), body condition (BCI in g) and developmental time in days from Bombina variegata at metamorphic climax (Gosner 42) separated by two seasons (early season: May-August; late season: July-September).

<table>
<thead>
<tr>
<th>Season</th>
<th>Source of Variance</th>
<th>SVL in mm</th>
<th></th>
<th>BCI in g</th>
<th></th>
<th>Developmental Time in Days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sum of squares</td>
<td>df</td>
<td>F-ratio</td>
<td>p-value</td>
<td>Sum of squares</td>
<td>df</td>
</tr>
<tr>
<td>early</td>
<td>origin</td>
<td>21.03</td>
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<td>78.73</td>
<td>&lt; 0.001</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>0.90</td>
<td>1</td>
<td>3.37</td>
<td>0.08</td>
<td>&lt; 0.01</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>origin x treatment</td>
<td>0.20</td>
<td>1</td>
<td>0.73</td>
<td>0.40</td>
<td>&lt; 0.01</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>error</td>
<td>9.61</td>
<td>36</td>
<td>-</td>
<td>-</td>
<td>0.03</td>
<td>36</td>
</tr>
<tr>
<td>late</td>
<td>origin</td>
<td>0.07</td>
<td>1</td>
<td>0.58</td>
<td>0.46</td>
<td>0.02</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>2.05</td>
<td>1</td>
<td>16.49</td>
<td>&lt; 0.001</td>
<td>0.03</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>origin x treatment</td>
<td>0.00</td>
<td>1</td>
<td>0.00</td>
<td>1.00</td>
<td>&lt; 0.01</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>error</td>
<td>1.99</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
<td>16</td>
</tr>
</tbody>
</table>

**Note:** Analyses were based on the mean of each tank (early n = 40, late n = 20), df= degrees of freedom, p-values in bold show significant differences.
Table 5 Temperature (minimum (min), maximum (max), mean and standard deviation (sd) of water in °C) and developmental data (size: size at metamorphosis in mm; time: developmental time in days; BCI: body condition index at metamorphosis in g, each mean and standard deviation) for early and late season and the corresponding treatment groups, where the first pictogram always indicates the origin of *Bombina variegata* eggs (sun = quarry; tree = forest), the second pictogram indicates the tadpoles’ treatment conditions (sun versus shade = tree).

<table>
<thead>
<tr>
<th>season</th>
<th>Temperature (°C)</th>
<th>Tadpole development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>group</td>
<td>min</td>
</tr>
<tr>
<td>early</td>
<td></td>
<td>9.14</td>
</tr>
<tr>
<td>May-August</td>
<td></td>
<td>8.13</td>
</tr>
<tr>
<td>(n = 40)</td>
<td></td>
<td>8.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.11</td>
</tr>
<tr>
<td>late</td>
<td></td>
<td>7.10</td>
</tr>
<tr>
<td>July-September</td>
<td></td>
<td>7.60</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
<td>6.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.57</td>
</tr>
</tbody>
</table>
**Figure 1** Interaction plot showing the effect of origin and treatment on mortality (number of non-metamorphosed tadpoles per tank) of yellow-bellied toads (*Bombina variegata*) at metamorphic climax (Gosner 42) during two experimental periods (early season: May to August, plot on the left, $n = 10$ per treatment/origin, total $n = 40$; late season: July to September, plot on the right, $n = 5$ per treatment, total $n = 20$) and corresponding treatments. The shaded area corresponds to the 95% confidence interval. Symbols: $\triangle =$ quarry origin, $\bullet = $ forest origin.

**Figure 2** Interaction plot showing the effect of origin of population and treatment on developmental time (days; a, b), snout-vent-length (SVL in mm; c, d) and body condition index (BCI in g; e, f) of yellow-bellied toads (*Bombina variegata*) at metamorphic climax (Gosner 42) during two experimental periods (early season: May to August, plots on the left, $n = 10$ per treatment/origin, total $n = 40$; late season: July to September, plots on the right, $n = 5$ per treatment, total $n = 20$) and corresponding treatments. The shaded area corresponds to the 95% confidence interval. Symbols: $\triangle = $ quarry origin, $\bullet = $ forest origin.
Figure 1 Interaction plot showing the effect of origin and treatment on mortality (number of non-metamorphosed tadpoles per tank) of yellow-bellied toads (*Bombina variegata*) at metamorphic climax (Gosner 42) during two experimental periods (early season: May to August, plot on the left, \( n = 10 \) per treatment/origin, total \( n = 40 \); late season: July to September, plot on the right, \( n = 5 \) per treatment, total \( n = 20 \)) and corresponding treatments. The shaded area corresponds to the 95% confidence interval.

Symbols: \( \triangle \) = quarry origin, \( \bullet \) = forest origin.

211x151mm (300 x 300 DPI)
Figure 2 Interaction plot showing the effect of origin of population and treatment on developmental time (days; a, b), snout-vent-length (SVL in mm; c, d) and body condition index (BCI in g; e, f) of yellow-bellied toads (Bombina variegata) at metamorphic climax (Gosner 42) during two experimental periods (early season: May to August, plots on the left, $n = 10$ per treatment/origin, total $n = 40$; late season: July to September, plots on the right, $n = 5$ per treatment, total $n = 20$) and corresponding treatments. The shaded area corresponds to the 95% confidence interval. Symbols: $\Delta =$ quarry origin, $\bullet =$ forest origin.

297x420mm (300 x 300 DPI)