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Carry-over effects of the larval environment on the post-metamorphic performance of *Bombina variegata* (Amphibia, Anura)

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Metamorphs of the yellow-bellied toad, *Bombina variegata*, vary widely in size at metamorphosis in the field. We performed a replicated outdoor mesocosm study to simulate the environmental factor combinations in permanent and ephemeral breeding sites and to quantify their effects on tadpole development (duration of the larval period, metamorph size and body condition). Looking for potential carry-over effects of the larval environment, we quantified locomotor performance of all metamorph phenotypes originating from the mesocosms immediately after metamorphosis under controlled conditions. In contrast to the prediction of life-history theory, tadpoles were unable to adjust developmental rate to water availability, but metamorphs originating from the ephemeral pond treatment were smaller and had a lower body condition than those from the permanent pond treatment. Size-dependent carry-over effects included the length of the first jump following tactile stimulation, burst performance (total length of spontaneous jumps) and endurance (total distance covered in 10 forced jumps). A size-independent effect of larval environment was the prolonged locomotor effort to escape (5.7 consecutive jumps following initial stimulus) of metamorphs from the ephemeral pond treatment compared to same-sized ones (3.7 jumps) from the permanent pond treatment. Thus, we demonstrate that carry-over effects of larval environment on metamorph phenotype and behaviour cause a considerable variation in fitness in the early terrestrial stage of *B. variegata*. Informed conservation management of endangered populations in the northern range should therefore include the provision of small permanent breeding ponds promoting larger and fitter metamorphs.

Keywords: complex life cycles, mesocosm, plasticity of metamorph phenotype, post-metamorphic jumping performance, delayed density dependence, delayed life-history effects

INTRODUCTION

Developmental plasticity is the property of a genotype to produce different phenotypes in response to the environmental conditions experienced during development (Denver & Middlemis-Maher, 2010; Gritti & Barbot, 2019). In organisms with complex life-history such as amphibians, environmental conditions experienced during the aquatic larval stage influence the timing of metamorphosis as well as morphological and behavioural features of the terrestrial metamorph (Alford & Harris, 1988; Crespi & Warne, 2013). In natural tadpole habitats, often subject to desiccation at unpredictable rates, environmental factor combinations vary considerably in time and space during tadpole development, e.g. nutrient resources and available space decrease, whereas tadpole density and the amplitude of temperature fluctuations increase simultaneously (Newman, 1998; Turner et al., 2000). The consequences of the complex interactions among these and other factors on metamorph features are hard to predict from experimental approaches focusing on the impact of a

single environmental factor (Brady & Griffiths, 2000). For example, tadpole density (=crowding; Richards, 1958) does not only affect morphology (metamorph size), but also physiology and behaviour (stress hormone concentrations; corticotropin-releasing hormone, glucocorticoids) (Denver, 1997; Brady & Griffiths, 2000; Crespi & Warne, 2013). Thus, metamorph plasticity and its possible consequences for fitness/survival of juveniles and adults remain a major challenge for ecologists studying amphibians that inhabit variable environments.

Available evidence suggests that environmental conditions in the aquatic habitat may cause variation in adult phenotypic expression of several life-history traits (Alford & Harris, 1988; Boes & Benard, 2013; Yagi & Green, 2018; Moore & Martin, 2019). The variation of traits such as size at and timing of metamorphosis often implies carry-over effects from larval conditions to the future fitness of juveniles and adults (Pechenik, 2006; Van Allen et al., 2010). Keeping in mind that stage-specific environmental effects are often not independent from each other and may combine in complex ways to shape fitness aspects, the correlation of single

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traits to adult fitness has to be proven experimentally (Beckerman et al., 2002; De Block & Stoks, 2005; Van Allen et al., 2010). In amphibians, there is substantial evidence for the link between large metamorph size and increased dispersal abilities, survival and reproductive success (e.g., Altwegg & Reyer, 2003; Vonesh, 2005; Chelgren et al., 2006; Reading, 2007; Briggs, 2008; Tarvin et al., 2015; Yagi & Green, 2018). In some frog species, however, the growth rate and fitness of juveniles did not only depend on the metamorphs' phenotype, but also independently on features of the larval habitat (Morey & Reznick, 2001; Álvarez & Nicieza, 2002a, b; Richter-Boix et al., 2006; Van Allen et al., 2010; Bredeweg et al., 2019; Zamora-Camacho et al., 2019). Interactions among the aquatic conditions experienced, variation of metamorph features, and fitness of the terrestrial stages seem to occur frequently in pond-breeding amphibians.

The yellow-bellied toad (*Bombina variegata*) is an excellent model to analyse these interactions because size of metamorphs varies in a remarkably broad range in the wild (Kapfberger, 1984; Barandun & Reyer, 1997a; Miesler & Gollmann, 2000; Di Cerbo & Biancardi, 2010; Dittrich et al., 2016; Schäfer et al., 2018). The ecological features of breeding ponds are comparably broad and range from water bodies with a short hydroperiod (high risk of desiccation before metamorphosis) to permanent ponds (numerous predators, competing tadpoles of other species) (Barandun & Reyer, 1997b; Hartel et al., 2007; Hantzschmann & Sinsch, 2019). Yet, there is no evidence that environmental factor combinations experienced during aquatic development are the proximate causes of plasticity in metamorph traits. Böll (2002) attempted to induce phenotypic plasticity in developmental rate and metamorph size under controlled conditions, but results were inconsistent with field observations. Tadpoles reared in desiccating habitats metamorphosed as expected at a small size, but developmental rate decreased instead of speeding up. The presence of carry-over effects from larval history to the performance of metamorph phenotypes has not been tested yet in *B. variegata*.

Therefore, we designed a replicated mesocosm study to establish the causal relationship between aquatic environment and metamorph features and to quantify potential carry-over effects on post-metamorphic performance mediated by metamorph features. To maximise impact, we tested the most contrasting larval environments used by free-ranging *B. variegata*: (1) permanent pond conditions, i.e. constant water level and volume during the complete larval development, and (2) ephemeral pond conditions, i.e. initial water level and volume successively decreasing as during the desiccation of a natural pond. Rearing conditions aimed to mimic the factor combinations acting at the extremes of the natural hydroperiod continuum used for breeding. Life-history theory predicts that the permanent pond environment should yield large metamorphs at slow developmental rate and the ephemeral treatment small metamorphs at fast growing rates (Wilbur & Collins, 1973; Stearns, 2000). We hypothesise that the aquatic environment experienced shapes the plasticity of metamorph

features and subsequently the locomotor performance of the metamorphs (indicator of fitness). We predict: (1) pond conditions determine timing of metamorphosis and metamorph phenotype (size, body condition). (2) metamorph phenotypes differ in jumping performance. (3) similar phenotypes resulting from either larval treatment have similar locomotor performance.

METHODS

Ethics statement

Permits for the collection of yellow-bellied toad *Bombina variegata* tadpoles were issued by the Struktur- und Genehmigungsdirektion Nord (Az 425-104.1711). All applicable institutional and/or national guidelines for the care and use of animals were followed (Kreisverwaltung Mayen-Koblenz, Az 39183-04). All toad metamorphs were released at the site of capture after testing jumping performance.

Animal collection

On 3 July 2017, we collected 360 tadpoles from four ponds at the former military training area Schmidtenhöhe near Koblenz (50.201-50.346°N, 7.393-7.644°E, 279-298 m a.s.l.; Rhineland-Palatinate, Germany; Schäfer et al., 2018; Hantzschmann & Sinsch, 2019). Three of the ponds sampled dried out during the next two weeks; the fourth had a hydroperiod long enough to warrant successful larval development. We immediately transferred all tadpoles to University of Koblenz, where we placed them altogether into a 100 L indoor plastic pan filled with 50 L of aged tap water. At the day of capture, we measured the developmental stage (range: 26-35; Gosner, 1960) and body length (to the nearest 0.5 mm; range: 4.5-15 mm) of each tadpole using a dissecting microscope (Zeiss Stemi 508). We randomly constituted 20 groups of 18 tadpoles that were assigned to plastic boxes with 1L aged tap water. The groups of tadpoles were transferred to outdoor mesocosms (plastic pans) for experimental treatment.

Experimental conditions of tadpole rearing

The experimental set-up consisted of two rows of 10 fully sun-exposed, outdoor mesocosms (60 x 40 x 20 cm) each, filled with initially 40 L of tap water (16 cm water height). We filled the mesocosms with tap water one week before the start of the experiment. In each mesocosm, we added a 3 mm ground layer of soil collected at the Schmidtenhöhe and a pasture sod (10 x 10 cm) to mimic the conditions at the natural breeding sites. Food available to tadpoles consisted, apart from an initial dose of 1 g Tetraphyll, of bacteria, algae and other microorganisms introduced with soil and sod. We covered the mesocosm surface completely with gauze to prevent litter from falling into the water, interference with airborne predators (e.g., birds, dytiscid beetles) and escape of metamorphs at the end of the experiment. On the day of capture, we randomly assigned the 20 groups of tadpoles to one of two regimes mimicking the extremes of hydrological breeding pond variation: (1) Permanent pond treatment: PP=constant water level

at 16 cm height plus one large free-ranging dragonfly-larva (*Aeshna cyanea*) as a potential aquatic predator. Potential additional effects of interspecific competition were not considered because in the permanent ponds of the study area used by *B. variegata*, tadpoles of the sympatric *Pelophylax esculentus*, *Hyla arborea* and *Alytes obstetricans* were rare to absent. (2) Ephemeral pond treatment: EP=decreasing water level from initially 16 cm to 11, 8, and 5 cm at weekly steps (final water volume 12 L) without predators. Two mesocosms of each treatment group were fitted with temperature loggers (Tinytag Plus 2 -TGP 4017) recording hourly water temperature. Note that the two treatments were chosen deliberately to evaluate the combined impact of the factors predator presence, tadpole density and natural water temperature fluctuations on tadpole development and metamorph features, not to disentangle the isolated effects of the single factors, which always interact in natural breeding ponds.

Tadpole development was monitored during six weeks. The number of surviving tadpoles was counted weekly, body length and Gosner-stage were measured bi-weekly. When we recorded the first metamorphic stages, we checked the mesocosms every two days to avoid drowning of metamorphs. We defined Gosner-stage 42 as the endpoint of complete aquatic development, removed these individuals from the rearing mesocosm and placed them into same-sized pans (one per treatment group) within a temperature-controlled chamber at 20 °C. These pans contained 2 L water and were inclined to allow tadpoles to leave the water at will.

Experimental testing of metamorph jumping behaviour

Resorption of the tail bud occurred usually within two days. Prior to testing, snout-vent length (SVL) of metamorphs was measured using a calliper (rounded to the nearest 0.5 mm). Following gently removing water drops on skin surface with a paper towel, we measured the corresponding body mass using a Sartorius electronic balance (to the nearest mg). Metamorphs were not fed because body mass during testing should reflect exclusively larval growth performance. The body condition of an individual is calculated as the studentised residual of the SVL-mass relationship using a multiplicative model $\ln(\text{mass}) = a + b \times \ln(\text{SVL})$, with a = intercept and b = slope (residual index, Băncilă et al., 2010; Scheele et al., 2014; Mikoláš, 2016). We transferred metamorphs immediately following measurements to a temperature-controlled chamber at 20 °C with artificial light. Thermal acclimation lasted two hours before testing.

Trials were performed in a white plastic box (60 x 40 x 20 cm) without any structure. The ground of the pan was cleaned and moistened with a paper towel before introducing the test individual. A digital camera was fixed 50 cm above the ground level of the pan to record the movements of the metamorph during the trial. Before any trial, the scale was calibrated by recording a 50 cm ruler at the ground of the pan. Each metamorph was tested once. A trial consisted of placing a metamorph into the pan and pushing it gently at the urostyle using a pencil. This tactile stimulus elicited a series of jumps in most

individuals. Unresponsive metamorphs were excluded from data analysis. If the initial stimulus elicited fewer than 10 jumps, the individual was stimulated again to complete 10 jumps. The trial was finished after 10 jumps and the metamorph was removed from the testing pan. The digital record of each trial was labelled with number of the individual tested, SVL, body mass and prior larval treatment. Every two days, the tested individuals were released again at the collecting site.

The locomotor performance of metamorphs was analysed using the program Tracker (Open Source Physics, version 4.11.0; <https://physlets.org/tracker/>). We quantified four parameters to describe the jumping behaviour of metamorphs (Tejedo et al., 2000; Richter-Boix et al., 2006): (1) number of spontaneous jumps following the tactile stimulus (=intensity of locomotor escape behaviour). (2) distance [mm] covered by each spontaneous jump (snout tip of the metamorph was defined as start and end point of a jump). (3) total distance [mm] covered in all spontaneous jumps (=burst performance). (4) total distance [mm] covered in 10 successive jumps following one or more tactile stimuli (=endurance; McGinley et al., 2013).

Statistical analyses

Data distributions were tested for normality determining standardised kurtosis and skewness. Since distributions often deviated from normality, all data were log10-transformed to normalise them for parametric procedures. We tested for carry-over effects of the larval treatment on the response variables using ANCOVAs with the continuous co-variables duration of larval treatment, SVL, body condition, depending on which response variable was analysed. Note that we did not account for potential differences among the 10 pans within a treatment due to logistical problems. Therefore, a potential increase of variability caused by a single pan remained statistically undetected. If one or two co-variables correlated significantly with a response variable, we used linear regression models (simple and multiple) to quantify the effect in each larval treatment. Differences of models with respect to slope or intercept were tested for using an ANOVA on the conditional sum of squares. Statistical significance was set at $\alpha=0.05$. Statistical analyses were conducted using the software package StatGraphics 18.

RESULTS

Tadpole performance under permanent and ephemeral pond treatments

Water temperatures fluctuated between 13.1 °C and 35.3 °C during the EP treatment and 13.5 °C and 35.9 °C during the PP treatment (Fig. S1). Minimum diel water temperature (log10-transformed) did not differ significantly between EP and PP (ANOVA, $F_{1,151}=1.89$, $P=0.1715$). In contrast, maximum diel water temperature and the range of diel fluctuations (all response variables log10-transformed) were significantly higher in the EP mesocosms than in the PP mesocosms (ANOVA, maximum: $F_{1,151}=4.59$, $P=0.0338$; range: $F_{1,151}=6.14$,

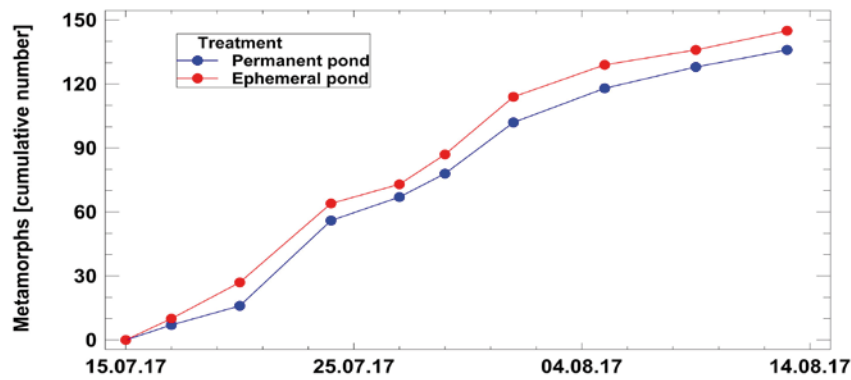


Figure 1. Time course of metamorphosis in *B. variegata* tadpoles exposed to constant water level (permanent pond treatment) or decreasing water level (ephemeral pond treatment). Data pooled for the 10 replicates per treatment.

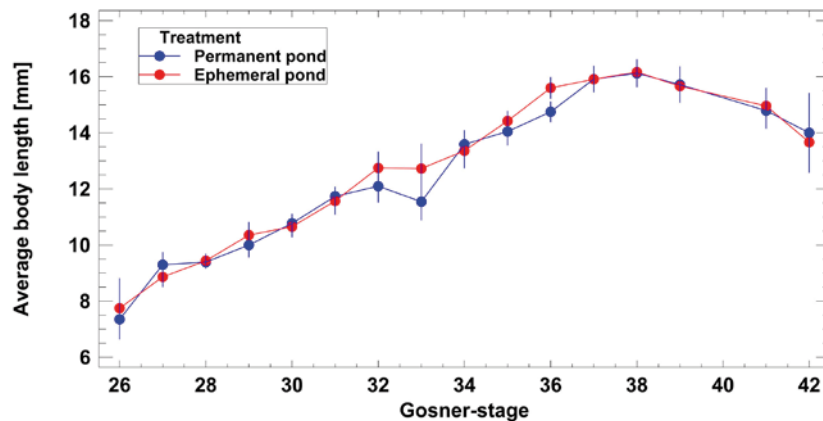


Figure 2. Growth pattern of *B. variegata* tadpoles in the two experimental treatments. Development is staged according to Gosner (1960), body size is given as the average body length [mm] and corresponding 95 % Bonferroni confidence interval. Data pooled for the 10 replicates per treatment.

$P=0.0143$), reflecting the effect of decreasing water level.

During the experimental treatments, 19 tadpoles (10.6 %) died in EP and 23 tadpoles (12.8 %) in PP. The mortality rate of tadpoles was not significantly affected by treatment (ANCOVA, $F_{1,79}=0.02$, $P=0.8931$) and did not vary in time (ANCOVA, $F_{1,79}=0.30$, $P=0.5837$). During the EP treatment, 145 tadpoles (80.6 %) reached the metamorphic stage 42, during the PP treatment 136 (75.6 %) did. The number (log10-transformed) of metamorphs per mesocosm was not significantly affected by treatment (ANCOVA, $F_{1,19}=0.73$, $P=0.4038$). The time course of metamorphosis was similar in the EP and PP tadpoles (Fig. 1). The difference between the cumulative number of metamorphs in the EP and PP mesocosms did not increase significantly with the duration of the experiment (all regression models tested: $P>0.05$). At the end of the experimental treatments, 16 EP and 21 PP tadpoles had not reached the metamorphic stage.

Tadpole body length increased continuously during the pre-metamorphic stages (26–38; linear regression model: $BL [mm] = -10.03 + 0.70 \cdot \text{Gosner-stage}$, $R^2=82.1\%$, $P<0.0001$; Fig. 2). During metamorphosis (stages: 39–45) body length shrank significantly (linear regression model: $BL [mm] = 40.86 - 0.64 \cdot \text{Gosner-stage}$, $R^2=47.6\%$, $P<0.0001$; Fig. 2). Stage-adjusted pre-metamorphic tadpole size (log10-transformed) did not differ between

the experimental treatments (ANCOVA, $F_{1,738}=2.15$, $P=0.1425$), but increased with Gosner-stage (ANCOVA, $F_{1,738}=2845$, $P<0.0001$).

Metamorph size decreased with date of emergence and was greater in the PP treatment (Fig. 3A). The linear regression models for EP metamorphs ($\log_{10}[\text{SVL}] = 1.15 - 0.0033 \cdot \text{day}$; $R^2=41.6\%$, $F_{1,124}=87.58$, $P<0.0001$) and PP individuals ($\log_{10}[\text{SVL}] = 1.16 - 0.0020 \cdot \text{day}$; $R^2=17.3\%$, $F_{1,114}=23.67$, $P<0.0001$) differed significantly with respect to slope (ANOVA for the conditional sum of squares, $F_{1,3}=5.51$, $P=0.0197$) and to intercept (ANOVA for the conditional sum of squares, $F_{1,3}=35.8$, $P<0.0001$). If the effects of exposure time to the experimental treatment were accounted for, PP metamorphs (13.4 mm) were significantly larger than EP metamorphs (12.7 mm) (ANCOVA, $F_{1,239}=35.13$, $P<0.0001$).

The body condition of PP metamorphs averaged $0.253 (\pm 0.09 \text{ SE})$ regardless of the date of metamorphosis, whereas that of EP metamorphs decreased continuously with the exposure time to treatment (linear regression model: $\text{Condition index} = 0.346 - 0.0412 \cdot \text{day}$; $R^2=10.5\%$, $F_{1,124}=14.44$, $P=0.0002$) (Fig. 3B). If the effects of exposure time to the experimental treatment were accounted for, PP metamorphs had a significantly higher condition than EP metamorphs (least square mean: $-0.238 \pm 0.06 \text{ SE}$, ANCOVA, $F_{1,239}=15.75$, $P=0.0001$).

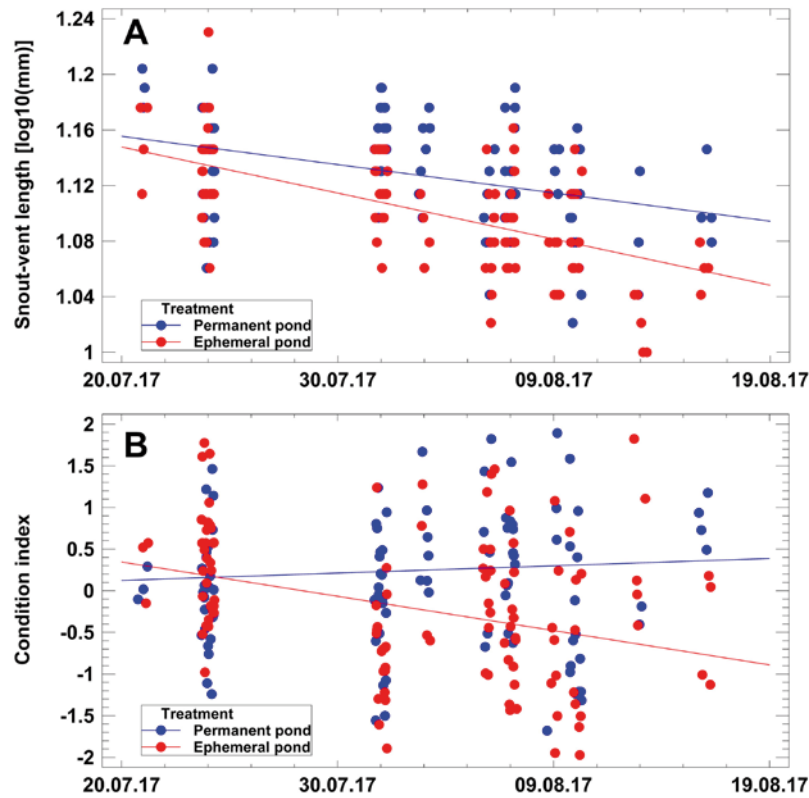


Figure 3. Temporal variation of snout-vent length (A) and condition index (B) in *B. variegata* metamorphs originating from the two experimental treatments. Each symbol represents a metamorph individual, the regression lines are given separately for the PP (blue) and for EP (red) treatments. For statistical details see text.

Metamorph jumping performance

During test trials, 113 out of 115 PP metamorphs and 92 out of 125 EP metamorphs responded to the initial tactile stimulus with at least one jump. Consequently, 2 PP metamorphs and 33 EP metamorphs were excluded from further analyses.

Intensity of locomotor escape behaviour

The number of spontaneous jumps was significantly greater in EP metamorphs (5.7 on average) than in PP metamorphs (3.7; ANCOVA, $F_{1,204}=17.93$, $P<0.0001$). Body condition was positively related to the number of spontaneous jumps (ANCOVA, $F_{1,204}=10.34$, $P=0.0015$), whereas SVL was not (ANCOVA, $F_{1,204}=1.04$, $P=0.3083$). The number of spontaneous jumps increased slightly, but significantly with body condition in PP metamorphs (linear regression model: $\log_{10}(n \text{ jumps})=0.460+0.098*\text{body condition}$; $R^2=8.8\%$, $F_{1,112}=10.65$, $P=0.0015$), but not in EP metamorphs ($R^2=1.7\%$, $F_{1,91}=1.57$, $P=0.2136$).

Length of first jump

The distance covered with the first jump was independent from the larval treatment (ANCOVA, $F_{1,204}=0.76$, $P=0.3842$), but increased significantly with metamorph size (ANCOVA, $F_{1,204}=46.10$, $P<0.0001$) and body condition (ANCOVA, $F_{1,204}=9.17$, $P=0.0028$). The multiple regression models for the length of the first jump were $\log_{10}(\text{jump1 length})=1.072+0.059*\text{SVL}+0.037*\text{body condition}$ (PP metamorphs; $R^2=29.2\%$, $F_{2,112}=22.73$, $P<0.0001$), and $\log_{10}(\text{jump1 length})=1.122+0.054*\text{SVL}$

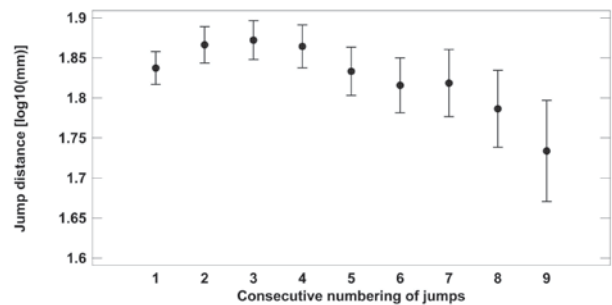


Figure 4. Distance covered by spontaneous, consecutive jumps of *B. variegata* metamorphs originating from the two experimental treatments. Each symbol represents the least square mean (with corresponding 95 % Bonferroni confidence interval) calculated in a 2-factor ANCOVA with jump number and treatment as fixed factors and SVL and condition index as continuous co-variables. For statistical details see text.

$+0.022*\text{body condition}$ (EP metamorphs; $R^2=12.2\%$, $F_{2,91}=6.21$, $P=0.0030$). The first four spontaneous jumps were significantly longer than the subsequent jumps (2-factor ANCOVA, $F_{8,918}=5.39$, $P<0.0001$; Fig. 4).

Burst performance

The total distance covered in all spontaneous jumps was significantly shorter in PP metamorphs than in EP metamorphs (least square mean \pm SE: 204 \pm 1mm vs. 332 \pm 2mm, ANCOVA, $F_{1,204}=15.81$, $P=0.0001$), and

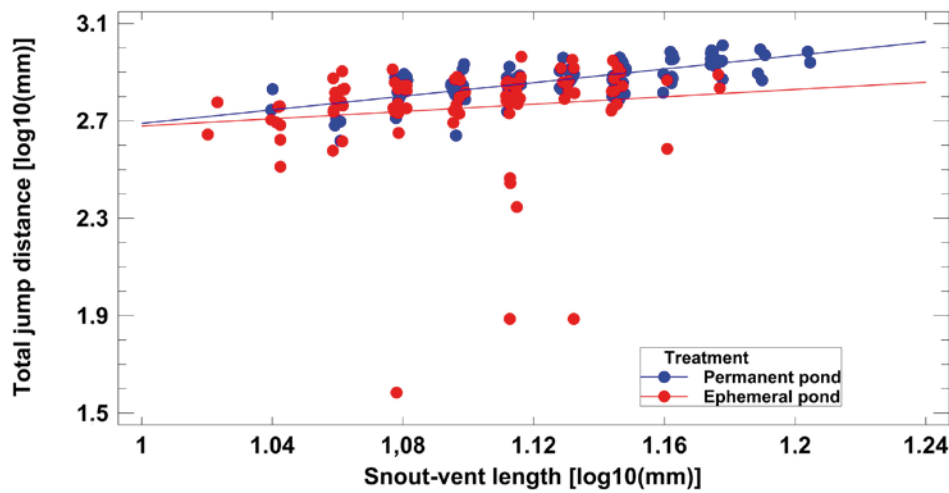


Figure 5. Variation of the cumulated distance covered within 10 jumps (spontaneous and forced) with respect to the snout-vent length of *B. variegata* metamorphs originating from the two experimental treatments. Each symbol represents a metamorph individual, the regression lines are given separately for the PP (blue) and for EP (red) treatments. For statistical details see text.

increased significantly with metamorph size (ANCOVA, $F_{1,204}=14.07$, $P=0.0002$) and body condition (ANCOVA, $F_{1,204}=17.38$, $P<0.0001$). The multiple regression models for the total distance covered in all spontaneous jumps were $\log_{10}(\text{burst performance})=1.213+0.083*\text{SVL}+0.131*\text{body condition}$ (PP metamorphs; $R^2=17.3\%$, $F_{2,112}=11.51$, $P<0.0001$), and $\log_{10}(\text{burst performance})=1.163+0.073*\text{SVL}+0.076*\text{body condition}$ (EP metamorphs; $R^2=12.2\%$, $F_{2,91}=3.41$, $P=0.0375$).

Endurance

The distance covered in 10 consecutive jumps was significantly larger in PP metamorphs than in EP metamorphs (least square mean \pm SE: 703 \pm 1mm vs. 598 \pm 1mm, ANCOVA, $F_{1,191}=8.25$, $P=0.0045$), and increased significantly with metamorph size (ANCOVA, $F_{1,191}=17.06$, $P=0.0001$), but not with body condition (ANCOVA, $F_{1,191}=3.44$, $P=0.0651$). A closer inspection on size dependence showed that endurance and size were positively correlated in PP metamorphs ($\log_{10}(\text{total distance})=1.29+1.40*\log_{10}(\text{SVL})$; $R^2=48.2\%$, $F_{1,99}=91.14$, $P<0.0001$), but not in EP metamorphs ($R^2=1.7\%$, $F_{1,91}=1.57$, $P=0.2136$; Fig. 5).

DISCUSSION

Our study evidences that the environment experienced during tadpole development affects predictably the phenotype of *B. variegata* metamorphs. Size and performance of most metamorphs originating from the permanent pond treatment were superior to those of metamorphs from the ephemeral pond treatment. These carry-over effects might influence survival of the early terrestrial stage because small metamorphs experience a greater predation risk than large ones (Schäfer et al., 2018). Despite the fact that the hydroperiod of water bodies chosen for reproduction is unpredictable for the adult toads, they show a remarkable philopatry instead of spreading the risk by depositing clutches in different ponds (Kapfberger, 1984; Seidel, 1987; Barandun & Heyer,

1997b, 1998). Consequently, the same breeding water body may change between permanent pond conditions and desiccating puddle during the reproduction period depending on the unpredictable rainfall regime (Hantzschmann & Sinsch, 2019). As a spatial bet-hedging strategy seems to be absent in *B. variegata*, the range of variation of water level at the breeding pond is crucial for the survival of the aquatic stages and determines the chance that an individual successfully completing metamorphosis reaches sexual maturity. We discuss the consequences of changing water levels and associated environmental factors for the fitness of offspring reaching the terrestrial stage.

Prediction 1: Larval conditions determine timing of metamorphosis and metamorph phenotype

In contrast to the predictions of life-history theory, but in agreement with Kapfberger (1984) and Böll (2002), *B. variegata* tadpoles were unable to accelerate larval development in response to simulated EP conditions. An adaptive trade-off between timing of metamorphosis and size at metamorphosis during the EP treatment was absent, but smaller metamorph size than that of PP metamorphs indicates that EP conditions implies additional costs for development possibly due to increased stress (crowding effect mediated by CRH; Kapfberger, 1984; Denver, 1997; Brady & Griffiths, 2000; Crespi & Warne, 2013; Bredeweg et al., 2019). The high risk of death in desiccating ponds and developmental constraints leading to low-quality metamorph phenotypes (small size, low body condition) raise the question why *B. variegata* often reproduces in water bodies prone to drying-out. Our experimental data support the field observation that predator avoidance or reproductive failure in ponds containing aquatic predators is not a major factor in breeding site choice (Barandun & Reyer, 1997b; Hartel et al., 2007). The presence of competing tadpoles from other species in permanent ponds does not seem to matter either (Barandun & Reyer, 1997b). High-quality metamorphs emerge mainly from ponds with long hydroperiod, in

sunny patches of the forest (Hartel et al., 2007; Scheele et al., 2014; Dittrich et al., 2016). Therefore, we propose that the surprising variety of water bodies used by *B. variegata* for spawning is simply a side product of a temporal bet-hedging strategy to increase reproductive success in rainy years when the hydroperiod of ephemeral ponds allows for successful metamorphosis (Buschmann, 2002). Since larval conditions in fact predict metamorph phenotype, carry-over effects with respect to size and body condition are crucial for the vulnerability of metamorphs to terrestrial predators (Schäfer et al., 2018).

Prediction 2: Metamorph phenotypes differ in jumping performance

The locomotor performance of anuran metamorphs during dispersal from the breeding water body to suitable juvenile habitats is often related to conditions experienced during the larval stage and to metamorph size (e.g., John-Alder & Morin, 1990; Goater et al., 1993; Tejedo et al., 2000; Charbonnier & Vonesh, 2015; Bredeweg et al., 2019). Our study corroborates that there are complex interactions among larval environment, metamorph size, body condition and locomotor performance of *B. variegata*. Metamorph size and condition predict partially different aspects of locomotor performance (length of first jump, burst performance, endurance) demonstrating the importance of phenotype for metamorph fitness. The burst performance of EP metamorphs exceeded that of PP metamorphs, suggesting that their energy expenditure for escape jumps is greater. In EP metamorphs, low body condition and probably stress experienced during the larval stage seem to mediate a stronger sensitivity to a tactile stimulus than in PP metamorphs, similar to findings in *Lithobates sylvaticus* (Crespi & Warne, 2013), but in contrast to *Pelobates cultripes* (Zamora-Camacho & Aragon, 2019). If the assumed stress-induced activity indeed overrides the limitations posed by muscle mass (e.g., Choi et al., 2003), the locomotor escape behaviour of EP metamorphs is less affected by size or condition than in PP metamorphs. Endurance showed the opposite pattern suggesting an overall greater locomotor performance of PP metamorphs than of same-sized EP metamorphs, as also shown for *Rana aurora* (Bredeweg et al., 2019). Finally, we provide evidence that the intensity of locomotor escape behaviour (number of consecutive spontaneous jumps) of *B. variegata* metamorphs has a size-independent component, which relates directly to the conditions experienced during the larval stage. Thus, morphological and behavioural traits of metamorphs determine in part their locomotor performance, i.e. dispersal distance and response to predator attacks.

Prediction 3: Similar phenotypes resulting from either larval treatment have similar post-metamorphic performance

Conditions experienced during the larval stage can have delayed effects on post-metamorphic growth not captured in the size of the metamorph when leaving the aquatic environment (Van Allen et al., 2010; Bredeweg et al., 2019). Therefore, similar phenotypes may differ

in their performance, if they have experienced different larval environment. Here, we provide clear evidence that the locomotor component of escape behaviour (frequency of jumps, endurance) is not predictable from metamorph size or condition alone but differs in same-sized metamorphs between the larval treatments. We conclude that metamorphs originating from distinct larval environments are not equivalent in post-metamorphic performance and have most probably different survival rates, even if they share similar phenotypes. Since locomotor performance is also a proxy for dispersal ability, the bearing on the structure and demography of the adult population is straightforward.

In summary, the outdoor mesocosm experiment gives deeper insight in the consequences of breeding site features for aquatic development and subsequent carry-over effects on the early terrestrial stages of yellow-bellied toads. The time course of water level fluctuation, unpredictable at clutch deposition, may lead to high- and low-quality metamorphs in the same pond during the prolonged reproduction period depending on the rainfall regime. Thus, a breeding water body may alternate between sink and source state within one year and between years indicating that the observed breeding site fidelity is a temporal bet-hedging strategy.

Implications for conservation management

Since *B. variegata* has recently experienced local extinctions, habitat fragmentation and population declines in western and north-western parts of its range, informed conservation management requires deeper insight in the ecological consequences of breeding habitat design (e.g., Cayuela et al., 2011; Scheele et al., 2014; Guicking et al., 2017; Weihmann et al., 2019). Larval mortality due to desiccation is probably more frequent than previously perceived and promoted by the focus on very shallow artificial ponds in breeding site management (e.g., Wagner, 1996; Zahn & Niedermeier, 2004). Water bodies used for breeding are not equivalent in habitat quality, even if their hydroperiod is long enough to allow tadpoles to complete metamorphosis. Metamorphs hatching from ephemeral ponds have a lower fitness due to carry-over effects on phenotype and behaviour. Thus, timing of and size at metamorphosis of *B. variegata* does not follow strictly the predictions of the Wilbur and Collins (1973) model suggesting that larval growth trajectories in this species are not adapted to conditions in ephemeral breeding sites with short hydroperiods. Conservation management of endangered *B. variegata* populations should thus include the construction of shallow, but permanent water bodies when attempting to optimize breeding habitats.

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