Testing the impact of environmental conditions and matriline on tadpole developmental traits in the European Common Frog, *Rana temporaria*, in the field

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Abstract. Phenotypic trait variation among populations is frequently studied to explore evolutionary mechanisms of adaptation processes. However, most studies are conducted over large spatial scales making gene flow between organisms unlikely. Here we explored differentiation of developmental traits among individuals within a small spatial area and asked if differences were environmentally induced and / or had a genetic basis. In a common garden approach we compared the development of *Rana temporaria* tadpoles, developing either in ponds where the clutch had been originally deposited or in alternative ponds. Forest ponds were small to medium sized, geographically close, and all used as breeding sites by the species. Our main findings were: The variation among pond characteristics was sufficient to generate differences in larval developmental traits among ponds. Non-sibling tadpoles developing in a common environment differed in developmental traits. Sibling tadpoles developing under different conditions showed differences in developmental traits. Tadpoles developing in their native environment did not always perform better than ‘foreign’ tadpoles. These results indicate that environmental conditions shape developmental plasticity to some extend (siblings raised under different conditions differed) but that matriline x environment interactions likewise influence the plasticity of developmental traits (non-siblings differed in their reactions within a common environment).

Key words. Amphibia, Anura, matriline x environment interaction, phenotypic divergence, within-population variation.

Introduction

Apart from the genetic disposition of an organism, its fitness is highly influenced by its environment. In order to maximize fitness, individuals should therefore choose habitats which best meet their physiological requirements and resource demands. For species lacking parental care in post-ovarian stages and restricted mobility in early development, such as amphibians with aquatic tadpoles, this also applies to the choice of the developmental environment of the offspring. The parents’ decision where to breed impacts their own reproductive fitness, since the oviposition site affects survival and performance of the offspring (e.g. Resetarits & Wilbur 1989, Thompson & Pellmyr 1991, Blaustein 1999, Refsnider & Janzen 2010). Therefore, selection of oviposition sites is an important part in the evolution of life-histories (Resetarits 1996).

The selection of particular oviposition sites has been shown to be influenced by a variety of environmental (biotic and abiotic) parameters (e.g. predation: Resetarits & Wilbur 1989, Petranka et al. 1994: pond characteristics: Strijbosch 1979, Egan & Paton 2004). Like many other organisms, amphibians react sensitively to their environment, especially during development (Wilbur & Collins 1973, Lindström 1999) and individuals respond to factors such as predators, conspecifics, climate and food abundance by changes in their morphology, behavior and development (e.g. Leips & Travis 1994, Benard 2004, Van Buskirk 2009). These responses affect their later life-history and fitness (Berven 1988, Semlitsch et al. 1988).

Since 2005 we examine a population of the European common frog, *Rana temporaria* Linnaeus, 1758, in southern Germany (Grözinger et al. 2012). This population exhibits a distinct long-term preference for a subset of avail-
able breeding ponds, indicating a choice of breeding habitats, rather than a random use of sites. However, the parameters investigated, although known to be influential in other studies (see above), could not explain oviposition site selection and the basis on which frogs used or avoided ponds for breeding remained unclear (Grözinger et al. 2012). In a subsequent study we revealed that tadpoles developing in the studied ponds showed an extreme divergence in larval metamorphic traits among and within breeding ponds that could not be explained by any of the examined biotic and / or abiotic pond characteristics. Froglings emigrating from the same pond on the same day varied in size by over 30% and the larger ones had up to twice the weight of smaller ones. These differences in body size and weight were even more pronounced among ponds (Grözinger et al. 2014).

The apparent lack of a clear environmental signal for oviposition site selection and tadpole development in our population seeks for an explanation beyond the above mentioned rationales. Here, we ask whether differences in genotype by environment interaction might result in the observed extreme differences in metamorphic traits. As a substitute for genotype, we herein use matrilines, i.e. tadpoles originating from the same clutch and thus having the same mother (but not necessarily the same farther; see e.g. Laurila & Seppä 1998, Vieites et al. 2004).

In order to address the question how matriline and / or environment influence the tadpoles’ development, we used a common garden approach to test whether 1) tadpoles from different clutches (non-siblings) develop differently in a common environment, 2) different ponds within the study area induce differences in the development between siblings (tadpoles from the same clutch), 3) tadpoles develop better in their native pond contrasted to tadpoles from an alternative pond. A divergence in developmental traits of siblings raised in different ponds would underline an environmental impact on developmental traits. A divergence in developmental traits of non-siblings raised under identical conditions would imply a genetic basis of differences, potentially indicating different life-history strategies. A better performance of the native tadpoles i.e. tadpoles raised in the ponds which was chosen by the female for oviposition, compared to "foreign" ones, would indicate optimal choice of ponds by breeding pairs.

### Material and methods

#### Study site

We conducted common garden-experiments in ponds in a forested area in the Steigerwald near the village Fabrikschlechach (49°55.4’ N, 10°33.3’ E), southern Germany. These forests are dominated by beech with patches of mixed forest. A large number of small artificial ponds (< 100) was established by the local forestry management within the last forty years (for pond characteristics see Grözinger et al. 2012, Table 1 and Supplementary Table S1). Since 2005, we annually monitored all ponds in this area for oviposition site use by *Rana temporaria*. In this population clutches are deposited within a few days around the end of March or beginning of April (Dtttrich et al. 2018). Females deposit only one clutch per breeding season.

#### Experimental set-up

In 2009, we selected nine geographically close ponds (distance < 1 km) based on the positive presence of *Rana temporaria* breeding activity in the previous years. From each of these ponds, one fertilized clutch was collected on April 8 or 9, ensuring equal age of the eggs. We hatched the eggs in plastic boxes (LWH: 35×31.5×22.5 cm) filled with rain water and placed outdoors in a partly shaded garden of a nearby field station (same climatic conditions as in the study area). As soon as tadpoles were free-swimming and feeding (developmental stage 25 according to Gosner 1960), 150 tadpoles of each clutch were randomly assigned to five tanks (three transfer-cages in different ponds, two control-tanks under standardized conditions, see below and Fig. 1). Thirty of these tadpoles were raised in each tank, resulting in 1530 tadpoles being studied in this experiment (150 tadpoles per clutch x nine clutches, each from a different pond). In the following we refer to each clutch as a ‘matriline’ and to tadpoles from the same clutch as “siblings”. We are aware that multiple paternity may occur in *R. temporaria* (Laurila & Seppä 1998, Vieites et al. 2004) thus possibly increasing genetic diversity within clutches.

#### Table 1. Summary of the environmental characters assessed in the study ponds (n = 9). Given are mean and standard deviation (mean ± sd), minimum and maximum values of the respective parameter (for details see Appendix A and Table S1). * = parameters were not used in further analyses since seven or more ponds showed the same values.

<table>
<thead>
<tr>
<th>environmental parameter</th>
<th>mean±sd</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
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<tr>
<td>pH</td>
<td>6.9±0.6</td>
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<tr>
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<td>3.50</td>
</tr>
<tr>
<td>presence of inflow [0;1]</td>
<td>0.44±0.53</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>volume [m³]</td>
<td>25.96±40.96</td>
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</tr>
<tr>
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<td>0.10</td>
</tr>
<tr>
<td>phosphate (PO₄) [mg/l]</td>
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<tr>
<td>water depth [mm]</td>
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<tr>
<td>bottom layer depth [m]</td>
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<tr>
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<tr>
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<tr>
<td>*nitrate (NO₃) [mg/l]</td>
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</tr>
<tr>
<td>*nitrite (NO₂) [mg/l]</td>
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<tr>
<td>*presence of sapropel [0;1]</td>
<td>1.00±0.00</td>
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<td>1.00</td>
</tr>
<tr>
<td>*pond bottom [0;1]</td>
<td>1.00±0.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
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</table>
In each of the nine ponds we placed three transfer-cages (wedge shaped, 80×50 cm basal area, max. 40 cm height, 80 l total volume) next to each other, one week before the start of the experiment. Cages were covered with gauze (mesh size: 1.4 mm) simultaneously ensuring perfusion with pond water and excluding predators, but not predators cues as these have been shown to influence tadpoles’ phenotypic plasticity (Luquet et al. 2011). External floats ensured a constant water level and volume in the cages (~30 l) and accessibility to atmospheric oxygen. We equipped the cages with soil and leaf litter (1.75 l) from the ponds’ surrounding to provide hiding places and food for the tadpoles. Within a pond one cage contained 30 native larvae, originating from this pond (native = N). The other two cages contained larvae from two other ponds. Due to size constraints of the ponds a full factorial design with transfer of larvae from all nine clutches to every pond was not possible.

In order to check for potential developmental differences under standardized conditions, additional control tanks (C) were set-up at the field station (see above) and filled with rain water (14 l). There, tadpoles of each clutch were raised in two replicates. The control tadpoles were fed ad libitum with commercial fish food (Tetra Pond Flakes®, Tetra GmbH, Melle, Germany). Every fourth day, the position of these tanks relative to each other was randomly shifted (thus accounting for potential random differences in micro-climate) and half of the water was replaced with aged spring water and faeces and food remains removed.

Figure 1. Scheme of the experimental set up exemplarily shown for one clutch of the common frog, Rana temporaria. A total of nine clutches from nine ponds were collected and tadpoles were raised in plastic tanks till Gosner-stage 25 (Gosner 1960). Subsequently, tadpoles of each clutch were assigned to either one of three transfer-cages located in three different ponds (pond the clutch was originally laid in and two foreign ponds) and to two control tanks (compare text). Each cage/tank comprised a total of 30 tadpoles from one clutch.

When the first tadpole in the control tanks completed metamorphosis we terminated all experimental trials and determined the survival and developmental traits (developmental stage according to Gosner (1960), snout–vent length (SVL ± 0.5 mm), and body mass (± 0.002 g) of all tadpoles (transfer and control, June 15–17 2009). In control tanks, only a randomly chosen subset of tadpoles (minimum 15 individuals per tank) was measured. Data of the two control tanks containing tadpoles of the same clutch were pooled for further analysis.

To assess the impact of ‘genotype’, developmental traits of different matrilines (tadpoles of different clutches) were compared within common environments, i.e. across control tanks and within ponds. In contrast, the comparison of Gosner-stage and body mass of siblings across ponds provides information on the impact of the environment on the development. For tadpoles raised in control tanks we assessed the amount of the variation in developmental traits within-clutches in contrast to between-clutches using a one-way random effects ANOVA with clutch as random effect. Further, the coefficient of variation (CV), describing the extent of variability of values in relation to the mean value, was assessed in three approaches to investigate the variability of traits of matrilines: First, CV of Gosner-stage and body mass of siblings raised in the control tank and their native pond (i.e. pond chosen by the female for oviposition). We expected that the more complex natural environment would induce a higher variability of traits within genotypes resulting in a higher CV in tadpoles in native ponds. Second, it was assessed if the amount of variation of traits differed among different environments of development (different ponds). Therefore, CVs of developmental traits of each matriline in the three pond cages (native pond and two foreign ponds) were calculated separately. The mean of these three CVs was compared to the CV of the genotype in the control tank. Third, to investigate if overall variability increases when tadpoles of one matriline develop in different environments, an overall CV was calculated over developmental traits of tadpoles per matriline from all pond cages.

Characterization of ecological parameters of breeding ponds

To characterize potential differences in the developmental environment for the larvae 18 habitat characteristics, including physical and chemical parameters, as well as a description of vegetation, were recorded in the ponds in May 2009 (see Supplementary Table S1 for further details). Daily mean water-temperature within cages and control tanks was recorded. Due to the low variation in some pond characteristics, only 11 parameters were used in further analyses (see Table 1). We tested for potential geographic autocorrelation of environmental parameters between the studied ponds (Borcard & Legendre 2012). A Mantel test (Manel 1967) did not reveal a correlation of pond
characteristics and spatial distance ($r = -0.13$, $p = 0.78$, 999 permutations, based on Euclidean distance). Further, pond parameters were summarized using Principal Component Analysis (PCA) and the first three axes were correlated to tadpoles’ developmental response. All analyses were conducted using R v3.0.2 (R Development Core Team 2013).

Results
Survival and general development in control tanks and transfer cages

In our control tanks, survival rate was very high (98.1%) as only 10 of 540 tadpoles died. We analysed body mass and snout–vent length (SVL) for Gosner-stages ≤ 40, since body proportions change dramatically with onset of metamorphosis at Gosner-stage 41 (Gosner 1960). Therefore, a total of 23 tadpoles that were already developed further (stages > 40, clutch 1: 8, clutch 2: 6, clutch 4: 2, clutch 8: 7 tadpoles), were excluded.

Due to damages of two transfer-cages and low survival in one pond, four replicates were excluded from further analysis (see Supplementary Table S2). Survival rate in the remaining transfer-cages was very high (94.8%), resulting in 654 measured individuals in the remaining 23 transfer-cages (Supplementary Table S2).

As SVL and body mass were highly correlated (control and transfer-cages, analysed for stages < 41, Spearman-rank correlation, $S = 27076636$, $p < 0.001$, $\rho = 0.78$) further analyses were conducted only for body mass.

We could not detect a correlation between the mean of developmental traits (Gosner-stage and body mass) of tadpoles that remained in the ponds where the clutch was initially laid (“native tadpoles”) and the prevalent temperature in these respective ponds (Spearman-rank correlation, $S_{\text{Gosner}} = 162$, $p = 0.36$, $\rho = -0.35$, $n = 9$; $S_{\text{mass}} = 136; p = 0.74$, $\rho = -0.13$, $n = 9$). Further, there was no correlation between developmental traits and the first three axes of the PCA of pond characteristics, which explained 76.7% of environmental variance of ponds (Axis 1: 36.4%, Axis 2: 24.8%, Axis 3: 15.4%).

Comparison of development of tadpoles developing in a common environment

Despite standardized environmental conditions in the control tanks, developmental traits differed significantly in some cases between replicates of the same clutch: Gosner-stage differed in three clutches (Mann-Whitney U test; clutch 2: $W = 194.5$, $p = 0.003$; clutch 3: $W = 61$, $p = 0.03$, clutch 9: $W = 227.5$, $p < 0.001$), body mass in two others (clutch 1: $W = 102$, $p = 0.005$, clutch 8: $W = 59$, $p = 0.019$, Fig. 2). When development of tadpoles among control tanks was compared, tadpoles of different clutches showed significant differences in both traits, Gosner-stage and body mass (Kruskal-Wallis $\chi^2_{\text{Gosner}} = 74.29$, df = 8, $p < 0.001$ for Gosner ≤ 40, $\chi^2_{\text{mass}} = 88.78$, df = 8, $p < 0.001$).

Comparison of development of tadpoles from different clutches within the same pond

When developmental responses of tadpoles from different clutches within one pond (same environment) were compared, we detected significant differences among clutches

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Figure 2. Development of common frog, *Rana temporaria*, tadpoles in control tanks. Given are Gosner-stage and body mass of the two replicates of each clutch. Replicates are grouped by the respective clutch identity. Asterisks indicate the level of significance between replicates of the same clutch. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Note the comparatively low developmental stage (Gosner-stage) and comparatively high body mass of tadpoles of clutch 3. Clutches marked with different letters differ significantly in Gosner-stage or body mass respectively ($p < 0.05$; Wilcoxon Rank sum test with fdr correction).
in the majority of the ponds (p < 0.05; Gosner: differences in 5 ponds; body mass: 5, n = 8; see Supplementary Table S3). Tadpoles developing in pond 5 showed fastest development (highest values in Gosner-stages: mean ± sd, 36.9 ± 1.7), whereas body mass was highest in pond 1 (539 mg ± 134). Lowest values for these traits were observed in pond 2 (Gosner: 30.4 ± 2.8; body mass: 302 mg ± 70; pond 6 had to be excluded in this analysis due to two damaged transfer cages, Fig. 3, see also Supplementary Tables S2 and S5).

The variability of traits within ponds (across clutches), described by the coefficient of variation (CV) was on average 0.07 ± 0.02 for Gosner-stages and 0.22 ± 0.06 for body mass (Supplementary Table S5). The variance within clutches was higher than the variance between clutches, with 18% of the total variance being explained by differences between clutches for Gosner-stage (variance between clutches: 0.938; within clutches 4.234) and 24% for body mass (variance between clutches: 394.935; within clutches 920.813).

Development of siblings developing in different environments
Developmental traits of siblings raised in different environments (control and transfer-cages in the ponds) differed significantly in all traits and in all ponds (Fig. 4, for post-hoc analysis see Supplementary Table S6) showing plastic reaction within matrilines towards different environments. The analysis of the CVs showed no significant difference when variation of developmental traits of siblings developing in control and in their native ponds was compared (Wilcoxon-signed-rank test; V = 14, p > 0.05 for Gosner-stage and body mass). Further, the average CV of siblings in different pond environments did not differ from the CV of siblings developing in the control (Wilcoxon-signed rank test, Gosner: V = 16, p = 0.50; body mass: V = 8, p = 0.10), indicating that the extent of trait variation did not differ among different raising environments. However, when the CV across pond cages (siblings in different environments pooled) was calculated, it was significantly higher compared to the CV of the respective matriline in the control tanks (Wilcoxon-signed rank test, Gosner: V = 4, p = 0.03; body mass: V = 4, p = 0.03; Fig. 4 and Supplementary Table S4). Thus, the extent of variation did not differ among environments (e.g. the smallest tadpoles were always around 10% smaller than the mean irrespective of environment) but the mean developmental response differed among environments, resulting in an overall larger variation for a matriline when pooled over all environments.

Performance of tadpoles in native ponds in comparison to alternative breeding ponds
If breeding pairs chose the optimal pond for development of their offspring we expected that performance should be best in the native pond and lower in alternative ponds. However, tadpoles from only two clutches had highest body mass in the native pond in comparison to alternative ponds and development was faster in only a single pond (Fig. 4). When native and foreign cages (different clutches/matrilines) were compared within ponds, the native matriline developed best only in three ponds with respect to Gosner-stage and body-mass (Fig. 3).
Discussion

In this study, we explored if divergence in developmental traits of *Rana temporaria* tadpoles in natural ponds, observed by Grözinger et al. (2014), is induced by the environment and/or by genotype. To this end, we investigated the reaction of related and non-related tadpoles developing in common, as well as in different environments.

Impact of matriline on developmental traits

To test for the impact of the matriline (genotype), we assessed if tadpoles from different clutches develop differently in a common environment. We detected a divergence in development both when matrilines were compared among control tanks and within ponds, emphasizing the impact of the genotype on the tadpoles’ responses to a certain environment. We expected only little differences in developmental traits among tadpoles developing in control tanks, which offered uniform developmental environments with reduced environmental stressors (e.g. no predators, no food limitation). Still, even there clutches differed significantly in their development, clearly indicating that genotypes impacted the development of tadpoles. Increased genetic variability within a clutch due to multiple paternity (Laurila & Seppä 1998) could explain such significant differences as within-clutch trait variation should be increased. No matriline was a “super-genotype” developing fastest or acquiring the highest body mass in all cages and tanks.

In addition to genotype, maternal effects could contribute to observed variability in performance (Mousseau & Fox 1998, Monaghan 2008). Depending on age, nutrition, number of eggs produced and the environment, females may differ in their egg provisioning (Gibbons & McCarthy 1984, Girish & Saidapur 2003). The impact of egg size on tadpole performance is, however, not straightforward and depends on the developmental conditions (e.g. Crump 1984, Gibbons & McCarthy 1984, Parichy & Kaplan 1992, Semlitsch & Schmiedehausen 1994, Loman 2002, Girish & Saidapur 2003, Räsänen et al. 2005). Maternal effects could therefore further contribute to the observed variation of tadpole development, but have not been quantified within this study.

Impact of environment on developmental traits

All matrilines exhibited phenotypic plasticity as siblings developing in different trials (control tanks as well as ponds) differed in Gosner-stage and body mass. However, the extent of trait variation did not differ between tadpoles from control tanks in comparison to those kept in transfer cages in ponds, indicating that a more complex environment does not induce a higher variation in developmental traits among siblings. Yet, we detected an increase of overall variability per clutch when siblings were pooled across environments (three different ponds), showing that the observed variation occurs around different average values depending on the environment.

Figure 4. Development of *Rana temporaria* tadpoles (grouped by clutch identity) in different environments. Given are Gosner-stage and body mass of tadpoles raised in control tanks (shaded column C), native pond (N) and two alternative ponds. Results of Kruskal-Wallis rank-sum test of 23 transfer-cages comparing developmental stage (Gosner-stage) and body mass of *R. temporaria* tadpoles of the same clutch developing in different ponds (control tanks were excluded from this analysis) are represented by asterisks above boxes per clutch. If only two cages were available, a Mann-Whitney U-Test was performed (significance levels of respective tests: * = p < 0.05; ** = p < 0.01; *** = p < 0.001). Due to low survival in two transfer-cages, results for pond 6 were excluded.
The impact of environment differed among matriline, emphasizing the importance of ‘genotype’ x environment interactions in shaping developmental traits. A genotype x environment interaction can be defined as a change in relative performance or size of a trait of two or more genotypes measured in at least two different environments (DeWitt & Scheiner 2004). Since the order of mean values of developmental traits among clutches changed, we can conclude that depending on the matriline, tadpoles reacted differently to a common environment. For example, within pond 3, clutches differed significantly in their Gosner-stage, with clutch 7 showing the lowest developmental stage compared to clutch 3 and 1 (Fig. 3). When Gosner-stages of these clutches were compared in the control tanks, however, tadpoles of clutch 7 showed an intermediate value (Fig. 2).

Such differences in genotype x environment interactions may also explain why we were unable to correlate environmental parameters to the observed divergence of developmental traits within and among ponds in the present experimental study as well as in a large scale study of natural ponds conducted before (Grözinger et al. 2014). This is in contrast to other studies describing tadpole reaction to different environmental cues, such as predators, desiccation, or vegetation structure (e.g. Newman 1989, Van Buskirk & Relyea 1998, Skelly et al. 2002). However, these studies focused on phenotypic responses when a single or few parameters are exemplarily contrasted (e.g. presence vs. absence of predators). Here, we investigated developmental traits in a continuous gradient in a natural habitat. This and the potentially contrasting effects of multiple environmental factors might have impeded the detection of environmentally induced patterns in development of the tadpoles.

Performance of tadpoles in their native ponds

In order to maximize their fitness, females should choose breeding ponds which match best the requirements and demands of their offspring. In our study area, a consistent preference of particular ponds and avoidance of others by breeding Rana temporaria was detected (Grözinger et al. 2012, unpubl. data). The basis for this choice, however, could not be explained by the assessed habitat parameters, comprising the whole set of factors known to influence amphibian breeding site choice. Hence, the question arose, whether this choice might mirror favourable conditions for the respective offspring. High level of site fidelity has been previously reported for R. temporaria (Blab 1986), i.e. the adult frogs return to the ponds where they developed as larvae. Site fidelity would only be an optimal strategy though if ponds were optimal or at least suitable for larval development over time. Alternatively, female choice of breeding pond could be influenced by the aggregations of calling males in potential breeding ponds (Knopp et al. 2008) rather than local environmental parameters.

In this study, only a minority of clutches developed best in the breeding pond chosen by ovipositing females while performance was better in other ponds that the tadpoles were transferred to. When we compared development of non-related tadpoles within ponds, native tadpoles showed higher values for development and body mass in one case only, compared to foreign tadpoles raised in the same environment. We are aware that only a random sample (one clutch, deposited in each of the nine study ponds; summing to a sample size of nine) was taken into account for this analysis. Nevertheless, our results suggest that the choice of the breeding habitat by the female does not reflect better developmental conditions for the respective offspring.

Conclusion

Rana temporaria larvae show a tremendous amount of within-clutch as well as within-population phenotypic plasticity in our study population influenced by matriline effects, as well as environmental factors and genotype/matriline x environment interactions. This large amount of phenotypic plasticity likely contributes to the ability of this habitat generalist to make use of a large variety of breeding ponds locally spanning the gradient from ephemeral to permanent ponds within the same geographic area (Van Buskirk 2005), but also along altitudinal and latitudinal gradients (Miaud et al. 1999, Laugen et al. 2003, 2005). Several studies have contrasted the amount of phenotypic plasticity among populations along a broad geographic range (e.g. Laugen et al. 2003, 2005) or among populations that are connected by gene flow but with highly divergent habitat parameters (e.g. Van Buskirk & Arioli 2005, Mür et al. 2014). Populations of R. temporaria living in harsher environments such as high altitude or high latitude show stronger canalization in developmental traits (Merilä et al. 2004), which is interpreted as an adaptation to seasonal time constraints. The maintenance of high levels of phenotypic plasticity in developmental time as found in our study population may not incur a high fitness cost (Lind & Johansson 2009) but may contribute to the long-term maintenance of the population and should allow a highly opportunistic use of available breeding sites.

Authors’ contributions

All authors conceived and designed the experiments. FG performed the experiments and analyzed the data. FG, HF, and MOR wrote the paper. All authors read and approved the final manuscript.

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References


Impact of environmental conditions and matriline on *Rana temporaria* tadpoles


**Supplementary material**

6 Supplementary Tables:
Table S1: Values of all environmental pond parameters assessed.
Table S2: Gosner-stage and body mass of *Rana temporaria* tadpoles developing in transfer cages.
Table S3: Results of the pairwise comparison of Gosner-stage and body mass of tadpoles raised in the same pond.
Table S4: Variation of Gosner-stage and body mass of tadpoles of different clutches developing in different environments.
Table S5: Variation of Gosner-stage and body mass of different clutches developing in a common pond.
Table S6: Comparison of Gosner-stage and body mass of tadpoles of the same clutch developing in different environments.

**Appendix A**

Provided is a list, definitions, and units of all environmental pond parameters assessed (compare Table 1 and Supplementary Table S1).

<table>
<thead>
<tr>
<th>environmental parameter</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>pH-value below surface (0.1 m) close to transfer cage using Water Tester HI98204 HANNA Instruments, accuracy ± 0.2 pH, Kehl am Rhein, Germany</td>
</tr>
<tr>
<td>temperature [°C]</td>
<td>mean water temperature calculated from continuous recordings (0.15 m) below water surface using data loggers (IButtons, Maxim ± 0.5°C, 2 h interval) between April 18 and May 20 2009</td>
</tr>
<tr>
<td>electric conductivity [µS]</td>
<td>electric conductivity below surface (0.1 m) close to transfer cage using Water Tester HI98204 HANNA Instruments, accuracy ± 2% F.S., Kehl am Rhein, Germany</td>
</tr>
<tr>
<td>canopy openness [%]</td>
<td>canopy openness calculated using Gap Light Analyzer Version 2.0 (Frazer et al. 1999)</td>
</tr>
<tr>
<td>duckweed cover [%]</td>
<td>% of water surface covered by duckweed, <em>Lemna</em> sp.</td>
</tr>
<tr>
<td>structuring vegetation [%]</td>
<td>% of water surface structured by plants</td>
</tr>
<tr>
<td>underwater vegetation [%]</td>
<td>% of water surface structured by underwater vegetation</td>
</tr>
<tr>
<td>turbidity [category:1-4]</td>
<td>visual inspection of turbidity [1=clear, 2=lightly turbid, 3=turbid, 4=highly turbid]</td>
</tr>
<tr>
<td>presence of sapropel [0;1]</td>
<td>absence or presence of sapropel in pond [0;1]</td>
</tr>
<tr>
<td>presence of inflow [0;1]</td>
<td>absence or presence of inflow [0;1]</td>
</tr>
<tr>
<td>pond bottom [0;1]</td>
<td>leaf litter or mud on pond bottom [0;1]</td>
</tr>
<tr>
<td>volume [m³]</td>
<td>pond volume, based on 0.5 pond length × 0.5 pond breadth × water depth in centre × 2/3 π.</td>
</tr>
<tr>
<td>nitrate (NO₃) [mg/l]</td>
<td>nitrate content below surface (0.1 m) close to transfer cages on May 23, analysis using Aquanal®-Ökotest Water Laboratory (0–80 mg/l), Sigma-Aldrich, Seelze, Germany</td>
</tr>
<tr>
<td>ammonium (NH₄) [mg/l]</td>
<td>ammonium content below surface (0.1 m) close to transfer cages on May 23, analysis using Aquanal®-Ökotest Water Laboratory (≤0.05–10.0 mg/l), Sigma-Aldrich, Seelze, Germany</td>
</tr>
<tr>
<td>nitrite (NO₂) [mg/l]</td>
<td>nitrite content below surface (0.1 m) close to transfer cages on May 23, analysis using Aquanal®-Ökotest Water Laboratory (≤0.02–1.0 mg/l), Sigma-Aldrich, Seelze, Germany</td>
</tr>
<tr>
<td>phosphate (PO₄) [mg/l]</td>
<td>phosphate content below surface (0.1 m) close to transfer cages on May 23, analysis using Aquanal®-Ökotest Water Laboratory (0–26 mg/l), Sigma-Aldrich, Seelze, Germany</td>
</tr>
<tr>
<td>water depth [m]</td>
<td>mean water depth calculated from 13 points (centre, as well as 0.1 m, 0.5 m, 1 m in all cardinal directions from centre)</td>
</tr>
</tbody>
</table>