

ADAPTIVE PLASTICITY IN AMPHIBIAN METAMORPHOSIS: RESPONSE OF *SCAPHIOPUS HAMMONDII* TADPOLES TO HABITAT DESICCATION

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Abstract. Amphibians exhibit extreme plasticity in the timing of metamorphosis, and several species have been shown to respond to water availability, accelerating metamorphosis when their ponds dry. In this study we analyzed the plasticity of the developmental response to water volume reduction in the western spadefoot toad, *Scaphiopus hammondi*. Also, we attempted to identify the environmental cue(s) that may signal a desiccating larval habitat. We spawned adults in the laboratory and raised tadpoles in aquaria in a controlled environmental chamber. Water levels of aquaria were gradually reduced by removing water at the rate of 0.5–1 L/d; water in control aquaria was similarly disturbed but not removed. Tadpoles subjected to water volume reduction showed significant acceleration of metamorphosis. The developmental acceleration depended on the rate of reduction of the water level; i.e., tadpoles exhibited a continuum of response. This developmental response did not result from thermal differences between treatments. Furthermore, the response was reversible in that refilling of the aquaria to the starting water level at various times following the onset of volume reduction resulted in restoration of body mass and a tendency to decelerate metamorphosis. Several lines of evidence suggest that the developmental response is due neither to the concentration of compounds in the water nor to chemical or physical interactions among conspecifics. Rather, the response appears to be related to the reduced swimming volume and perhaps the proximity to the water surface. When the water level is reduced, tadpoles reduce foraging, and food restriction of prometamorphic tadpoles maintained in a constant high water environment accelerated metamorphosis. Spadefoot toad tadpoles are a valuable model system for explaining both the proximate mechanisms (environmental cues and physiological responses) and the ultimate causes for adaptive phenotypic plasticity in amphibian metamorphosis.

Key words: amphibian; desert species; developmental plasticity; environmental variation; habitat desiccation; metamorphosis; desert amphibians; phenotypic plasticity; western spadefoot toad.

INTRODUCTION

The lower and upper limits to the length of the larval period and body size at metamorphosis are central amphibian life history traits (Wilbur and Collins 1973, Werner 1986, Smith 1987). Within these limits, larvae exhibit extreme plasticity in age and size at metamorphosis, which vary in relation to changes in both biotic and abiotic factors present in the larval habitat. For instance, biotic factors that influence amphibian development include, but are not limited to, food availability (D'Angelo et al. 1941, Travis 1984, Alford and Harris 1988, Berven and Chadra 1988, Crump 1989a), predation (Skelly and Werner 1990, Wilbur and Fauth 1990, McCollum and VanBuskirk 1996), population density (Richards 1958, Licht 1967, Brockelman 1969, Wilbur 1972, 1976, 1977a, b, Gromko et al. 1973, Wilbur and Collins 1973, John and Fenster 1975, Smith-Gill and Gill 1978, Smith-Gill and Berven 1979, Sem-

litsch and Caldwell 1982, Newman 1987, 1994, Berven and Chadra 1988, Scott 1990), and inter- and intraspecific chemical signaling (see Smith-Gill and Berven 1979, Werner 1986). Some abiotic factors that influence metamorphosis are water temperature (Smith-Gill and Berven 1979, Newman 1989, Hayes et al. 1993), photoperiod (Wright et al. 1990), and pond duration (reviewed by Newman 1992).

Many amphibian species breed in temporary ponds that are sporadically filled by rain and then dry at different rates. The rate of pond drying depends both on the geological characteristics of the pond (i.e., the substrate) and climatic factors (i.e., the frequency of rainfall and rate of evaporation; see Bragg 1965, Newman 1989). In such unpredictable habitats, desiccation is arguably the single most important environmental factor affecting larval survivorship, and species that breed in such ponds have evolved several traits that allow successful development. For instance, these species tend to have short periods of development compared with species that breed in more permanent environments (see Low 1976, Denver 1997a). In addition, lar-

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vae of several species have been shown to accelerate metamorphosis in response to habitat desiccation; i.e., they exhibit phenotypic plasticity in development time (*Bufo americanus*, Wilbur 1987; *Ambystoma talpoideum*, Semlitsch and Wilbur 1988; *Scaphiopus couchii*, Newman 1988, 1989, Morey and Janes 1994; *Hyla pseudopuma*, Crump 1989b). Plasticity in development may be adaptive in such ephemeral environments (see Stearns 1989, Newman 1992).

For species that breed in variable environments there are obvious trade-offs between mortality in the larval habitat and size at metamorphosis. Plasticity in development time results in variation in size at metamorphosis (see Wilbur and Collins 1973, Werner 1986, Newman 1992), and this size differential could result in a fitness differential in the terrestrial habitat. Tadpoles that metamorphose early and at a smaller size should benefit by escaping mortality in the desiccating larval habitat, but they may incur costs such as reduced juvenile survivorship, physiological performance, and size at first reproduction (Martof 1956, Turner 1962, Clarke 1974, Berven 1982, Pough and Kamel 1984, Taigen and Pough 1985, Werner 1986, Smith 1987, Semlitsch et al. 1988, John-Alder and Morin 1990, Goater et al. 1993, Newman and Dunham 1994). Models of amphibian metamorphosis that attempt to evaluate optimal size at transformation (Wilbur and Collins 1973, Werner 1986) predict that when conditions are favorable for larval growth (i.e., in long-duration ponds), tadpoles should delay metamorphosis and transform at a larger size. The ability to respond rapidly to changes in water availability by accelerating development or growth rate (i.e., when the pond is drying) or slowing them down (i.e., when the drying pond is refilling) may be selected for in unpredictable habitats.

Because they exhibit a rapid development time and have strong responses to environmental variation, tadpoles of the genus *Scaphiopus* (spadefoot toads) provide an excellent model system to study all aspects of adaptive developmental plasticity (see Newman 1992). In this study we examined, in a controlled laboratory setting, phenotypic plasticity in development time of the western spadefoot toad *Scaphiopus hammondi* as influenced by changes in the water volume of the larval habitat. Our goal was to manipulate single aspects of the environment that accompany habitat desiccation in order to determine their relative contributions to accelerated metamorphosis. We determined the degree of plasticity in the developmental response (i.e., whether the rate of development varies as a continuous function of the rate of decline in the water level) and whether the response is reversible once initiated. While the developmental response of spadefoot toad tadpoles to habitat desiccation has been examined in field studies and experiments with artificial ponds (*S. couchii*, Newman 1988, 1989) little attention has been paid to the identification of the proximal environmental cues that signal a drying pond (Newman 1994). We tested several

potential environmental cues that tadpoles might use to respond to deleterious changes in their larval habitat (e.g., temperature, tadpole density-dependent effects, resource level) and we discuss other environmental factors that may be involved in the developmental response to habitat desiccation.

METHODS

Experimental design

Male and female western spadefoot toads were collected in the late winter in various years along streams and roadways near Livermore, California, and maintained in a breeding colony. Adults were housed in polypropylene cages of varying size depending on animal density and size class. Cages were filled with potting soil, which was moistened periodically. Juveniles and breeding stock were maintained on a 12L:12D photoperiod; the temperature ranged from 15° to 22°C, and animals were fed crickets dusted with DIAGLO vitamins (Syntex). One week prior to breeding, the temperature was raised to 23°C and the soil was moistened daily to simulate rainfall and stimulate emergence.

Breeding pairs were set up in transparent polystyrene cages containing 2 L of 10% Holtfreter's solution (see Rugh 1962) and sphagnum moss to act as a substrate for spawning. Gravid females were identified by looking for yolked eggs through transparent areas of the abdominal skin. Breeding males were chosen based on nuptial pad development and color (larger and darker when in breeding condition) and darkness of throat color. To induce amplexus and oviposition, both males and females (weighing ~15–20 g) were given a single injection of 1 µg gonadotropin-releasing hormone agonist (GnRH_a dissolved in 0.6% NaCl; Sigma) in the dorsal lymph sac. Females usually oviposit within 24 h using this protocol. Fertilization varies from 0 to 90% depending on the quality of the male and the maturity of the eggs.

After hatching, *S. hammondi* tadpoles were maintained in polystyrene tanks (24 cm wide × 45 cm long × 20 cm deep [rat cages]); for experiment 6, smaller tanks were used (17 cm wide × 28 cm long × 12 cm deep [mouse cages]). The tanks were filled with distilled H₂O containing 10% Holtfreter's salt solution, and were provided with airstones; water was changed every 2–3 d. Both stock and experimental tadpoles were fed ad libitum with tadpole chow (a mixture of rabbit pellets, agar, and Knox gelatin; see Rugh 1962) and boiled lettuce and spinach.

All experiments were done in a ConViron walk-in environmental chamber, which controls temperature ±1°C. Water temperature in the first experiment was 23°C. Subsequent experiments were done at slightly lower temperatures (18°–20°C) in order to slow development rate so as to provide greater resolution in developmental measurements. The photoperiod was kept constant at 12L:12D in all experiments. The de-

velopmental stage of spadefoot toad tadpoles was determined using the Gosner (1960) staging system. Terms from Etkin (1968) are also used to describe the general stages of amphibian development: premetamorphosis (early larval stage that precedes hindlimb development), prometamorphosis (period of rapid hindlimb development), and climax (frontlimb emergence [FLE] and other dramatic morphological change).

Each experiment was done with single sibships to increase statistical power. The first experiment was initiated when tadpoles reached stage 26. All other experiments were initiated when tadpoles were in stages 28–32 (early prometamorphosis). At the start of each experiment tadpoles were selected haphazardly and distributed to experimental tanks. With the exception of experiment 1, tadpole densities were 8–10 animals per tank. Each treatment was replicated three to four times. In each experiment tanks were arranged in randomized arrays to homogenize the thermal background with respect to treatment. In addition, a rotational system for replicate tanks within a treatment was arranged in which tanks were randomly redistributed to different locations within the environmental chamber each time the water was changed. To control for simple disturbance effects, water was removed from and then replaced in tanks whose water level was maintained constant.

Experiment 1.—This experiment tested whether tadpoles of *S. hammondi* can accelerate metamorphosis in response to a decline in the water level in the aquarium in which they are reared (hereafter referred to as “volume reduction”). The experiment examined a single factor (water level) with two levels (constant or decreasing water volume) and had four replicates. One gravid female was spawned within 24 h of capture in the wild. The experiment was initiated when tadpoles had reached stage 26 (1 wk after hatching). The stocking density was 20 tadpoles per tank. Tadpoles in the constant high water treatment group were maintained in a constant volume of 10 L. Water in the tanks of tadpoles in the decreasing-water treatment (starting volume 10 L) was removed daily in 0.5–1 L increments until the water level reached 0.5 L; the rate of decline in the water level was arbitrarily chosen. When tadpoles reached stage 42 (FLE) they were scored and removed from the tanks. Water temperatures (taken at both the bottom of the tank and at the water surface) were recorded daily in the morning.

Experiment 2.—This experiment tested the degree of plasticity in the response to volume reduction; e.g., can tadpoles coordinate their rate of metamorphosis with the rate of water decline? Initial stocking density was 10 animals per tank, and the experiment had a single factor (water level) with five levels (water volume reduction regimes; see Fig. 3, bottom left panel) and three replicates. The volume reduction regimes were: constant high water (10 L; regime 1), “slow” decline to

0.5 L (regime 2), “intermediate” decline to 0.5 L (regime 3), “rapid” decline to 0.5 L (regime 4), and “intermediate” decline to 0.5 L (regime 5; tadpoles were transferred from the stock tank where they were raised from hatching [in 10 L of water] to 0.5 L at the start of the experiment).

Experiment 3.—This experiment was designed to simulate the ecological release of tadpoles from the stress of habitat loss (water volume reduction). We tested whether the acceleration in development rate of tadpoles subjected to a decreasing water level could be reversed by returning the water level to the starting, high water point (10 L). The volume reduction regime used in this experiment was the same as the intermediate regime (regime 3) used in experiment 2. Stocking density was eight tadpoles per tank; the experiment examined a single factor (water level) with seven levels and had three replicates. The water level of tadpoles subjected to the decreasing water volume regime was restored to 10 L at 2-d intervals beginning 5 d after the initiation of the water decline (see Fig. 4). Mean body mass was monitored at 2-d intervals throughout the experiment.

Experiment 4.—This experiment was designed to evaluate whether a change in swimming volume is sufficient to cause accelerated metamorphosis. The experiment differs from experiments 2 and 3 in that the swimming volume was decreased while the dilutional volume was maintained constant (i.e., experiments 2 and 3 could have resulted in a change in the concentration of some compound in the water). Screens (fashioned out of 6-mm polypropylene mesh fastened to a Plexiglas frame) were gradually moved vertically from the bottom to the top of the tank. These tanks had 10 L water throughout the experiment; the rate of change in swimming volume was calculated to be comparable to that of regime 3 of experiment 2 (see Fig. 3). For either the constant water volume treatment or decreasing water volume treatment (regime 3 of experiment 2), the screens remained on the bottom of the tank throughout the experiment. For this experiment, rectangular polystyrene cages (32 cm wide \times 36 cm long \times 17 cm deep) with straight sides were used; the tanks were filled with 10 L water at the start of the experiment, as with the other tanks. Tadpoles were stocked at 10 animals per tank. This experiment had a single factor (swimming volume), three levels, and three replicates.

Experiment 5.—In this experiment swimming volume was reduced (with the dilutional volume maintained constant as in experiment 4) while the water depth was maintained constant. This experiment repeated experiment 4 and included an additional treatment in which a screen was placed in the side of the tank and was moved horizontally from one side to the other. The rate of change in swimming volume created by this horizontally manipulated screen was calculated

to be identical to that of the decreasing water and the vertically manipulated screen treatments.

Experiment 6.—This experiment tested whether tadpoles raised individually exhibit a developmental response to volume reduction; i.e., whether the response depends on interactions among conspecifics. Tadpoles were raised individually (8 replicates) and exposed to either a constant high water or a decreasing water environment. The tanks used were smaller than previous experiments (17 cm wide \times 28 cm long \times 12 cm deep [mouse cages]); however, while the total water volume differed with the smaller tanks (3 L vs. 10 L in the large tanks), the rate of change in the water level was identical to that used in previous experiments with the large tanks (i.e., regime 3, experiment 2). The experiment had one factor (water level) with two levels.

Experiment 7.—This experiment was designed to test whether the cessation of feeding due to restricted locomotion contributes to the developmental acceleration induced by volume reduction. In previous experiments we observed that when the water level was reduced (or the swimming volume was reduced with the vertically manipulated screens) tadpoles reduced and then ceased foraging behavior. Because resource limitation is known to influence in complex ways age and size at metamorphosis in other species (see *Discussion*), we reasoned that the cessation of feeding caused by restricted locomotion could influence metamorphosis (rather than the depletion of resources, since tadpoles cease foraging even when the resource level is kept constant). We raised tadpoles at constant density (eight animals per tank; three replicates) and constant water volume (10 L), and removed food at three developmental stages: Gosner stages 30–32, 34–35, and 36–37; controls received food throughout the experiment. The experiment had a single variable (food availability) with four levels; however, the early stage (stages 30–32) food-restricted group was excluded from statistical analyses because these tadpoles failed to grow and did not develop beyond stage 34.

Measurements and statistical analyses

In most experiments the following variables were measured: mean age at metamorphosis, and mean wet mass (body mass, BM) and snout–vent length (SVL) at metamorphic climax (Gosner stage 42). For body size measurements and staging, tadpoles were anesthetized by immersion in 0.01% benzocaine (Sigma) and blotted on paper towels. Body mass was measured using a digital balance (accurate to 0.1 g), and SVL was measured with a digital micrometer (accurate to 0.1 mm). Mean age at metamorphosis was measured from the time of hatching to the time of FLE (Gosner stage 42).

Statistical analyses were performed using the computer software packages SPSS and Statistica. Aquaria containing 8–20 tadpoles were replicated three to four times within a treatment, and statistical analyses were

conducted on population (tank) means. All data were \log_{10} transformed before analysis to reduce skewness. The mean age at metamorphosis in experiment 1 was analyzed by Student's *t* test. All other experiments analyzed single variables with multiple levels, and MANOVAs of the three variables—age at metamorphosis, BM, and SVL—were done for each experiment. If the MANOVAs indicated significant overall differences then separate univariate contrasts were analyzed (summary statistics for the one-way ANOVAs are presented). In all analyses planned pairwise comparisons were made with Scheffé's multiple-contrast test ($P < 0.05$; presented in graphs as lowercase letters; Day and Quinn 1989). To determine if body size and age at metamorphosis were positively correlated, regression analyses were performed in several experiments using either pooled data from the different treatments or measurements within a treatment (each data point represents a measurement from an individual tadpole).

RESULTS

Tadpoles of Scaphiopus hammondi accelerate metamorphosis in response to water volume reduction (experiment 1).—Exposure of *S. hammondi* tadpoles to a decreasing water level resulted in a significant reduction in age at metamorphosis ($t = 8.12$, $P < 0.001$ [Student's *t* test]; Fig. 1). Tadpoles reached metamorphic climax (Gosner stage 42; FLE) as early as 22 d after hatching in the decreasing water level group.

We monitored bottom and surface water temperatures throughout the experiment to determine if the accelerated development could be explained by an elevation in water temperature in the decreasing water treatment (see Newman 1992). While there was some variation of $\sim 0.8^\circ\text{C}$ over the course of the experiment, the water temperatures did not differ significantly, either between the bottom and the surface within a treatment or between treatments (see Fig. 2). This experiment was done with a water temperature range of $22^\circ\text{--}23^\circ\text{C}$. In order to gain better resolution of the developmental acceleration induced by volume reduction, subsequent experiments were done at a lower temperature ($18^\circ\text{--}20^\circ\text{C}$) to slow the “baseline” rate of development.

Tadpoles exhibit a continuum of response to water volume reduction (experiment 2).—In experiment 1 the rate of water decline was arbitrarily chosen. Experiment 2 was designed to determine whether the rate of development is influenced by the rate at which the water level declines using five different volume reduction regimes. Age at metamorphosis was significantly reduced by volume reduction (volume reduction regimes 1–5; Table 1); tadpoles exhibited a continuum of response to volume reduction (Fig. 3). Similarly, body size at metamorphosis (BM and SVL) differed significantly among the groups (Table 1). Accelerated development resulted in smaller size at metamorphosis, and body size at metamorphosis was positively cor-

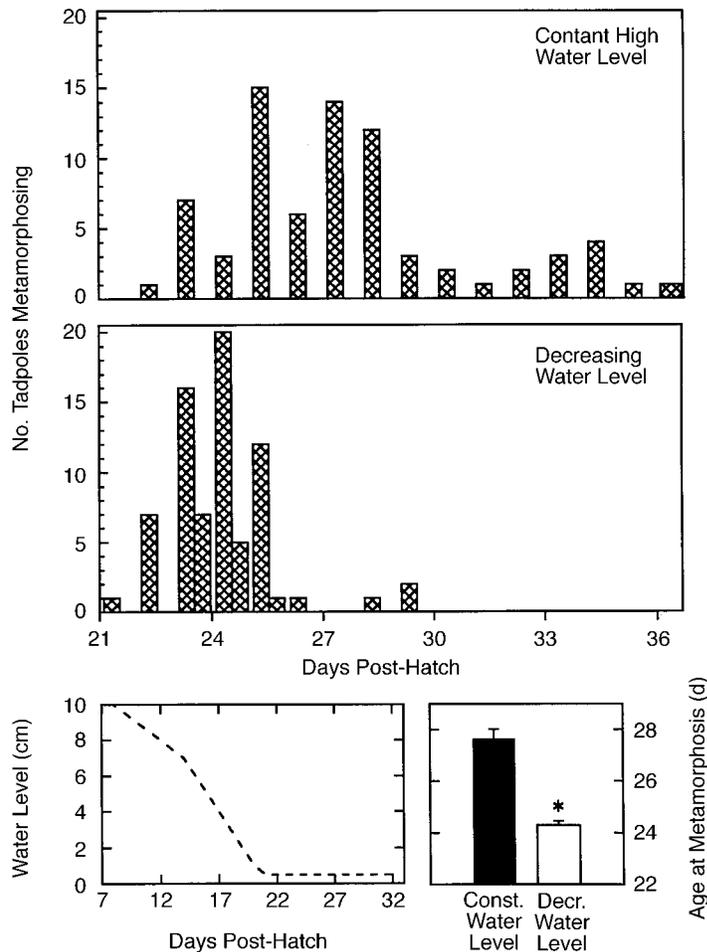


FIG. 1. Acceleration of metamorphosis of *S. hammondi* tadpoles by water volume reduction. The water level was maintained at either a constant high level (10 L) or decreasing (from 10 to 0.5 L; see bottom left panel). The top two panels show the frequency distributions of the two treatments for the numbers of animals metamorphosing by time since hatching. The bottom right panel shows the mean age at metamorphosis for the two treatments (mean \pm 1 SE; $n = 4$ tanks/treatment; the asterisk indicates significant difference at $P < 0.001$ [t test]).

related with age at metamorphosis (BM, $R^2 = 0.63$, $P < 0.001$; SVL, $R^2 = 0.43$, $P = 0.009$).

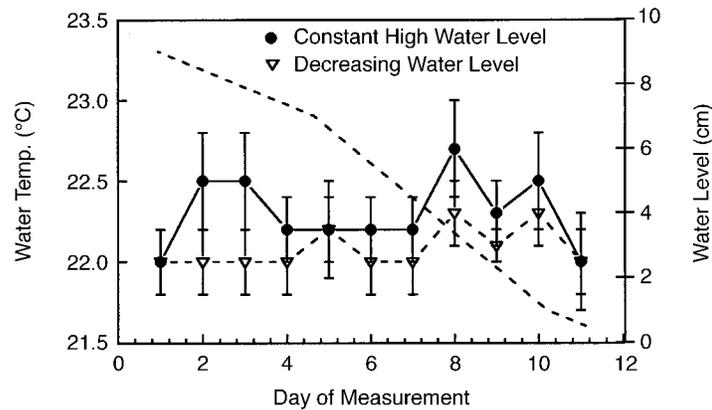
Tadpoles were capable of responding to the immediate water decline treatment (regime 5) because they were in early to mid-prometamorphosis (Gosner stages 30–32). In an earlier experiment we tested whether tadpoles in early prometamorphosis (Gosner stages 26–28) were capable of accelerating metamorphosis in response to an immediate drop in the water level. These early-stage tadpoles did not grow or develop (data not shown). Thus, there appears to be a minimum degree of differentiation (between Gosner stages 30 and 32) or a minimum body size required for tadpoles to become competent to respond to volume reduction by accelerating development.

Ecological release from desiccation stress restores body mass (experiment 3).—This experiment tested whether tadpoles could respond adaptively (by restoring body mass and decelerating metamorphosis) to the simulated ecological release from the stress of habitat loss that may occur in ephemeral ponds that are periodically refilled by rainfall. Analysis of univariate contrasts for BM revealed significant differences between

treatments from day 36 onward (see Table 2). When shifted back to the high water (10 L) environment, tadpoles previously subjected to water volume reduction restored body mass to the level of the constant high water animals within 2–3 d of the shift (Fig. 4). At metamorphic climax, tadpoles from each of the “shift” treatments were all as large as the constant high water controls; tadpoles subjected to the continued decline in water level metamorphosed at significantly smaller body size (Fig. 5; Table 1). Tadpoles shifted from the decreasing to the high water environment exhibited a trend toward deceleration of metamorphosis; however, this effect was not statistically significant (see Fig. 5).

Metamorphosis is accelerated by reducing the swimming volume without changing the dilutional volume (experiment 4).—Tadpoles subjected to the vertically manipulated screen treatment accelerated development. Indeed, the acceleration was even greater than that observed with tadpoles subjected to the decreasing water level (Fig. 6; Table 1), although the rate of decline in the swimming volume was identical in both treatments. Body size at metamorphosis was significantly reduced

FIG. 2. Water temperatures over an 11-d period in experiment 1. Temperature was recorded at the bottom of the tank daily in the morning. Points on the graph represent the mean, and vertical lines represent ± 1 SE ($n =$ four tanks/treatment). The dashed line without symbols represents the rate of water decline in the decreasing treatment.



in tadpoles subjected to the decreasing water and the vertically manipulated screen treatments (Table 1). Results from this experiment suggest that tadpoles do not use the concentration of compounds in the drying pond as cues for accelerated metamorphosis, but may sense a reduction in swimming volume or an increased proximity to the water surface.

Reduced locomotion may be involved in the developmental response to water volume reduction (experiment 5).—Based on the results of experiment 4, we hypothesized that tadpoles may respond to either a reduction in swimming volume (which may well result in reduced locomotion and increased physical interactions among conspecifics) or increased proximity to the surface (which might be assessed visually). We repeated experiment 4 and included a fourth treatment using a side screen (horizontally manipulated), which allowed us to test whether a reduction in swimming area per se was a sufficient cue for accelerated metamorphosis. As in experiment 4, tadpoles subjected to the vertically manipulated screen treatment accelerated metamorphosis, and the acceleration rate was similar

to that of tadpoles whose water level was reduced by removing water from the aquaria (Fig. 7; Table 1). Tadpoles subjected to the horizontally manipulated screen treatment also accelerated development relative to the constant high water controls; however, this acceleration of development was significantly less than that observed with the other two treatments. As in other experiments, BM at metamorphosis was reduced in tadpoles that had accelerated development (Fig. 7); body mass at metamorphosis and age at metamorphosis were positively correlated (pooled data from different treatments: $R^2 = 0.56$, $P < 0.001$). We attempted to test whether proximity to the surface is important to the response to volume reduction by placing screens in the top of the aquaria and gradually moving them to the bottom (thus reducing swimming volume but increasing distance from the water surface). However, we learned that tadpoles of this species require access to the water surface to gulp air (i.e., they probably begin to develop lungs early in prometamorphosis) and so the experiment could not be done.

The acceleration of metamorphosis by water volume reduction does not depend upon chemical or physical interactions among conspecifics (experiment 6).—To determine whether interactions among tadpoles (either physical or chemical) are necessary to the developmental response to volume reduction, we raised tadpoles individually and manipulated the water level of their aquaria. Tadpoles raised individually metamorphosed earlier and at a smaller body size when the water level of their aquaria was reduced (Table 3), and the magnitude of this response ($\sim 20\%$) was comparable to that observed in other experiments (e.g., see Fig. 3, regime 3).

The developmental acceleration induced by volume reduction may be related to the cessation of feeding (experiment 7).—We observed in our experiments that the intensity of foraging behavior declined when the water volume (or the swimming volume in the screen experiments) was reduced (we did not specifically define the volume at which the behavior ceased). We hy-

TABLE 1. Summary statistics for ANOVAs for age and body size (BM = body mass; SVL = snout-vent length) at metamorphosis for experiments 2–5 and 7 (see *Methods* for descriptions).

Experiment	Source	MS	F	df	P
2	Age	0.0337	12.82	4, 14	0.0006
	BM	0.2493	9.66	4, 14	0.0018
	SVL	0.0143	5.39	4, 14	0.014
3	Age	0.0266	7.0	6, 20	0.0013
	BM	0.5267	20.94	6, 20	0.0001
	SVL	0.0419	12.8	6, 20	0.0001
4	Age	0.1196	326.59	2, 8	<0.0001
	BM	0.5418	21.43	2, 8	0.0019
	SVL	0.0294	29.44	2, 8	0.0008
5	Age	0.0784	64.79	3, 11	<0.0001
	BM	0.3427	61.2	3, 11	<0.0001
7	Age	0.0157	14.94	2, 8	0.0047
	BM	0.3748	17.61	2, 8	0.0031
	SVL	0.0347	20.06	2, 8	0.0022

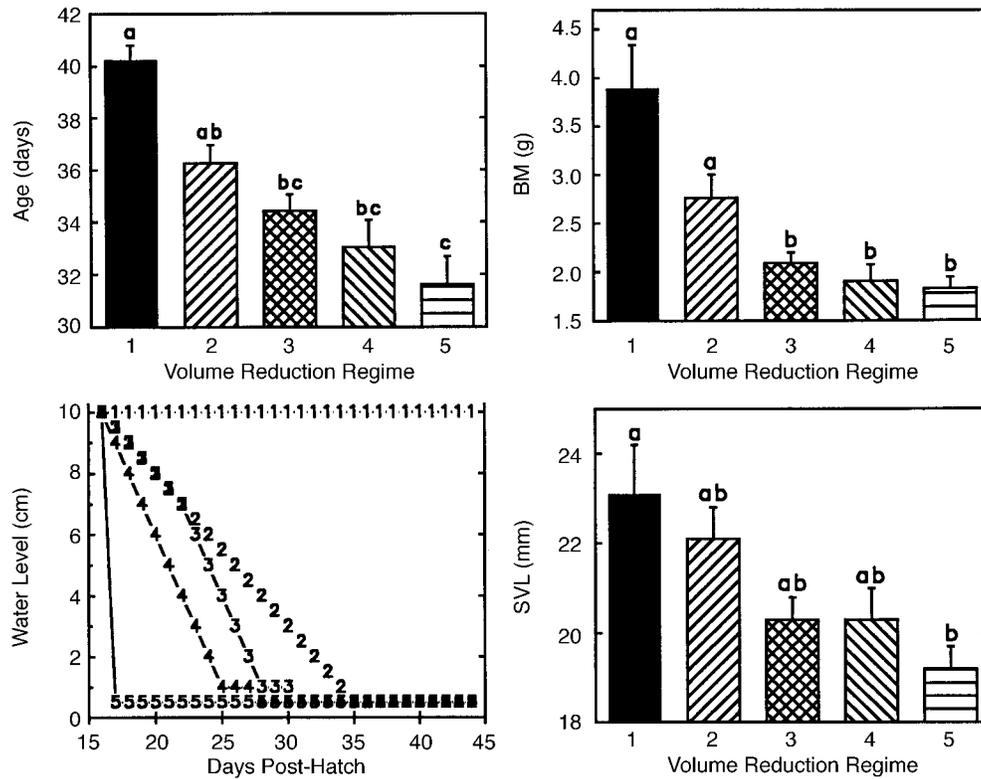


FIG. 3. Spadefoot toad tadpoles exhibit a continuum of developmental response to a decreasing water level. Prometamorphic tadpoles (10 animals/tank) were exposed to various volume reduction regimes as outlined in the bottom left panel (regimes 1–5). The bar graphs show mean age, BM, and SVL at metamorphosis ($n = 3$ tanks/treatment); error bars represent $+1$ SE.

pothesized that the developmental acceleration induced by volume reduction might be related to both the reduced locomotion and the cessation of feeding (caused by the restriction in movement). Food restriction of early prometamorphic tadpoles (stage 30–32) produced inhibition of growth and developmental stasis (tadpoles developed to stage 34 but then underwent stasis; data not shown). Food restriction of tadpoles in mid- to late prometamorphosis (stages 34–35 or 36–37) significantly accelerated metamorphosis relative to fed controls (these tadpoles were all maintained in a constant high water environment; Fig. 8, Table 1). However,

food-restricted tadpoles did not accelerate development to the same extent as tadpoles whose water level was reduced but whose food level was maintained (for comparison, data from a parallel experiment done under identical environmental conditions at the same time using tadpoles from the same spawn are presented in Fig. 8); i.e., the acceleration of development of food-restricted tadpoles was ~55% of that of fed controls subjected to volume reduction. Differences in body size at metamorphosis closely paralleled the length of the larval period (Fig. 8); i.e., both BM and SVL were positively correlated with age at metamorphosis (pooled data from different treatments: BM, $R^2 = 0.47$, $P = 0.042$; SVL, $R^2 = 0.51$, $P = 0.031$).

TABLE 2. Summary statistics of ANOVAs for body mass in experiment 3.

Days post-hatch	MS	F	df	P
27	0.0181	0.65	6, 20	0.69
31	0.0006	0.03	6, 20	0.99
34	0.0372	1.32	6, 20	0.312
36	0.0989	4.29	6, 20	0.0116
38	0.2951	5.03	6, 20	0.006
40	0.2831	6.25	6, 20	0.0023
42	0.3894	14.48	6, 20	<0.0001
44	0.3042	13.31	6, 20	<0.0001

DISCUSSION

The model system we have developed using tadpoles of *S. hammondi* allows us to address how and to what extent water volume reduction influences plasticity in development rate. By conducting experiments in a controlled setting, we have systematically evaluated, in the absence of other correlates of pond drying, several environmental factors that could function to alter development rate in response to habitat desiccation. Because this is a laboratory experiment, statements regarding

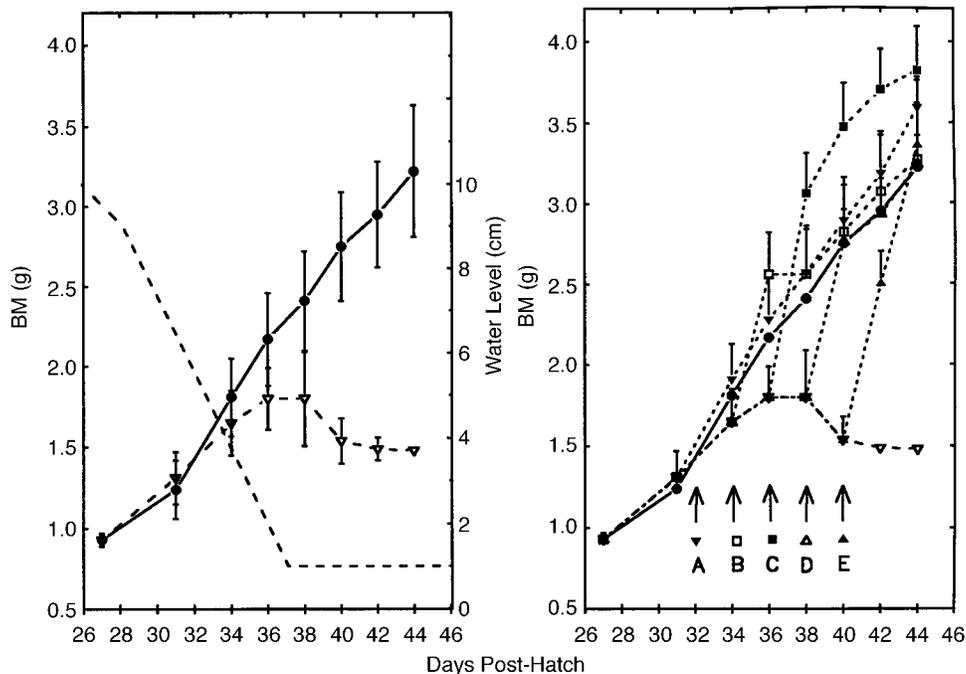


FIG. 4. Effects of ecological release from the stress of habitat loss on body mass in tadpoles of *S. hammondi*. Tadpoles were shifted from a decreasing water environment to a constant high water environment at various times. Prometamorphic tadpoles (eight animals per tank; three tanks per treatment) were maintained in either a constant high water or a decreasing water environment (equivalent to regime 3, experiment 2; see Fig. 3). The left panel shows the change in mean body mass (BM) (± 1 SE) of tadpoles in the two control groups (constant high water level [filled circles] and decreasing water level [open inverted triangles]). The right panel shows the change in BM of tadpoles subjected to a decreasing water level and then shifted back to the high water level at 2-d intervals beginning 5 d after the onset of volume reduction (days post-hatch treatment was shifted: A = 32 d, B = 34 d, C = 36 d, D = 38 d, E = 40 d). Standard errors of the controls are excluded for clarity. Arrows under the graph indicate the time points at which each of the treatments A–E were shifted.

the precise connections between the effects of changes in the single variables we tested and the complex changes that occur in the drying pond are speculative. Nevertheless, our results clearly demonstrate that tadpoles can respond to a reduction in water level by accelerating metamorphosis, and that this response is not all-or-none, but instead varies as a continuous function of the rate of change in the water level. Furthermore, our experiments allow us to rule out, or at least reduce the likelihood, that several environmental factors such as chemical cues, physical interactions among conspecifics, and increased water temperature function as the primary cues for accelerated development (although each of these factors could contribute to alterations in development rate in the wild).

Phenotypic plasticity in age and size at metamorphosis is found in all amphibian families (see Wilbur and Collins 1973, Werner 1986, Newman 1992). Several species from diverse families have been shown to be capable of accelerating metamorphosis as their pond dries (*Bufo americanus*, Wilbur 1987; *Ambystoma talpoideum*, Semlitsch and Wilbur 1988; *Scaphiopus couchii*, Newman 1988, 1989, Morey and Janes 1994; *Hyla pseudopuma*, Crump 1989b). In general, these species (and *S. hammondi*; this study) breed in tem-

porary ponds. In our experiments, western spadefoot toad tadpoles exhibited plasticity in development rate in response to a changing water level, and this response varied in direct relation to the magnitude of the environmental signal. Both the rate of development and body size at metamorphosis were positively correlated with the rate of decline in the water level; i.e., tadpoles exhibited a continuum of response to volume reduction in both timing of metamorphosis and size at metamorphosis. Furthermore, when released from desiccation stress, tadpoles were capable of capitalizing on conditions that were favorable for growth. For instance, tadpoles exposed to volume reduction exhibited a rapid and dramatic restoration of body mass (as much as 220% of starting BM) and tended to decelerate metamorphosis when their aquaria were refilled to the starting level. The restoration of body mass is likely to be due to a combination of restoration of growth and the rapid refilling of the gut upon the resumption of feeding. Because the gut comprises a very large proportion of a tadpole's total mass it is conceivable that the majority of change in body mass is a result of this refilling of the gut. However, our finding that growth can be significantly restored in a relatively short time is supported by the observation that "shifted" animals (see

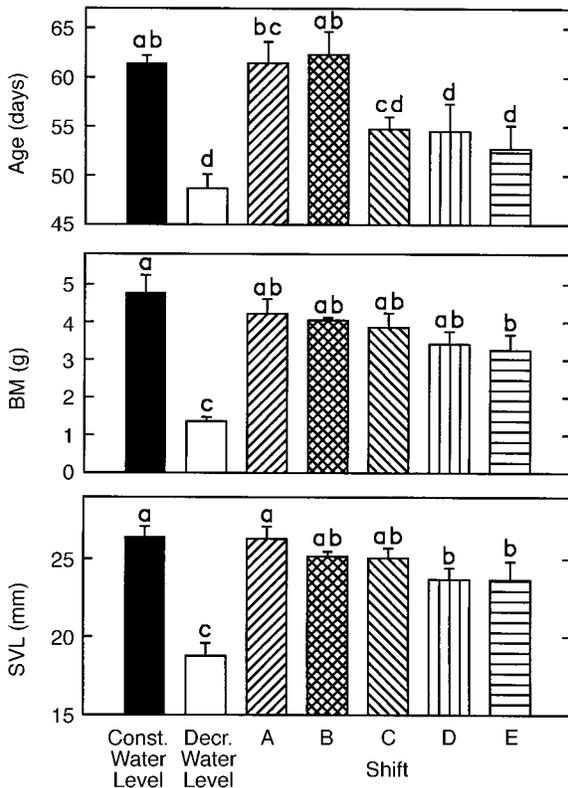


FIG. 5. Effects of ecological release from the stress of habitat loss on age and body size at metamorphosis in *S. hammondii*. Mean age, body mass (BM), and snout-vent length (SVL) at metamorphosis were recorded for tadpoles in experiment 3 (the same animals measured in Fig. 4). See Fig. 4 for shift periods A-E. Bars represent means +1 SE (eight tadpoles per tank; three tanks per treatment). Different lowercase letters indicated statistically significant differences among the means ($P < 0.05$; Scheffé's multiple-contrast test).

Ecological release from desiccation stress . . . , above) were significantly larger at metamorphosis (in both BM and SVL) than animals exposed to the decreasing water level (see Fig. 5).

Similar to the findings of others with *Scaphiopus* spp. (*S. couchii*, Newman 1989, Morey and Janes 1994; *S. multiplicatus*, Pfennig et al. 1991), our results show a positive correlation between age and body size at metamorphosis in *S. hammondii*. The size differences may be related to variation in muscle and bone mass as well as body fat. *Scaphiopus couchii* tadpoles, which develop rapidly in response to habitat desiccation, metamorphose smaller and with leaner somatic condition (Morey and Janes 1994, Newman and Dunham 1994). These size differentials at metamorphosis could result in fitness differentials in the terrestrial habitat. For instance, large size at transformation can result in greater juvenile survivorship, enhanced physiological performance, and a larger size or younger age at reproductive maturity (Martof 1956, Turner 1962, Clarke 1974, Berven 1982, Pough and Kamel 1984, Taigen and Pough

1985, Werner 1986, Smith 1987, Semlitsch et al. 1988, John-Alder and Morin 1990, Goater et al. 1993, Newman and Dunham 1994).

Proximal environmental cues that signal a desiccating larval habitat

While phenotypic plasticity for development time in response to habitat desiccation has been described in several amphibian species, the proximal environmental cues that tadpoles use to monitor the quality of the larval habitat have not been identified. The developmental response to habitat desiccation in *S. hammondii* tadpoles could result either from abiotic factors specific to the drying pond (e.g., increased pond temperature, increased salt concentration, decreased water depth, etc.), or from biotic factors such as changes in tadpole density (e.g., resource limitation, accumulation of waste products), species interactions (Werner 1992), or the presence of predators (Skelly and Werner 1990,

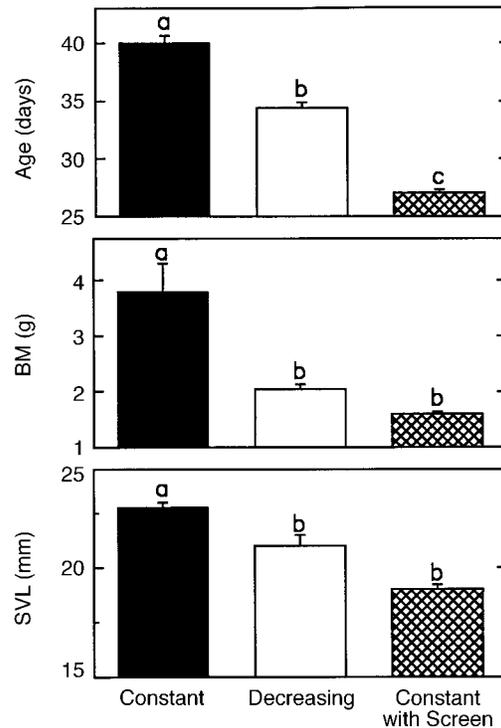


FIG. 6. Developmental response in *S. hammondii* to reduced swimming volume without a change in the dilutional volume. Prometamorphic tadpoles (10 tadpoles per tank; three tanks per treatment) were subjected to constant high water volume or to a volume reduction regime (regime 3, experiment 2; see Fig. 3) or had screens placed in their tanks that were moved vertically to the water surface in increments. The screen treatments were designed to mimic the change in swimming volume of the decreasing water regime while maintaining the dilutional volume at the level of the constant high water regime. Graphs show mean age, body mass (BM), and snout-vent length (SVL) at metamorphosis. Bars show means +1 SE, and different lowercase letters indicate statistically significant differences among the means ($P < 0.05$; Scheffé's multiple-contrast test).

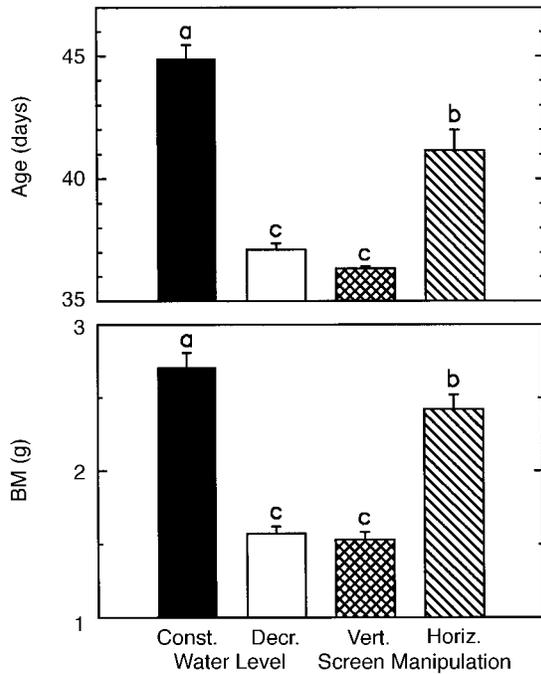


FIG. 7. Developmental response to reduced swimming volume (with constant dilutional volume) with and without increasing proximity to the water surface. This experiment repeated experiment 5 (shown in Fig. 6) except that an additional treatment was included in which a screen was placed in the side of the tank and moved horizontally. Graphs show the mean age (top) and mean body mass (bottom) at metamorphosis. Bars are means +1 SE (eight tadpoles per tank; three tanks per treatment), and different lowercase letters indicate statistically significant differences among the means ($P < 0.05$; Scheffé's multiple-contrast test).

Wilbur and Fauth 1990, McCollum and Van Buskirk 1996). Most likely there is a complex interaction of biotic and abiotic components. We can also distinguish between effects that are dependent on chemical concentrations (i.e., of waste products or hormonal/pheromonal substances) and those that are not (e.g., physical interactions among tadpoles, reduction in swimming volume). Little is known about the proximal environmental cues that signal a desiccating habitat (Newman 1994). While pond temperature and tadpole density (both of which are known to influence development rate) were positively correlated with short-duration ponds (Newman 1989, 1994), a causative role

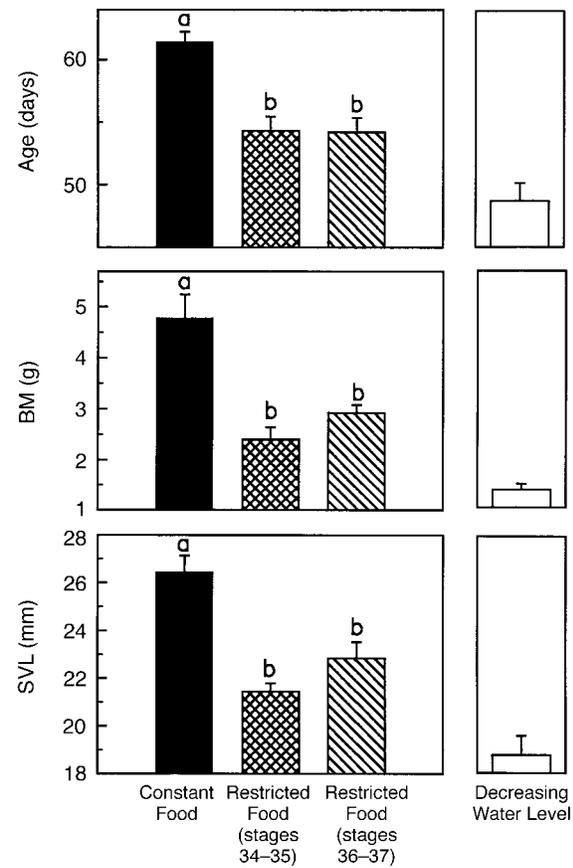


FIG. 8. Food restriction of prometamorphic tadpoles maintained in a constant high water environment accelerates metamorphosis. Prometamorphic tadpoles were distributed to tanks (eight tadpoles per tank; three tanks per treatment) and maintained in a constant high water environment. Food was either maintained constant or restricted at different stages of development. For comparison data for tadpoles exposed to a decreasing water level (identical to volume reduction regime 3, experiment 2; see Fig. 3; food was constantly available) derived from a parallel experiment with the same sibship are shown. Graphs show mean age, body mass (BM), and snout-vent length (SVL) at metamorphosis; bars show means ± 1 SE. Different lowercase letters indicate statistically significant differences among the means ($P < 0.05$; Scheffé's multiple-contrast test).

for these environmental factors in the response to habitat desiccation was not determined.

Because the drying pond is a complex environment and no two drying ponds are likely to be exactly alike,

TABLE 3. Age (in days), body mass (BM, in grams), and snout-vent length (SVL, in centimeters) from experiment 6, showing that tadpoles maintained individually accelerate metamorphosis and metamorphose at a smaller body size in response to volume reduction. Values are means ± 1 SE. Summary statistics are also presented for age and body size at metamorphosis.

Source	Constant high water vol.	Decreasing water vol.	MS	F	df	P
Age	54.6 \pm 0.8	43.9 \pm 0.4	0.1769	197.04	1, 14	<0.0001
BM	2.8 \pm 0.2	1.7 \pm 0.1	0.7769	23.8	1, 14	0.0003
SVL	2.4 \pm 0.1	2.1 \pm 0.1	0.0095	15.96	1, 14	0.0015

tadpoles must either be capable of detecting a signal that is common to all drying ponds or they must be able to respond to multiple signals. If the latter is correct, then these multiple cues may function in an additive or synergistic manner, resulting in variation in the response between ponds. There are several potential ways in which tadpoles could monitor pond duration or depth to allow for a physiological response leading to accelerated metamorphosis. The developmental response could result directly from decreased water volume where tadpoles monitor water depth through the use of special senses (e.g., the visual system). Alternatively, the response could be due to factors correlated with decreased water volume (i.e., concentration or tadpole density-dependent effects).

Thermal effects

Newman (1989) found that as outdoor experimental ponds dry, their thermal profiles change dramatically. That is, shallow ponds (<10 cm depth) are significantly warmer at the bottom (as much as 12°C warmer) compared with deeper ponds (>20 cm depth). The rate of metamorphosis is strongly influenced by temperature, which can be directly related to the thermal sensitivities of the rate functions of biochemical and physiological processes that underlie morphogenesis (see Dodd and Dodd 1976, Smith-Gill and Berven 1979, Petranka 1984, Hayes et al. 1993). Thus, the developmental response to habitat desiccation could result from unavoidable environmental effects (i.e., increased pond temperature) rather than natural selection (i.e., an adaptive response). However, this conclusion is not supported by our results, where water temperatures did not differ between experimental groups (see *Results*) yet tadpoles showed dramatic developmental responses to the decreasing water level. Thus, while elevated pond temperature may contribute to and may be sufficient for accelerated metamorphosis, it is not necessary for the morphogenic response to habitat desiccation.

The concentration of compounds in the drying pond

If the thermal effects caused by habitat desiccation are not a necessary condition for accelerated metamorphosis, then what are the environmental cues that tadpoles use to alter development rate? As the pond dries, one predicts that salts, waste products (e.g., fecal matter, CO₂), tadpole secretions, etc. become concentrated in the water. Hormones and hormone metabolites are known to be both secreted and taken up by tadpoles (see Hayes and Licht 1993, 1995), and the concentration of hormonal products that stimulate metamorphosis could be responsible for accelerated morphogenesis. Alternatively, a critical compound dissolved in the water (e.g., O₂) could be depleted and become limiting, thus triggering metamorphosis. Our experiments with screens placed in the bottom of the tanks and moved to the water surface argue against such an explanation since in these experiments the dilutional volume re-

mained the same while the swimming volume (or the proximity to the surface) decreased. Thus, while the concentration of compounds in the drying pond could play a role in accelerating metamorphosis, it is probably not essential to the response.

Swimming volume

To test whether a simple reduction in swimming volume (rather than an increased proximity to the water surface) could account for the developmental response, we moved a screen from one side of the tank to the other, again keeping the dilutional volume constant while reducing the swimming volume. While tadpoles subjected to this treatment metamorphosed sooner than constant high water controls, the acceleration of development was much less than that observed with either the decreasing water or the vertically manipulated screen treatments (absolute swimming volume was maintained the same in each of these treatments). The results of this experiment suggest that the developmental response is influenced by a decrease in swimming volume. The difference in the magnitude of the response between the vertically and horizontally manipulated screen treatments may indicate that tadpoles also monitor proximity to the surface (although we were unable to test this; see *Results*). It may also be that the restriction in swimming volume produced by the vertically manipulated screen treatment is qualitatively different from the horizontally manipulated treatment (i.e., foraging may be restricted to a greater degree in the former than the latter).

Physical interactions among conspecifics

As the pond dries, tadpole density is likely to increase as swimming volume decreases (see Newman 1989, 1994), providing for greater physical interactions among conspecifics. In most amphibian species, crowding of the larvae tends to slow both growth and development rates (Richards 1958, Licht 1967, Brockelman 1969, Wilbur 1972, 1976, 1977a, b, Gromko et al. 1973, Wilbur and Collins 1973, John and Fenster 1975, Smith-Gill and Gill 1978, Smith-Gill and Berven 1979, Semlitsch and Caldwell 1982, Berven and Chandra 1988, Scott 1990). However, Newman (1994) showed that metamorphosis was accelerated as tadpole density increased in a laboratory experiment with *S. couchii*, suggesting that density stress can function as a positive metamorphic stimulus in species that breed in ephemeral habitats. Because a chemical concentration effect is unlikely to explain the response to volume reduction (see experiments 4 and 5), the response might depend on increased physical interactions among conspecifics brought about by the reduced swimming volume. In this study, tadpoles raised individually responded to the decreasing water level (see Table 3), which argues against physical interactions being necessary to the initiation of accelerated metamorphosis. Although tadpole density-dependent effects could con-

tribute to alterations in the rate of development in the natural habitat (e.g., Newman 1994), such effects do not explain the fundamental developmental/physiological response to a drying pond.

Resource limitation

In ephemeral breeding ponds, resource availability is likely to change in proportion to tadpole size and density; i.e., as tadpoles grow and their density increases due to the decrease in water volume, the amount of food available to each animal most probably decreases (Newman 1994). Food level is known to influence age and size at metamorphosis in anurans (D'Angelo et al. 1941, Travis 1984, Alford and Harris 1988, Berven and Chadra 1988, Crump 1989a, Newman 1994). Models of amphibian metamorphosis have attempted to evaluate optimal size at transformation, taking into account the relative risk of mortality vs. the relative opportunity for growth in the aquatic and terrestrial habitats (Wilbur and Collins 1973, Werner 1986). These models suggest that in ephemeral breeding environments where food level is initially high and then declines, metamorphosis should be initiated earlier and at a smaller body size relative to animals that develop in an environment characterized by a sustained high level of resources. Newman (1994) provided support for these models by showing that a declining food level (all else being equal) results in a shortening of time to metamorphosis in *S. couchii*.

In our studies we observed that as the water level was reduced (or as the vertically moved screen approached the surface of the water), tadpoles reduced foraging behavior. This reduction in foraging could be related to the direct, physical inhibition of locomotion, or could be indirectly related to the induction of metamorphosis, at which time feeding ceases due to dramatic remodeling of the intestine and mouth to prepare the animal for an exclusively carnivorous lifestyle (reviewed by Shi and Ishizuya-Oka 1996). Thus, while resource availability in the drying pond probably decreases in parallel with increasing tadpole density, causing food intake to taper off (see Newman 1994), feeding may cease abruptly as locomotion becomes impossible. We found that metamorphosis was accelerated if we removed food at various stages of development of *S. hammondi* tadpoles maintained in a constant high water environment. Our results agree with those of D'Angelo et al. (1941), who showed that starvation after the limb bud developed accelerated metamorphosis in *Rana sylvatica* and *R. pipiens*, whereas starvation before this stage retarded development. Alford and Harris (1988) also showed that differentiation rate is slowed if food is restricted early in the larval period of *Bufo woodhousei fowleri*; however, food restriction late in the larval period did not alter age at metamorphosis. Thus, the rate of differentiation may respond to alterations in resource availability that occur throughout the larval period (contra Travis [1984], who

proposed that differentiation rates may be fixed early in the larval period; however, such effects could vary among species). Whether the response to food restriction is positive (acceleration of differentiation) or negative (deceleration of differentiation) may depend on the developmental stage at which the resource limitation occurs, and also on the species under investigation. In *Scaphiopus* tadpoles the cessation of feeding, as a consequence of either restricted locomotion (due to reduced water volume) or the induction of metamorphosis (i.e., in response to some as yet unidentified environmental factor) could contribute to the accelerated development associated with the response to volume reduction. At the physiological level, it is of interest that the neurohormone that accelerates metamorphosis (corticotropin-releasing hormone and its related peptides; interestingly, this is the primary vertebrate stress neurohormone; Denver and Licht 1989, Gancedo et al. 1992, Denver 1993, 1996, 1997a, b) has been shown to function in the control of appetite and feeding behavior in several vertebrates (York 1992, De Pedro et al. 1993, 1995, Spina et al. 1996). It is worth noting that this neuropeptide is also involved in the central control of locomotory behavior in amphibians (Lowry et al. 1990).

Conclusions

Adaptive phenotypic plasticity allows tadpoles of *S. hammondi* to exert fine control over the rate of metamorphosis in response to changes in their larval habitat. Our experiments in a controlled laboratory setting provide important insights into both the degree of plasticity and the proximal environmental cues operating in the response of spadefoot toad tadpoles to pond drying, insights that can be used to develop and test hypotheses in the natural environment. We have shown that the rate of metamorphosis varies as a continuous function of the rate of decline in the water level. Furthermore, by simulating an ecological release from the stress of habitat loss, we showed that the response is partially reversible, and this reversibility depends on the stage of development.

Our results do not allow us to define the precise environmental cue(s) that tadpoles use to respond to a desiccating habitat. Nevertheless, they do allow us to eliminate several variables as being necessary for the developmental response (e.g., elevated temperature, chemical cues, and physical interactions among tadpoles). Our findings suggest that the restriction of swimming volume in the desiccating habitat constrains foraging, and that the cessation of feeding contributes to the accelerated development. However, this effect can explain only ~55% of the response; other mechanisms must contribute to the developmental response. It is possible that tadpoles perceive, through the use of the visual system, their proximity to the water's surface and activate neuroendocrine centers that control metamorphosis (see Denver 1997a, b). Our ability to ma-

nipulate the rate of metamorphosis in a controlled laboratory setting allows us to test hypotheses regarding the proximal environmental cues and the physiological/developmental mechanisms that underlie the metamorphic response to the desiccating habitat (see Denver 1995, 1997a, b).

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