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Observations on the intraspecific variation in tadpole morphology in natural ponds

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Abstract. Intraspecific morphological variation of anuran tadpoles occurs in response to several factors. Causes and consequences of this variation have been largely studied hitherto in controlled environments, but data from natural habitats is clearly less abundant. Here, we present a series of observations on the morphology – mainly tail depth – of three tadpole species from NE Iberian Peninsula across different pond typologies. According to experimental data on tadpole morphology and selective pressures along the pond permanency gradient, we should expect that tadpoles inhabiting ponds with a short hydroperiod – mainly facing desiccation risk – have shallower tail fins than tadpoles from ponds with longer hydroperiod – mainly facing predation risk. Thus, we expected that the link between these complementary selective pressures – predation risk, desiccation risk – and hydroperiod could make possible to detect intraspecific variation in tadpole morphology among different typologies of natural ponds. Morphological differences were found in all studied species, and variation, when present, agreed with theory: tadpoles had deeper fin tails as they were collected in ponds with a longer hydroperiod. Interestingly, in most cases these morphological differences were more marked as tadpoles were larger in size. Although distances among the studied ponds were generally short – posing phenotypic plasticity as the most plausible proximate mechanism – specifically designed studies would be needed to disentangle the relative role of other processes like local adaptation.

Keywords. *Alytes obstetricans, Hyla meridionalis, Rana temporaria,* predation risk, desiccation risk, phenotypic plasticity.

INTRODUCTION

Intraspecific morphological variation of anuran tadpoles occurs in response to several factors and is created through different mechanisms. Phenotypic plasticity and various processes creating population-level genetic changes (Van Buskirk and McCollum, 1999; Pfennig and Murphy, 2000; Relyea, 2004; 2005) have been listed as natural sources of this variation. Usually, a series of both biotic and abiotic stressors – desiccation and predation risk, tadpole competition and density – combined with the particular life history characteristics of each species, creates a set of predictable tadpole morphologies (Relyea, 2004; Richter-Boix et al., 2006a; 2006b; 2007; Touchon and Warkentin, 2008; Van Buskirk, 2009). Importantly, these morphologies have been proved to correlate with individual fitness during larval stages (Johnson et al., 2008; Dijk et al., 2016; Pujol-Buxó et al., 2017) and to influence also post-metamorphic morphology and fitness in turn (Tejedo et al., 2010; Johansson and Richter-Boix, 2013; Pujol-Buxó et al., 2013). Causes, effects and consequences of intraspecific morphological variation in tad-

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poles have been largely studied so far, but mainly using laboratory experimental procedures or controlled garden experiments (e.g., Relyea, 2004; 2005; Touchon and Warkentin, 2008). Hence, in this field of study, morphological data of tadpoles from natural ponds is clearly less abundant (but see Van Buskirk, 2009; 2014; Johnson et al., 2015). This data is crucial to confirm the trends observed in laboratory or garden experiments and to spur novel research questions and hypotheses.

The pond permanency gradient – ranging from ephemeral pools to permanent water bodies (Skelly, 1995; Schneider and Frost, 1996; Wellborn et al., 1996) – correlates with most selective pressures acting on tadpoles in the Mediterranean area. Predation and pond desiccation are arguably the most important selective pressures acting on tadpole populations, and they tend to create a trade-off along the pond permanency gradient (Skelly, 1995): the mean time a pond contains water each year negatively correlates with its desiccation risk, but it is also commonly linked to an increasing number or diversity of predators (Smith, 1983; Pearman, 1995; Schneider and Frost, 1996; Richter-Boix et al. 2006b; 2007). Interestingly, as showed by laboratory experiments, both selective pressures also create opposite morphological outcomes in the tail shape of tadpoles. Thus, tadpoles under predation risk display deeper tail fins to lure predators away from lethal surfaces in case of attack (Van Buskirk et al., 2003; Johnson et al., 2008), while tadpoles under desiccation risk display shallower tails, investing more energy in the feeding and digesting structures located in the main body (Vences et al., 2002; Richter-Boix et al., 2006a). Therefore, assuming an inverse correlation between predation and desiccation risk along the pond permanency gradient, we can expect from experimental data that tadpoles inhabiting ponds with a long hydroperiod should usually display – either by phenotypic plasticity or other mechanisms – deeper tail fins than tadpoles from ponds with a short hydroperiod (Smith, 1983; Richter-Boix et al., 2006a; 2006b; 2007; Van Buskirk, 2009). Here, we explore this assumption re-analysing simple morphological data – tail depth and total length of tadpoles – on three European species inhabiting more than one pond typology.

MATERIALS AND METHODS

We gathered available morphological data of tadpoles of three anurans inhabiting different pond typologies in two Natural Parks (NP) located near Barcelona (Catalonia, Spain), namely *Alytes obstetricans* (Anura, Alytidae) and *Hyla meridionalis* (Anura, Hylidae) from Garraf NP; and *Rana temporaria* (Anura, Ranidae) from Montseny NP. Data from Garraf NP was initially collected as part of a monitoring of the parks' anuran populations during spring of year 1991, and data from Montseny NP is from a PhD thesis by Campeny (2001) on tadpole trophic ecology made during years 1985 and 1986. In both cases, tadpoles had been dip-netted from natural ponds along several weeks or months of spring, being the ponds in Montseny NP the same for both years (Tables S1, S2 and S3). Since all tadpoles were euthanized for other purposes within each study, they could not be possibly sampled twice. Although tadpoles were measured differently in both studies – using a caliper Garraf NP, and using a binocular microscope in Montseny NP – we did not perform comparisons across species or parks, and therefore we can discard possible biases due to the measurement methods. In both cases, we assigned ponds to a certain category – ephemeral, temporary or permanent – according to criteria by Richter-Boix et al. (2006b) and each pond's usual hydroperiod during the years of sampling. According to these criteria, *Alytes obstetricans* in Garraf NP chooses mainly permanent water bodies as reproduction ponds, using temporary and even ephemeral ponds occasionally (Montori et al., 2015), while *Hyla meridionalis* mostly uses temporary ponds, breeding also in all pond typologies present in Garraf NP (Montori et al., 2015). On the other hand, *Rana temporaria* in Montseny NP breeds in most types of water bodies, from permanent streams to temporary or occasionally ephemeral ponds (Campeny, 2001).

Since necessary assumptions for parametric tests were not met – mainly due to important differences in the numbers of specimens measured in each pond –, differences in tail depth (Fig. S1) were analysed using non-parametric randomization tests implemented in the package *lmPerm* (Wheeler and Torchiano, 2016), using 1000 randomizations in each case. Tests were run separately for each species: tail depth as response variable, pond as factor and total length of the tadpole as a covariate, allowing for interactions. When there were multiple ponds to test for the same species, we used the same procedures in pairwise tests to detect statistically homogeneous groups if global differences were found. Experimental data for comparison using the same measurements (in this case on *Discoglossus pictus* and *Pelodytes punctatus*) was re-analysed from a study on inducible defences (Pujol-Buxó et al., 2013). In this case we used linear mixed models instead of permutation tests – using the same model structure – to account for lack of independence, by adding a random intercept depending on tank. All statistical analyses and figures were done in R v3.2.3 (R core team, 2015).

RESULTS

The relationship between tail depth and total length of *A. obstetricans* tadpoles significantly differed in slope (that is, effects of the interaction were significant: $F_{4,423} = 6.44$, $P < 0.001$) and intercept ($F_{4,423} = 21.6$, $P < 0.001$) when testing all five ponds together. However, there were clearly two types of ponds according to posterior pairwise analysis: on one hand, *A. obstetricans* tadpoles from permanent ponds displayed the steepest slopes, not differing in slope among them ($F_{1,391} = 0.01$, $P = 0.863$) but having the pond G6 a higher intercept than pond G1

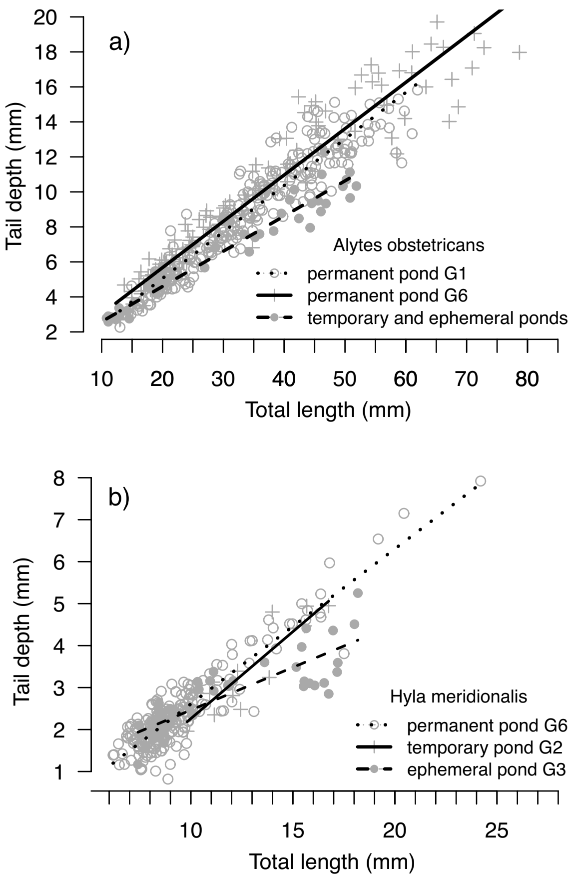

Fig. 1. Intraspecific morphological variation among different nearby natural ponds from Garraf NP (for pond information see supplementary material): a) *Alytes obstetricans*, b) *Hyla meridionalis*.

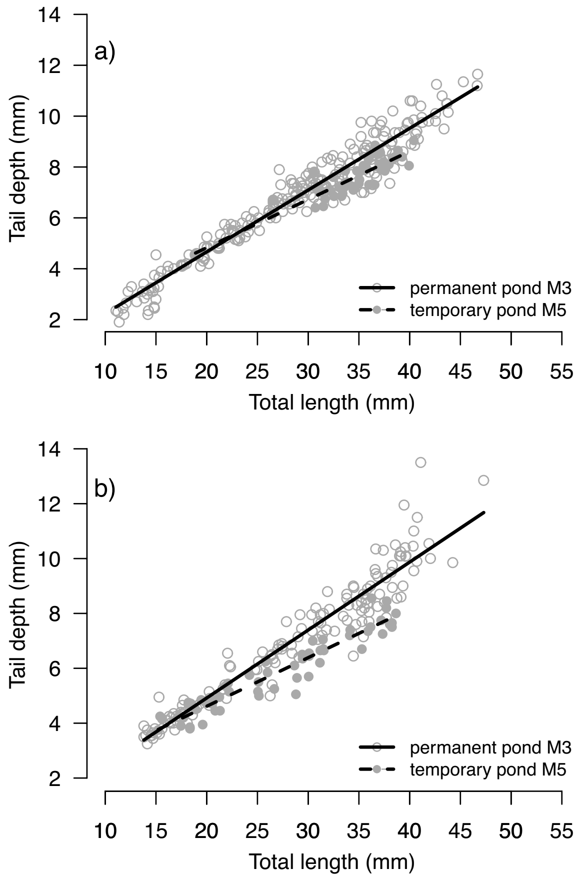

Fig. 2. Intraspecific morphological variation in *Rana temporaria* from two nearby natural pools of Montseny NP in consecutive years (for pond information see supplementary material): a) year 1985, b) year 1986.

($F_{1,392}$ = 25.6, P < 0.001). On the other hand, tadpoles from temporary and ephemeral ponds showed more gentle slopes, not differing among them neither in slope ($F_{2,32}$ = 0.06, P = 0.883) nor in the intercept ($F_{2,34}$ = 2.65, P = 0.131) (Fig. 1, both pond typologies grouped together for clarity).

Relationship between tail depth and total length of *H. meridionalis* tadpoles differed in slope ($F_{2,286}$ = 36.2, P < 0.001) and intercept ($F_{2,286}$ = 8.44, P = 0.039) among the three studied ponds when tested all together (Fig. 1). According to pairwise tests, the slope of the ephemeral pond is significantly more gentle than the ones of permanent ($F_{1,275}$ = 70.7, P < 0.001) and temporary ($F_{1,56}$ = 11.69, P = 0.001) ponds. Tadpoles from the permanent and temporary ponds did not differ in slope ($F_{1,241}$ = 0.71, P = 0.261). Differences in the intercept disappeared in pairwise analyses (all P > 0.05).

Differences in morphology between *R. temporaria* tadpoles from the temporary and permanent ponds (Fig. 2) were significant in both studied years, being the slope between tail depth and total length of tadpoles always steeper in the permanent pond ($F_{1,266}$ = 6.48, P = 0.003 for 1985, and $F_{1,189}$ = 29.84, P < 0.001 for 1986). Differences in the intercept were also found in both cases ($F_{1,266}$ = 51.1, P < 0.001 for 1985, and $F_{1,189}$ = 70.3, P < 0.001 for 1986).

Differences in experimental morphology between *D. pictus* tadpoles under or without predation risk from *Anax sp.* included as well a significant interaction ($F_{1,84}$ = 10.93, P = 0.001), being the slope between tail depth and total length of tadpoles steeper when a caged predator was present (Fig. S1). The same applies for experimental data on *P. punctatus* ($F_{1,85}$ = 6.29, P = 0.014), being again the slope steeper when a caged predator was present (Fig. S2).

DISCUSSION

Morphological differences among ponds were found in all studied species, and variation, when present, agreed with theory: tadpoles had deeper fin tails as they were collected in ponds with a longer hydroperiod. Thus, observations coincide with theoretical predictions, arguably posing the trade-off among desiccation and predation risk (Skelly, 1995) as the possible underlying cause of the observed intraspecific morphological differences. Unluckily, given that these observations were not originally taken to explore this hypothesis, we lack data on predator density and diversity in the studied ponds – among other potentially useful data –, making impossible to assess if the observed morphological trends are in each case rather a consequence of desiccation risk, predation risk, or both.

Interestingly, morphological differences among pond typologies were always expressed through a significant interaction between pond type and total length, that is, as changes in the relationship among both measures along growth (i.e., slope differences seen in Fig 1 and Fig 2). Thus, when morphological differences are found among pond typologies, these become more exaggerated as tadpoles are larger in size, coinciding with the re-analyzed experimental data on anti-predator morphology from Pujol-Buxó et al. (2013), and being consistent with similar studies examining tadpole morphology along wide size ranges (Relyea, 2003). Morphological differences between *Alytes obstetricans* tadpoles from the two permanent ponds, where differences were found in the intercept, represent the only exception to this pattern. The exaggeration of morphological differences with size might be consistent with previous works reporting that behavioural defences are, in relative terms, more used in the first stages of tadpole life, while morphological differences become more marked as tadpoles grow larger (Relyea, 2003; Pujol-Buxó et al., 2017).

Which is the process creating the variation we observe in these ponds? The two ponds from Montseny NP are separated less than 1km, and the mean distance among studied ponds in the other study area (Garraf NP) is approximately 3.15 km (Tables S1 and S2). Given these distances, we cannot discard gene flow and therefore we suggest a role of phenotypic plasticity in shaping the observed morphological differences (DeWitt and Scheiner, 2004; Van Buskirk, 2009). However, another complementary option is that, even assuming moderate rates of gene flow (Lind et al., 2011), after several generations of natural selection the sub-populations breeding in the different ponds have also constitutively departed in their morphology (Ledón-Rettig et al., 2008; Lind et al., 2011; Van Buskirk, 2014). This could be expressed in a default production of – or a greater tendency to produce – deep-tailed tadpoles in populations usually breeding in permanent ponds and shallow-tailed tadpoles in populations from temporary and ephemeral ponds. Interestingly, our data of *R. temporaria* in different consecutive years from the same two ponds shows that although general patterns may repeat year after year, exact results – the degree of morphological divergence – may vary across years (Fig. 2). Thus, in both areas, neither microevolutionary processes among nearby ponds – mediated by processes like genetic accommodation (Ledón-Rettig et al., 2008; Wund et al., 2008) – nor a prominent role of phenotypic plasticity cannot be totally disregarded. Further studies specifically designed to disentangle the relative role of these mechanisms would be needed. Finally, it is necessary to highlight that, although results agreed with prediction and the number of tadpoles sampled was high in some cases, our observations are based on too few ponds to be conclusive, and other additional studies would be needed to confirm the observed pattern.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www.unipv.it/webshi/appendix> manuscript number 20894.

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