

Effect of habitat drying on the development of the Eastern spadefoot toad (*Pelobates syriacus*) tadpoles

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Abstract. Amphibians exhibit plasticity in the timing of metamorphosis, and tadpoles of many species respond to pond drying by accelerating their development. In the present study we investigated the phenotypic plasticity of the developmental response to water volume reduction in tadpoles of Eastern spadefoot toad *Pelobates syriacus*. The response of tadpoles to the simulated drying conditions was evaluated by gradually reducing the water level in the experimental containers under controlled laboratory conditions. Four water level treatments were used: constant high, slow decrease, fast decrease and constant low level. We tested if (i) tadpoles can speed up their development in a drying aquatic habitat, and (ii) if the accelerated development causes a reduced body size at metamorphosis. Our results showed that *P. syriacus* tadpoles were able to respond to pond drying by speeding up their metamorphosis and that metamorphosis was not influenced by water level, but by water level decrease rate. The accelerated development caused by the decreasing water level resulted in smaller body size at metamorphosis. The smallest size at metamorphosis was in tadpoles raised in constant low water level treatments and was probably induced by the crowding effect. We compared our results to similar studies which show that the response of the Eastern spadefoot toad tadpoles to pond drying is less impressive, especially if compared to the response of the North American spadefoot toads inhabiting desert environments.

Keywords: amphibians, larval period, metamorphic climax, metamorphosis, phenotypic plasticity, temporary ponds.

Introduction

One of the major threats to the survival of amphibians that breed in temporary ponds is the drying of these aquatic habitats before metamorphosis. This means that slower growth rates and/or longer larval periods in unpredictable environments will decrease the chances of tadpoles to reach metamorphosis before the pond disappears (Wells, 2007). According to the model proposed by Wilbur and Collins (1973), amphibians that use temporary ponds for breeding display more variability in both larval period and size at metamorphosis than those breeding in permanent water bodies.

Phenotypic plasticity in species that breed in variable environments may involve trade-offs between different life history traits, especially larval mortality and size at metamorpho-

sis. For example, lower larval mortality due to faster development may result in a smaller size at metamorphosis, which in turn may have consequences for later survival. Smaller metamorphs have lower locomotory capacity (Goater, Semlitsch and Bernasconi, 1993; Richter-Boix, Llorente and Montori, 2006a), lower tolerance to dehydration (Newman and Dunham, 1994), reduced resistance to parasites (Goater, 1994), weaker immune system (Gervasi and Foufopoulos, 2008) and in general lower juvenile survivorship (Reques and Tejedo, 1997; Morey and Reznick, 2001; Altwegg and Reyer, 2003), and lower reproductive success later on (Smith, 1987; Howard, 1988; Scott, 1994). However, when larval mortality risk increases due to pond drying, earlier metamorphosis may be favoured despite the negative trade-offs associated with a smaller size.

Tadpoles of several anuran species accelerated metamorphosis in response to habitat desiccation (e.g., Crump, 1989; Loman, 1999; Richter-Boix, Llorente and Montori, 2006b). Many studies on larval phenotypic plasticity were done on North American spadefoot toads

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(*Scaphiopus* sp. and *Spea* sp.), which often breed in ephemeral ponds and have extremely rapid rates of development (Newman, 1992; Denver, Mirhadi and Phillips, 1998; Morey and Reznick, 2004). In contrast, European spadefoot toads (*Pelobates* sp.) usually prefer to breed in permanent ponds, have longer larval periods and metamorphose at larger sizes (Buchholz and Hayes, 2002, 2005). However, the Eastern spadefoot toad (*P. syriacus*) populations inhabiting the dry Black Sea coastal area of Dobruja (Romania) with unpredictable and varied rainfall patterns, are forced to breed in a wide range of both permanent and temporary ponds (e.g., Székely et al., 2009).

In the present study we investigated the phenotypic plasticity of *P. syriacus* tadpoles from a temporary pond exposed to different water volume reduction rates to test (i) if tadpoles will express a faster development rate when facing decreasing water levels; and (ii) if the accelerated development affects size at metamorphosis.

Materials and methods

Eastern spadefoot toad (*P. syriacus*) tadpoles, in stage 25 (Gosner, 1960), were collected from a temporary pond complex near Vadu, Constanța County, Dobruja, Romania (44°26'12"N, 28°44'13"E) and reared in 8 litre opaque plastic containers (27 × 27 × 12 cm). The containers were filled with aged tap water, according to the assigned drying regime. Healthy looking tadpoles of similar sizes were randomly chosen from the holding tanks and put into experimental containers. Due to the spadefoot toad tadpoles large size we appointed only four larvae per container, using a total of 96 tadpoles in 24 experimental containers. The developmental stage of the tadpoles was determined according to the Gosner (1960) staging system. All tadpoles were fed ad libitum a standard diet, consisting of rabbit pellets (containing 17.2% protein) supplemented with TetraMin fish flakes (47% protein).

The tadpoles were exposed to the following water reduction treatments: 1 – constant high, control treatment, with 6000 ml water/container (90 mm depth), 2 – slow decreasing treatment, from 6000 to 800 ml, the water level being reduced by 200 ml every four days for the first 20 days and every third day afterwards (from 90 mm to 16 mm depth), 3 – fast decreasing treatment, from 6000 to 800 ml, the water level being reduced by 300 ml every four days for the first 20 days and every three days afterwards (from 90 mm to 16 mm depth) and 4 – constant low volume with 1500

ml water/container (27 mm depth). Each of these four treatments was replicated six times. We used two distinct drying regimes because we hypothesized that the phenotypic response to desiccation may depend on the rate at which the pond dries. To minimize the effect of waste accumulation on growth and development, the water was completely changed every three or four days. The tadpole wet mass was recorded with an electronic balance (Kern model ABJ) to the nearest 0.01 g, while snout-vent length (SVL) was measured to the nearest 0.1 mm with a digital calliper at both forelimb protrusion (Gosner stage 42) and metamorphosis (Gosner stage 45). In addition, the wet mass of all tadpoles was measured each time the water was changed in the containers.

The experiment started on May 17, 2009 (day 1 of the experiment) and ended on August 6 (day 82) when the last tadpole completed its metamorphosis. The containers were kept in a laboratory room with natural day-night photoperiod. To homogenize the thermal conditions the containers were randomly redistributed to different locations each time the water was changed. Water temperature and conductivity were regularly recorded with a Consort C561 multi-parameter analyzer prior to changing water and in addition two temperature data-loggers were kept in two randomly selected containers during the whole period to check for potential changes in temperature. No significant temperature variations between the treatments were observed during the experiment, the temperature varying between 19.4°C to 25.5°C. The conductivity varied from 1.00 mS/cm to 1.41 mS/cm, highly correlated with the water level from the treatments.

We measured time to forelimb protrusion (the beginning of metamorphic climax, stage 42), as the time (days) elapsed since the start of the experiment until the emergence of the first forelimb; and time to metamorphosis (end of the metamorphic climax, stage 45), as the number of days elapsed since the start of the experiment until the metamorphs have a tail which was shorter than body length. After the first tadpole with forelimbs was observed, the containers were checked daily for the new metamorphs.

Data analysis

All data were log₁₀ transformed prior to analysis to reduce skewness. To avoid pseudoreplication, we used in analyses the mean of individual responses for each container. We used one-way Analysis of Variance (ANOVA) to check for the collective effects of the different experimental variants on the age of tadpoles, body mass and SVL at forelimb protrusion and metamorphosis. When significant differences were detected post-hoc, multiple comparisons versus the control group (treatment 1) were performed using the Holm-Sidak test. The differences between the specific treatments and the control in age at forelimb protrusion and metamorphosis were analyzed with Student's *t* test. Partial growth rate was computed as the ratio between the difference in body mass during two consecutive measurements and the number of days elapsed. All the tests were performed using the SigmaPlot 11 computer software package.

Table 1. Summary statistics for length of the larval period and body size at the forelimb emergence and at metamorphosis (ANOVA).

Source of variation	At Gosner stage 42				At Gosner stage 45				
	df	MS	F	P	df	MS	F	P	
									Larval period length
Treatment	3	0.000424	1.615	0.218	3	0.000430	1.942	0.155	
									Body mass
Treatment	3	0.0292	22.952	<0.001	3	0.0166	18.089	<0.001	
									Snout-vent length
Treatment	3	0.00268	9.336	<0.001	3	0.00174	16.415	<0.001	

Results

Survival rate

Larval survival was very high; of the initial 96 tadpoles used in the experiment, only two died before completion of the metamorphosis and both died after the emergence of forelimbs. One tadpole from the slow decrease treatment completed its development with more than one month delay and was excluded from data analysis. For all other tadpoles, metamorphosis was completed within 54 days of the start of the experiment, but as early as 39 days.

Time of metamorphosis

There was no collective effect detected in the time of metamorphosis between the four treatments neither at Gosner stage 42 nor at Gosner stage 45 (table 1). However, the tadpoles from the fast decrease treatment (3) accelerated their metamorphosis in response to water reduction when compared with the control (treatment 1) (fig. 1) ($t = 3.174$, $P = 0.010$, Student's t test). They had a reduction of two days, or 4.4% of the 45 days larval period in constant water. A similar effect was observed at forelimb emergence but it was not statistically significant ($t = 2.205$, $P = 0.052$, Student's t test). The response of tadpoles from the slow decrease treatment (2) and the constant low treatment (4) did not differ from the control ($P > 0.1$, Student's t test).

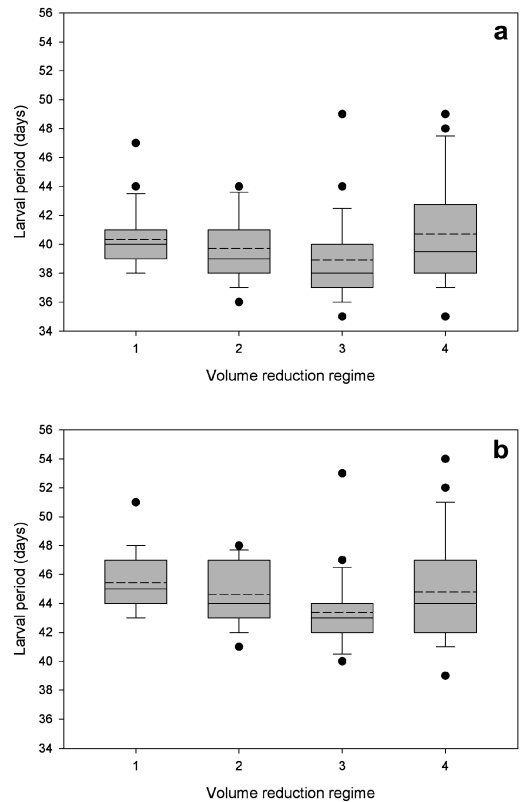


Figure 1. Development time for the four desiccation levels at (a) Gosner (1960) stage 42 and (b) stage 45. The mean for each treatment is represented with dashed line.

Size at metamorphosis

Water level had a significant effect on the body mass and SVL of tadpoles at metamorphosis both at Gosner stage 42 and Gosner stage 45 (table 1, fig. 2). The metamorphs were significantly smaller in the fast decrease treatment than in the constant high and slow decrease treatments both at forelimb emergence (body

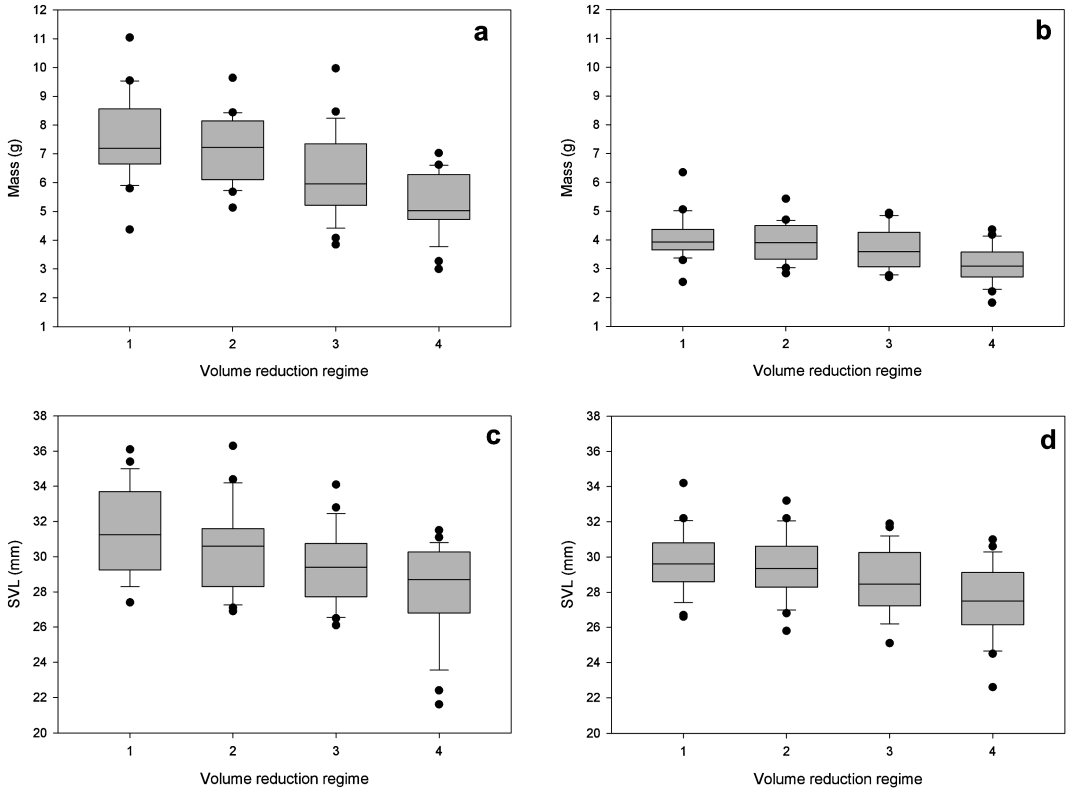


Figure 2. Tadpoles body mass at Gosner (1960) stage 42 (a) and 45 (b) and SVL at stage 42 (c) and 45 (d) for the four desiccation levels.

mass $P < 0.001$, SVL $P < 0.008$, Holm-Sidak test) and end of metamorphosis (body mass $P = 0.022$, SVL $P < 0.005$). However, the smallest size was observed in the tadpoles reared in constant low water levels (fig. 2), both at forelimb emergence (body mass $P < 0.001$, SVL $P < 0.001$) and end of metamorphosis (body mass $P < 0.001$, SVL $P < 0.001$, Holm-Sidak test), even if the length of their larval period was not significantly different from the constant high one.

Partial tadpole growth rates displayed a two-stage pattern: a period of intensive growth followed by a phase of decrease in the tadpole's body mass (fig. 3). The maximum mass of the metamorphosing tadpoles was reached a few days before the forelimb protrusion. The tadpoles from the constant low treatment showed significant differences in body mass after day 27 ($P < 0.001$, Holm-Sidak test), whereas in

the case of tadpoles reared in the fast decrease treatment, these differences appeared after day 33, at a 3300 ml water volume ($P = 0.017$).

We recorded a significant correlation between the increase of tadpole mass and water conductivity, the containers with the constant low water level having the highest values. Conductivity increased as the water level decreased and the tadpole body mass in each container grew (fig. 4).

Discussion

Time to metamorphosis was influenced by water level. The fast reduction of water level in treatment 3 influenced the timing of metamorphosis, as tadpoles reduced their developmental time by 4.4% compared to the control group. Comparing this response with that of several other species (table 2), the differences in timing of metamorphosis are considerably less impres-

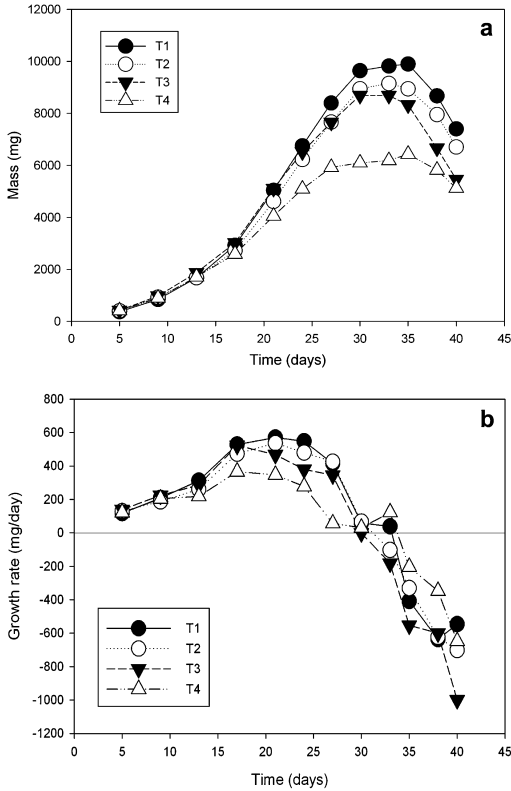


Figure 3. Dynamics of average tadpole mass during the experiment (a) and of the daily growth rates of the tadpoles in the four experimental treatments (b).

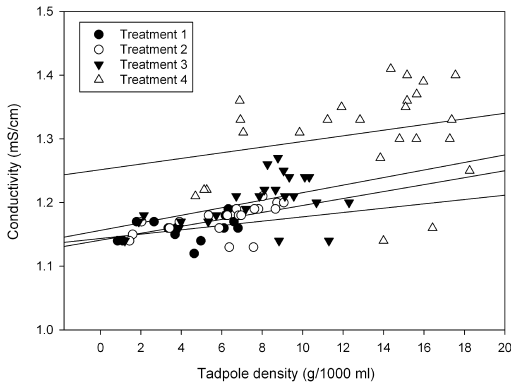


Figure 4. The relationship between water conductivity and tadpole density in the experimental containers.

sive, particularly when compared with the North American spadefoot toads inhabiting desert areas. *P. syriacus* is a large spadefoot toad species reaching up to 9 cm, and it requires for breeding larger and deeper ponds. However, in our study

area there is a scarcity of permanent fishless ponds, so they have to use for breeding ponds with unpredictable duration.

Furthermore, the entire length of the larval development in our experiment was very short, typical to species breeding in rapidly drying ponds. The larval development lasted on average 44 days and adding about 10-14 days (the time required to reach stage 25) this period is still much shorter than the 3-4 months (83-110 days) reported by Gomez-Mestre and Buchholz (2006). Our results support the hypothesis that phenotypic plasticity in response to pond drying is higher in species breeding in ephemeral desert ponds than those using other types of temporary ponds (Wells, 2007). It is interesting that the tadpoles from the constant low treatment did not complete their development sooner (e.g., Denver, Mirhadi and Phillips, 1998; Spieler, 2000) or later (e.g., Crump, 1989) than the control treatment, but at almost the same time; the constant low water level affected only the size, but not the time of metamorphosis.

Since a faster development in amphibians is usually associated with reduced size at metamorphosis (Wilbur and Collins, 1973; Newman, 1992), we expected to find differences in sizes between the different treatments. Indeed, the reduction of the developmental time in the fast decreasing treatment was accompanied by a smaller metamorphic size, representing a significant reduction with 9% of mass and 4% of SVL at metamorphosis as compared to the control. The smallest size was observed in the tadpoles reared in constant low water levels (treatment 4) that suffered a reduction of 23% of mass and 8% of SVL at metamorphosis. This reduction could be explained by the changes in water chemistry (Morey and Reznick, 2004) and increased crowding effect caused by tadpole growth (Semlitsch and Caldwell, 1982; Berven and Chadra, 1988; Scott, 1990).

In our experiment, the comparison of the two metamorphic climax moments (sensu McDiarmid and Altig, 1999) revealed that while

Table 2. Summary of similar studies in which anuran species responded to the pond drying by accelerating their metamorphosis. In the pond permanency column are presented the pond types from which the tadpoles used in the experiments originated. The pond duration variables represent the different number of water volume reduction regimes used (e.g., 3 is usually associated with constant, slow decreasing and fast decreasing water treatments). Time of metamorphosis represents the moment (Gosner, 1960 stage) used by the authors to designate the completion of the metamorphosis in their studies. Size at metamorphosis indicates the stage when the size measurements were done and the parameters used (body mass – BM or snout-vent length – SVL). The value of adaptive plasticity was calculated as a percentage of the larval period reduction compared to the control treatments (Wells, 2007).

Species	Family	Pond permanency	Experiment type	Pond duration variables	Time of metamorphosis	Size at metamorphosis	Adaptive plasticity	Effect on tadpole size	Source
<i>Alytes obstetricans</i>	Alytidae	temporary + permanent	laboratory	2	42	46/BM	19%	yes	Richter-Boix, Llorente and Montori, 2006b
<i>Pelobates syriacus</i>	Pelobatidae	temporary	laboratory	4	45	42 + 45/BM + SVL	4%	yes	present study
<i>Pelodytes punctatus</i>	Pelodytidae	temporary + permanent	laboratory	2	42	46/BM	15%	yes	Richter-Boix, Llorente and Montori, 2006b
<i>Scaphiopus couchii</i>	Scaphiopodidae	temporary	field	2	42	42/SVL	up to 50%	yes	Newman, 1989
<i>Spea hammondi</i>	Scaphiopodidae	temporary	laboratory	5	42	42/BM + SVL	up to 20%	yes	Denver, Mirhadi and Phillips, 1998
<i>Spea hammondi</i>	Scaphiopodidae	temporary	field	2	42	42/BM	up to 62%	yes	Morey and Reznick, 2004
<i>Bufo (Epidalea) calanita</i>	Bufoinae	temporary	mesocosm	2	42	46/BM	3%	no	Tejedo and Reques, 1994
<i>Bufo (Pseudipidalea) viridis</i>	Bufoinae	temporary	field	2	42	46/SVL	up to 32%	yes	Hussein and Darwish, 2000
<i>Bufo (Amietophrynus) maculatus</i>	Bufoinae	temporary	laboratory	4	46	46/BM	up to 10%	yes	Spieler, 2000
<i>Bufo (Anaxyrus) americanus</i>	Bufoinae	temporary	mesocosm	3	42	42/BM	up to 29%	no	Wilbur, 1987
<i>Bufo (Rhinella) spinulosa</i>	Bufoinae	temporary	field	3	46	46/SVL	up to 17%	yes	Márquez-García et al., 2009
<i>Hyla meridionalis</i>	Hylidae	temporary + permanent	laboratory	2	42	46/BM	29%	yes	Richter-Boix, Llorente and Montori, 2006b

Table 2. (Continued).

Species	Family	Pond permanency	Experiment type	Pond duration variables	Time of metamorphosis	Size at metamorphosis	Adaptive plasticity	Effect on tadpole size	Source
<i>Pseudacris triseriata</i>	Hylidae	temporary + permanent	field	3	42-46	42-46/SVL	up to 14%	no	Skelly, 1995
<i>Pseudacris crucifer</i>	Hylidae	temporary + permanent	field	3	42-46	42-46/SVL	up to 16%	yes	Skelly, 1995
<i>Isthmohyla (Hyla) pseudopuma</i>	Hylidae	temporary	laboratory	3	42	42 + 45/BM + SVL	3%	yes	Crump, 1989
<i>Rana temporaria</i>	Ranidae	temporary	laboratory	3	42	42/BM	3%	yes	Merilä et al., 2000
<i>Rana temporaria</i>	Ranidae	temporary + permanent	laboratory	3	42	42/BM + SVL	3%	yes	Laurila, Karttunen and Merilä, 2002
<i>Rana temporaria</i>	Ranidae	temporary + permanent	mesocosm	2	45	45/SVL	6%	yes	Loman and Claesson, 2003
<i>Rana temporaria</i>	Ranidae	temporary	mesocosm	2	45	45/BM + SVL	up to 6%	no	Loman, 1999
<i>Rana temporaria</i>	Ranidae	-	laboratory	2	42	42/BM + SVL	up to 6%	yes	Merilä, Laurila and Lindgren, 2004
<i>Rana temporaria</i>	Ranidae	temporary	laboratory	2	42	42/BM	up to 7%	yes	Laurila and Kujasalo, 1999
<i>Rana (Pelophylax) perezi</i>	Ranidae	permanent	laboratory	2	42	46/BM	17%	no	Richter-Boix, Llorente and Montori, 2006b
<i>Rana (Lithobates) blairi</i>	Ranidae	temporary	mesocosm	2	42	46/BM	8%	yes	Parris, 2000
<i>Rana (Lithobates) sphenoccephala</i>	Ranidae	temporary	mesocosm	2	42	46/BM	4%	yes	Parris, 2000
<i>Rana (Lithobates) sphenoccephala</i>	Ranidae	temporary	mesocosm	3	46	46/BM + SVL	up to 20%	no	Ryan and Winne, 2001
<i>Rana (Lithobates) sylvatica</i>	Ranidae	temporary	laboratory	4	42	46/BM + SVL	up to 20%	no	Gervasi and Fofopoulos, 2008

the effect of decreasing water level on the time of metamorphosis was evident only at the end (stage 45, when metamorphosis is almost completed), the effect on the size of metamorphs was similar in both moments. Nevertheless, after the beginning of metamorphic climax (the forelimb protrusion at stage 42) the differences tend to be harder to detect as the general size of metamorphs becomes considerably smaller. This final observation, along with several other arguments presented by Walsh (2010), supports his recommendation to use the moment of forelimb emergence (stage 42) as the conclusion of the larval phase.

Although numerous studies attempted to explain the mechanisms which are responsible for the accelerated development in short-duration ponds, the results are contradicting. While some studies suggested that tadpoles responded to the elevated temperatures in the drying ponds (Newman, 1992; Tejedo and Reques, 1994; Gotthard and Nylin, 1995), other indicated no response to temperature (Loman, 1999; Laurila and Kujasalo, 1999; Márquez-García et al., 2009). Another set of studies suggested that a lower growth rate due to decreasing food level may explain the accelerated development (Travis, 1984; Alford and Harris, 1988; Newman, 1994). Since we fed the tadpoles *ad libitum*, the decreasing food resource is therefore a less likely explanation. We can also dismiss the hypothesis that the tadpoles may sense an increased density of conspecifics and respond to this (Wilbur and Collins, 1973; Newman, 1989, 1994; Loman, 1999) because the larvae from treatment 4 were more crowded and started to sense much earlier the increased density and still metamorphosed later than those from the decreasing treatments. This observation is also supported by the results published by Denver, Mirhadi and Phillips (1998).

Another possible explanation is the changes in water chemistry (Denver, 1997; Morey and Reznick, 2004; Gerlanc and Kaufman, 2005). We also recorded in our experiment an increase in conductivity as the water level decreased. It is

possible that the acceleration of metamorphosis can be triggered by a hormone-mediated mechanism, as proposed by some authors (Denver, 1997; Boorse and Denver, 2004). It is also possible that the tadpoles may somehow directly sense the rate of decrease in water level (Loman, 1999) and thus it is not the water quantity that is determinant, but the actual decrease of the water level, which is the cue that triggers the special response. Since temperature, food level and the increased crowding effect did not affect our experimental treatments, we conclude that the timing of tadpole development was influenced only by hydroperiod.

In conclusion, our results indicate that *P. syriacus* tadpoles are able to respond to pond drying by speeding up their development and thus increase their survival rate. This explains their ability to maintain viable populations in dry areas with pond types of unpredictable duration.

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