# The relationship between habitat permanence and larval development in California spadefoot toads: Field and laboratory comparisons of developmental plasticity

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# The relationship between habitat permanence and larval development in California spadefoot toads: field and laboratory comparisons of developmental plasticity

**Steven R. Morey and David N. Reznick**

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We evaluated differences in larval habitats and life history of three species of spadefoot toads, then compared their life histories in a common garden study. Our field work defined the selective regime encountered by each species. Our Great Basin spadefoot (*Spea intermontana*) bred asynchronously in permanent streams and springs where there was no risk of larval mortality due to drying. The water chemistry remained fairly stable throughout the larval period. The western spadefoot toad, *Sp. hammondii*, bred fairly synchronously following heavy spring rains in temporary pools that remained filled an average of 81 d. Fifteen % of the breeding pools dried completely on or before the day the first larvae metamorphosed. The desert spadefoot toad, *Scaphiopus couchii*, bred synchronously after heavy summer showers in very short duration pools; 62% of the breeding pools dried completely on or before the day the first larvae metamorphosed. The concentration of ammonium nitrogen and $CaCO_3$ increased markedly as the *Sp. hammondii* and *S. couchii* pools dried. *S. couchii* attained metamorphosis at a much earlier age and smaller size than the other two species. *S. couchii* also showed little variation in the age at metamorphosis but considerable variation in the size at metamorphosis, while the other two species varied in both age and size. The results identify some variables that could serve as cues of pool drying and demonstrate an association between breeding pool duration, breeding synchrony, development rate, and larval development. Our laboratory study yields information about the genetic basis of the differences in development and controlled comparisons of phenotypic plasticity. We manipulated food supply to study the plastic response of age and size at metamorphosis and hence construct the reaction norm for these variables as a function of growth rate. The growth rates ranged from below to above those observed in natural populations. As in the field, in the lab *S. couchii* attained metamorphosis at an earlier age and smaller size than the other two species. All three species had a similarly shaped reaction norm for size(y-axis) and age (x-axis) at metamorphosis, which was a concave upward curve. A consequence of this shape is that age at metamorphosis changes more readily at low levels of food availability and size at metamorphosis changes more readily at high levels of food availability. If we restrict our observations to just those growth rates that are seen in nature, then *S. couchii* has almost no variation in the age at metamorphosis but considerable variation in size at metamorphosis, while the other two species vary in both age and size at metamorphosis. All three species increased in size at metamorphosis with increased food levels. Our comparative reaction norm approach thus demonstrates that *S. couchii* has adapted to ephemeral environments by shifting its growth rate reaction norm so that age at metamorphosis is uniformly fast and is not associated with growth rate. The realized variation is concentrated in size rather than age at metamorphosis.

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Organisms in nature often exist as metapopulations, or as networks of sub-divided populations that are connected by migration (Hanski and Gilpin 1997). To understand adaptation, it is necessary to understand the frequency distribution of habitats that are experienced by the organism, rather than to characterize a single unit within the metapopulation. Breeding populations of amphibians often fit this metapopulation concept well because they are sub-divided by the utilization of widely dispersed breeding sites, but have long-lived, mobile adults that can move among sites (Murphy 2003). Here we report on a comparative study of three species of spadefoot toads, each of which is represented by multiple populations clustered in a larger geographic area. Each species is thus represented by a frequency distribution of breeding environments, rather than a single locality, to provide a characterization of the selective environment and the larval life histories of natural populations. We then compare the three species in a "common garden" laboratory environment so that we can characterize the genetic components of field differences in the life history.

The larval habitats of many species of amphibians are temporary, of variable duration, and offer pronounced variation in growth opportunity (Bragg 1965, Licht 1974, Shoop 1974, Newman 1987, Wilbur 1987). Larvae from temporary habitats are notable for their plastic response of size and age at metamorphosis (Travis 1984, Alford and Harris 1988, Pfennig et al. 1991, Blouin 1992, Leips and Travis 1994, Morey and Reznick 2000, Doughty 2002). Both life history traits have been linked to fitness (Berven and Gill 1983, Smith 1987, Semlitsch et al. 1988, Berven 1990, Scott 1994, Morey and Reznick 2001) through variables like the probability of survival to maturity, age at maturity, or fecundity. More importantly, both traits and the plasticity in these traits are hypothesized to represent adaptations to the larval environment (Wilbur and Collins 1973). Do interspecific differences in patterns of plastic response (norms of reaction) illustrate adaptive responses to larval environments? Among closely related species, do those from more stable environments show levels of responsiveness to a changing environment that are distinguishable from the pattern of those that regularly encounter variability? One way to evaluate a potential adaptation is a comparative study, either among populations within a species or among closely allied species that are thought to be subject to different selective regimes with respect to the potential adaptation (Gotthard and Nylin 1995, Doughty and Reznick 2002).

Excellent examples of variation in the larval environment are found within the North American spadefoot toads (Pelobatidae: *Scaphiopus*, *Spea*). Some species are associated with extremely short duration breeding and larval habitats where aquatic larvae often face a high risk of mortality due to the drying up of their pools. Risk of drying has been identified as an important selective feature of the spadefoot environment (Gosner and Black 1955, Bragg 1965, Pomeroy 1981, Newman 1988a, 1992, 1994a, 1989, Pfennig 1990a, 1992a) and is the basis for recent adaptive explanations for their patterns of larval development (Newman 1988a, b, Pfennig 1990a, 1992b, Morey and Reznick 2000). Most earlier work concentrated on individual species. Some recent studies (Buchholz and Hayes 2000, 2002, Leips et al. 2000) have involved comparisons among related species. We follow this more recent work by making comparisons among species in larval environment. We do so by sampling a series of breeding sites, or subunits of a metapopulation, for each species to yield a statistical distribution of the properties of larval environments. These distributions define the adaptive regime of the local populations, at least in the near-term. Associations between the environment and larval development potentially yield clues about the extent to which differences among species in development represent adaptations to the larval habitat. We thus present a comparative study of larval ecology and development.

We studied the larvae of three species of spadefoot toads from California, *Spea intermontana*, *Sp. hammondii* and *Scaphiopus couchii;* there is no overlap in the ranges of these species. Our *S. couchii* populations bred during the summer monsoon rains in pools of extremely short duration. Our *S. hammondii* populations bred in pools filled by winter rains that were of intermediate duration. Our *S. intermontana* populations bred in permanent bodies of water that were fed by snowmelt. In the field component of our study, we characterized larval development and the selective regime with two classes of variables: those that describe the larval habitat and those that describe the larvae themselves. Our environmental variables included: 1) permanence of the aquatic habitat, because habitat duration is related to risk of mortality due to desiccation and is believed to be an important force shaping the evolution of larval life history characteristics, such as age and size at metamorphosis and the plasticity of development in response to habitat variation (Newman 1988a, b, 1992, Semlitsch et al. 1988, Crump 1989, Pfennig 1990a, 1992b). 2) water temperature and chemistry, because amphibian larvae have been shown to alter development in response to drying conditions (Newman 1988a, b, Semlitsch and Wilbur 1988, Crump 1989) yet the specific cues that larvae detect are unknown. Wilbur and Collins (1973) offer recent growth history as a plausible cue. Newman (1992) suggested that a suite of correlated changes in drying ponds, such as temperature and water chemistry, may also be important. We thus measured changes in the aquatic environment over the course of larval development.

Our second set of variables that characterize the larvae include the development rate and age and size at metamorphosis. Age and size at metamorphosis are

important life history characteristics that have been linked to survival and reproductive success in the adult life stage (Berven and Gill 1983, Smith 1987, Semlitsch et al. 1988, Berven 1990, Pfennig 1992b, Scott 1994, Morey and Reznick 2001). We measured both traits for individuals from field populations, allowing us to evaluate the correspondence between these characteristics and important features of the larval habitat (e.g. pool duration, risk of mortality due to drying).

We also evaluated larval growth rate. This variable is very sensitive to resource availability and can thus serve as a surrogate for assessing variation in growth opportunity in the larval habitat when considering variation among ponds within a species. There may also be inherent differences among species in growth rate. Our goal here was to assess the range of growth rates offered by natural pools. In addition, Wilbur and Collins (1973) hypothesized that recent growth history is a crucial variable that determines the timing of amphibian metamorphosis.

In the laboratory, we evaluated how larval development differs among species when compared in a common environment, but also performed a comparative study of plasticity in larval development. Most prior research on amphibian metamorphosis has focused on a single population of one species. If larval development is an adaptation, then we argue that perceiving it as such requires mapping differences among populations within a species or among species on differences in the larval environment. As far as we are aware, only Blouin (1992), Leips and Travis (1994, 2000), and Buchholz and Hayes (2000, 2002) have made interspecific comparisons of plasticity in larval age and size among anurans that utilize larval habitats that differ in permanence. Our laboratory experiments evaluate the relationship between permanence of the larval habitat and plasticity in age and size at metamorphosis. We evaluate the relationship between these life history traits at different levels of food availability and hence different growth rates. Our range of food availability enabled us to generate reaction norms for a range of growth rates that exceed what is observed in nature. We can thus compare the entire reaction norm for each species, then just the range of growth rates seen in nature, based on our observations on natural populations. The purpose of considering the whole reaction norm, versus the sub-set that is expressed in nature, is to evaluate whether or not the norm itself has changed in some fundamental way (lab) and/or if it has changed in a fashion that restricts the sort of variation that is expressed in nature (field). The species considered here are a sub-set of those studied by Buchholz and Hayes (2002) and our methods are similar. The studies differ because theirs was a laboratory study that compared growth and metamorphosis among species in the family Pelobatidae while our study was paired with field investigations that characterized a spectrum of natural populations. A consequence of this difference is that our methods and interpretations could be associated with the environment from which the subjects were derived.

## Material and methods

We surveyed natural larval habitats of *Sp. intermontana* in the western Great Basin Desert of California, east of the Sierra Nevada (Inyo and Mono Counties) during the spring and summer of 1989 and 1990. We sampled two clusters of pools, one in the Long Valley Caldera, which drains into the Owens River and one in the Mono Basin, which has no hydrological outlet. They are separated by approximately 30 miles. *Sp. hammondii* habitats were surveyed in coastal central and southern California (Riverside and San Luis Obispo Counties) during the spring of 1991–1993. There was one cluster of pools on the western flank of the Box Springs Mountains, near the Univ. of California Campus, a second on the Motte-Rimrock Reserve, which is part of the Univ. of California Reserve System, a third in San Timoteo Canyon, and a fourth near San Luis Obispo. The first three clusters may well have once been part of a diffuse network of breeding sites but are now separated by development. We surveyed *S. couchii* in the Colorado Desert of extreme southeastern California (Imperial County) during the summer of 1991. The pools were distributed across the west and east side of the Chocolate Mountains from just east of the town of Glamis to the Milpitas Wash. Most of the pools are in arroyos that drain into the Colorado River to the east. Others are in arroyos that drain west into the Algodones Dunes. There is no obvious barrier between any of the pools, other than distance. The pools range from less than a mile apart to more than five miles apart, although there could easily be other breeding pools interdispersed among those that were sampled. *Sp. intermontana* and *Sp. hammondii* pools containing larvae were visited weekly following the first bout of breeding. Pools inhabited by *S. couchii* were visited at two to three day intervals: once on the day following the breeding bout, once mid-way through larval development, and for a third time on the day of metamorphosis (emergence of the first forelimb). The larval pools of *Sp. intermontana* and *Sp. hammondii* were sampled weekly after the first bout of breeding. We continued to visit pools after metamorphosis to establish the date of drying.

### Larval characteristics

During each visit to each pond, a sample ($n = 10$) of the oldest cohort of larvae were staged (Gosner 1960), and weighed, either in the field with a hand-held balance (to the nearest 0.1 g), or in the laboratory on an electronic

balance (to the nearest 0.001 g). Each sample contained individuals of more than one developmental stage. We used this information to construct curves of growth and development and to establish larval growth rates. The growth rates reported here were estimated as the mass at metemorphosis divided by the larval period.

## Habitat characteristics

We characterized three aspects of the physical environment: permanence, temperature, and water chemistry. The permanence of the habitat was evaluated as: 1) the average number of days a pool persisted after the first bout of breeding, and 2) the relationship between drying of the pool and the completion of development of the oldest cohort of larvae. Pools that dried on the day of metamorphosis, or before, were classified as high-risk larval habitats.

Water temperatures were recorded and water samples were collected periodically during the period of larval development. Here the temperature of the larval environment is characterized by averaging the daytime temperatures recorded early in larval development, mid-way through the larval period, and late in larval development. We report the temperature of shallow water near the shore (depth 1 cm) as well as at the deepest part of the pool near the bottom. The characterization of water chemistry included pH, ammonium nitrogen, sodium chloride, and $CaCO_3$ hardness. Determinations were made with titration colorimetry using the methods given with the Fish Farmer's Water Quality Test Kit (Hach 1982). The values reported are the averages of readings taken early, in the middle and late in larval development.

## Laboratory experiments

The experiments were conducted sequentially by species: *S. couchii* (August, 1991), *Sp. hammondii* (February, 1992); *Sp. intermontana* (June, 1992). All experiments were conducted in the same environmental chamber, which maintained a constant water temperature of 27°C over the entire period. This temperature falls within the range of temperatures experienced by all three species in nature (Table 2) and represents a compromise among the average values experienced by each species. It was higher than the average daytime temperature experienced by larvae of *Sp. intermontana* and *Sp. hammondii*, and lower than average daytime temperatures experienced by *S. couchii* (Table 2). Each experiment was a simple full sibling, split-brood design. Larvae were obtained from fertilized eggs produced by amplectant pairs of spadefoot toads collected in the field. We evaluated three families each from *Sp. hammondii* and *S. couchii* and two families from *Sp. intermontana*. As a consequence, the sample sizes for *Sp. intermontana* are smaller than those for the other two species for all laboratory results. Each pair of toads produced a large clutch of eggs from which 72 full sibling hatchlings (stage 25, Gosner 1960) were reared in groups of three, in small plastic tubs containing one liter of aged tap water. There were four tubs per sibship for each level of food availability. Each tub was assigned to one of several food levels and to one of four shelves in the environmental chamber (food levels summarized in Table 3). We used a randomized block design, assigning tubs in a way that did not confound food level or sibship with shelf (block). The response of larval development was evaluated via food manipulations that were used as a surrogate variable for growth opportunity. In an attempt to remain true to the ''common garden'' design (Tansley 1917, Turesson 1930), the food rations were identical in each experiment. Because a wide range of growth rates were generated, interspecific comparisons of responses at specific food levels as well as responses at common growth rates were possible. The food was a mixture of commercial tropical fish food, commercial guinea pig chow, freeze dried tubifex worms, puppy vitamins and finely sifted silt. Larvae were fed once a day immediately after a complete change of water. With the exception of the earliest larval stages, all food was consumed shortly after it was offered.

Table 1. Characteristics of larval spadefoot toads in the wild. *Spea intermontana* are represented by the combined samples from 1989 and 1990. The results reported for *Spea hammondii* and *Scaphiopus couchii* are from 1991. Values shown are the means for all pools samples for each species. The range is shown in parentheses.

| Larval trait | *Spea intermontana* | *Spea hammondii* | *Scaphiopus couchii* |
|---|---|---|---|
| Larval period (days) | 48 (36–60) | 58 (30–79) | 7.4 (7–8) |
| No. populations | 4 | 9 | 8 |
| Mass (g) | 3.6 (1.8–6.5) | 3.3 (1.5–10.4) | 0.5 (0.2–0.8) |
| No. populations | 4 | 12 | 8 |
| Growth rate (mg/d) | 83 (37–181) | 64 (32–152) | 68 (30–103) |
| No. populations | 4 | 9 | 8 |
| Risk of drying (%) | 0.0 | 15.0 | 62.0 |
| No. populations | 8 | 20 | 13 |

Table 2. Some characteristics of the natural habitats of spadefoot toad larvae. The values reported for *Spea intermontana* habitats are combined samples from 1989 and 1990. The values reported for *Spea hammondii* and *Scaphiopus couchii* are from 1991. The values shown are means of all pools sampled for each species. The range is shown in parentheses. For temperature and water chemistry, the period of larval development has been devided into early, mid, and late periods to illustrate temporal changes.

| | *Spea intermontana* | *Spea hammondii* | *Scaphiopus couchii* |
|---|---|---|---|
| Pool duration (d) | Permanent | 81 (36–127) | 10.3 (7–19) |
| No. pools | 7 | 9 | 8 |
| Water temp. (C) | | | |
| Early-larval period | | | |
| Shallow | 20.2 (13.0–26.9) | 23.0 (16.0–27.0) | 28.3 (26.2–30.0) |
| Deep | 20.4 (15.5–26.9) | 17.8 (11.0–25.0) | 28.3 (26.2–30.0) |
| Mid-larval period | | | |
| Shallow | 23.5 (17.4–31.0) | 26.8 (24.5–32.0) | 28.3 (24.5–33.0) |
| Deep | 22.2 (15.6–31.0) | 24.8 (14.0–32.0) | 27.2 (24.2–32.0) |
| Late-larval period | | | |
| Shallow | 25.0 (23.8–26.5) | 27.6 (22.0–32.0) | 27.5 (24.2–32.5) |
| Deep | 24.6 (23.6–25.5) | 26.0 (22.0–32.0) | 23.9 (21.3–29.0) |
| No. pools | 4 | 6 | 12 |
| Water chemistry | | | |
| pH | | | |
| Early-larval period | 8.3 (7.7–9.0) | 8.2 (7.5–9.8) | 7.2 (7.0–7.4) |
| Mid-larval period | 8.2 (7.9–8.5) | 8.1 (7.3–10.1) | 7.3 (6.8–7.6) |
| Late-larval period | 7.5 (7.1–7.9) | 8.1 (7.1–9.3) | 7.3 (6.8–7.5) |
| Ammonium ion (mg/l) | | | |
| Early-larval period | 0.6 (0.6–0.7) | 1.3 (0.8–2.2) | 1.5 (1.2–2.1) |
| Mid-larval period | 0.7 (0.4–1.0) | 2.1 (1.0–4.2) | 1.9 (1.8–3.5) |
| Late-larval period | 1.3 (1.3–1.3) | 2.5 (1.3–4.2) | 4.6 (2.1–15.6) |
| NaCl (mg/l) | | | |
| Early-larval period | 69.4 (12.5–133.3) | 23.7 (12.5–58.3) | 15.0 (12.5–18.8) |
| Mid-larval period | 66.7 (18.8–125.0) | 28.8 (15.6–62.5) | 19.4 (12.5–25.0) |
| Late-larval period | 50.0 (37.5–62.5) | 44.6 (28.1–93.8) | 21.9 (18.8–31.3) |
| $CaCO_3$ hardness (mg/l) | | | |
| Early-larval period | 98.8 (79.8–108.3) | 120.3 (51.3–205.2) | 114.6 (85.5–171.1) |
| Mid-larval period | 88.4 (85.5–94.1) | 145.4 (85.5–256.5) | 202.3 (136.8–427.8) |
| Late-larval period | 124.0 (94.1–153.9) | 243.0 (149.6–316.4) | 273.6 (171.1–513.0) |
| No. pools | 3 | 9 | 7 |

Table 3. The eight food levels and the mean dry mass $\pm 1$ SE at metamorphosis. Food level is the dry mass in mg of food offered per tub (3 larvae) per day. The number of replicate tubs per food level is shown in parentheses. Not all species were reared at all food levels.

| | *Spea intermontana* | *Spea hammondii* | *Scaphiopus couchii* |
|---|---|---|---|
| Food level (mg/tub) | Dry mass (mg) | Dry mass (mg) | Dry mass (mg) |
| 6 | … | … | $9.0 \pm 1.0$ (9) |
| 12 | $36.0 \pm 1.0$ (7) | $58.0 \pm 8.0$ (6) | $11.0 \pm 1.0$ (12) |
| 25 | $49.0 \pm 3.0$ (8) | $42.0 \pm 2.0$ (12) | $15.0 \pm 1.0$ (12) |
| 49 | $73.0 \pm 5.0$ (8) | $74.0 \pm 4.0$ (12) | $19.0 \pm 1.0$ (12) |
| 103 | $109.0 \pm 6.0$ (8) | $102.0 \pm 5.0$ (12) | $27.0 \pm 2.0$ (12) |
| 206 | $193.0 \pm 25.0$ (8) | $196.0 \pm 7.0$ (12) | $32.0 \pm 3.0$ (12) |
| 412 | 388.0 (1) | $325.0 \pm 2.0$ (3) | … |
| 515 | $403.0 \pm 4.0$ (2) | $357.0 \pm 14.0$ (3) | … |
| 618 | $515.0 \pm 39.0$ (4) | $362.0 \pm 12.0$ (6) | … |

## Response variables and statistical analyses

All of the analyses are based on results obtained from the first larva to initiate metamorphosis in each tub. In nature, spadefoot larvae usually live in fairly dense populations and often aggregate (Bragg 1968, Pfennig 1990b). There can be a noticeable "Allee effect" (Wilbur 1977) on growth in spadefoot toads. Semlitsch and Caldwell (1982) found poorer growth performance among isolated *S. holbrookii*, so we reasoned that it was realistic to provide a social context for growth and development in the laboratory. In some tubs in the low food treatments the growth and development of either one or two larvae were severely inhibited. This inhibition, coupled with mortality of some of the inhibited individuals, resulted in the survivors and dominant individuals receiving a disproportionately large share of food intended for three individuals. This disproportionate consumption of food resulted in increased variance within a replicate and reduced treatment effects. We

also analyzed the response of all three individuals in each tub. At the higher food levels (Food levels 3–8, Table 3) the conclusions conformed well with those from analyses that included only the first larvae to initiate metamorphosis.

We evaluated the effect of food supply on the larval period and total dry mass at metamorphosis. Larval period was the number of days from hatching to the emergence of the first forelimb. Transforming larvae were weighed and preserved in formalin after receiving a lethal overdose of anesthetic (MS222). Each individual was dissected and all the food was removed from the intestine. The empty intestine was placed with the carcass, then they were dried to a constant mass at 55°C, then weighed.

Larval period and total dry mass as a function of food supply (growth opportunity) were analyzed with separate analyses of variance (PROC GLM, SAS 1989) with family (sibship) and food level as the independent variables. Type-III sums of squares were used in each analysis. The original variates were transformed to meet the assumptions of normality and homogeneity of variance as follows: body mass was transformed using natural logarithms and the reciprocal transformation was used on larval period (Zar 1984). 1/larval period has been interpreted as the average developmental rate (Smith-Gill and Berven 1979, Blouin 1992). Preliminary analyses revealed that "blocks" (the shelf in the laboratory) were never significant, so they were not included in any of the analyses reported below. The overall effects of food, family, and the interaction (food x family) on the bi-variate response profiles of developmental rate and mass at metamorphosis were analyzed with multivariate analysis of variance (PROC GLM, SAS 1989).

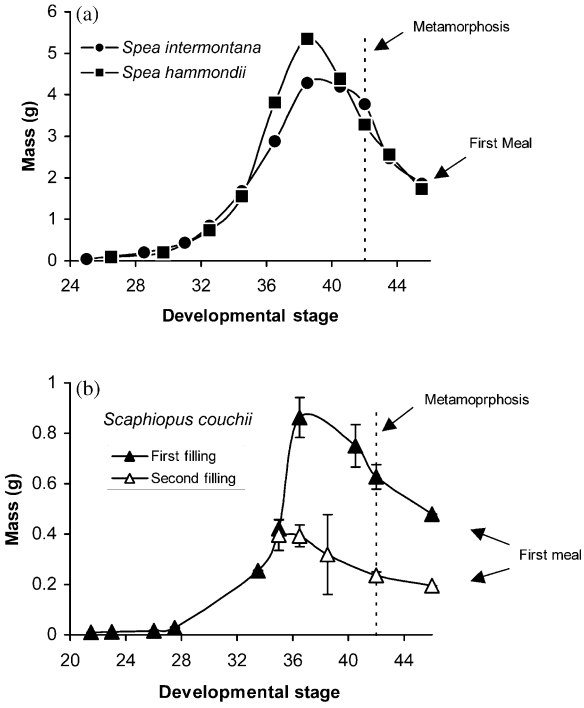


Fig. 1. Growth curves based on wet (live) mass of larvae collected in the wild. Symbols are the means of all pools from which larvae of a given developmental stage were captured (*Spea intermontana*, 1 – 5 pools; *Spea hammondii*, 1 – 12 pools; *Scaphiopus couchii*, 1 – 5 pools). For clarity error bars are not shown on the combined *Spea intermontana* and *Spea hammondii* curves (a). In (b), *Scaphiopus couchii*, error bars are $\pm$1SE of the mean. Metamorphosis (emergence of the first forelimb, stage 42) is almost complete at the initiation of terrestrial feeding.

## Results

### Field observations

*Larval growth and development*

The patterns of growth and development in the field (Fig. 1) were similar in all three species. *Sp. intermontana* and *Sp. hammondii* were much larger than *S. couchii* at all larval stages, but the general pattern was one of very rapid growth during the mid-larval stages (prometamorphosis) followed by loss of mass during the metamorphic climax. The external morphological changes associated with metamorphosis are complete when the tail is completely resorbed (Gosner stage 46). This is also when successful terrestrial feeding begins. At the initiation of successful terrestrial feeding the body mass was about half of the maximum larval mass, which is usually attained by Gosner stage 38 (Fig. 1).

In 1989 and 1990, breeding in *Sp. intermontana* was quite asynchronous. Breeding occurred sporadically in April, May and June and was not always associated with rainfall. In 1991, *Sp. hammondii* breeding bouts were restricted to March, a period of heavy rains that year. In 1992–1993, breeding was concentrated after warm heavy rains between January and March. For *S. couchii* in 1991, there were two distinct breeding periods associated with separate summer storms. Pools filled the first time in mid-August and were dry by early September. The second storm filled pools again in mid-September. Larvae born after the second filling were much smaller than similar-staged larvae born after the first storm (Fig. 1b) both half way through development and at metamorphosis.

Larval development (hatching to metamorphosis) took 48 days for *Sp. intermontana* with a range from 36 to 60 days, 58 days for *Sp. hammondii* with a range from 30 to 79 days, and only 7.4 days for *S. couchii*, with a range from 7 to 8 days (Table 1). The *S. couchii* were therefore both faster and less variable in development rate than the other two species. Size at metamorphosis was similar for *Sp. intermontana* (3.6 g) and *Sp. hammondii* (3.3 g); *S. couchii* were much smaller (0.5 g) at metamorphosis (Table 1). *S. couchii* and *Sp. inter-*

*montana* both had an approximately four-fold range of variation in size at metamorphosis, while *Sp. hammondii* had a seven-fold range. No strong relationship between larval period and mass at metamorphosis was observed within any species. The growth rate over the entire larval period was 83 mg/d for *Sp. intermontana*, 64 mg/d for *Sp. hammondii* and 68 mg/d for *S. couchii*. However, these are average growth rates; the highest growth rates observed among populations of *Sp. intermontana* were 80% higher and those for *Sp. hammondii* were 50% higher than the highest growth rates observed for *S. couchii* (Table 1).

*Pool duration, age and size at metamorphosis and risk of catastrophic mortality due to drying*

The larval habitats of *Sp. intermontana* were permanent water settings, usually quiet pools associated with springs or occasionally small streams. Here, risk of larval mortality due to drying was very low (Table 1). We surveyed a total of 8 breeding sites, none of which showed substantial declines in water level during the period of larval development. The larval habitats of the other two species were temporary and the larvae were sometimes subject to drying conditions. For *Sp. hammondii* in 1991, we observed that the average duration of the larval habitats that produced metamorphs (8 pools) was 83.1 days. Of the 11 pools surveyed that year, three (27%) dried on the day larvae completed larval development or before. When all three years are combined, we found that 15% (3/20) of the pools surveyed dried. The pools that produced at least some metamorphs lasted an average of almost three weeks longer than it took larvae to complete aquatic development, indicating substantial variation in risk of drying to larvae. The larval habitat of *S. couchii* is generally very short-lived. Of the pools from which at least some individuals survived to metamorphosis (8 pools), the average habitat duration was 10.3 d. Of 13 pools surveyed in 1991, eight (62%) of the pools dried completely on or before the day the first larvae metamorphosed. Of the pools that produced some metamorphs, the average duration was only three days longer than the completion of larval development.

The two species that bred in ephemeral pools differed in their response to pool duration. Of nine *Sp. hammondii* pools that produced some metamorphs in 1991, extreme drying was a factor in only one pool. Here, metamorphosis occurred on the day the pool dried and transforming individuals weighed 2.2 g. This mean is far smaller than average size of metamorphs from longer-lived pools (4.2 g) that year. More generally, there was a significant positive correlation between pool duration and the larval period ($r = 0.86$, $p = 0.007$, d.f. = 8) and mass at metamorphosis ($r = 0.72$, $p = 0.042$, d.f. = 8, Fig. 2a, c). Age and mass at metamorphosis of *S. couchii* was not correlated with pool duration (Fig. 2b, d). After the first filling in 1991, one short-lived pool produced metamorphs that weighed 0.66 g on the day the pool dried. These individuals were about the same size as metamorphs from longer-lived pools (mean = 0.64 g, range 0.53–0.82 g). After the second filling, the shortest-duration pool that produced metamorphs dried 12 hours after metamorphosis of the first larvae. Those metamorphs weighed 0.22 g, which was smaller than the average size of transforming toadlets from longer-lived pools (0.33 g, range 0.25–0.40 g).

*The changing abiotic environment*

The daytime temperature of pools containing *Sp. intermontana* and *Sp. hammondii* tended to increase as the period of larval development progressed. Pool temperature was fairly stable across the short larval period of *S. couchii* (Table 2); we did not observe marked differences in temperature between rapidly drying and more stable pools. However, the deepest pools were cooler near the bottom than shallow pools. Across species, the average pool temperatures increased from *Sp. intermontana* to *Sp. hammondii* to *S. couchi*, which follows a trend of decreasing pool permanence.

The permanent springs and streams utilized by larvae of *Sp. intermontana* were relatively stable in water chemistry throughout development. In contrast, the temporary pools used by *Sp. hammondii* and *S. couchii* showed substantial increases in the concentration of dissolved substances as the ponds dried, notably ammonium nitrogen and total $CaCO_3$ hardness (Table 2). The increase in total hardness is an index of pool drying while the increase in ammonium nitrogen represents the concentration of nitrogenous wastes produced by the tadpoles. This increase in ammonium nitrogen is likely to be a product of the reduced water volume, plus the increased average body size of the tadpoles and hence the increased production of ammonia. Among pools used by *Sp. hammondii* and *Sp. couchii*, temporal changes in pool chemistry were most pronounced in pools that dried substantially, exposing larvae to a high risk of mortality due to desiccation (Fig. 3).

## Laboratory experiment

*Reaction norm*

We first consider the responses to the full range of food availability experienced in the laboratory (Fig. 4). All three species took longer to attain metamorphosis at the lowest levels of food availability; the larval period then plateaued at higher food levels (Fig. 4a). *S. couchii* and *Sp. intermontana* showed progressive reductions in body mass at metamorphosis at food level declined, while *Sp. hammondii* seemed to reach a small size limit for successful metamorphosis at 25 mg of food/tub/day (Fig. 4b). The size at metamorphisis of all three species leveled off at the highest levels of food availability (Fig.

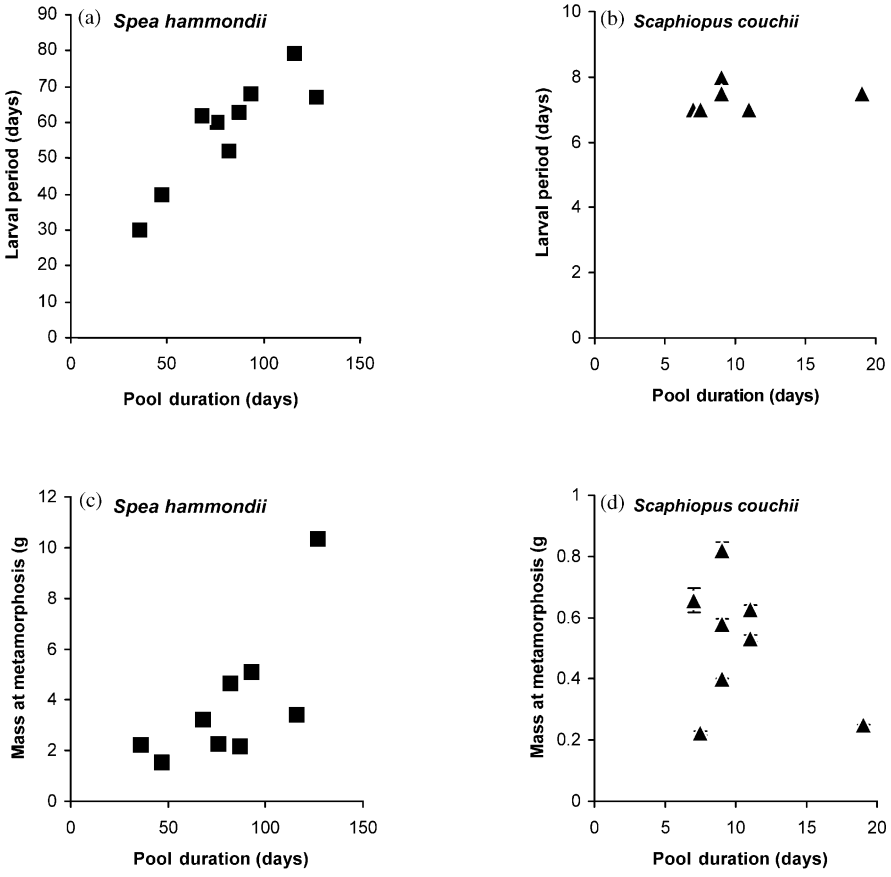


Fig. 2. Relationship of pool duration with larval period (a, b) and mass at metamorphosis (c, d). Pool duration is the number of days from the deposition of the first eggs to complete drying. Larval period is the number of days from hatching to metamorphosis (emergence of the first forelimb) of the first cohort of larvae. Each symbol is the mean from a cohort at a single pool (*Spea hammondii*, 9 pools; *Scaphiopus couchii*, 8 pools). Two symbols are hidden behind other symbols in (b). On the lower panels, error bars are $\pm$1SE of the mean.

4b). These results suggest that there is a maximum body size at metamorphosis, as postulated by Wilbur and Collins (1973). The univariate responses (Fig. 4a, b) and the overall shape of the bi-variate response profiles for age and size at metamorphosis (Fig. 4c) are quite similar for all three species when viewed across this wider range of environments.

*Comparisons of growth in lab and nature*

Laboratory food rations of 25–206 mg/tub/day (levels 2–5) generated larval growth rates comparable with those observed in nature (Table 1, Fig. 5); growth rate increased progressively with food level. Fool levels 6–8 supported growth rates higher than those seen in nature (Fig. 5). The response of *S. couchii* to higher food levels was not tested because the larvae could not consume more than 206 mg/tub/day (the quantities offered at food levels 6–8). The maximum growth rates displayed by *S. couchii* in the lab were never as high as those seen in the field (Fig. 5). The lower growth rates and lower maximum rates of food consumption may both be attributable to the lower average laboratory temperatures relative to field temperatures. Prior research has shown that lower temperature generally results in delayed metamorphosis at a larger size (Blouin 1992, Newman 1994b, 1998, Alvarez and Nicieza 2002), but does not address growth rate.

At food levels that yielded growth rates comparable to those observed in nature (levels 2–5) the mortality was very low: *Sp. intermontana*, 1/96 (1%); *Sp. hammondii*, 0/144 (0%); *S. couchii*, 2/144 (2%). Below the range of growth rates observed in nature (6.0–12.0 mg/tub/day; food levels 0–1), all three species showed increased mortality (2/24 (8%) for *Sp. intermontana*; 16/36 (44%) for *Sp. hammondii*; 16/36 (44%) for *S. couchii* ).

*Plastic response of developmental rate*

We restrict our comparisons to responses to food levels 2–5 since these yielded results that were comparable to those observed in natural populations (Fig. 6). The overall differences in rates of development for the three species, as measured in the laboratory, are consistent with the differences in larval habitat longevity in nature. Larvae of *S. couchii* developed the fastest from hatching to metamorphosis ($x = 9.02$ days, $SE = 0.27$), *Sp. hammondii* was substantially slower ($x = 17.73$ days, $SE = 0.35$), and larvae of *Sp. intermontana* developed most slowly ($x = 20.13$ days, $SE = 0.25$). The differences were more pronounced at the lowest food ration

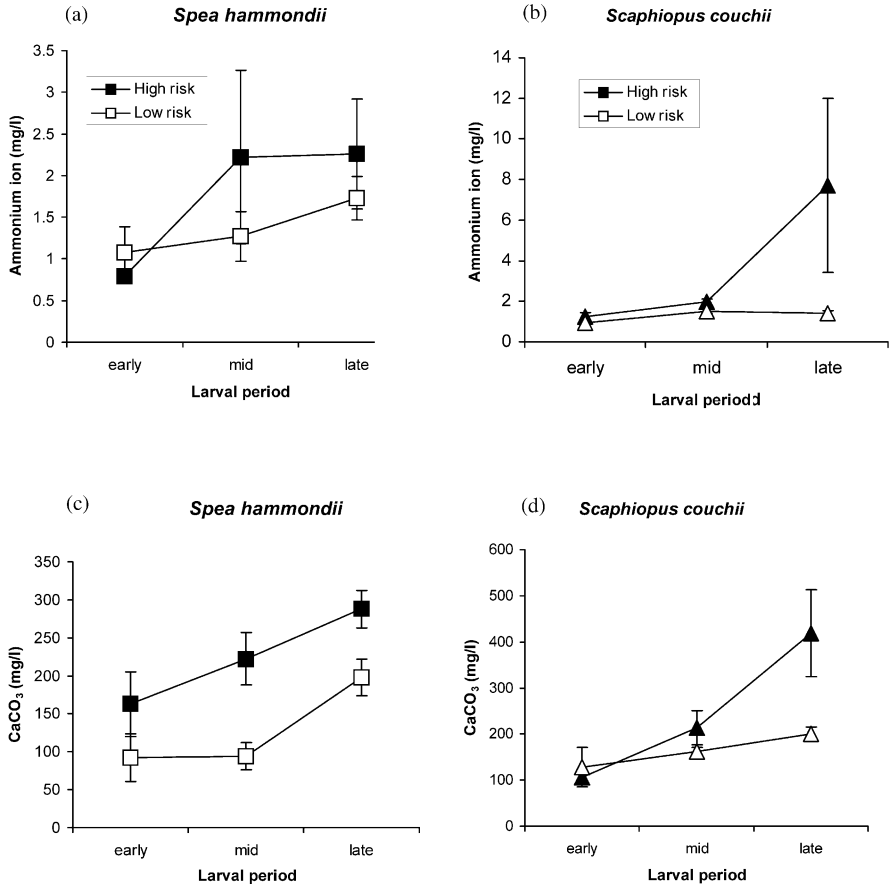


Fig. 3. Changing levels of ammonium nitrogen and $CaCO_3$ hardness in long-lived (low risk) and short-lived (high risk) pools. Symbols are the means from several pools (*Spea hammondii*: high risk, 2 pools, low risk, 7 pools; *Scaphiopus couchii*: high risk, 4 pools, low risk, 3 pools). Error bars are $\pm$1SE of the mean.

and tended to diminish at higher rations (Fig. 6a). Increased larval food availability was associated with substantially shorter larval periods in *Sp. intermontana* and *Sp. hammondii* (Table 3, Fig. 6a). For larval *S. couchii*, the development rate declined and age at metamorphosis actually increased at higher food levels but the trend was not statistically significant. Larvae reared at 25 mg/tub/day versus those reared at 206 mg/tub/day took 38% (10.0 d) longer to metamorphose in *Sp. intermontana* and 26% (5.4 d) longer in *Sp. hammondii*. *S. couchii* reared on the lower food level took 12% less time (1.2 d) than those reared on higher food levels. A consequence of restricting the comparison to just those food levels that yield growth rates that correspond to what we observed in nature is that there are substantial differences in the shape of the norm of reaction for *S. couchii* versus the two species of *Spea*. This occurs because natural conditions encompass just that portion of the complete reaction norm (Fig. 6) that has little variation in development rate for *S. couchii*.

Identity in a particular sibship was not associated with variation in mean larval period, nor were there statistically significant interactions involving sibships (Table 4). Compared to the laboratory results, the larval periods in natural populations averaged substantially longer in *Sp. intermontana* ($x = 48$ days, range 36–60 days) and *Sp. hammondii* ($x = 58$ days, range 30–79 days) and slightly shorter in *S. couchii* ($x = 7.4$ days, range 7–8 days). These patterns are consistent with the cooler average water temperatures in nature for the former two species and warmer average temperatures for *S. couchii* (Table 2). These patterns do not suggest lower food availability in nature for *Sp. intermontana* or *Sp. hammondii* vs *S. couchii* because the natural populations for the former two species had similar average and higher maximum growth rates (Table 1).

*Age and size at metamorphosis*

There was a substantial increase in mass at metamorphosis at higher food levels in all three species (Table 3 and 4; Fig. 6b). The two larger species display a large and nearly identical increase in body size (about 4-fold) over the food levels tested. Over the same range of food levels the scope of the response of *S. couchii* was only about half as great. These increases in mass were accompanied by proportionately larger increases in fat stores (Morey 1994).

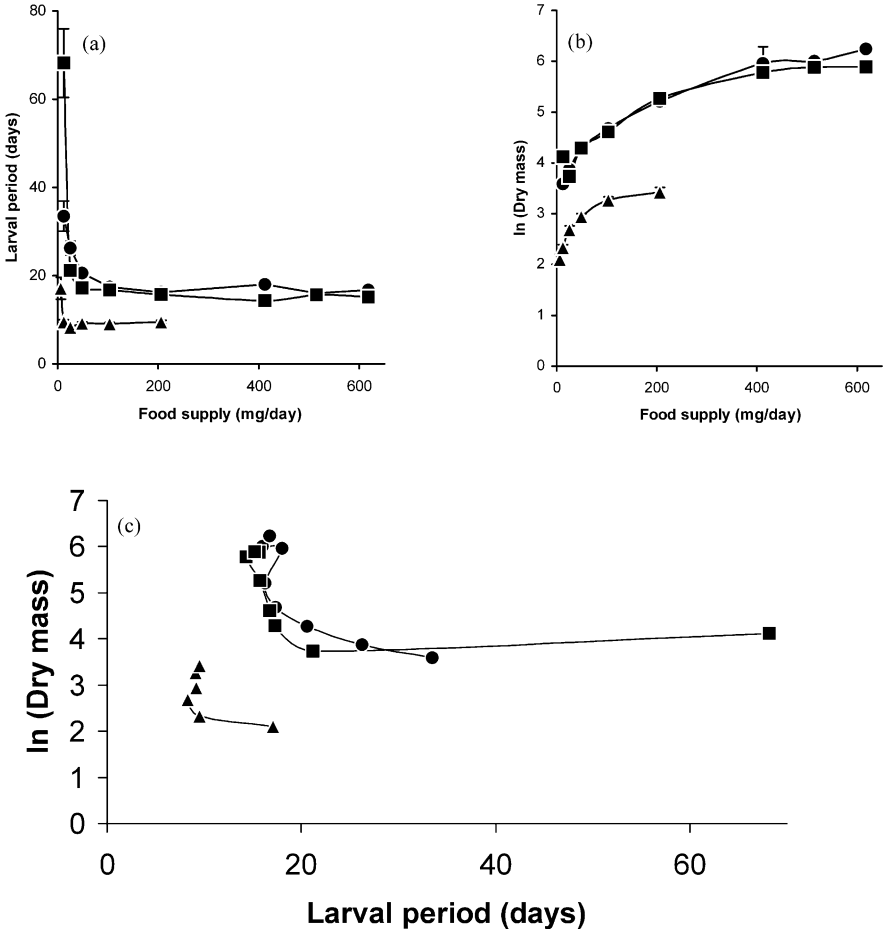


Fig. 4. The plastic response of developmental rate and mass at metamorphosis across a wide range of growth rates. Variation in growth rate was generated by manipulating food supply as indicated on the x-axis of (a) and (b). (a) illustrates the effects of increasing food on the duration of the larval life stage. (b) illustrates the effects of increasing food on the dry mass at metamorphosis (mg). (c) illustrates the bi-variate responses in age and mass at metamorphosis. Circles = *Spea intermontana*, squares = *Spea hammondii*, and triangles = *Scaphiopus couchii*. Symbols indicate the mean and the vertical bars show $\pm$1SE. Error bars are not shown in (c). Error bars frequently do not appear because they are smaller than the symbols.

Identity in a sibship was not generally associated with variation in mean mass at metamorphosis, the exception being a statistically marginal effect in *S. couchii* (Table 4). However there was a significant interaction between sibship and food level in *Sp. intermontana* and *Sp. hammondii* (Table 4). The responses of individual sibships were qualitatively similar, but not parallel, at all food levels (Fig. 7a, b).

It is instructive to view these results as the bi-variate relationship between age and size at metamorphsis (Fig. 6) because it reveals differences among the species in their reaction norms. In *Sp. intermontana* larvae, both developmental rate and body mass were quite responsive to changes in food availability (Fig. 6a, b). At low growth conditions (25 mg/tub/day) this species was the largest and had the slowest rate of development. When growth conditions were high (206 mg/tub/day), *Sp. hammondii* performed similarly to *Sp. intermontana* (Fig. 6c). *S. couchii* was smaller and took less time to develop than the other two species at each food level. Variation in food availability did not affect development rate of *S. couchii*; larvae reared on the lowest food ration could develop just as fast, if not faster, than larvae reared on higher rations. Similarly, the scope of plastic response of body mass was smaller than that of the other species, even when scaled by the smaller average size of *S. couchii*.

A qualitative comparison of the bi-variate response profiles (Fig. 6c) illustrates a shift from a shallow negative correlation (*Sp. intermontana* and *Sp. hammondii* ) to no phenotypic correlation (*S. couchii* ) between developmental rate and mass at metamorphosis across laboratory food levels. *Sp. intermontana*, which typically does not experience limited growth due to drying conditions in nature, is the species whose larval development is most severely slowed by low growth conditions in the laboratory. At the other extreme, the rate of development of *S. couchii* larvae, which regularly experiences drying conditions in nature (Table 1), is not slowed at all by low food availability in the laboratory.

The overall relationship between family identity and the bi-variate response (Fig. 6c) was not statistically significant in *Sp. intermontana* (Wilks' Lambda $F2,23 = 0.92$, $p = 0.413$), but was marginally so in *Sp. hammondii* (Wilks' Lambda $F4,70 = 2.31$, $p = 0.067$),

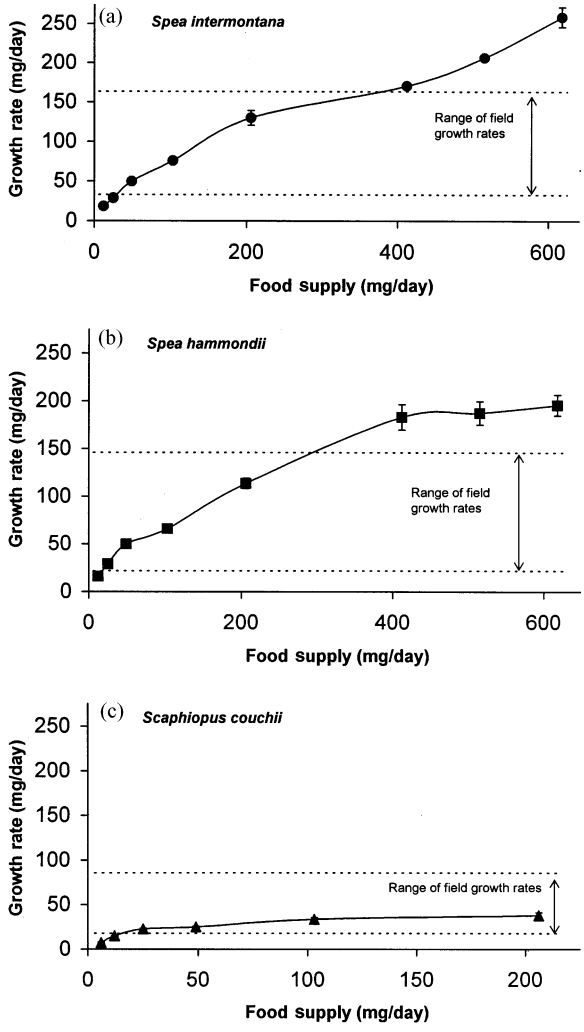


Fig. 5. Growth rates generated by the food supplies used in the laboratory experiments. Symbols are means based on 3 full-sibling families for *Spea hammondii* and *Scaphiopus couchii*, and 2 full sibling families for *Spea intermontana*. For comparison, the dotted lines indicate the maximum and minimum growth rates observed in the wild. "Growth rate" equals the mass at metamorphosis divided by the age at metamorphosis, or the average weight gained per day.

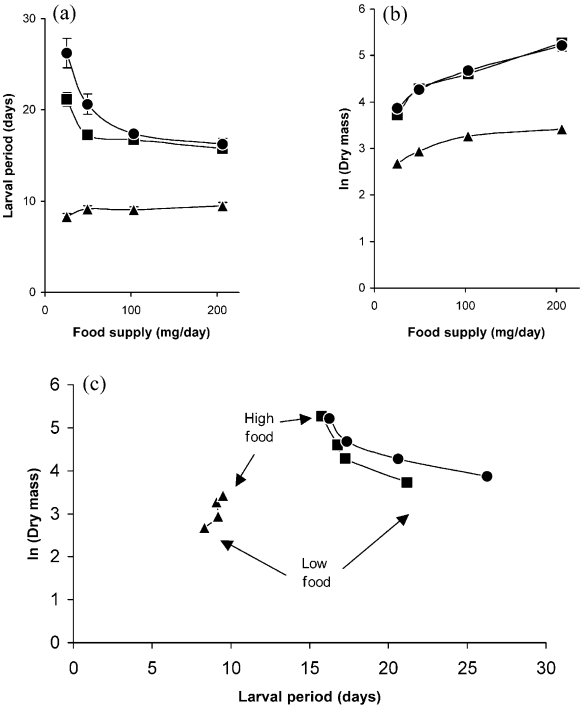


Fig. 6. The plastic response of developmental rate and mass at metamorphosis across the range of growth rates observed in the wild. For the purposes of this paper these are "realistic growth rates." These data are the sub-set of those in Fig. 4 that correspond to food levels 2–5, which are in turn the food levels that sustain the range of growth rates observed in nature. (a) illustrates the effect of increasing food on the duration of the larval life stage. (b) illustrates the effects of increasing food on the dry mass at metamorphosis (mg). (c) illustrates the bi-variate responses shown separately in (a) and (b). Circles = *Spea intermontana*, squares = *Spea hammondii*, and triangles = *Scaphiopus couchii*. Symbols indicate the mean and the vertical bars show $\pm$ 1SE. Error bars are not shown in (c).

and *S. couchii* (Wilks' Lambda $F_{4,70} = 2.61$, $p = 0.043$). The family $\times$ food interaction was statistically significant in *Sp. intermontana* (Wilks' Lambda $F_{6,46} = 2.73$, $p = 0.024$) and *Sp. hammondii* (Wilks' Lambda $F_{12,70} = 2.76$, $p = 0.004$) indicating that the response profiles (Fig. 7) represent an average of family profiles that are not parallel to one another. The food $\times$ family interaction term was not significant for *S. couchii* (Wilks' Lambda $F_{12,70} = 1.47$, $p = 0.157$) (not shown in Fig. 7).

## Discussion

### Comparative ecology and life histories

Our field observations characterize a series of breeding sites and larval biology for each species so that we can describe the comparative larval ecology of each and make inferences about the evolution of larval life history traits. We demonstrate that there is a gradient from most to least permanent larval habitats in the series *Sp. intermontana*, *Sp. hammondii*, and *S. couchii*. There are correlated differences in breeding biology, growth rate, development rate, and variability in age and size at metamorphosis. The laboratory experiments demonstrate a genetic basis for these differences. All of them may thus represent adaptations to the differences in larval habitat.

One form of adaptation to the ephemeral environment is represented in the association between habitat permanence and breeding synchrony. In general, the

Table 4. Factor level responses (least square means $\pm 1$ SE) and significance levels of *Spea intermontana*, *Spea hammondi* and *Scaphiopus couchii* to food level and full-sibling identity. The untransformed values for dry mass are shown for ease of interpretation.

| | Level | n | 1/Larval period (days) | Dry mass (mg) |
|---|---|---|---|---|
| | | | Response of *Spea intermontana* | |
| Food level | 2 | 8 | $.039 \pm .002$ | $48.6 \pm 10.3$ |
| | 3 | 8 | $.049 \pm .002$ | $72.8 \pm 10.3$ |
| | 4 | 8 | $.058 \pm .002$ | $108.6 \pm 10.3$ |
| | 5 | 8 | $.062 \pm .002$ | $193.1 \pm 10.3$ |
| | | | $F_{3,3} = 20.11$ | $F_{3,3} = 15.00$ |
| | | | $P = 0.017$ | $P = 0.026$ |
| Sibship | 1 | 16 | $0.52 \pm .002$ | $95.2 \pm .007$ |
| | 2 | 16 | $0.52 \pm .002$ | $116.3 \pm .007$ |
| | | | $F_{1,24} = 0.02$ | $F_{1,24} = 1.92$ |
| | | | $P = 0.882$ | $P = 0.179$ |
| Food × sibship | | | $F_{3,24} = 1.11$ | $F_{3,24} = 5.44$ |
| | | | $P = 0.365$ | $P = 0.005$ |
| | | | Response of *Spea hammondii* | |
| Food level | 2 | 12 | $.048 \pm .001$ | $42.0 \pm 4.4$ |
| | 3 | 12 | $.058 \pm .001$ | $74.1 \pm 4.4$ |
| | 4 | 12 | $.060 \pm .001$ | $102.3 \pm 4.4$ |
| | 5 | 12 | $.064 \pm .001$ | $196.2 \pm 4.4$ |
| | | | $F_{3,6} = 16.56$ | $F_{3,6} = 74.99$ |
| | | | $P = 0.003$ | $P = 0.0001$ |
| Sibship | 1 | 16 | $.056 \pm .001$ | $98.7 \pm 3.8$ |
| | 2 | 16 | $.059 \pm .001$ | $103.5 \pm 3.8$ |
| | 3 | 16 | $.058 \pm .001$ | $108.7 \pm 3.8$ |
| | | | $F_{2,36} = 2.43$ | $F_{2,36} = 1.74$ |
| | | | $P = 0.102$ | $P = 0.190$ |
| Food × sibship | | | $2\ F_{6,36} = 1.83$ | $F_{6,36} = 3.96$ |
| | | | $P = 0.12$ | $P = 0.004$ |
| | | | Response of *Scaphiopus couchii* | |
| Food level | 2 | 12 | $.122 \pm .004$ | $15.0 \pm .002$ |
| | 3 | 12 | $.111 \pm .004$ | $19.3 \pm .002$ |
| | 4 | 12 | $.112 \pm .004$ | $27.0 \pm .002$ |
| | 5 | 12 | $.107 \pm .004$ | $32.3 \pm .002$ |
| | | | $F_{3,6} = 3.47$ | $F_{3,6} = 10.74$ |
| | | | $P = 0.091$ | $P = 0.008$ |
| Sibship | 1 | 16 | $.120 \pm .004$ | $22.2 \pm .001$ |
| | 2 | 16 | $.109 \pm .004$ | $26.7 \pm .001$ |
| | 3 | 16 | $.110 \pm .004$ | $21.3 \pm .001$ |
| | | | $F_{2,36} = 2.37$ | $F_{2,36} = 2.84$ |
| | | | $P = 0.108$ | $P = 0.071$ |
| Food × sibship | | | $F_{6,36} = 0.64$ | $F_{6,36} = 2.18$ |
| | | | $P = 0.698$ | $P = 0.068$ |

spadefoot toads of the North American southwestern deserts breed synchronously following heavy seasonal rains (Bragg 1965, Pomeroy 1981, Newman 1992). *S. couchii* conforms to this pattern, since it does almost all of its breeding on the night following a heavy summer shower that fills breeding pools. Rarely, some reproduction occurs the second night after the pools fill. In contrast, *Sp. hammondii* continues to breed over a period of two to three weeks following warm, late winter or early spring rains. *Sp. intermontana* can extend breeding over several weeks in spring and summer, and breeding is not necessarily associated with rainfall. The permanent habitats of *Sp. intermontana* reduce the risks of larval mortality due to drying, resulting in weaker selection for breeding synchrony than that experienced by *Sp. hammondii* and *S. couchii*. Breeding synchrony increases in *Sp. hammondii* and *S. couchii* in concert with declining pool duration. At the extreme in *S. couchii*, a delay of a single day can result in heavy larval mortality (Newman 1987, this study). There is thus a positive association between increased risk of pool drying and increased breeding synchrony, suggesting that pool duration and risk of larval death due to desiccation may be a significant factor that selects for breeding synchrony.

In spite of the differences among the three species in the age and size at metamorphosis, the general shape of the relationship between developmental stage and mass is similar in all three species (Fig. 4), suggesting that *S. couchii* is simply a speeded up version of the two larger forms. Arendt (1997) has argued that growth rate is an evolvable trait, but that there might be costs associated with an increase in growth rate or, more generally, that growth rate is associated with other features of development. In our case, he has hypothesized (Arendt, pers. comm.) that the rapid development of *S. couchii* might

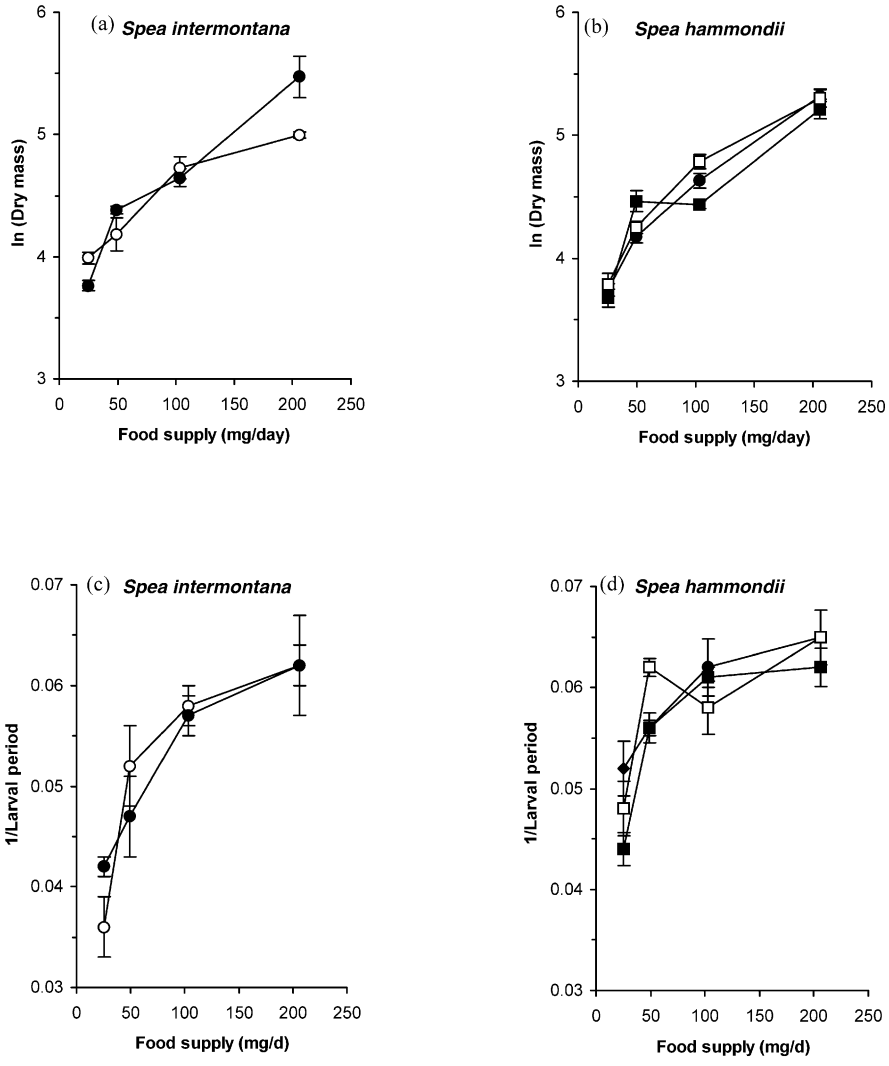


Fig. 7. Non-parallel univariate responses in developmental rate and dry mass (mg) at metamorphosis by different full-sibling families in *Sp. intermontana* and *Sp. hammondii* to illustrate the statistical interaction between sibship and food availability. Variation in growth rate was generated by manipulating food supply as indicated on the x-axis. Shapes of symbols indicate family identity. The symbols are means for sibships and the vertical bars show $\pm 1$ SE.

have been attained at the cost of reduced maximum growth rate.

*Sp. hammondii* and *S. couchii* also differ in their developmental plasticity in response to pool duration. In *Sp. hammondii*, larval period and mass at metamorphosis were positively correlated with pool duration. Delays in metamorphosis associated with increased pool duration result in larger size at metamorphosis, a feature related to higher fitness in temperate amphibians (Berven and Gill 1983, Smith 1987, Semlitsch et al. 1988, Berven 1990, Scott 1994). While this pattern suggests that *Sp. hammondii* is better adapted than *S. couchii* to exploit an unpredictable environment, other research demonstrates that such field observations mask important differences between these species. Denver (Denver 1997a, b, Denver et al. 1998) showed that *Sp. hammondii* can respond to a deteriorating environment by accelerating development. Morey and Reznick (2000) found that all three species accelerate development in response to a cessation of feeding if they have surpassed a developmental threshold. Individuals that had not yet exceeded the threshold entered developmental stasis. Those that have surpassed the threshold accelerate metamorphosis if growth conditions are poor, but defer metamorphosis and metamorphose at a larger body size if conditions are favorable for continued, rapid growth. Reduced food availability and reduced growth rates are usual consequences of the crowding associated with drying pools. *S. couchii* attained this threshold at an earlier stage of development and accelerated development more dramatically than the other two species. Its adaptations to a brief and uncertain larval environment are thus extraordinarily rapid development plus an enhanced ability to accelerate development in response to specific environmental cues.

In conclusion, larval ecology is correlated with and possibly a causal factor in selecting for many differ-

ences in the development of these larvae. This inference is based on the sampling of multiple breeding ponds for each species and the nature of the variation among these populations.

## Changes in the larval environment

Recent lab and enclosure studies have shown that amphibian larvae can alter development to reduce the time to metamorphosis in response to drying (Wilbur 1987, Semlitsch and Wilbur 1988, Crump 1989, Newman 1989, Denver et al. 1998, Doughty 2002). We measured the daytime temperature and chemical characteristics of pools during the period of larval development to identify factors that change in a predictable way as pools dry. One candidate is water temperature. We observed no consistent differences in daytime water temperature among pools occupied by *Sp. hammondii* and *S. couchii* in association with risk of drying. Other investigators (Zweifel 1968, Semlitsch and Wilbur 1988, Newman 1989) found that shallow and deep pools in the same region will have similar mean temperatures, but that shallow pools have greater daily fluctuations in temperature. Therefore, daily variance in temperature might provide a reliable cue of impending loss of habitat.

The water chemistry characteristics of the permanent aquatic habitats inhabited by larvae of *Sp. intermontana* were fairly stable compared to the temporary habitats of *Sp. hammondii* and *S. couchii*. In spite of the important effects of photosynthesis and respiration, the pH of the temporary pools was fairly stable through time. In contrast, the concentrations of ammonium nitrogen and calcium carbonate hardness tended to increase as larvae developed and pools dried. Increases in hardness are probably a simple consequence of drying. Increases in the concentration of ammonia are caused by excretion by spadefoot larvae. The levels of ammonia recorded in this study generally exceed the lethal levels reported for some freshwater fishes and are sufficient to produce a variety of pathological effects including inhibition of growth in fishes and freshwater invertebrates (Spotte 1979). The increases that were observed were very pronounced in the cases where drying was most extreme, so it is possible that these changes represent chemical cues to imminent drying of the larval habitat. It is also likely that there was an increase in other metabolites associated with pool drying.

Denver (1996, 1997a, b) and Denver et al. (1998) have found that *Sp. hammondii* accelerate development in response to a reduction in water depth, independently of any other cues. Doughty (2002) made similar observations for the Australian frog *Crinia georgiana*. Denver hypothesized that this response is mediated by an increase in the titre of corticotropic releasing hormone, which in turn modifies development. The inevitable increase in crowding associated with pool drying might enhance this stress response. Our observation of shorter larval periods and smaller sizes at metamorphosis in short-duration pools of *Sp. hammondii* is consistent with Denver's hypothesis.

In summary, increased daily variation in temperature, increased ammonia (and other metabolites), increased hardness, and decreased depth are all correlated with pool drying. Other variables considered by other authors are increased crowding and decreased growth rate. Any combination of these variables could serve as predictors of pool drying.

An interesting and unexpected observation with respect larval development relates to the late season storm filled pools utilized by *S. couchii* a second time. Pool duration was about the same, but the larvae transformed at less than half the size of those associated with the first filling. This observation raises the question of whether the earlier cohort of larvae reduced the productivity of the pools for the second filling by reducing the potential food supply (Seale 1980, Loring et al. 1988), or whether the quality of the second batch of larvae was influenced by the quality of the adult toads breeding during the second filling (Woodward 1987). These observations do not permit any explanation, but they do illustrate that pronounced variation in size at metamorphosis can occur in a single population.

## Laboratory results

The most striking aspect of our results is the contrast between the complete reaction norm for the three species versus the component of the reaction norm that is expressed in nature. All three species have a similarly shaped reaction norm when compared across a wide range of food availability (growth rates) in the laboratory (Fig. 4); however, *S. couchii* differs substantially from the other two species if we restrict our comparisons to just the conditions seen on our survey of natural populations (Fig. 6). In this sub-set of the data, *S. couchii* has little variation in the age at metamorphosis but substantial variation in the size at metamorphosis. The other two species display similar ranges of variation in both the age and size at metamorphosis.

The virtue of our comparative approach in combination with laboratory experiments and field observations is that it makes explicit how some of these differences in larval development evolved. The differences among species in how they respond to a natural range of variation in growth rate may represent an adaptive shift in the reaction norm of *S. couchii* relative to the two species of *Spea* since it preserves their high rate of development in an ephemeral environment. Low growth rates result in smaller metamorphs but no increase in development time. In the other two species, lower growth rates cause an increase in the age at metamorphosis and a decline in size at metamorphosis

(Fig. 6). Adaptation in *S. couchii* is thus manifested as a shift in the shape of the reaction norm (Gotthard and Nylin 1995, Doughty and Reznick 2002). Their adaptation to ephemeral environments also includes their more dramatic acceleration in development in response to a deteriorating environment and the earlier the threshold stage of development after which this acceleration is possible (Morey and Reznick 2000).

## Association between larval habitat permanence and plasticity in larval development

The fixed larval period of *S. couchii* thus increases the probability of successful metamorphosis in drying ponds. The decrease in size and increase in time to metamorphosis in the other two species is a common pattern seen in other species of anurans in which food supply, density, interspecific competition, or predation are manipulated (Morin 1983, Alford and Wilbur 1985, Alford and Harris 1988, Morin and Johnson 1988, Pfennig et al. 1991, Hensley 1993, Leips and Travis 1994, Leips et al. 2000, Doughty 2002). In this study, the species with the lowest risk of catastrophic loss of larval habitat, *Sp. intermontana*, has the largest proportional response in development rate, while *Sp. hammondii*, the species with intermediate risk, is somewhat less responsive than *S. intermontana*.

At the lowest food levels, which produced growth rates below those observed in nature, larvae of all three species showed a precipitous reduction in developmental rate (Fig. 4a). The general shape of the response profiles and the elevated mortality rates at the lowest food levels suggests that successful metamorphosis in the wild is unlikely at growth and developmental rates as low as those associated with the lowest food levels. At very high growth rates, developmental rates level off and apparently reach a rate that cannot be exceeded. This upper limit in development rate indicates that there is a minimum amount of time (about 16 days for *Sp. intermontana*, 14 days for *Sp. hammondii*, and 8 days for *S. couchii* ) in which larval development can be completed under the conditions of this experiment.

The rapid increase in mortality in *Sp. hammondii* and *S. couchii* larvae when reared at the lowest food levels also suggests a minimum mass for successful metamorphosis. Conversely, at the highest growth rates, mass at metamorphosis appears to asymptotically approach an upper limit. These limits are similar to the upper and lower size limits for metamorphosis (b and b + c) postulated by Wilbur and Collins (1973).

## Trade-off between age and mass at metamorphosis

The invariant development rate of *S. couchii* means that lower growth rates will always be tightly linked to smaller size at metamorphosis. In contrast, the decelerated rate of development in response to low growth rate in *Sp. hammondii* and *Sp. intermontana* results in metamorphosis at a later age but at a larger size than would be possible with a fixed rate of development, even if this size is smaller than that attained at higher growth rates. Larger size at metamorphosis has been linked to higher terrestrial fitness in spadefoot toads (Pfennig et al. 1991, Pfennig 1992a, Newman 1994a, Morey and Reznick 2001) and other amphibians (Berven and Gill 1983, Smith 1987, Berven 1990, Scott 1994). While such a delay could be disastrous for *S. couchii*, the other two species are not under such strict time constraints so they can take advantage of retarded development and become larger.

We contrast fixed versus flexible development rates in Fig. 8 by showing how much smaller *Sp. intermontana* would be if it had the fixed developmental response of *S. couchii*. We selected a low growth rate of 0.038 g/day because it falls in the range observed in natural populations of all three species. Fixed development in a hypothetical *Sp. intermontana* larva growing at 0.038 g/day

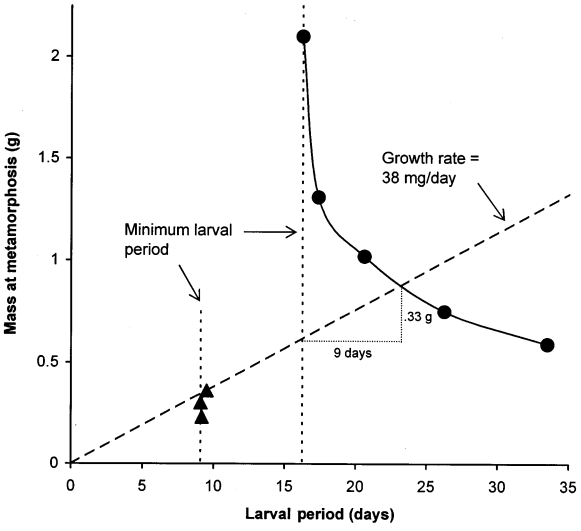


Fig. 8. Trade-offs between age and size at metamorphosis result from different shaped reaction norms for age and mass at metamorphosis. The concave curving reaction norm of *Spea intermontana* (circles) is contrasted with the vertical reaction norm of of *Scaphiopus couchii* (triangles). The patterns of live mass and larval period are actual results generated by rearing larvae at growth rates comparable to those observed in the wild (food levels 2–5 for *Spea intermontana* and food levels 3–5 for *Scaphiopus couchii* ). The diagonal dashed line indicates an average growth rate of 38 mg/day, a growth rate sometimes encountered by both species in the wild. Vertical lines indicate the age at metamorphosis for both species when the developmental rate is fixed at or near the minimum larval period. At the growth rate illustrated here, the curved shape of the norm of reaction for *Spea intermontana* allows larvae to transform at 0.83 grams in 25 days. The alternative fixed pattern would result in metamorphosis at a younger age (16 days) and smaller mass (0.50 grams).

results in metamorphosis at 0.50 g and 16 days, but flexible development results in metamorphosis at 0.83 g (a 63% increase) and 25 days (a 56% increase). The increase in development time may thus trade off with a proportionately larger increase in size at metamorphosis. More generally, the shape of the norms of reaction for age and size at metamorphosis at different growth rates for all three species (Fig. 4) suggests that the same sort of trade-off occurs at both low and high growth rates. A consequence of this profile shape is that, at high growth rates, the cost of a small increase in the age at metamorphosis is matched by the benefit of a large increase in the size at metamorphosis. At low growth rates, the cost of a small decrease in size at metamorphosis is paired with the benefit of a large decrease in the age at metamorphosis. This adaptive interpretation of the shape of the reaction norm was originally proposed by Wilbur and Collins (1973) and was further developed by Reznick (1990) with reference to age and size at maturity in guppies. The potential fitness consequences of this trade-off between age and size at metamorphosis represent a testable, but as yet untested hypothesis.

## A comparative view of the association between plasticity and larval environment

This association between the relative plasticity of age and size at metamorphosis and the larval environment is similar to Wilbur and Collins' (1973) prediction that the range of body sizes at metamorphosis will be larger in species that breed in more ephemeral habitats (their Fig. 10); this larger size range is presumably matched by a smaller range in age at metamorphosis. If reduced plasticity in development rate is an adaptation to risk of drying or a correlated response related to selection for rapid larval development, then similar patterns should be detectable within other anuran families. Not all of the available data are consistent with our results.

Blouin (1992) evaluated age and size at metamorphosis in *Hyla cinerea* (permanent pools), *H. gratiosa* (infrequent drying) and *H. squirrella* (frequent drying of larval habitat). Leips and Travis (1994, 2000) extended the work on just *H. cinerea* and *H. gratiosa*. None of these studies were consistent with our pattern because *H. squirella* and *H. gratiosa* both tended to have more plastic ages and sizes at metamorphosis than *H. cinerea*. Among the Ranidae, *R. sylvatica* (generally more ephemeral habitats), *R. pipiens* (longer-lived larval habitats) and *R. spenocephala* (temporary to permanent larval habitats) offer a gradient of larval environments. DeBenedictis (1974) found that the larval period of *R. sylvatica* varied little across a wide range of growth rates and did not vary consistently with growth rate. In contrast, increased growth rate caused a dramatic decrease in larval period in *R. pipiens* (DeBenedictis 1974). *R. spenocephala* had a relatively invariant developmental rate across a wide rage of higher growth rates, but in lower growth conditions developmental rate declines dramatically (Wilbur et al. 1983). Under low growth conditions, this species often overwinters as larvae (Morin 1983, Wilbur et al. 1983, Alford and Wilbur 1985). Interspecific comparisions in the genus *Rana* are thus more similar to our findings for *Scaphiopus* and *Spea*.

The available literature thus does not reveal a consistent association between larval habitat and the relative plasticity of age versus size at metamorphosis. The lack of consistent associations among species may be due in part to an inadequate description of the selective regime of the larval environment. Our studies (this paper, Morey and Reznick 2000), Newman's (1987, 1988a, 1988b, 1989), Pfennig's (1990a, 1990b, 1991) and Murphy's (2003) represent the only efforts that we are aware of to evaluate the statistical distribution of larval habitats for a given geographic range in concert with evaluations of plasticity in age and size at metamorphosis. Other authors have relied instead on general descriptions of the breeding environment. An association between the range of larval environments and larval development is necessary to evaluate the potential for a cause-and-effect relationship between the two and hence support the conclusion that these patterns of development represent adaptations to the larval environment (Reznick and Travis 1996). Secondly, the studies cited here were often executed for different purposes and utilized a diversity of methods, so they are not necessarily comparable. Finally, habitat permanence is only one factor that might influence the evolution of development rate or age and size at metamorphosis. Competition and predation also clearly play important roles (Wilbur 1987). The species of *Scaphiopus* and *Spea* we chose to study may yield a clearer pattern because habitat permanence plays such a dominant role in discriminating among the larval environments experienced by each species.

## Adaptation versus phylogenetic constraint

Buchholz and Hayes (2002) found a strong relationship between phylogeny and development rate in the Pelobatidae, including *Scaphiopus* and *Spea*, but an apparent absence of an association between development rate and the environment in which the frogs are found. *Scaphiopus* (*S. couchii* and *S. holbrookii* ) develop faster than *Spea* (*Sp. bombifrons*, *Sp. hammondii*, *Sp. intermontana* and *Sp. multiplicata*), yet *S. holbrooki* is found in the temperate eastern USA, whereas *Sp. hammondii* is found in the coastal hills of California, and the remaining species are found primarily in southwestern deserts. Our comparative study makes a strong case for the differences between *S. couchii* and the two

species of *Spea* being adaptive. How can we reconcile these interpretations?

First, a more detailed consideration of preferred larval habitats suggests that the differences in development rate may be adaptive. In spite of *S. holbrookii*'s being found in the temperate east coast, they select breeding sites that are very similar to those chosen by *S. couchii*, which are ephemeral pools in which there is high risk of death due to desiccation, but which are also not utilized by many other anurans and have a greatly reduced risk of predation (Bragg 1945, 1961, 1965, H. Wilbur, pers. comm.). Breeding in *S. holbrookii* can be delayed as much as seven days following rains (Gosner and Black 1955) and, in association with the very low water temperatures and lower evaporation rates that are sometimes encountered in the spring, the larval period can be long by *Scaphiopus* standards. Laboratory and outdoor enclosure larval periods of 10–60 days have been reported (Gosner and Black 1955, Semlitsch and Caldwell 1982, Morin 1983, Wilbur et al. 1983). Thus, *S. holbrookii* is similar to *S. couchii* in habitat choice and maximum development rate under standard conditions (Buchholz and Hayes 2002) and differ in the temperatures encountered during the breeding season. *Sp. bombifrons* and *Sp. multiplicata*, which are sympatric in portions of the southwestern deserts of the United States and may also co-occur with *S. couchii*, breed in pools that may last from only a few days to several months. Pools sometimes dry before larvae have completed metamorphosis (Pomeroy 1981, Pfennig 1990a, Simovitch et al. 1991). Pfennig (1990a) found that 4 of 37 natural pools used by *Sp. multiplicata* dried before tadpoles transformed, so their risk of desiccation was comparable to our observations for *Sp. hammondii*. They displayed a broad range of ages and sizes at metamorphosis (14–44 days and 12–29 mm Pfennig et al. 1991), which is again similar to *Sp. hammondii*. We have not been able to find similar data for desiccation risk for *Sp. bombifrons*, other than that their breeding ecology is similar to and sometimes overlapping with *Sp. multiplicata* (Simovitch et al. 1991, Pfennig 1992a), so they may face similar risks of death due to desiccation. The larval period is also quite plastic (13 days to several weeks, Simovitch et al. 1991). There thus appears to be a good correspondence between risk of desiccation and the patterns of development of the larvae.

More generally, invoking phylogenetic constraint as an explanation for anything incurs the risk of confusing correlation and causation. There is indeed a correlation between phylogeny and development rate in *Scaphiopus* and *Spea*, but there also appears to be a correlation between development and larval environment. To say that the species of *Scaphiopus* and *Spea* are similar within a sub-genus but differ among sub-genera because of phylogenetic constraint implies that the evolution of larval characteristics is limited by some unknown genetic mechanism. The limited quantitative genetic information on age and size at metamorphosis that is available (Berven et al. 1979, Berven and Gill 1983, Berven 1987, Travis et al. 1987, Newman 1988b, Blouin 1989, Simovitch et al. 1991, Pfennig 1992a) does not support the idea that the evolution of these traits is consistently constrained. The similarity in larval environments and patterns of development in *Scaphiopus* and *Spea* may instead suggest that they inherited both traits from a common ancestor. The reason that a *Scaphiopus*, rather than a *Spea*, was successful in invading a temperate region may be that it was preadapted to invade a larval environment that was similar to that of the southwest in being ephemeral with few competitors or predators. The phylogenetic signal may thus reflect a causal relationship between pre-existing qualities and opportunities for range expansion rather than some form of limitation in the ability to evolve. Any such correlation along phylogenetic lines is explainable by either a genetic constraint or by shared adaptations that promote successful invasion of new environments. Only more detailed genetic and adaptive analyses can discriminate among these alternatives.

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