LARVAL GROWTH PLASTICITY IN WILD POPULATIONS OF THE BETIC MIDWIFE TOAD, *ALYTES DICKHILLENI* (ANURA: DISCOGLOSSIDAE)

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We studied plasticity in larval development and growth in the endangered toad *A. dickhilleni* in south-eastern Spain. The species is strongly dependent upon artificial breeding sites. The relation between growth and differentiation of larvae is variable across breeding sites. Tadpoles in fountains showed a linear growth/development relationship ("fast trajectory"), whereas in streams and tanks the relationship was non-linear("slow trajectory"). This variation is interpreted as developmental plasticity that allows the species to maximize the range of breeding habitats colonizable in areas characterized by scarce and unpredictable precipitation, as occurs across most of its distribution.

Key words: amphibian conservation, development, reproduction, Spain, tadpoles

INTRODUCTION

Phenotypic plasticity and environmental heterogeneity are subjects of interest in modern evolutionary biology (Berven & Gill, 1983; Via & Lande, 1985; Relyea, 2001). The different phenotypes a single genotype can produce under certain conditions enhance survival probabilities of organisms that live in unpredictable environments with differential environment-dependent responses. Comprehensive studies about phenotypic plasticity should start, according to Newman (1988*a,b*), with preliminary identification of selective environmental factors, variable in both a temporal and a spatial scale, and phenotypic variability in morphological traits. The next step concerns the study of the possible relationships between this phenotypic variability and the differential fitness of each variant under each distinct set of environmental conditions. Eventually, quantitative genetic analyses of different cohorts within a single species can provide an evaluation of the heritability of the traits studied, which can be used to estimate the extent to which these can be affected by natural selection.

Amphibian populations inhabiting areas characterized by scarce, highly unpredictable precipitation have proved to be good models for studying genotype-environment interactions (Newman, 1988*a,b*, 1989, 1994; Tejedo & Reques, 1994; Reques & Tejedo, 1997). This paper describes phenotypic variability in developmental traits of the endangered Betic midwife toad, *Alytes dickhilleni*, which is endemic to a few mountain ranges of the south-eastern Iberian Peninsula. In the karstic limestone mountains inhabited by *A. dickhilleni*, water availability for reproduction is limited, discontinuous and unpredictable both within and between years, being in most cases restricted to human-made water contain-

ing constructions such as water tanks, fountains or troughs (Antúnez *et al.*, 1982; García-París & Arntzen, 1997; own data). This, considering the relatively long larval period of the species, which is a general condition in the genus *Alytes* (Crespo, 1982), imposes problems for the survival of *A. dickhilleni* in these regions. Phenotypic variability in larval traits is explained in this context as a product of different developmental strategies that would result in a better performance in the population-environment interaction.

We have studied the reproductive phenology and larval development of *A. dickhilleni* in the northernmost mountain range within its distribution area, the Sierra de Alcaraz, in the Province of Albacete (Castilla-La Mancha, south-eastern Spain), where the species maintains one of the largest and healthiest groups of populations overall. We followed the development of larvae of *A. dickhilleni* from hatching to metamorphosis in a selection of breeding sites (artificial and natural) and analysed the relationship between growth and differentiation across sites.

MATERIALS AND METHODS

We found evidence of reproduction of *A. dickhilleni* at 65 sites across the study area. As cited for Sierra Tejeda populations (Antúnez *et al.*, 1982), *A. dickhilleni* populations from the Sierra de Alcaraz mainly use three types of breeding sites, two of which are related to human activities and constitute more than 50% of the total number of sites used (Table 1). Brooks are temporary or permanent courses of water whose duration is related to each year's weather conditions and varies both within and between years. Occasionally they are associated with artificial dams that can hold water throughout the year. Fountains have a constant, low water level for most of the year, but sometimes they dry out or are emptied for cleaning. Finally, water tanks are relatively stable aquatic environments that have a constant, high level of water during most of the breeding period, but they are also emptied at least once a year for cleaning, which makes them unpredictable environments.

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TABLE 1. Breeding site categories and their properties as aquatic systems used for amphibian reproduction (see explanations in text).

Type	Stability	Predictability
Water tanks	Intermediate	Low
Brooks	Low	Low
Fountains	High	Intermediate

At some breeding sites sampling was carried out once a month from August 1995 to October 1996. Those selected included representatives of the three categories most commonly used by *A. dickhilleni*: brooks, water tanks and fountains. We chose two sites of each type: brooks (Rio Turruchel and Arroyo del Saz), water tanks (Venta Mendoza and El Bellotar) and fountains (Las Raigadas-La Guitarra and Riópar Viejo). On each visit larvae were captured by dipnetting. Total length (TL), snout-vent length (SVL) and developmental stage (GSD), following Gosner (1960), were determined and measured for all the larvae captured. Totals of 2508 larvae from water tanks, 828 from brooks, and 1203 from fountains were measured. All the specimens were released immediately at their place of capture. Statistical analyses were performed with the packages SPSS 8.0 and STATISTICA 5.5. Mann-Whitney U-tests were performed to test differences in means of post-metamorphic (GSD>42) SVL measurements across reproduction categories.

RESULTS

The calling period of males of *A. dickhilleni* extended from March to August during the study. Empty egg sacs were detected in the water from May to September. A proportion of the tadpole population overwinters. We have not observed an obvious relationship between hatching date and probability of overwintering. Overwintering larvae were found throughout the year (except in fountains, see below). Metamorphic juveniles were found from August to October. We did not observe reproductive activity during the autumn and winter periods.

A large proportion of the total number of tadpoles were at Gosner's stage of development (herein referred to as GSD) 26 (Fig. 1). In fountains, the proportion of tadpoles at GSD 26 gradually decreases after the peak of maximum reproductive activity. However, in water tanks and fountains there are several additional peaks of high proportion of tadpoles at GSD 26 throughout the year.

SVL descriptive statistics for each GSD across breeding sites are shown in Table 2. Larvae from brooks metamorphosed at a larger size than larvae from water tanks or fountains (Mann-Whitney test, GSD's 42 to 46 pooled, water tanks *vs.* brooks, U=649.5, P<0.001; water tanks *vs.* fountains, U=2438.5, P=0.939; brooks *vs.* fountains, U=500, P<0.001). In addition, overwintering larvae were present only in water tanks and brooks, and not in the fountains. The results for the SVL comparisons among breeding site types of stages 26-41 are shown in Table 3. There were no significant differences
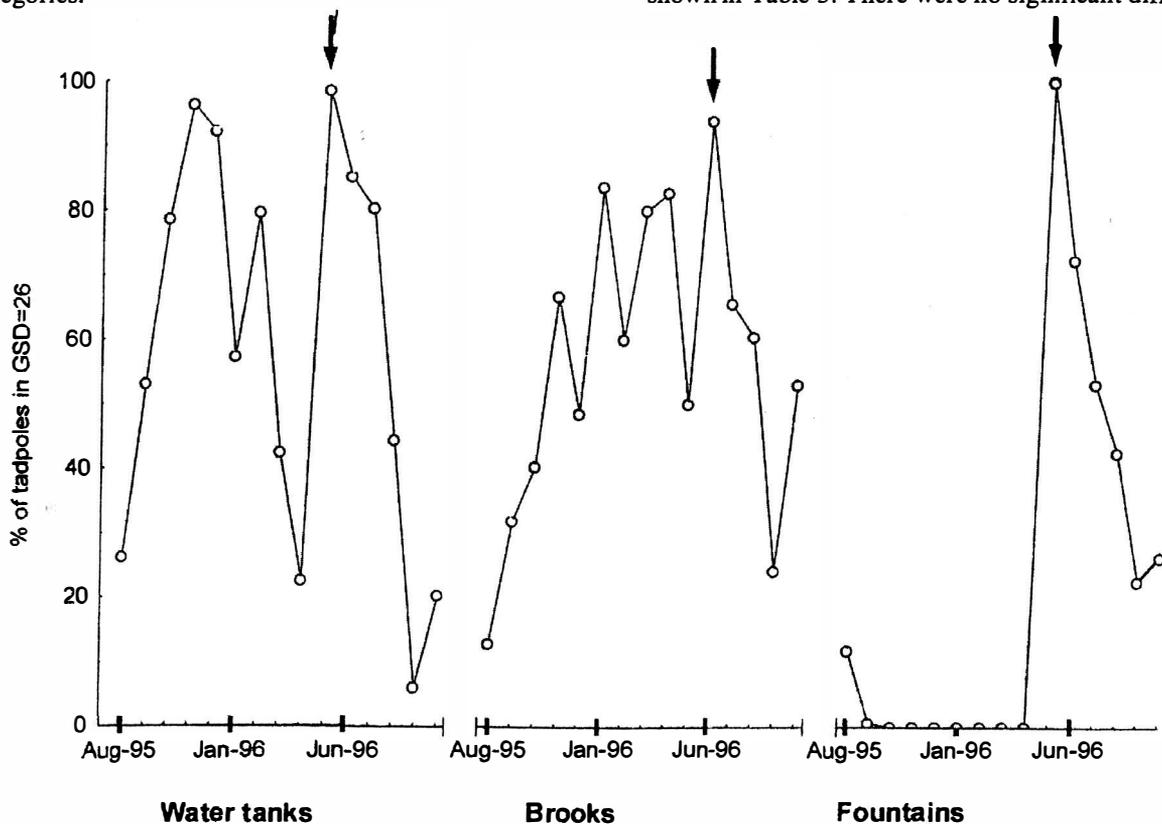

FIG. 1. Percentage of tadpoles at GSD 26 throughout the year in each of the types of breeding sites. 100% = total number of tadpoles sampled in each month. Arrows indicate the period of maximum reproductive activity. In water tanks and brooks, tadpoles in early stages of development are present throughout the year, whereas in fountains they are only present during the breeding season.

TABLE 2. Descriptive statistics for snout-vent length (SVL) measurements of larvae of *A. dickhilleni* at different stages of development (GSD, Gosner, 1960) in three different categories of reproduction. *N* = sample size.

GSD	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
FOUNTAINS																					
Mean	9.31	13.13	14.86	16.10	17.22	17.11	16.38	16.92	17.34	16.90	17.68	17.92	18.91	19.18	20.80	18.04	18.28	17.57	18.35	18.00	18.50
SD	2.46	2.25	2.22	2.45	2.35	2.39	1.72	1.74	1.88	1.83	1.49	1.57	1.88	1.99	1.30	2.11	1.93	2.48	2.37	2.00	0.70
Range	13.5	9.5	10	13	11	13	7	9	9	7	5	7	6	6	3	7	6	12	8	4	1
N	501	137	114	64	43	51	22	57	46	20	16	25	12	11	5	25	14	21	14	3	2
WATER TANKS																					
Mean	12.03	16.70	17.12	16.75	17.74	18.00	17.00	17.87	18.63	18.18	18.69	19.42	20.97	20.19	20.47	18.97	17.38	17.61	18.19	18.2	19.50
SD	4.80	3.83	3.64	2.46	2.76	2.23	1.89	1.65	2.38	1.80	2.03	2.19	1.74	1.32	1.80	1.87	2.19	1.90	1.50	1.10	0.70
Range	21	17	14	13	11	13	9	7	11	6	9	9	6	4	7	8	7	7	5	4	1
N	1646	190	112	70	67	59	20	27	22	29	41	33	36	21	19	24	22	27	21	17	2
BROOKS																					
Mean	13.33	14.24	17.00	17.10	19.06	18.78	20.00	20.41	22.21	21.97	20.78	20.95	20.50	19.75	19.60	18.00	19.71	20.20	22.14	21.00	
SD	4.40	3.50	4.26	3.14	2.56	3.07	3.09	3.08	2.86	3.92	1.53	1.70	1.88	1.42	0.84	2.82	1.49	1.43	3.99	1.58	
Range	18	17	18	9	10	13	8	12	10	18	6	6	6	4	3	4	5	6	18	5	
N	449	60	54	10	38	21	6	17	14	18	23	24	12	12	10	2	7	20	14	17	

in size at each GSD for stages 29, 31, 39, 40 and 41. Stages 26 and 37 were the most variable between sites. In most cases, post-hoc comparisons showed that the significant differences detected were related to a larger size at brooks with respect to water tanks and fountains (Table 3).

In order to evaluate the influence of overwintering larvae on the analyses – and considering that in fountains (where no such tadpoles were detected during the surveys) the maximum SVL recorded was 17 mm – we arbitrarily considered tadpoles ≥ 18 mm at GSD 26 to be overwintering larvae in water tanks and brooks, and excluded them from the analyses. Differences between types of breeding sites were again significant at that stage (ANOVA: $F=24.6$, $P<0.0001$), but in this case the differences were related to significantly smaller tadpoles at GSD 26 in water tanks with respect to brooks and fountains, according to post-hoc comparisons.

We also analysed the relationship between size and differentiation across breeding site types. Mean SVL of *A. dickhilleni* larvae are correlated with GSD in all categories (all correlations significant at $P<0.01$; Spearman's $r_s = 0.842$ in fountains vs. 0.636 and 0.531 for brooks and water tanks, respectively). In brooks and water tanks correlation coefficients were lower than in fountains, probably as a result of the coexistence of newly hatched larvae with overwintering larvae. When these values are excluded from the analyses, corrected correlation coefficients increase to 0.770 and 0.791 in water tanks and brooks, respectively, values that are very close to those obtained for fountains.

DISCUSSION

We found differences in larval phenology and growth history that were related to the type of breeding site used by males of *A. dickhilleni* for the release of their larvae. It has to be stressed, however, that we have not analysed

possible effects of other factors (for example, water quality or density-related crowding effects) that might also partly explain the observed differences in larval growth between breeding site categories. Experiments focused on these interactions should be of help in further testing our hypotheses.

Overwintering larvae were found in water tanks and brooks, but not in fountains. At these sites, low water level was associated with higher predation pressures and a risk of total freezing during cold months in the Sierra Tejeda (Antúnez *et al.*, 1982). Thus, the possibility of surviving winter in fountains may be low, due to the existence of a temporal limit marked by freezing. This might impose restrictions upon individual developmental trajectories, as will be discussed below. Brooks and water tanks do not freeze completely, facilitating tadpole overwintering. Water tanks are cleaned periodically (usually once or twice a year), but sometimes they are not completely emptied during the process, allowing the presence of larvae all along the year.

Variance in developmental traits was detected in the ranges of SVL associated with each GSD. Earlier stages (especially GSD 26, Fig. 2) show significant differences when compared to other stages: their associated ranges of SVL are much larger. We observed that most of the overwintering individuals are at early stages of development. Crespo (1982) noted that earlier stages of development in *A. cisternasii* and *A. obstetricans boscai* represent more than 50% of the time spent at the larval stage. He pointed out that at low temperatures, *Alytes* larvae can stay several months without appreciable morphological development: growth is faster than differentiation at lower temperatures, and vice versa at higher temperatures.

These differences in SVL ranges across GSD's are the consequence of the coexistence of newly-hatched in-

TABLE 3. Results of ANOVAs comparing size at each GSD among different breeding site types. Post-hoc comparisons were based on Tukey's HSD tests for unequal sample sizes (Spjotvol/Stoline test).

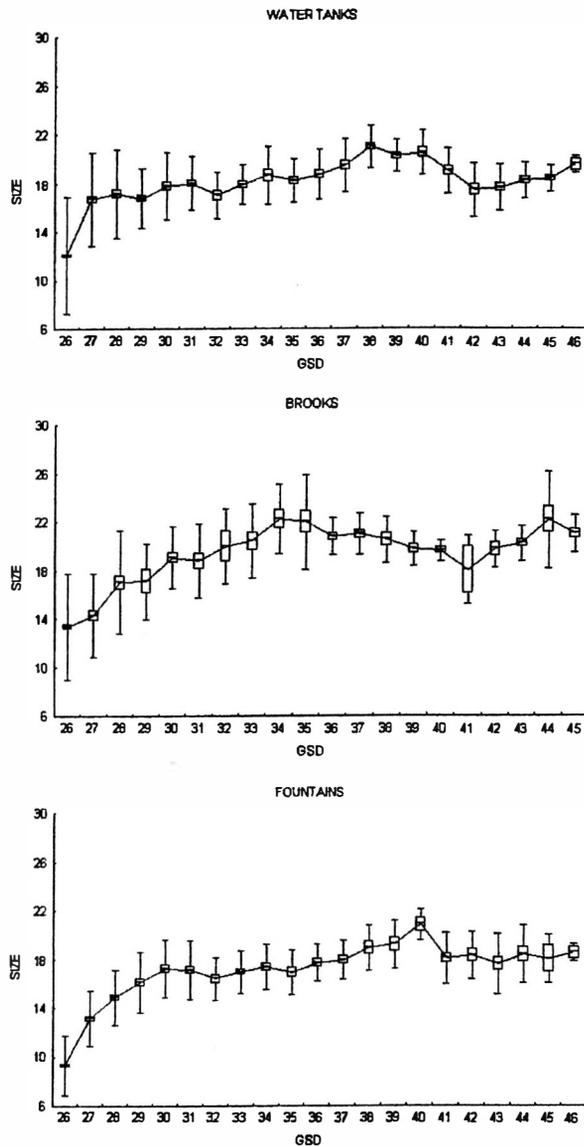| GSD	F	P	Post-hoc comparisons significant
26	110.41	<0.01	All significant
27	48.70	<0.001	Water tanks vs brooks and fountains
28	15.35	<0.001	Brooks vs. water tanks and fountains
29	1.45	N.S.	-
30	5.39	<0.01	Brooks vs. fountains
31	3.88	N.S.	-
32	7.79	<0.01	Brooks vs. water tanks and fountains
33	19.90	<0.001	Brooks vs. water tanks and fountains
34	26.05	<0.001	Brooks vs. water tanks and fountains
35	20.25	<0.001	Brooks vs. water tanks and fountains
36	15.86	<0.001	Brooks vs. water tanks and fountains
37	15.89	<0.001	All significant
38	5.86	<0.01	Water tanks vs. fountains
39	1.56	N.S.	-
40	1.43	N.S.	-
41	1.37	N.S.	-

FIG. 2. Mean (line), standard error (box) and standard deviation (vertical bars) of SVL (size) of tadpoles at each stage of development (GSD) in the different types of breeding site.

hypothesize that at these plastic stages there would be two possible developmental trajectories: a "fast" one characterized by a linear relationship between size (SVL) and GSD, and a "slow" trajectory where most of the growing takes place during an extended period of time in earlier stages of development, thus the relationship between size and GSD being non-linear. As development progresses past the earliest stages, the plasticity for growing without differentiation would be gradually minimized and the linear ("fast") trajectory would prevail until metamorphosis.

Selection of either strategy might be mediated by differential behavioural responses of developing larvae. In brooks and water tanks tadpoles can select their individual positions along a temperature gradient, since these are established between shallow waters directly exposed to sun radiation and deeper waters where temperatures remain colder. The relation of growth and developmental rates is temperature-dependent (Smith-Gill & Berven, 1979). Thus, it is possible that larvae developing in shallow waters of brook margins or in superficial waters of water tanks, where temperature is higher throughout the summer, could display higher activity and maintain higher metabolic rates than larvae developing in colder waters. In situations where resources are not expected to be limiting, models of resource allocation predict that in early stages these resources will be allocated primarily for development, and after the developmental trajectory is fixed, for growth (Leips & Travis, 1994). Thus, growth and development would be highly correlated, adjusting to the "fast" strategy. The constantly low water level of fountains would prevent the establishment of temperature gradients, and thus only the "fast " strategy would be possible. The differences in the percentage of tadpoles at GSD 26 throughout the year appears to be related to differences in the presence of overwintering individuals in the different breeding sites. At fountains, where no over-wintering individuals were found, the gradual decrease in the percentage of tadpoles at GSD 26 can be explained by the progressive differentiation of tadpoles into more advanced stages over time (Fig. 2). On the other hand, larvae spending most of their time at the bottom of deep pools in brooks, for example, where temperatures are expected to be relatively lower, would also show lower metabolic rates, with the result of delayed development, thus producing deviations from linearity in the growth-development relationship, following a "slow" strategy. Differentiation would even cease in larvae in early stages while they are still growing (though at a slow rate) during the overwintering period. Thus, the additional peaks of high relative numbers of tadpoles at GSD 26 in water tanks and brooks might represent the persistence of tadpoles at early stages of development through the winter period. The steepest declines in the percentage of larvae at GSD 26 in water tanks when compared with brooks, might be related to the periodic cleaning of water tanks, which usually takes place during the summer.

dividuals and overwintering larvae, both in early stages of development. Apparently, growth and differentiation is decoupled in earlier stages of development such that in some individuals differentiation ceases during winter, while growing still proceeds for several months. Differences in size range associated with each GSD are not expressed equally in all breeding types. In fountains, SVL ranges are of the same magnitude across GSD's, including earlier stages (26-27).

Developmental constraints might limit the possible responses of *A. dickhilleni* larvae to different stability and predictability conditions in the bodies of water in which they grow. In the hylid frogs *Hyla cinerea* and *H. gratiosa,* timing of metamorphosis appears not to be equally plastic at each stage of development, but after a particular threshold is reached, developmental rate appears to be unalterable (Leips & Travis, 1994). In *A. dickhilleni,* our data suggest that earlier stages of development are characterized by a higher plasticity. We

We have shown that variability exists in larval traits across different reproduction sites and across stages of development. Is this variability a by-product of other evolutionary processes or is it the result of selection on developmental traits? If so, what selective advantages would have this variability in a context of variable and often unpredictable environments? This is an important point because costs of maintaining variability in life-history traits must be overcome by its associated benefits (De Witt *et al.*, 1998). The coexistence of two different developmental strategies in populations of a single species may confer upon them higher probabilities of local persistence by allowing a better exploitation of reproductive habitats (Esteban *et al.* 1999). This would be especially important when the availability of reproduction sites is limited, as is the case in south-eastern Spain. *A. dickhilleni* populations are able to reproduce mainly in permanent waters (the usual condition in the genus *Alytes*), but also in temporary ones, such as fountains and some brooks. In these sites, developmental time is shorter than in permanent ponds, which imposes long-term costs in terms of fitness derived from lower sizes at metamorphosis (see, for example, Leips *et al.*, 2000). However, colonization of these sites allows *A. dickhilleni* to maximize the number of ponds in which it breeds, an important issue since sites available for reproduction are a limiting resource in most of its range. Breeding also in permanent waters allows them to prolong the larval period and metamorphose at larger sizes, as observed in brooks. Thus, variability in growth patterns could be related to a better exploitation of heterogeneous habitats by *A. dickhilleni* populations by increasing the range of potential reproductive sites.

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