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LACK OF ADAPTIVE PLASTICITY IN *RANA TEMPORARIA* TADPOLES CAUSED BY STARVATION: AN EXPERIMENTAL STUDY

Introduction

Environmental variability affects processes at all levels and scales of the ecological organization. Phenotypic plasticity is an important potential means of adaptation to varying (heterogeneous) environments for both plants[1] and animals[2].

Amphibians lay their eggs in ponds that range from permanent to temporary ones. Temporary ponds frequently dry out before or during the time of metamorphosis. A key to survive in areas where only temporary ponds are available for reproduction is the ability of larvae for extreme plasticity in age and size at metamorphosis as a response to deterioration in habitat quality. Biotic factors that influence amphibian development include food availability[3], predation[4], density[5] and inter and intraspecific chemical signalling[6]. Part of the plastic responses are adaptive, enhancing the survival probabilities of the tadpoles, but the adaptive phenotypic responses often have a cost, that is, a lower size at metamorphosis.[7] Therefore, variations in growth rate and timing of metamorphosis generate variations in size at metamorphosis, which may affect fitness in later ages.[8] In amphibians there is a positive correlation between size at metamorphosis and fitness[9] so, the smaller juveniles are more vulnerable in terrestrial life stages than larger ones. According to the model of Wilbur and Collins[10], there is a threshold larval body size/developmental stage that must be attained to be capable for initiating and completing metamorphosis, in response to environmental deterioration (see also Morey and Reznick[11]).

Previous studies demonstrate that the larvae of *R. temporaria* and *R. arvalis* fail to metamorphose in crowded conditions.[12] In this study, we assume that reaching threshold larval size or a developmental stage that allows tadpoles to accelerate metamorphosis depends on early larval development, and therefore also on the environment that influences development. Hence, our hypothesis was that the constant low level of food during the whole larval period will hamper the adaptive plastic response of larvae to decreasing water volume.

Materials and Methods

We studied the tadpoles of the common frog *Rana temporaria* in Sighişoara (Romania). *R. temporaria* is an explosive breeder (*sensu* Wells[13]), that frequently breeds in small temporary waters, with high risk of desiccation. In Târnava Mare Valley, breeding occurs in early March – middle of April.[14] The population from which the egg clump was collected reproduces in a number of small ponds in a mixed deciduous forest, at about 500-m altitude. The risk of desiccation of the temporary ponds is unpredictable. In 2001–2002 no metamorphs were observed (the ponds disappeared until the end of May), in 2003 the precipitation maintained the hydroperiod until the end of the metamorphosis in some of the ponds.[15]

In this experiment we used tadpoles from one clutch produced by a pair of frogs in a temporary pond. After hatching (stage 20[16]), we collect approximately 200 tadpoles and placed them in a 50 l holding tank for the next 7 days until they reach stage 25.[17] At this stage, the tadpoles measured on average

[1] e.g. SCHLICHTING, CD. – PIGLIUCCI, M. 1995.

[2] e.g. REZNICK, DN. 1990; NEWMAN, RH. 1992; JORDÁN F. 2003.

[3] CRUMP, ML. 1989a; REZNICK, DN. 2000.

[4] SKELLY, DK. – WERNER, EE 1990; PETRANKA, JW. – RUSHLOW, AW. – HOPEY, ME. 1998; PETRANKA JW.– KENNEDY, CA. 1999.

[5] SEMLITSCH, CD. – CALDWEL, JP. 1982; SCOTT, DE. 1990; SMITH, GR. 1998.

[6] WERNER, EE. 1986.

[7] CRUMP, ML. 1989b; LAURILA, A. – KUJASALO, J. 1999.

[8] SEMLITSCH, RD. – SCOTT, DE. – PECHMANN, JHK.

1988; SCOTT, DE. 1990. 1994.

[9] SEMLITSCH, RD. – SCOTT, DE. – PECHMANN, JHK. 1988; NEWMANN, RH. – DUNHAM AE. 1994; SCOTT, DE. 1990, 1994; MOREY, S. – REZNICK, D. 2001.

[10] WILBUR, HM. – COLLINS, JP. 1973.

[11] MOREY, S. – REZNICK, D. 2000.

[12] LOMAN, J. 2002.

[13] WELLS, KD. 1977.

[14] HARTEL T. 2005.

[15] HARTEL T., personal observations

[16] GOSSNER, KL. 1960.

[17] GOSSNER, KL. 1960.

16.37 mm in body length (SD: 2.04) and 0.04 g in body weight (SD: 0.012). After this, we placed 4 tadpoles from the holding tank into each of the 23 plastic vessels (totally 92 tadpoles). The experiment was 2x3 factorial design with two temperature treatments (constant and fluctuating) and three water volume treatments: constantly high (= CH; 3000 ml/vessel); decreasing (= D; from 3000 ml to 200 ml and kept constant until the end of metamorphosis); and constantly low (=CL; 200 ml). The initial density was 1.33 individuals/L in the constant high water treatment and 20 individuals/L in the constant low water level treatment. The density of the individuals in the decreasing volume treatment increased gradually with the water removal. Water removal started after five days (Fig 1). The water temperature was measured at the bottom two days every week.

The tadpoles were fed with 0.02 g Tetra Min (fish food) per plastic bowl (0.005 g *per capita*) in every three or four days. The amount of food was constant during the experiment. Approximately 50 % of the water was changed weekly. When metamorphs appeared, we anaesthetized them with MS222 and measured the SVL (0.1 mm precision) and body weight (0.01 g precision). For the estimation of the developmental time (days) the median value was used.

The level of significance for statistical tests was set at 5%. Means were tested by one-way ANOVA after testing data for equality in variances and normal distribution. Spearman rank correlation was used to test the strength of relationship between different variables.

Results

Timing of metamorphosis differed significantly between temperature treatments ($F_{1,59}$=4.16, p=0.04) while among the different water level treatments there were no significant differences ($F_{2,58}$=0.13, p=0.72). It was a temperature effect on the developmental time, the larvae growing in fluctuating temperature metamorphosed slightly after the constant temperature group. (Fig. 2.) In the constant temperature group, the age until metamorphosis being the shortest for those in constant high water level, intermediate in the decreasing water level treatment and longest for those in constant low water level treatment, but there is no significant difference among them ($F_{2,58}$=0.16, p=0.85). In the fluctuating temperature treatment, the decreasing water treat-

ment group has the longest larval period and the metamorphs from the constant high water level were the youngest, but also there is no significant difference ($F_{2,25}$=0.13, p=0.49). No significant differences among body length or weight were recorded among different treatments and groups. (Table 1., Fig. 3.) Timing of metamorphosis correlates negatively with both body length and body weight but the relationship is nonsignificant. (Table 2.)

The mortality of larvae was higher in the fluctuating temperature group than in the constant water temperature group. (Fig. 4.) The highest mortality was observed between the larvae in 34–38 Gossner stage[18] in both temperature treatments. The body length of the death larvae was 27.1 mm (SD=1.25), the SVL was 8.86 mm (SD=2.05) and the wet body weight was 10.16 mg (SD=0.76).

Discussion

Several studies demonstrate the capacity of amphibian larvae to respond to decreasing water volume by earlier metamorphosis. This plasticity might have adaptive value in allowing tadpoles to escape the drying habitat.[19] The available food quantity is a significant factor that influences the larval growth and development in amphibians.[20] Wilbur and Collins[21] predict that the age at metamorphosis in amphibians is a function of the individual's size and growth rate. The metamorphosis is possible only if the individuals reach a minimal, or threshold body size (see also Morey and Reznick[22]). If the growth rate is high, the threshold body size is quickly attained and the consequence of this is a delay in metamorphosis. This is because fast growth implies good conditions; the individual continue to grow up to maximum larval sizes that result in a large size after completing the metamorphosis. Slow growers will initiate metamorphosis soon after they reach the threshold and by this, they metamorphose at a smaller size.

Our results indicate that the constant low food level hampers the capacity of tadpoles to respond adaptively to decreasing water level by earlier metamorphosis. Therefore, the age and size at metamorphosis are not in accordance with the findings of other studies referring to the common frog[23] or other amphibian species[24]. These studies demonstrate plasticity in response to different food and water treatments, and the observed plasticity was interpreted as adaptive. Laurila and Kujasalo[25] found that the larval

[18] GOSSNER, KL. 1960.
[19] CRUMP,ML. 1989b; LAURILA, A. – KUJASALO, J. 1999; MERILA, J. et alii 2000.
[20] NEWMAN, RH. 1994.
[21] WILBUR, HM. – COLLINS, JP. 1973.

[22] MOREY, S. – REZNICK, D. 2000.
[23] LAURILA, A. – KUJASALO, J. 1999; LOMAN, J. 1999; MERILA, J. et alii 2000.
[24] CRUMP, ML. 1989b; NEWMAN, RH. 1988.
[25] LAURILA, A. – KUJASALO, J. 1999

period and mass at metamorphosis was influenced by water volume, but also by food level. In their experiment, the larvae growing at low food level (2.5% of their body weight, kept constant during the experiment) were smaller than larvae growing at high food level (7.5% of their body mass, kept constant), but the adaptive plasticity in the first case still occured, showing that the larvae were still capable to make further progress toward a plastic response to water drying. In our experiment, the food level was about 0.005 g *per capita*, kept constant during the experiment. The constantly low food levels hamper the growth rate and by this, probably prevent the attainment of the minimal threshold size that enables metamorphosis.

Resource limitation has been shown to influence amphibian development. Morey and Reznick[26] studying the plasticity in three species of spadefoot toads found that after food elimination, larvae either completed development or entered a developmental stasis, making no further progress toward metamorphosis. They found that prior to some critical stage, no larvae were able to complete metamorphosis after food was eliminated. D'Angelo et al.[27], showed that in *Rana sylvatica* and *R. pipiens*, starvation before the early limb development stage retarded metamorphosis; whereas starvation after this stage accelerated metamorphosis. Denver et al.[28] observed a similar phenomenon in *Scaphiopus hammondii*. These studies suggest that there is a critical period of development for responding positively to starvation; *i.e.*, perhaps there is a minimum size or developmental stage for metamorphosis. We assume that the lack of adaptive plasticity in our experiment is due to the limited food resources. Since the water was changed weekly, the observed phenotypic traits could be the result of other possible environmental stress factors interacting with low food level, like density dependent effects: crowding, increased metabolic waste products, (including high CO_2 concentrations, decreased amount of O_2, other chemical cues, the extent of the swimming area etc.

An important environmental variable, which could drive an accelerated development, is pond temperature. Environmental temperature is positively correlated with the rate of metamorphosis and growth,[29] but, the thermal sensitivity of differentiation is more pronounced than that of growth.[30] Thermal effects are expressed through alterations in the rate functions of the biochemical processes, which drive morphogenesis. As it was expected, the tadpoles that experienced fluctuating temperature treatment metamorphose slightly after the tadpoles that develop at constant temperature.

In conclusion, the low food level could be a major limiting factor in tadpole growth and development. Therefore, the adaptive response to pond drying may be hampered by a prolonged starvation. However, detailed studies have to be conducted concerning developmental stages and capacity of adaptive response to pond drying in the case of the common frog.

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References

CRUMP, ML.
    1989a   Life history consequences of feeding versus non – feeding in a facultatively non – feeding toad larva, *Oecologia,* 78, 486–489.
    1989b   Effect of habitat drying on developmental time and size at metamorphosis in Hyla pseudopuma, *Copeia 1988,* 794–797.
DENVER, RJ.
    1997a   Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis, *Am. Zool.,* 37, 172–184.
    1997b   Environmental stress as a developmental cue: corticotropin releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis, *Hormones and Behaviour,* 31, 169–179.
DENVER, RJ. – MIRHADI, N. – PHILLIPS, M.
    1998   Adaptive plasticity in amphibian metamorphosis: response of Scaphiopus hammondii tadpoles to habitat desiccation, *Ecology,* 79, 1859–1872.
GOSNER, KL.
    1960   A simplified table for staging anuran embryos and larvae with notes on their identification, *Herpetologica,* 16, 183–190.
HARTEL, T.
    2005   Aspects of breeding activity of Rana dalmatina and Rana temporaria reproducing in a seminatural pond, *North-Western Journal of Zoology,* 1, 5–13.
JORDÁN, F.
    2003   From genes to nature conservation, [in Hungarian] *Természet Világa,* 134, 194–196.

[26] MOREY, S. – REZNICK, D. 2000.

[27] cited in DENVER, RJ. 1997a

[28] DENVER, RJ. – MIRHADI, N. – PHILLIPS, M. 1998.

[29] NEWMAN, RH. 1994; DENVER, RJ. 1997a.

[30] DENVER, RJ. 1997a.

LAURILA, A. – KUJASALO, J.
    1999   Habitat duration, predation risk and phenotypic plasticity in common frog (Rana temporaria) tadpoles, *J. Anim. Ecol.,* 68, 1123–1132.

LOMAN, J.
    1999   Early metamorphosis in common frog Rana temporaria tadpoles at risk of drying: an experimental demonstration, *Amphibia-Reptilia,* 20, 421–430.
    2002   When crowded, tadpoles (Rana arvalis and R. temporaria) fail to metamorphose early and thus fail to escape drying ponds, *Herpetological Journal,* 12, 21–28.

MERILA, J. – LAURILA, A. – PAHKALA, M. – RASANEN, K. – TIMENES LAUGEN, A.
    2000   Adaptive phenotypic plasticity in timing of metamorphosis in the common frog Rana temporaria, *Ecoscience,* 7, 18–24.

MOREY, S. – REZNICK, D.
    2000   A comparative analysis of plasticity in larval development in three species of spadefoot toads, *Ecology,* 81, 1736–1749.
    2001   Effects of larval density on postmetamorphic spadefoot toads (Spea hammondii), *Ecology,* 82, 510–522.

NEWMAN, RH.
    1988   Adaptive plasticity in development of Scaphiopus couchii tadpoles in desert ponds, *Evolution,* 42, 774–783.
    1992   Adaptive plasticity in amphibian metamorphosis, *BioScience,* 42, 671–678.
    1994   Effects of changing density and food level on metamorphosis of a desert amphibian, Scaphiopus couchii, *Ecology,* 75, 1085–1096.

NEWMAN, RH. – DUNHAM, AE.
    1994   Size at metamorphosis and water loss in a desert anuran (Scaphiopus couchii), *Copeia,* 372–381.

PETRANKA, JW. – KENNEDY, CA.
    1999   Pond tadpoles with a generalized morphology: is it time to reconsider their functional roles in aquatic communities? *Oecologia,* 120, 621–631.

PETRANKA, JW. – RUSHLOW, AW. – HOPEY, ME.
    1998   Predation by tadpoles of Rana sylvatica on embryos of Ambystoma maculatum: implications of ecological role reversals by Rana (predator) and Ambystoma (prey), *Herpetologica ,* 54, 1–13.

REZNICK, DN.
    1990   Plasticity in age and size at maturity in male guppies (Poecilia reticulata): an experimental evaluation of alternative models of development, *J. Evol. Biol.,* 3, 185–203.

SCHLICHTING, CD. – PIGLIUCCI, M.
    1995   Lost in phenotypic space: environment – dependent morphology in Phlox drummondii (Polemoniaceae), *Int. J. Plant. Sci.,* 156, 542–546.

SCOTT, DE.
    1990   Effects of larval density in Ambystoma opacum: an experiment in large scale field enclosures, *Ecology,* 71, 296–306.
    1994   The effect of larval density on adult demographic traits in Ambystoma opacum, *Ecology,* 75, 1383–1396.

SEMLITSCH, RD. - CALDWELL, JP.
    1982   Effects of density on growth, metamorphosis, and survivorship in tadpoles of Scaphiopus holbrooki, *Ecology,* 63, 905–911.

SEMLITSCH, RD. – SCOTT, DE. – PECHMANN, JHK.
    1988   Time and size at metamorphosis related to adult fitness in Ambystoma talpoideum, *Ecology,* 69, 184–192.

SKELLY, DK. – WERNER, EE.
    1990   Behavioural and life historical responses of larval American toads to an odonate predator, *Ecology,* 71, 2313–2322.

SMITH, GR.
    1998   Abundance and water volume effects in amphibian larvae, *Bull. Of the Maryland Herpetological Society,* 34, 113–117.

WELLS, KD.
    1977   The social behaviour of anuran amphibians, *Anim. Behav.,* 25, 666–693.

WERNER, EE.
    1986   Growth rate, predation risk, and the optimal size at transformation, *Am. Nat.,* 128, 319–341.

WILBUR, HM. – COLLINS, JP.
    1973   Ecological aspects of amphibian metamorphosis, *Science,* 182, 1305–1314.

A gyepibéka (Rana temporaria)-lárvák alkalmazkodási plaszticitásának hiánya a táplálékszegény környezethez. Kísérleti tanulmány
(Kivonat)

Jelen dolgozatban a gyepibéka lárvák fenotípusos válaszait mutatjuk be, változó környezeti feltételek mellett (hőmérséklet és vízszint), amikor a táplálék a limitáló tényező. Az állandó hőmérséklet gyors fejlődést tett lehetővé, míg a csökkenő vízszint nem hatott lényegesen a lárvák fejlődésére. A különböző csoportok átalakult lárváinak a testméretei nem különböztek szignifikánsan. A metamorfózisig eltelt idő (napok), valamint a kisbékák testhossza és -súlya között negatív korrelációt találtunk. Legnagyobb volt a mortalitás a fluktuáló hőmérsékleten tartott lárvák között. Eredményeink azt mutatják, hogy a táplálékhiány fontos limitáló tényezője lehet az adaptív fenotípusos plaszticitásnak.

Lipsa plasticității adaptive a larvelor de *Rana temporaria* față de foamete
Un studiu experimental
(Rezumat)

În acest studiu am testat experimental răspunsurile fenotipice ale larvelor de *Rana temporaria* la condițiile de mediu schimbătoare (temperatură și scăderea nivelului apei) în condițiile unui consum mic de energie. Temperatura constantă a permis dezvoltarea mai rapidă, scurtând perioada larvară, pe când scăderea nivelului apei nu a afectat perioada larvară până la metamorfoză. Nu am găsit diferențe semnificative în lungimea și greutatea corporală a exemplarelor metamorfozate aparținând la diferitele grupuri. Perioada trecută până la metamorfoză este corelată negativ cu lungimea și greutatea corporală. Mortalitatea larvelor este cea mai mare în grupul expus fluctuațiilor de temperatură comparativ cu cea a larvelor, care s-au dezvoltat în condiții de temperatură constantă. Rezultatele noastre arată că în condițiile ostile de mediu, când hrana este un factor limitant, larvele de *R. temporaria* nu se adaptează condițiilor schimbătoare de mediu.

Comparisons	F	*Df*	*p*
Constant temperature			
SVL			
CL – D – CH	0.95	2.30	0.95
Mg			
CL – D – CH	0.11	2.30	0.89
Fluctuating temperature			
SVL			
CL – D – CH	0.54	2.28	0.590
Mg			
CL – D – CH	1.63	2.28	0.213
Among temperature treatment			
SVL	0.18	1.59	0.670
Mg	0.02	1.59	0.871

Table 1. The comparison of the body size of the individuals among volume treatments and temperature treatments. (CHC = constant high volume, constant temperature; CHFL = Constant high volume, fluctuating temperature; DC = Decreasing volume, constant temperature; DFL = Decreasing volume, fluctuating temperature; CLC = Constant low volume, constant temperature; CLFL = Constant low volume, fluctuating temperature).

Variable	*r(s)*	*t (N-2)*	*P*
(a) Constant temperature			
CL	-0.47	-1.31	0.23
D	-0.59	-2.08	0.07
CH	-0.09	0.34	0.73
(b) Fluctuating temperature			
CL	-0.05	-0.12	0.9
D	-0.11	-0.30	0.77
CH	-0.54	-2.03	0.069

Table 2. The Spearman correlation between the age and body weight of the newly metamorphosed frogs. See Table 1. for abbreviations.
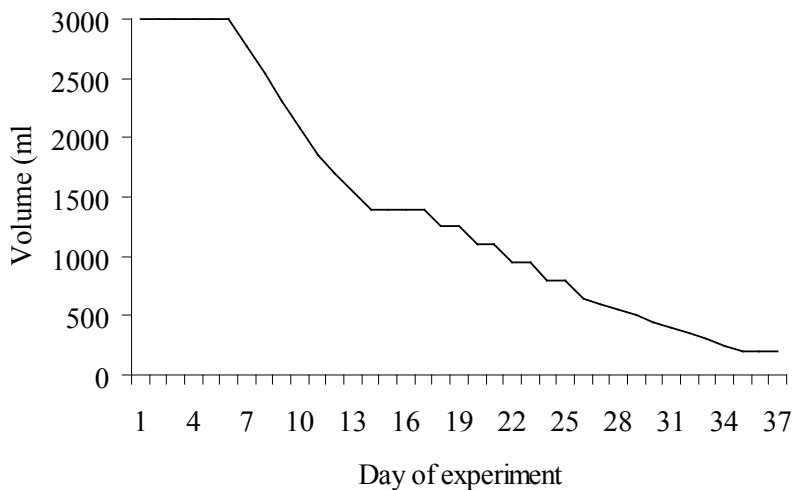
Figure 1. The dynamics of water removal in the decreasing volume experiment.
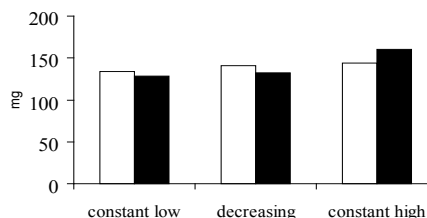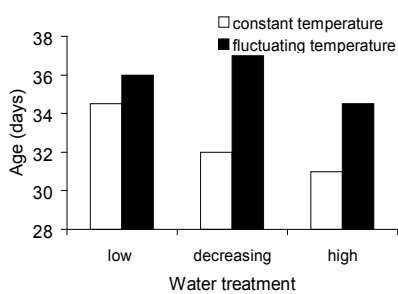

Figure 2. The median of the age at metamorphosis in the three water treatments and two temperature treatments.
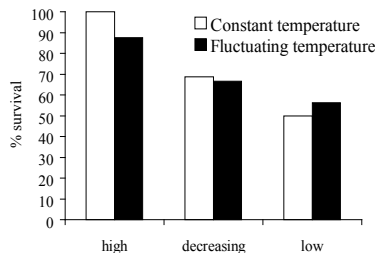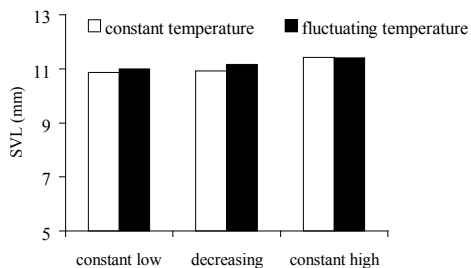

Figure 3. The length (SVL) and weight (mg) of the newly metamorphosed frogs.

Figure 4. The percentage of survival among tadpoles under different water level.

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