

Early metamorphosis in common frog *Rana temporaria* tadpoles at risk of drying: an experimental demonstration

Jon Loman

Dept. Animal Ecology, University of Lund, Ecology building S-223 62 Lund, Sweden
e-mail: jon.loman@zoekol.lu.se

Abstract. Does the development rate of common frog tadpoles accelerate if their habitat dries? To study this, the water level in experimental tanks was reduced shortly before time of metamorphosis. Water level remained high in control tanks. The experiment was performed at two different tadpole densities and replicated four times, with tadpoles from different source ponds. The experimental treatment, simulating a drying pond, resulted in earlier metamorphosis while no significant difference in size at metamorphosis was found. Resources per capita decreased as a result of the decreased water level so the increase in development rate was not an effect of feeding conditions. Temperatures in the tanks were such that it is unlikely that the increased development rate was due to temperature effects. I interpret the advancement of metamorphosis as an adaptive response to the threat of drying. This response has been documented for several other anuran species. All those breed in temporary water bodies, supporting the hypothesis that the trait is an evolved adaptation for breeding in such waters.

Introduction

Breeding frogs lay their eggs in ponds that range from permanent to temporary ones. In Sweden, the common frog (*Rana temporaria* L.) sometimes breeds in ponds that dry up before or during the time of metamorphosis. Thus, drying of the pond is a mortality factor of some importance for tadpoles of this species (Cooke, 1985; Loman, 1996). In such ponds early metamorphosis is at a premium. On the other hand, when there is no risk of drying, it may be better to take advantage of the pond habitat for a longer time.

Tadpoles have been shown to be plastic in several respects: morphology (Smith and Van Buskirk, 1995), behaviour (Skelly, 1995) and development rate (Crump, 1989). The optimum development rate may depend on information on quality of the breeding pond and on 'expected' quality of the future land habitat (Wilbur and Collins, 1973; Werner, 1986). In most cases, high development rate has been associated with slow growth rate (e.g. Crump, 1989).

Can a tadpole somehow individually adjust the balance between growth rate and development rate? The “choice” would be between an early metamorphosis, at a small size, or a later one at a larger size. If a pond is about to dry up shortly it is definitely of very poor quality for a tadpole and it would be adaptive to metamorphose sufficiently early.

Growth rate, the increase in weight per time, and development rate, the inverse of time required to reach metamorphosis (or a given developmental stage) tend to decrease with increased competition (density) (Wilbur, 1976, 1977; Dash and Hota, 1980; Morin, 1986) and to increase with higher temperatures. The former is likely to be an effect of reduced food resources. Thus, effects on development rate are not necessarily due to adaptive responses (Gotthard and Nylin, 1995). A similar point is made by Smith-Gill (1983) who separates between developmental conversion and phenotypic modulation. The former process, but not necessarily the latter, is considered adaptive by her.

If there is a positive effect on tadpole development rate from the drying rate of a pond, this may be due to several factors. **1.** The tadpoles may somehow directly sense the rate of decrease in water level. **2.** The tadpoles may sense an increased density of conspecifics and respond to this, either **2a.** because it is an indication of decreasing water volume or **2b.** because it by itself constitutes an adverse condition, motivating a quick escape onto land. **3.** Because the tadpoles’ main food is periphytic algae, decreased water volume (and thus usually less pond bottom area) means less available food per tadpole. The resulting decrease in feeding rate may, as in the previous case, **3a.** either be a cue to increased risk of desiccation **3b.** or by itself, reason enough for an increased development rate. The separation between case 2 and 3 is not clear-cut, and as far as the response under 2 relates to resources, not very important. **4.** Shallow water may have a temperature regime that differs from that of deeper water. At least during day-time, shallow water should be warmer than deep water. Possibly, lower night temperatures may compensate for this but this is not necessarily so. Thus, **4a.** a higher average temperature may speed up growth rate and/or development rate. **4b.** Alternatively, warm water may be another cue to future drying of the pond. All effects except #4a would be examples of an adaptive response. A review of amphibians’ developmental response to drying ponds is given by Newman (1992). He points out that such a tadpole response is an example of “norm of reaction” in the sense of Stearns (1983) and also discusses to what extent such responses represent *adaptive* plasticity. Denver (1997) discusses how the above mentioned cues may affect development rate through proximate hormonal mechanisms.

Earlier, I performed an experiment on the development rate of common frogs and moorfrogs (*Rana arvalis*) (Loman, unpublished). In that experiment, tadpoles were raised in tanks where water levels were experimentally decreased but no evidence of an adaptive, plastic development rate was found. This may have been due to the design of the experiment where the gradual decrease of water level made it difficult to separate effects of resource availability and tadpole density from those of water level per se.

Here I report on a similar experiment, now only with common frogs as subjects. The main difference between the experiment reported on here and the one performed earlier is

that the water level manipulation was now done late. This reduced the effect of increased competition in experimental tanks that, by itself, tends to delay metamorphosis and thus mask any adaptive increase in development rate.

The idea behind the new design is that a late change in resource availability is not likely to non-adaptively affect growth and development as much as is an early one. This is supported by the results of Leips and Travis (1994) and by Audo et al. (1995) in studies of *Hyla* species. They found that only early changes in food availability affected the timing of metamorphosis.

The experiment reported on here was thus designed with two objects in mind. Firstly, to test if common frog tadpoles can advance metamorphosis when at risk from drying. Second, to allow a separation of the adaptive nature of such a response from any secondary effects (like temperature, changes in competition or amount of food available) that might have a similar consequence on tadpole growth and development.

Methods

Experimental tanks. The 16 experimental tanks were made of plastic barrels, cut lengthwise. They were thus semicircular in one cross section and rectangular in the other. Original water surface was 80 times 60 cm and original depth was 25 cm. The tanks were set up outdoors, close to each other on a rectangular grid at the Lund University Ecology Department's field station, 17 km east of Lund (55° 40'N, 13° 30'E). Each tank was filled with 80 l of tap water and "inoculated" with about 2 l of pond water. Vegetation (strings of *Ranunculus aquatilis*) and about 25 g of dry beech (*Fagus silvatica*) leaves were added as a substratum (in addition to that provided by tank walls) for algae. This was done about 2 weeks before the introduction of the tadpoles. No more food was added. The tadpoles fed exclusively on resources produced in the containers. This was mainly algae on the wall of the container. The containers were covered with fine mesh to prevent colonization by predaceous insects. Above the tanks I suspended thin textile material as a sun shade to reduce temperature extremes.

Collection and introduction of tadpoles. Newly hatched common frog tadpoles were collected from four breeding ponds during the last week of April 1991. These ponds were situated within 30 km of each other in the province Skåne in southernmost Sweden. The samples contained tadpoles from several newly hatched clutches at each pond. After collection, all tadpoles from one site were allowed to mix and put in small 2 l tanks. After two days, healthy looking tadpoles were introduced into 16 large experimental tanks. There were 20 or 80 tadpoles in each tank.

Experimental treatments. The experimental setup was a 2 × 2 randomized block design with 4 replicates of each block; the 4 replicates came from the 4 different source ponds. Different ponds were used to increase generality but the effect of pond per se

was not within the scope of this study. The first treatment was tadpole density. High density tanks contained 80 tadpoles each and low density tanks contained 20 tadpoles each. The second treatment was water level. Water level in control tanks remained high (water depth 25 cm) throughout the experiment. In experimental tanks, water level was lowered to a depth of about 2 cm and maintained at that level until the conclusion of the study.

The water level reduction took place on the day when the front legs were first visible on the most early tadpole in the respective density category. This means that water was reduced in all low density tanks on June 23 and in all high density tanks on June 26. Most tadpoles reached this developmental stage one to two weeks later.

Measurements. When tadpoles started to metamorphose, containers were checked every or every second day. Tadpoles close to metamorphosis have a tail that is shorter than body length and were collected. They were weighed and their body and tail length was measured. Based on observations of tadpoles in the study population, relative tail length was used to correct more precisely for metamorphosis day. If relative tail length was less than 3% of body length, metamorphosis was considered to take place on the day of capture, if between 3% and 30% on the following day, and if 30% to 100% two days after capture. As weight and length of metamorphs were highly correlated ($n = 230$, $r = 0.92$), an index representing “size” was formed as the first principal component of length and weight. All statistical tests of effects on size and day of metamorphosis were performed using mean tank values. Residuals were tested for normality by the Lilliefors test.

Tank temperatures. During 14 days in early July, temperature was measured with six max-min thermometers, read daily. There was one at the surface and one on the bottom in each of two deep tanks with a water depth of 25 cm. There was one thermometer in each of two shallow tanks, because the depth was only 2 cm there was no point in separating bottom from surface in these.

Results

Effects on survival

Some tadpoles disappeared during the course of the experiment. Probably, most died but some may have escaped. In the low density treatment, all those remaining metamorphosed before the termination of the experiment on August 4 while only 61% metamorphosed in the high density treatment (table 1). There was much variation in the proportion metamorphosing before August 4 between the different source ponds but little between the different water level treatments (Mantel-Henzel $X^2 = 1.13$, $P = 0.29$).

Table 1. Performance of tadpoles in deep and shallow tanks. Each entry is the average of all tadpoles metamorphosing in the respective tank. Day refers to number of days after June 1st. “Not metamorphosing” are tadpoles that remained in the tanks on August 4th. “Metamorphosing (%)” refers to the proportion of those not lost that metamorphosed before August 4th.

Density and source pond	Metamorphosing		Day		Weight (mg)		Length (mm)		Not metamorphosing		Metamorphosing (%)	
	n	n	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow
High density												
AR13	45	37	45.9	44.0	79.9	72.4	9.3	9.0	22	26	67	59
HL7	18	9	46.0	38.1	64.1	59.7	8.7	8.5	57	46	24	16
HP27	40	41	39.4	42.2	84.4	79.9	9.5	9.3	14	13	74	76
M3	59	65	43.0	40.4	86.1	78.4	9.4	9.0	10	12	86	84
All	162	152	43.25	41.40	80.61	75.99	9.3	9.03	103	97	61	61
Low density												
AR13	20	15	36.1	31.9	191.2	168.0	12.4	11.8	0	0	100	100
HL7	16	15	33.8	31.4	192.7	220.7	12.6	12.5	0	0	100	100
HP27	16	17	30.6	30.1	188.6	155.7	12.0	11.0	0	0	100	100
M3	18	16	30.0	28.9	151.8	175.8	11.1	11.6	0	0	100	100
All	68	63	32.73	30.51	174.53	179.80	11.92	11.72	0	0	100	100

Table 2. Testing effects on *date* of and *size* at metamorphosis with a 3-way ANOVA where source POND is a blocking factor. ANOVAs with an interaction DENS*DEPTH yielded an insignificant effect for this interaction (time: $P = 0.89$, size: $P = 0.80$). The table presented gives the analysis without an interaction term. The residuals' distribution were tested for normality with the Lilliefors test. They were not significantly different (time: $P = 0.22$, size: $P = 0.38$).

	d.f.	Date		Size	
		F	P	F	P
Source pond	3	4.60	0.029	0.099	0.96
Tadpole density	1	157.5	<0.001	143.1	<0.001
Tank depth	1	7.40	0.022	0.777	0.399

Table 3. Tank temperatures ($^{\circ}\text{C}$) during the period July 1 to July 14. “Maximum” and “Minimum” are average of daily maximum and minimum temperatures, respectively. “Mean” is the mean of those two values. The significance of the difference between position was tested by a 3-way ANOVA, accounting for effects of tanks (nested under position) and date. Residuals were not significantly different from normal (Lilliefors test, $P = 0.11$ and 0.61 for maximum and minimum values respectively).

	Maximum	Minimum	Mean
Deep tanks — bottom	21.1	16.5	18.8
Deep tanks — surface	22.5	16.1	19.3
Shallow tanks	24.5	13.6	19.1
$F_{2,65}$	34.17	33.46	
P	<0.001	<0.001	

Effects on time of metamorphosis

Among those metamorphosing, there was considerable and significant variation in metamorphosis date between ponds and between density treatments; tadpoles metamorphosed earlier at low density (tables 1 and 2). However, when accounting for these effects, it was also found that tadpoles in shallow “experimental” tanks (with reduced water level) metamorphosed significantly earlier (tables 1 and 2) than those in deep “control” tanks.

Effects on size at metamorphosis

Tadpoles in high density tanks were significantly smaller at metamorphosis than those in low density tanks but there was no consistent difference between tadpoles from different source ponds (tables 1 and 2). Furthermore, when accounting for effects of density and source pond, there was no significant effect of water depth on size at metamorphosis (tables 1 and 2).

Temperatures in the tanks

The sun shades eliminated most of the temperature variations between tanks but some persisted. The highest temperature variations between night and day were recorded in the shallow tanks (table 3). The fluctuations were less in the deep tanks. In these, the

fluctuations were higher at the surface than at the bottom. The averages of maximum and minimum temperatures were very similar in all three positions (table 3).

Discussion

The experimental reduction of water level did produce an effect on time of metamorphosis. The development speeded up and was on average advanced by two days (table 1). This is a substantial reduction, considering that the water level manipulation was performed only about 7 days before the average time of metamorphosis in the low density tanks.

Was the advancement of metamorphosis adaptive?

The advance of metamorphosis was not due to increased resource levels. The main food of the tadpoles appeared to be periphytic algae, the availability of which decreased with decreasing water level (and thus decreasing tank wall). If anything, this should retard metamorphosis.

Neither was the effect likely to be due to a difference in temperature regime between the water level treatments. Average daily temperature was similar in shallow and deep tanks. The only reservation is that the maximum temperatures were slightly higher in the shallow tanks (table 3) so a development rate that is highly progressively temperature dependent could have contributed to the effect found. On the other hand, if tadpoles select an optimum temperature for development, they had more options in the deep tanks. Thus, staying at the surface during day and moving to the bottom at night would actually have resulted in a higher average temperature in deep than in shallow tanks. Thus, it is not likely that the effect on development was due to the temperature in the tanks.

So, I think non adaptive explanations for the response on development rate found can be refuted and conclude that the change in development rate of these tadpoles was actually was an adaption (*sensu* Gotthard and Nylin, 1995). Most other studies that have demonstrated plasticity in frog metamorphosis time as a response to drying (Crump, 1989; Newman, 1988, 1989; Pfennig, 1990; Tejedo and Reques, 1994; Denver and Denver, 1995) have not explicitly rejected the possibility that the experimental design can have allowed temperature effects that affect development rate. However, as stated by some of the authors, I believe that also these studies do constitute examples of adaptive plasticity. Laurila and Kujasalo (in press) did actually design their experiment in a way that excluded the possibility of any temperature and resource effects.

Trade-offs

Advancing metamorphosis should involve some sort of cost. The most likely cost would be reduced growth rate, demonstrated in other studies on tadpole development by Crump

(1989), Newman (1989), Tejedo and Reques (1992) and Laurila and Kujasalo (in press). In my study, any modifications of the relation growth/development had to take place during a short time only. Actually when water level was reduced, the most advanced tadpoles had begun emerging front legs and had ceased feeding. Those developing slower emerged front legs within one or two weeks. While feeding, canalizing additional resources to development could mean reduced growth. After beginning the first phases of metamorphosis, feeding ceases and restructuring of the body results in a size loss from maximum size to metamorphosis. If this period is shortened, a less efficient restructuring may take place, resulting in an even greater reduction of metamorph size. Both processes could result in a reduced size of fast developing tadpoles compared to slow developing ones. Actually a size reduction of fast developing tadpoles was indeed recorded in the high density tanks (table 1). However, the increased crowding in these tanks could (and most likely did) by itself reduce growth rate (Wilbur, 1976; Loman, unpubl.). So, the reduction in growth rate when water level decreased could be both due to reduced resources due to crowding and a consequence of increased development rate. However, there was no tendency for a size compensation in the low density tanks (table 1) and the overall effect (combining high and low densities) was not significant (table 3). So, in this study I cannot definitely demonstrate the trade-off hypothesized. This may be due to the short time available from start of drying to metamorphosis. Also, alternative trade-offs are possible. Such could, e.g., involve modifications of behaviour. It could be that desperate tadpoles in drying tanks feed faster, neglecting predator vigilance, and consequently develop faster without sacrificing growth rate (Laurila and Kujasalo in press). Usually, tadpoles that sense the presence of a predator do indeed grow slower, probably due to less efficient feeding (Skelly, 1992; Lardner, 1998; Van Buskirk and Yurewicz, 1998).

Which species respond adaptively to drying ponds?

Several other studies have previously demonstrated advanced metamorphosis as a response to drying (*Hyla pseudopuma*, Crump, 1989, *Scaphiopus couchii*, Newman, 1988, 1989, *Scaphiopus multiplicatus*, Pfennig, 1990, *Bufo calamita*, Tejedo and Reques, 1994, *Scaphiopus hammondi*, Denver and Denver, 1995, *Rana temporaria*, Laurila and Kujasalo in press). In all cases the authors explicitly state that their subject species do, invariable or commonly, breed in temporary ponds that may interrupt metamorphosis by complete drying. The only study I have found where the response under study has been tested for but not documented is *Rana esculenta* (Semlitsch and Reyer, 1992). Indeed, this species tends to breed in permanent ponds. It is not possible to draw any strong conclusions because there has been a study bias towards species breeding in temporary ponds. However, the collective results suggest that the ability to speed up development rate in response to pond drying is, not surprising, a capacity of tadpoles in temporary ponds.

Acknowledgements. I thank Ralph Tramontano for help with measuring tadpoles and metamorphs. Suggestions from Bodil Enoksson and James Sutherland improved the language. The work has been supported by the Swedish Council for Forestry and Agricultural Research.

References

- Audo, M.C., Mann, T.M., Polk, T.L., Loudenslager, C.M., Diehl, W.J., Altig, R. (1995): Food deprivation during different periods of tadpole (*Hyla chrysoscelis*) ontogeny affects metamorphic performance differently. *Oecologia* **103**: 518-522.
- Cooke, A.S. (1985): The deposition and fate of spawn clumps of the common frog *Rana temporaria* at a site in Cambridgeshire 1971-1983. *Biol. Conserv.* **32**: 165-187.
- Crump, M.L. (1989): Effect of habitat drying on developmental time and size at metamorphosis in *Hyla pseudopuma*. *Copeia* **1989**: 794-797.
- Dash, M.C., Hota, A.K. (1980): Density effects on the survival growth rate and metamorphosis of *Rana tigrina* tadpoles. *Ecology* **61**: 1025-1028.
- Denver, P., Denver, R.J. (1995): Plasticity of the developmental response to habitat desiccation in tadpoles of the western spadefoot toad. *Amer. Zool.* **35**: 67A.
- Denver, R.J. (1997): Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. *Amer. Zool.* **37**: 172-18.
- Gotthard, K., Nylin, S. (1995): Adaptive plasticity and plasticity as an adaption: a selective review of plasticity in animal morphology and life history. *Oikos* **74**: 3-17.
- Lardner, B. (1998): Plasticity or fixed adaptive traits? Strategies for predator avoidance in *Rana arvalis* tadpoles. *Oecologia* **117**: 119-126.
- Laurila, A., Kujasalo, J. (In press): Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *J. Anim. Ecol.*
- Leips, J., Travis, J. (1994): Metamorphic responses to changing food levels in two species of hylid frogs. *Ecology* **75**: 1345-1356.
- Loman, J. (1996): Övervakningsprogram för brungrödor i Skåne. Rapport från Miljöövervakningen i Malmöhus län **7**: 1-47.
- Morin, P.J. (1986): Interactions between intraspecific competition and predation in an amphibian predator-prey system. *Ecology* **67**: 713-720.
- Newman, R.A. (1988): Adaptive plasticity in development of *Scaphiopus couchii* tadpoles in desert ponds. *Evolution* **42**: 774-783.
- Newman, R.A. (1989): Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. *Ecology* **70**: 1775-1787.
- Newman, R.A. (1992): Adaptive plasticity in amphibian metamorphosis. *BioScience* **42**: 671-678.
- Pfennig, D. (1990): The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* **85**: 101-107.
- Semlitsch, R.D., Reyer, H.-U. (1992): Performance of tadpoles from the hybridogenetic *Rana esculenta* complex: Interactions with pond drying and interspecific competition. *Evolution* **46**: 665-676.
- Skelly, D.K. (1992): Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology* **73**: 704-708.
- Skelly, D.K. (1995): A behavioural trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. *Ecology* **76**: 150-164.
- Smith, D.C., van Buskirk, J. (1995): Phenotypic plasticity, and ecological performance in two tadpole species. *Amer. Natur.* **145**: 211-233.
- Smith-Gill, S.J. (1983): Developmental plasticity: Developmental conversion versus phenotypic modulation. *Amer. Zool.* **23**: 47-55.
- Stearns, S. (1983): The evolution of life-history traits in mosquitofish since their introduction to Hawaii in 1905: Rates of evolution, heritabilities, and developmental plasticity. *Amer. Zool.* **23**: 65-76.
- Tejedo, M., Reques, R. (1994): Plasticity in metamorphic traits of natterjack tadpoles: the interactive effects of density and pond duration. *Oikos* **71**: 295-304.

- Van Buskirk, J., Yurewicz, K.L. (1998): Effects of predators on prey growth rate: relative contribution of thinning and reduced activity. *Oikos* **82**: 20-28.
- Werner, E.E. (1986): Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Amer. Natur.* **128**: 319-341.
- Wilbur, H.M. (1976): Density-dependent aspects of metamorphosis in *Ambystoma* and *Rana sylvatica*. *Ecology* **57**: 1289-1296.
- Wilbur, H.M. (1977): Density dependent aspects of growth and metamorphosis in *Bufo americanus*. *Ecology* **58**: 196-200.
- Wilbur, H.M., Collins, J.P. (1973): Ecological aspects of amphibian metamorphosis. *Science* **182**: 1305-1314.

Received: September 11, 1998. Accepted: February 2, 1999.