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LARVAL PERIOD COMPARISON FOR THE SPADEFOOT TOADS *SCAPHIOPUS COUCHII* AND *SPEA MULTIPLICATA* (PELOBATIDAE: ANURA)

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ABSTRACT: The spadefoot toad *Scaphiopus couchii* lives in desert environments and has the shortest larval period known among anurans. We compared the larval period of *Sc. couchii* with that of a sympatric relative, *Spea multiplicata*, under identical laboratory conditions. It was possible that (A) *Sp. multiplicata* might metamorphose as fast as *Sc. couchii* when both taxa were reared under the same conditions, because larval periods are phenotypically plastic, or (B) *Sc. couchii* might metamorphose more quickly even under identical conditions due to taxon-specific physiological control of larval period length. We reared six clutches of *Sc. couchii* and *Sp. multiplicata* under laboratory conditions, varying in temperature, food type, and density to test for differences in growth and development between these taxa. Rearing conditions affected the larval period in both species, but within each condition, *Sc. couchii* developed 1.2–1.5 times faster and metamorphosed 4–6 days earlier than *Sp. multiplicata*. Also, *Sc. couchii* grew 2–3-fold slower in body and tail length and 10–20-fold slower in mass and metamorphosed at half the length and about 14% the mass compared to *Sp. multiplicata*. Because no rearing condition altered the rank order differences between taxa in growth and development, these taxa may differ in physiological mechanisms underlying larval period lengths. We discuss these consistent differences between taxa in terms of their physiological, ecological, and evolutionary significance.

Key words: Spadefoot toad; Larval period; Metamorphosis; Growth; Development

THE North American spadefoot toads *Scaphiopus couchii* and *Spea multiplicata* are sympatric, desert-adapted amphibians (Bently, 1966; Bragg, 1945; Low, 1976; Stebbins, 1985). The genera are sister taxa and are believed to have diverged in the Miocene during the formation of the southwestern deserts (Kluge, 1966). These taxa often share the same breeding pools, but *Scaphiopus* can also be found to breed in shallower pools than *Spea* (Bragg, 1965; personal observation). Comparing the larval periods of these two species may provide important implications for the evolution of life histories and metamorphic physiology in two closely related taxa with similar ecology.

Previous observations from natural breeding pools suggested that tadpoles of *Sc. couchii* are smaller than the tadpoles of *Sp. multiplicata* (Pfennig et al., 1991; Wright and Wright, 1949). In addition, both taxa develop from egg to juvenile remarkably quickly with a broad range of overlap (8–40 days versus 14–44 days, respectively) (Mayhew, 1965; Newman, 1989; Pfennig et al., 1991; Pomeroy, 1981; Wright and Wright, 1949). Larval period comparisons from these data may not be appropriate because of the potential for phenotypic plasticity. For example, these taxa may not have the same larval period length if reared under identical environmental conditions. Many environmental factors, such as temperature, tadpole density, food type, predators, and pond duration, greatly affect tadpole growth and development (Denver, 1997; Gromko et al., 1973; Hayes et al., 1993; Hota and Dash, 1986; Kupferberg, 1997; Newman, 1994; Steinwascher and Travis, 1983; Tejedo and Reques, 1994). Observations in which both species were present in the same natural pond suggested equivalent larval periods (Bragg, 1967), although dates of egg laying and measurements of tail resorption were not provided. Nevertheless, even for taxa growing in the same pond, differential microhabitat use, species competition, and differential predation may represent po-

tentially different conditions (Diaz-Paniagua, 1987; Loschenkohl, 1986; Smith and Van Buskirk, 1995; Tejedo, 1993).

Here, we asked whether the larvae of *Sc. couchii* and *Sp. multiplicata* differ in larval period length and size at metamorphosis under a range of controlled environmental conditions in the laboratory. The taxa were chosen for this study because they are closely related and sympatric. Also, these taxa share the same temperature tolerances (Brown, 1967), diets (Pomeroy, 1981), and pond ecology (Bragg, 1965). All pairs of both species were collected from the same locality (except one pair of *Sc. couchii* from a nearby locality) to reduce the chances of larval period differences due to adaptation to local environments. We chose environmental factors with known effects on fitness (temperature, density, and food type) to compare growth and development in these two taxa. We ruled out potential differences due to the environment, and reduced the possibility that observed differences in the larval periods would be due to differential response to a single growing condition. Consistent rank order differences between taxa across all laboratory conditions would suggest that these taxa evolved different strategies for surviving ephemeral desert pools. Lack of differences between taxa would suggest breeding pool choice by adults or microhabitat choice and behavior of larvae may explain size differences observed under natural conditions. We discuss potential physiological differences between taxa and why specific physiological changes may have evolved to underlie the differences between taxa.

Materials and Methods

Adult Care and Breeding

We collected five adult pairs of *Scaphiopus couchii* and six adult pairs of *Spea multiplicata* in the same ponds near Buenos Aires National Wildlife Refuge in Pima Co., Arizona on 3–5 July 1996. We collected an additional pair of *Sc. couchii* used for clutch 4 near Douglas, Cochise Co., Arizona on 20 August 1996. We maintained adults in covered plastic boxes (55 × 35 × 22 cm) in 7 cm of 50% utility sand and 50% potting soil and fed them twice per week with crickets dusted with $CaCO_3$. To induce breeding, we injected adults intraperitoneally once with 20–100 μl of 1 μg/100 μl GnRH agonist [des-Gly^{10},(D-His(Bzl)^{6})-luteinizing hormone releasing hormone ethylamide; Sigma] and placed them in 50-l tanks (50 × 30 × 40 cm) at 24 or 28 C with 20 l of filtered tap water or 10% Holtfreter's solution overnight. Several plastic strips, that mimicked natural egg deposition sites, were angled into the water at 45° from stiff plastic mesh at the water's surface. The morning after fertilization (day 1), we transferred embryos and water to 20-l tanks and aerated them. On day 3, when feeding began, we assigned tadpoles to experimental rearing conditions (for summary of all experimental conditions and sample sizes, see Table 1). Six clutches of tadpoles were reared from each species. Clutches 1 and 2 were obtained at the same time, and the other clutches (3, 4, 5, and 6) were obtained on separated breeding occasions. Tadpoles were reared in 10% Holtfreter's solution (except for clutches 1 and 2 which used filtered tap water to remove chlorine), and all feedings were ad libidum. Tadpole water was changed every other day with fresh 10% Holtfreter's solution (or tap water) acclimated to the rearing temperature. Tadpole tanks within each temperature room were kept on the same shelf to avoid the effects of temperature stratification. The photoperiod for all treatments was 12 h dark/12 h light (lights on at 0700 h).

Comparison of Temperature Effects

Three temperature-controlled rooms with water at 24 ± 1, 28 ± 1, and 32 ± 1 C were used to test the effects of temperature. We tested the effects of temperature using five clutches per taxon, and tadpoles from each clutch were reared at two or all three temperatures (Table 1). We reared tadpoles at 10, five, or one tadpole per tank and fed them fish food or rabbit chow, depending on clutch.

Comparison of Food Type Effects

The effects of food type were tested at 28 C by rearing tadpoles on three ad li-

TABLE 1.—Growing conditions, sample sizes, and conditions tested.

Clutch	Growing conditions					
	Temp. (C)	Food type	Density		Tanks/clutch	Condition tested
			Larvae/tank	Water volume		
Clutch 1, 2 (July 1996)	24	Fish food	10	51	3	Temperature
	28	Fish food	10	51	3	
Clutch 3 (Aug. 1996)	24	Fish food	10	51	3	Temperature
	28[a]	Fish food	10	51	3	
	32[b]	Fish food	10	51	3	
	28[a]	Fish food	10	51	3	Food type
	28	Rabbit chow	10	51	3	
	28	Spinach	10	51	3	
	32[b]	Fish food	10	51	3	Density
	32	Fish food	1	51	6	
Clutch 4 (Sept. 1996)	24	Rabbit chow	5	51	5	Temperature
	28[c]	Rabbit chow	5	51	5	
	32	Rabbit chow	5	51	5	
	28	Fish food	5	51	5	Food type
	28[c]	Rabbit chow	5	51	5	
	28	Spinach	5	51	5	
Clutch 5 (Nov. 1996)	28	Rabbit chow	1	51	6	Density
	28	Rabbit chow	5	51	3	
	28	Rabbit chow	10	51	3	
Clutch 6 (Feb. 1997)	24	Rabbit chow	1	51	10	Temperature
	28	Rabbit chow	1	51	10	
	32	Rabbit chow	1	51	10	

[a,b,c] Rows listed twice indicate use of same tanks in analysis of two growing conditions.

bidum diets: (1) fish food (TetraMin Large Flake Cichlid Food; Tetra, Morris Plains, New Jersey) which had 46% protein, 7% fat, and 2% fiber; (2) finely ground rabbit chow (Newman, 1994) which contained animal fat and had 14% protein, 1.5% fat, and 18.8% fiber; and (3) boiled spinach, which had 2.8% protein, 0.5% fat, and 3.3% carbohydrate (Weast, 1986). We tested the effects of food type using two clutches per taxon (Table 1). We reared tadpoles from each clutch on all three food types at 28 C at 10 or five tadpoles per tank, depending on clutch (Table 1).

Comparison of Density Effects

The effects of density were tested by rearing one, five, or 10 tadpoles in 5 l of water in mouse cages (28 × 18 × 12 cm). We tested the effects of density using two clutches per taxon (Table 1). We reared tadpoles at 28 or 32 C and fed them fish food or rabbit chow, depending on clutch (Table 1).

Measurements

Stage, mass, body length (snout–vent), and total length (snout–vent plus tail) were measured every other day for clutches 1–5 and daily for clutch 6. Stages 25–31, and 41–46 were assigned according to Gosner (1960). Stages 32–38 were assigned according to Gosner but made explicit for spadefoot toads (Busack and Zug, 1976). Stages 39 and 40 were assigned by the partial and complete degeneration of the cloacal tail piece, respectively. We monitored forelimb emergence (stage 42) every 12 h and tail resorption (stage 46) daily. Tadpoles and juveniles on the day of tail resorption were blotted and weighed to the nearest 0.01 mg. Body and total length were measured with a millimeter ruler with a measurement error <2 mm as diagrammed in Altig (1970).

Statistics

Data were analyzed using Statview and SuperANOVA statistical software (Abacus

Concepts, Berkeley, California). We tested for homogeneity of variances for all data for each analysis using the Equality of Variances *F* test in StatView. In the presence of significant heterogeneity, the data were natural log-transformed and tested for homogeneity of variances again. In the absence of significant heterogeneity, ANOVA was performed, and if significant heterogeneity persisted, a nonparametric Mann-Whitney or Kruskal-Wallis analysis was performed. First, the presence of significant differences in time to forelimb emergence, size at tail resorption, and growth and development rates between taxa was tested for each rearing condition. Next, a two factorial ANOVA with taxon and growing condition (temperature, food type, or density) was obtained to identify significant interactions. Then, effects of treatments were examined within taxon split by treatment. Scheffe post hoc tests were used to compare significant effects between treatments within taxa. We used three, five, six, or 10 tanks per taxon per treatment, and there was no significant tank effects within any treatment. All clutches were analyzed separately. In all treatments, mortality was <20% at forelimb emergence.

RESULTS

Comparison of Temperature Effects

Comparing taxa across temperatures, *Sc. couchii* metamorphosed significantly earlier than *Sp. multiplicata* (Fig. 1A,B). At 24 C, *Sc. couchii* metamorphosed 5.9 days earlier than *Sp. multiplicata* ($P < 0.0001$, $F_{1,24} = 505$). At 28 C, *Sc. couchii* metamorphosed 4.1 days earlier ($P < 0.0001$, $F_{1,36} = 482$). At 32 C, *Sc. couchii* metamorphosed 3.5 days earlier ($P < 0.0001$, $F_{1,46} = 751$). There was no significant taxon by temperature interaction for larval period length (Table 2). Within clutches for *Sc. couchii* and *Sp. multiplicata,* temperature had large effects on larval period lengths. For *Sc. couchii,* results of unifactorial ANOVA using ln-transformed data were $F_{2,70} = 521$, $P < 0.0001$, and Scheffe post hoc tests showed significant decreases in larval period length with increases in temperature ($P < 0.0001$ for each pairwise comparison of temperatures). Similar results for *Sp. multiplicata* were obtained. The temperature effects on larval period lengths in clutch 4 were observed for the other clutches in which effects of temperature were examined (clutches 1, 2, 3, and 6; data not shown).

Comparing across taxa, individuals of *Sp. multiplicata* were heavier and longer at tail resorption than *Sc. couchii* (Fig. 1C,D). At 24 C, *Sp. multiplicata* was 5.3 times heavier ($P < 0.0001$, $F_{1,41} = 3121$) and 1.8 times longer ($P < 0.0001$, $F_{1,41} = 999$) than *Sc. couchii.* At 28 C, *Sp. multiplicata* was 6.8 times heavier ($P < 0.0001$, $F_{1,35} = 1320$) and 1.9 times longer ($P < 0.0001$, $F_{1,35} = 1384$). At 32 C, *Sp. multiplicata* was 6.7 times heavier ($P < 0.0001$, $F_{1,44} = 1467$) and 1.8 times longer ($P < 0.0001$, $F_{1,44} = 1674$). Clutches 1, 2, 3, and 6 gave similar results (data not shown).

For mass and body length at tail resorption, the taxon by temperature interactions were not consistent. In clutch 4, there was a significant taxon by temperature interaction for both mass and body length at tail resorption (Table 2). Within clutch 4 of *Sc. couchii,* there was no significant effect of temperature on mass or body length at tail resorption. However, for *Sp. multiplicata* in clutch 4, mass increased significantly with increasing temperature ($P < 0.0001$, $F_{2,52} = 21.5$ and $P < 0.0001$ for all Scheffe pairwise comparisons), and body length was significantly higher at 28 C than 24 or 32 C ($P < 0.0001$, $F_{2,52} = 10.5$ and $P < 0.0001$ for all Scheffe pairwise comparisons). In clutch 3, no tadpoles of *Sc. couchii* survived to tail resorption, whereas in *Sp. multiplicata,* mass decreased significantly with temperature ($P < 0.0001$, $F_{2,79} = 8.9$ and $P < 0.0001$ for all Scheffe pairwise comparisons), and body length was not significantly affected by temperature ($F_{2,79} = 0.983$). In clutches 1 and 2, the significant interaction for mass ($P = 0.0225$, $F_{1,114} = 5.35$) was due to *Sc. couchii* increasing with temperature more than *Sp. multiplicata.* For body length in clutches 1 and 2, there was no significant taxon by temperature interaction because body length increased with temperature in

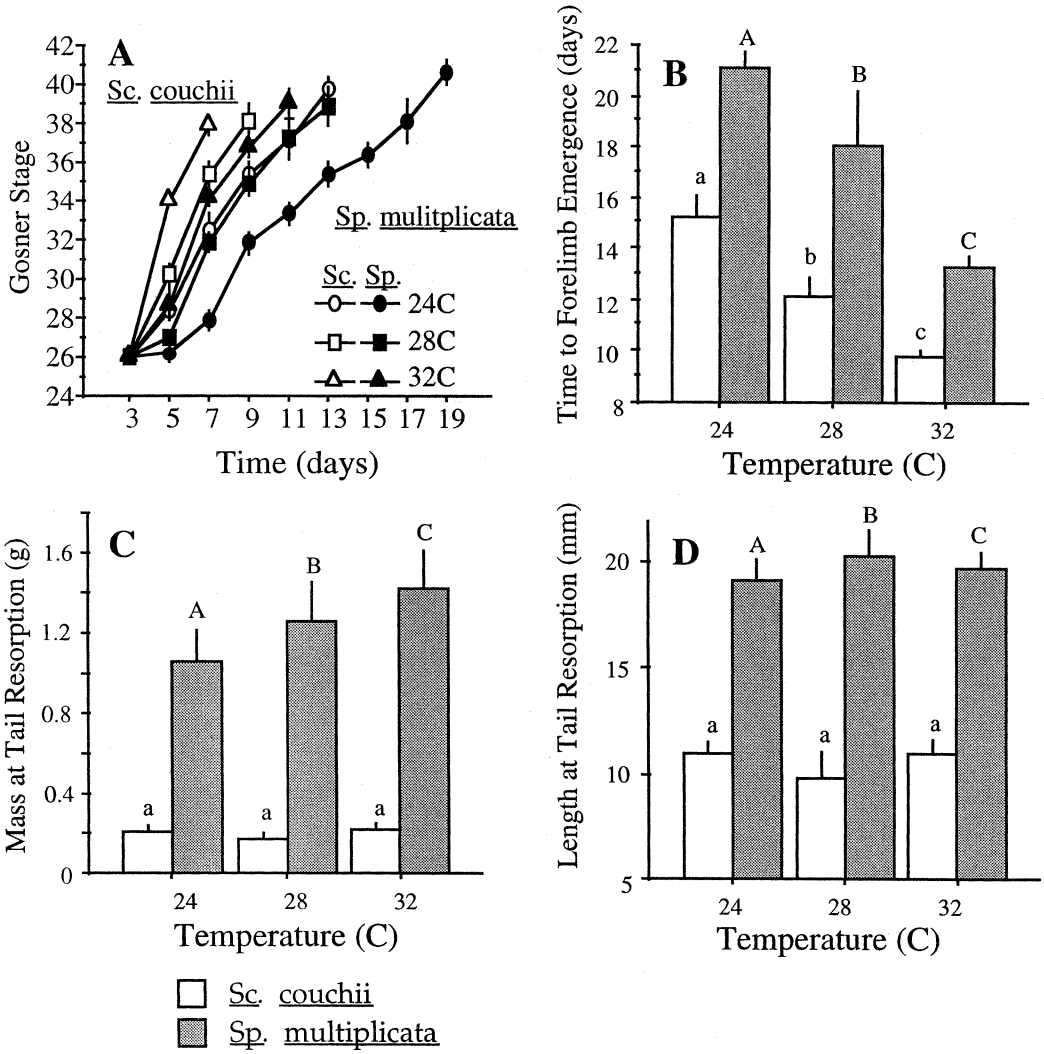

FIG. 1.—Comparison of temperature effects on *Sc. couchii* and *Sp. multiplicata*. (A) Developmental progression is compared as Gosner stage versus time for tadpoles grown at 24, 28, and 32 C. Temperature affected (B) time to forelimb emergence, (C) mass at tail resorption, and (D) and length at tail resorption. Data are from clutch 4 where each point shows the average and standard deviation from 25 individuals reared at five tadpoles/5-l tanks and fed rabbit chow ad libidum. ANOVA within each temperature showed that *Sc. couchii* was significantly different from *Sp. multiplicata* in time to forelimb emergence and mass and body length at tail resorption at $P < 0.0001$. ANOVA across each temperature split by taxon showed significant temperature effects. Big letters indicate significance groups across temperatures within *Sp. multiplicata*, and small letters indicate significance groups across temperatures within *Sc. couchii* at a significance level of $P < 0.0001$ as determined by Scheffe post hoc tests.

both taxa ($P < 0.0001$, $F_{1,56} = 39.0$ for *Sc.* and $P < 0.0001$, $F_{1,58} = 49.1$ for *Sp.*). In clutch 6, mass was not significantly affected in *Sp. multiplicata* (Kruskal-Wallis, $H = 5.594$, $P = 0.061$), but mass was significantly affected in *Sc. couchii* ($P = 0.0024$, $F_{2,22} = 8.033$). For body length in clutch 6, there was no significant taxon by temperature interaction because body length was not significantly affected by temperature in either taxon.

To obtain development and growth rates, 10 individuals were reared singly per taxon per temperature and measured daily

TABLE 2.—Analysis of variance table for the effects of temperature on ln-transformed time to forelimb emergence data (TTM), ln-transformed mass at tail resorption data, and untransformed body length at tail resorption data from clutch 4. See Table 1 for rearing conditions. S = source of variation, df = degrees of freedom, MS = mean square, F = F statistics, Tax = taxon, Tm = temperature, Res = residual.

	TTM				Mass				Body length			
S	df	MS	F	P	df	MS	F	P	df	MS	F	P
Tax	1	3.02	1656	0.001	1	100	3933	0.001	1	2376	3911	0.001
Tm	2	2.36	1293	0.001	2	0.344	13.6	0.001	2	4.5	7.4	0.001
T × T	2	0.002	0.94	0.392	2	0.204	8.1	0.001	2	6.6	10.8	0.001
Res	124	0.002			120	0.025			120	0.61		

until tail resorption (clutch 6). Development rate was calculated as the least squares slope of the linear portion (stages 27–46) of the stage versus time curves. We excluded stage 26 from these analyses where a lag in development between stages 26–27 occurred only in *Sp. multiplicata* (Figs. 3A, 4A). Developmental progression across time was linear (all r^2 values were >0.96) therefore justifying the use of the linear regression coefficient of stage versus time curves to compare taxa (Smith-Gill and Berven, 1979). *Scaphiopus couchii* developed about 1.5 times faster than *Sp. multiplicata* ($P < 0.0001$ for each temperature) (Fig. 2A). Higher temperatures significantly increased the rates of development within both taxa ($P < 0.0001$, $F_{2,23}$ = 157 for *Sc.*, $F_{2,22}$ = 495 for *Sp.*; $P < 0.0001$ for all Scheffe pairwise comparisons) (Fig. 2A). There was a significant taxon by temperature interaction (Table 3) because *Sc. couchii* increased faster than *Sp. multiplicata* across temperatures.

Growth rates were calculated as the slope of the linear portion (stages 27–38) of the mass, body length, and total length versus time curves. Although mass increased exponentially during the larval period, the growth rate was calculated as a linear function because r^2 values were rarely below 0.90 and never below 0.75. We excluded stage 26 from these analyses because a rapid increase in growth occurred between stages 26–27 only in *Sp. multiplicata* (Fig. 5A,B). *Spea multiplicata* increased significantly faster in mass than *Sc. couchii* (Fig. 2B). At 24 C, *Sp. multiplicata* increased 18 times faster than *Sc. couchii* ($P < 0.0015$, Mann-Whitney U' = 54). At 28 C, *Sp. multiplicata* increased 9.4 times faster ($P < 0.0002$, Mann-Whitney U' = 90). At 32 C, *Sp. multiplicata* increased 9.2 times faster ($P < 0.0006$, Mann-Whitney U' = 70). *Spea multiplicata* increased 2–3-fold faster than *Sc. couchii* in body length and total length ($P <$ 0.0001 for each temperature) (Fig. 2C,D), and there was no significant taxon by temperature interaction for rate of growth for body length or total length. Higher temperatures significantly increased the rates of growth within both taxa for mass ($P <$ 0.0001; $F_{2,23}$ = 19.8 for *Sc.* and $F_{2,22}$ = 31.1 for *Sp.*; $P < 0.0001$ for all Scheffe pairwise comparisons), body length ($P < 0.0001$; $F_{2,23}$ = 43.4 for *Sc.* and $F_{2,22}$ = 136 for *Sp.*; $P < 0.0001$ for all Scheffe pairwise comparisons), and total length ($P < 0.0001$; $F_{2,23}$ = 35.1 for *Sc.* and $F_{2,22}$ = 133 for *Sp.*; $P < 0.0001$ for all Scheffe pairwise comparisons).

Comparison of Food Type Effects

Food type had effects within and between taxa (Fig. 3A). Comparing across taxa, *Sc. couchii* always metamorphosed earlier than *Sp. multiplicata* (Fig. 3B). When fed fish food, *Sc. couchii* metamorphosed 5.8 days earlier than *Sp. multiplicata* ($P < 0.0001$, Mann-Whitney U' = 240). When fed rabbit chow, *Sc. couchii* metamorphosed 4.1 days earlier than *Sp. multiplicata* ($P < 0.0001$, Mann-Whitney U' = 325). When fed spinach, *Sc. couchii* metamorphosed 7.3 days earlier than *Sp. multiplicata* ($P < 0.0001$, Mann-Whitney U' = 475). Metamorphs of *Sp. multiplicata* were seven times heavier than *Sc. couchii* ($P < 0.0001$, Mann-Whitney U' = 120 when fed fish food, U' = 300 when fed rabbit chow, and U' = 400 when fed

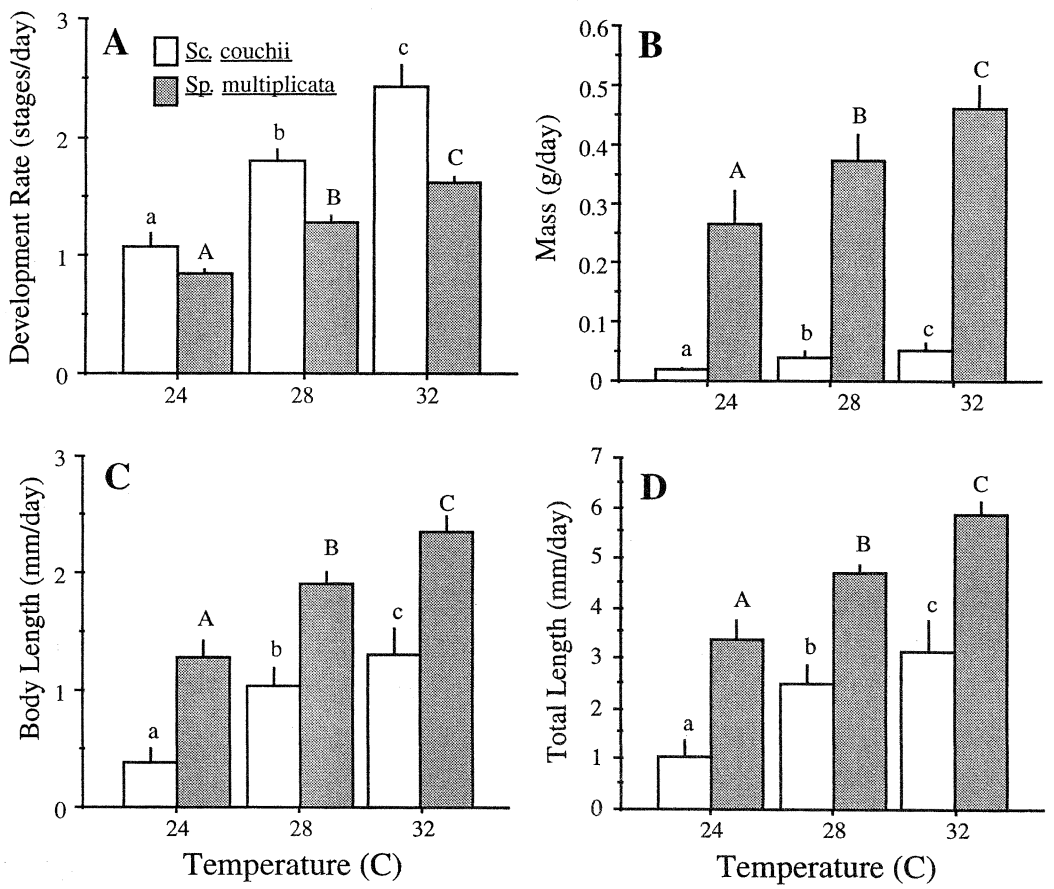

FIG. 2.—Comparison of tadpole development and growth rates between *Sc. couchii* and *Sp. multiplicata*. (A) Development rates are compared for tadpoles reared at 24, 28, and 32 C. Growth rates for (B) mass, (C) body length, and (D) total length were calculated from size versus time curves for tadpoles reared at 24, 28, and 32 C. Data are from clutch 6 where each point shows the average and standard deviation for 10 individuals reared singly in 5-l tanks and fed rabbit chow ad libidum. ANOVA within each temperature showed that *Sc. couchii* was significantly different from *Sp. multiplicata* in development and growth rates at $P < 0.0001$. ANOVA across each temperature split by taxon showed significant temperature effects. Big letters indicate significance groups across temperatures within *Sp. multiplicata*, and small letters indicate significance groups across temperatures within *Sc. couchii* at a significance level of $P < 0.0001$ as determined by Scheffe post hoc tests.

spinach) (Fig. 3C). Also, metamorphs of *Sp. multiplicata* were 2–3 times longer than *Sc. couchii* ($P < 0.0001$, $F_{1,20} = 1243$ when fed fish food, $F_{1,35} = 1383$ when fed rabbit chow, and $F_{1,39} = 1058$ when fed spinach) (Fig. 3D). Similar results were obtained in clutch 3 (data not shown).

Within taxa, food type significantly affected time to forelimb emergence in both taxa (Fig. 3B). In clutch 4, rabbit chow resulted in the shortest larval periods for both taxa and spinach the longest ($P = 0.0032$, Kruskal-Wallis $H = 11.5$ for *Sc.*; $P < 0.0001$, $H = 27.0$, for *Sp.*). In clutch 3, food type also had a significant effect on time to forelimb emergence, but in contrast to clutch 4, the taxa responded differently to food type. In *Sc. couchii*, rabbit chow and spinach gave similar larval periods whereas fish food resulted in longer larval periods. In *Sp. multiplicata*, rabbit chow and fish food gave similar larval periods whereas spinach gave longer larval periods (data not shown).

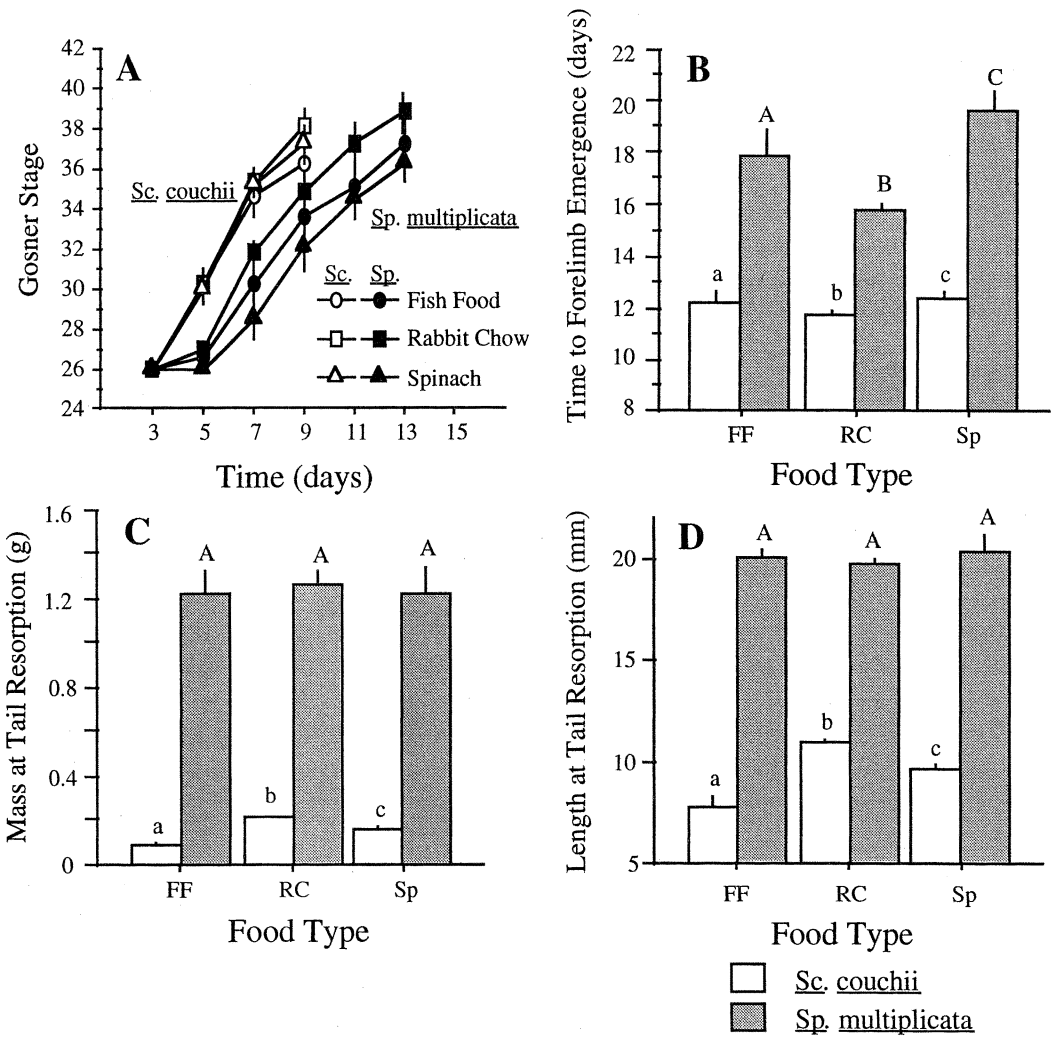

FIG. 3.—Comparison of food type effects on *Sc. couchii* and *Sp. multiplicata*. (A) Developmental progression is compared as Gosner stage versus time for tadpoles fed fish food, rabbit chow, and spinach ad libidum. Food type affected (B) time to forelimb emergence, (C) mass at tail resorption, and (D) length at tail resorption. Data are from clutch 4 where each point shows the average and standard deviation for 25 individuals reared at five tadpoles per 5-l tank at 28 C. ANOVA within each food type showed that *Sc. couchii* was significantly different from *Sp. multiplicata* in time to forelimb emergence and mass and body length at tail resorption at $P < 0.0001$. ANOVA across each food type split by taxon showed significant food type effects. Big letters indicate significance groups across food types within *Sp. multiplicata*, and small letters indicate significance groups across food types within *Sc. couchii* at a significance level of $P < 0.0001$ as determined by Scheffe post hoc tests.

At tail resorption, food type had no effect in *Sp. multiplicata* on mass ($P = 0.3623$, $F_{2,37} = 1.044$) or body length ($P = 0.4001$, $F_{2,37} = 0.939$) (Fig. 3C,D). In contrast, each food type gave significantly different results in *Sc. couchii*. For mass, rabbit chow resulted in the heaviest and fish food the lightest metamorphs ($P < 0.0001$, $F_{2,57} = 51.4$, $P < 0.0001$ for all Scheffe pairwise comparisons). Similarly, for body length, rabbit chow resulted in the longest and fish food the shortest metamorphs ($P < 0.0001$, $F_{2,57} = 93.1$, $P < 0.0001$ for all Scheffe pairwise comparisons) (Fig. 3C,D).

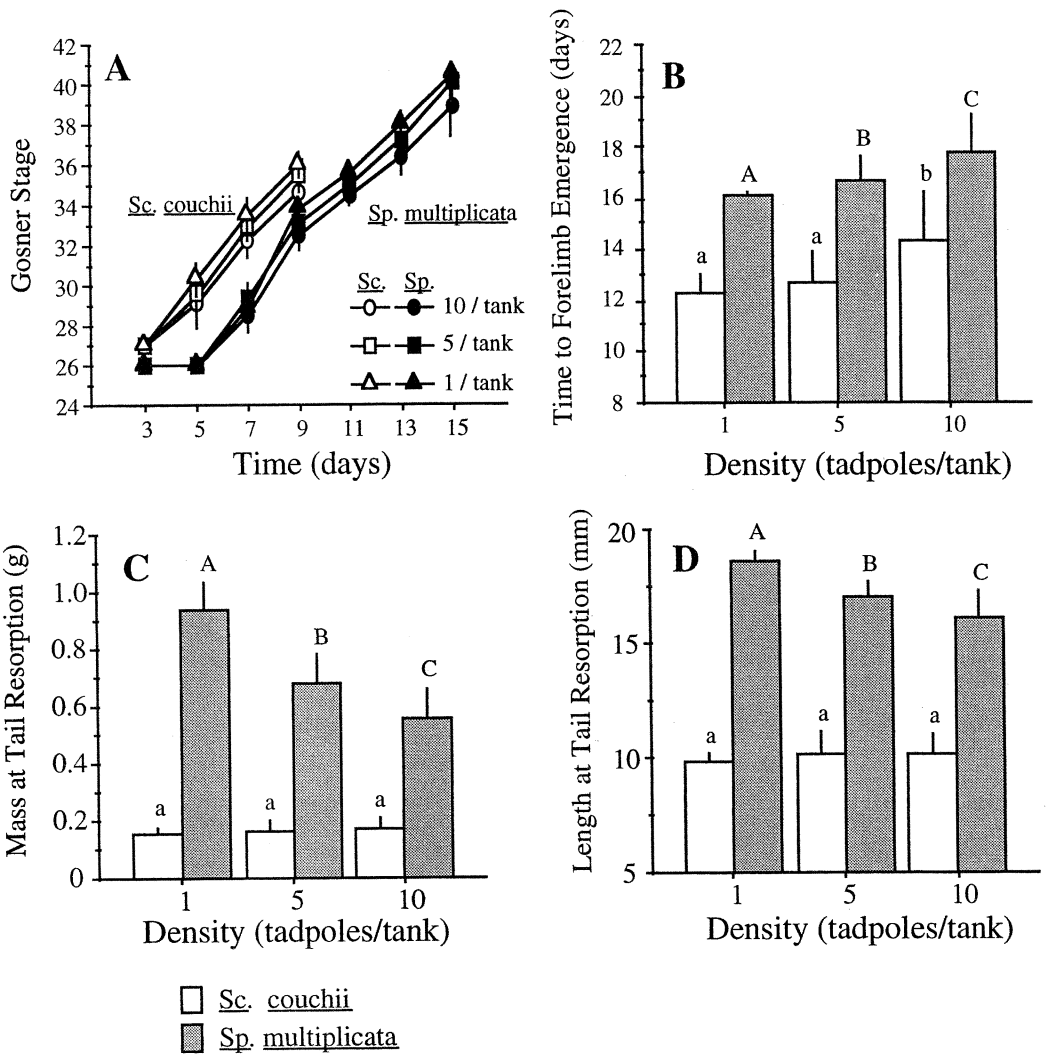

FIG. 4.—Comparison of density effects on *Sc. couchii* and *Sp. multiplicata*. (A) Developmental progression is compared as Gosner stage versus time for tadpoles reared at one, five, or 10 tadpoles per 5-l tank. Density affected (B) time to forelimb emergence, (C) mass at tail resorption, and (D) and length at tail resorption. Data are from clutch 5 where each point shows the average and standard deviation for 6–30 individuals reared at 28 C and fed rabbit chow ad libidum. ANOVA within each density showed that *Sc. couchii* was significantly different from *Sp. multiplicata* in time to forelimb emergence and mass and body length at tail resorption at $P < 0.0001$. ANOVA across each density split by taxon showed significant density effects. Big letters indicate significance groups across densities within *Sp. multiplicata*, and small letters indicate significance groups across densities within *Sc. couchii* at a significance level of $P < 0.0001$ as determined by Scheffe post hoc tests.

Clutch 3 gave different results. Rabbit chow resulted in significantly smaller metamorphs in mass and body length than fish food or spinach in *Sp. multiplicata*. In *Sc. couchii*, rabbit chow again resulted in larger metamorphs than spinach, but no tadpoles survived to tail resorption when reared on fish food (data not shown).

Comparison of Density Effects

Density affected larval period length within and between species (Fig. 4A). At all three densities (one, five, or 10 tadpoles/tank), the larval periods of *Sc. couchii* were four days shorter compared to *Sp. multiplicata* ($P < 0.0001$, $F_{1,10} = 123$ for one tadpole/tank, $F_{1,57} = 56.1$ for five

TABLE 3.—Analysis of variance table for the effects of temperature on ln-transformed development rate data from clutch 6. See Table 1 for rearing conditions and Table 2 for abbreviations.

Source	df	MS	F	P
Taxon	1	1.323	327	0.001
Temperature	2	2.085	516	0.001
T × T	2	0.026	6.44	0.004
Residual	45	0.004		

tadpoles/tank, $F_{1,28}$ = 93.5 for 10 tadpoles/tank) (Fig. 4B). There was no significant interaction, because both species had shorter larval periods at lower densities. For *Sc. couchii,* Scheffe post hoc comparisons revealed that the significant density effect (P = 0.0021, $F_{2,48}$ = 7.02) was due to shorter larval periods for one and five tadpoles/tank compared to 10 tadpoles/tank. For *Sp. multiplicata,* larval periods increased with increasing density (P = 0.003, Kruskal-Wallis H = 11.6). Taxon

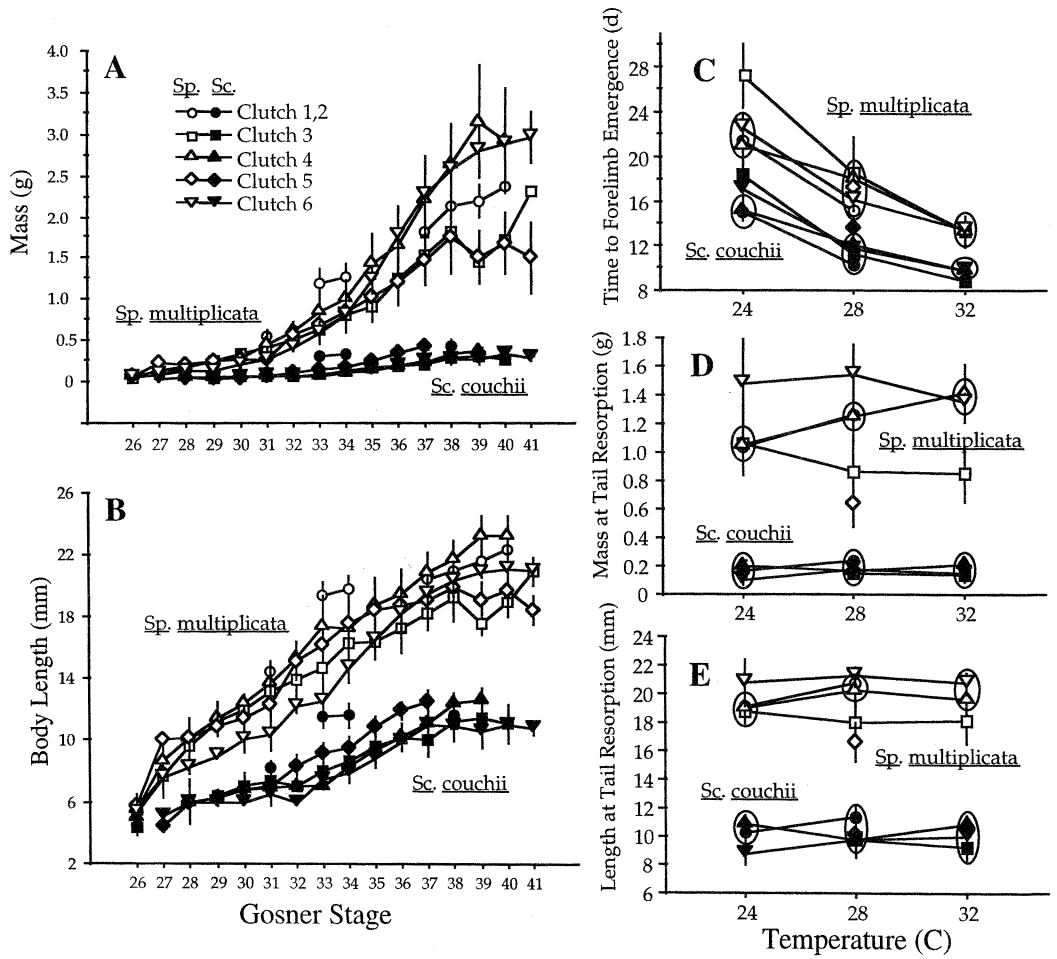

FIG. 5.—Comparison of the larval period and metamorphosis across clutches between *Sc. couchii* and *Sp. multiplicata.* Growth in (A) mass and (B) body length is compared across Gosner stage for tadpoles reared at 28 C across all six clutches. (C) Time to forelimb emergence, (D) mass at tail resorption, and (E) length at tail resorption are compared across all clutches for tadpoles reared at 24, 28, and 32 C. Each point shows the average and standard deviation of 6–90 individuals. The average incorporates all data from each clutch at each temperature, even though food type and density were sometimes different within temperature. In C, D, and E, uncircled data points and circled groups of data points represent significance groups based on Tukey-Compromise post-hoc tests at a significance level of $P < 0.01$.

differences were also observed in clutch 3, and there was a significant interaction effect. The larval period of *Sp. multiplicata* decreased with decreasing density ($P = 0.0205$, $F_{1,33} = 5.9$, $P < 0.0001$ for all Scheffe pairwise comparisons), whereas the significant effect of density on larval period of *Sc. couchii* ($P = 0.0061$, $F_{1,33} = 8.60$) was due to longer larval period at one tadpole per tank compared to five and 10 tadpoles per tank shown by Scheffe post hoc test (data not shown).

At all three densities, *Sc. couchii* was lighter and shorter than *Sp. multiplicata* at tail resorption (Fig. 4C,D). For mass, metamorphs of *Sp. multiplicata* were 6.1 times heavier than metamorphs of *Sc. couchii* reared singly ($P < 0.0001$, $F_{1,10} = 656$). At five tadpoles per tank, metamorphs of *Sp. multiplicata* were 4.2 times heavier ($P < 0.0001$, $F_{1,53} = 453$). At 10 tadpoles per tank, metamorphs of *Sp. multiplicata* were 3.3 times heavier ($P < 0.0001$, $F_{1,26} = 413$). For body length, metamorphs of *Sp. multiplicata* were 1.9 times longer than metamorphs of *Sc. couchii* reared singly ($P < 0.0001$, $F_{1,10} = 966$). At five tadpoles per tank, metamorphs of *Sp. multiplicata* were 1.7 times longer ($P < 0.0001$, $F_{1,53} = 378$). At 10 tadpoles per tank, metamorphs of *Sp. multiplicata* were 1.7 times longer ($P < 0.0001$, $F_{1,26} = 403$). There was a significant interaction for both mass and body length at tail resorption because lower densities led to a significant increase in size only in *Sp. multiplicata*. The repeat test of density effects in clutch 3 gave the same significant differences between species. However, there was no significant interaction because lower densities led to significant increases in size in both taxa.

DISCUSSION

Metamorphosing in as little as eight days, the spadefoot toad *Scaphiopus couchii* has the shortest larval period reported for any anuran (Bragg, 1961; Newman, 1989). Because tadpole growth and development are extremely phenotypically plastic (Alford and Harris, 1988; Hensley, 1993; Smith-Gill and Berven, 1979; Wilbur and Collins, 1973), one may hypothesize that tadpoles of any species grown under the same conditions as *Sc. couchii* would metamorphose as quickly. Alternatively, specialized developmental and physiological mechanisms may allow rapid metamorphosis in *Sc. couchii* compared to other species. We compared the larval periods of *Sc. couchii* and the related spadefoot toad *Spea multiplicata* to determine whether their larval periods differ when reared under comparable laboratory conditions. Our results established that these taxa differ in larval period length and size at metamorphosis and the plasticity of these traits in response to variations in the environmental variables tested. These results contribute to an understanding of the evolution of physiological systems and the concurrent life-history evolution.

Our rearing conditions were chosen so that we could address the question of larval period differences in the taxa under study. We reared tadpoles in the laboratory because identical conditions in nature are difficult to control due to daily temperature fluctuations, predators, and tadpole's choice of food types. The temperature range used (24–32 C) is within the range found in natural ponds (20–38 C) (Newman, 1989; Pomeroy, 1981). Because tadpoles of these species differ in size, we varied density within ranges used in previous experiments (Hall et al., 1997; Newman, 1994; Pfennig et al., 1991). Also, we varied food type because, in a preliminary study, we observed that *Sp. multiplicata* preferred fish food and *Sc. couchii* preferred spinach when both food types were given simultaneously. We used multiple rearing conditions to obtain results that are not dependent upon a single condition. Furthermore, our results apply to the locality where we collected the adults and may not extrapolate to the entire range of these taxa, because variation within species has been shown in other taxa (Pettus and Angleton, 1967, and references therein).

Within each laboratory condition tested, *Sc. couchii* developed faster and reached forelimb emergence earlier than *Sp. multiplicata*, whereas individuals of *Sp. multiplicata* grew faster and were larger at tail resorption. These results are so robust that combined data from all six clutches per

species for 28 C in the same analysis still resulted in significantly different larval periods even though density and food type were different between clutches (Fig. 5). In addition, there was no overlap in time to forelimb emergence or size at tail resorption between species within any growing condition. Thus, these populations of *Sc. couchii* and *Sp. multiplicata* differ in larval period characteristics.

Even though our results seem to contradict previous studies on these taxa which showed a large overlap in larval period length (Mayhew, 1965; Newman, 1989; Pfennig et al., 1991; Pomeroy, 1981; Wright and Wright, 1949), we saw overlapping larval period lengths if we compared taxa reared under different rearing conditions as in previous studies. For example, the development curve of *Sc. couchii* reared at 24 C overlaps the development curve of *Sp. multiplicata* reared at 28 C (Fig. 1A). Also, we found differences between pairs within species, as seen in other experiments on development time among sibships in *Sc. couchii* (Newman, 1988) and size differences among sibships in *Hyla gratiosa* (Travis, 1983). The reasons for significant differences between clutches in our studies are confounded because we cannot separate the effects of different food types, densities, parents, and clutch laying dates. However, the significant variation between clutches within taxa was much smaller than the variation between taxa signifying the robust differences between *Sc. couchii* and *Sp. multiplicata.*

Even though we reared tadpoles in the laboratory, the results were consistent with the development times observed under natural conditions. *Spea multiplicata* in our studies developed as fast as has been observed in other studies (Mayhew, 1965; Newman, 1989; Pfennig et al., 1991; Pomeroy, 1981; Wright and Wright, 1949). Also, even though *Sp. multiplicata* developed more slowly than *Sc. couchii,* its rate of growth was higher suggesting that the rearing conditions were not unfavorable for *Sp. multiplicata.* In addition, the rearing conditions were not unnaturally favorable for *Sc. couchii,* because such rapid metamorphosis has been observed under natural conditions (Newman, 1989). Thus, the large differences between the two taxa were not likely due to laboratory conditions that differentially favored one species over the other.

Not only did these taxa differ within all growing conditions, they responded differently across growing conditions. Temperature had a greater acceleratory effect on *Sc. couchii* than on *Sp. multiplicata* as shown by significant interaction in development rates, even though there was no taxa by temperature interaction for time to forelimb emergence. The basis for this difference between taxa is not known, but the results are in agreement with previous studies in which higher temperatures decreased the larval period (Hayes et al., 1993; Uhlenhuth, 1919). The effects of temperature on size at tail resorption were not consistent. Most often, there was no effect of temperature on mass or body length at tail resorption. Sometimes mass or body length increased with temperature, and in one case, mass increased with increasing temperature in clutch 4 for *Sp. multiplicata* (Fig. 5D). This observation contrasted with previous reports in which higher temperatures led to a decrease in size at metamorphosis (Hayes et al., 1993; Uhlenhuth, 1919). Within the same temperature, these taxa showed taxon-specific responses to density and food type. As seen in previous studies, lower densities resulted in increased size at metamorphosis compared to higher densities for both clutches of *Sp. multiplicata* (Gromko et al., 1973; Tejedo and Reques, 1994). A significant increase in size at metamorphosis at lower density was seen in only one of the two clutches in *Sc. couchii.* Similarly, the larval period decreased with decreasing density in *Sp. multiplicata* (Gromko et al., 1973; Tejedo and Reques, 1994). In contrast, the larval period of *Sc. couchii* reared singly was longer than larval periods at higher densities in clutches. This opposite effect of density has been seen when density was increased during the larval period; the larval period was shorter with higher density (Newman, 1994). The higher sensitivity of *Sp. multiplicata* to higher density likely reflects the larger tad-

pole size of this taxon. Both taxa consistently grew best on rabbit chow, but *Sp. multiplicata* tended to grow poorly on spinach and *Sc. couchii* grew poorly on fish food.

These plasticity differences between taxa may relate to their slight differences in ecology. *Scaphiopus couchii* tends to choose smaller, more ephemeral ponds than *Sp. multiplicata* (Bragg, 1965; personal observation). The smaller size may be advantageous in smaller pools where high densities may be commonplace. The greater response to temperature may be advantageous when small pools evaporate and reach higher temperatures. On the other hand, advantages for differences in diet are not clear. The deleterious effect of high protein fish food on *Sc. couchii* (the metamorphs died or were moribund) but not on *Sp. multiplicata* may relate to the ability of *Sp. multiplicata* to form carnivore morphs which consume animal prey (Pfennig, 1990; Pomeroy, 1981). Also, these taxa seem to direct energy resources differently, as suggested by the lag in development in *Sp. multiplicata* between stage 26 and 27. *Spea multiplicata* may commit more energy resources into growth during these stages, whereas *Sc. couchii* may emphasize development during these stages (compare Figs. 3A and 4A with Fig. 5B). The growth and development rate calculations gave significantly different rates between species even though they did not include these stages. Nevertheless, this early stage may be an important time in development to pursue differences in metamorphic physiology to explain differences between taxa.

This work also raises interesting physiological and evolutionary questions. The underlying mechanisms explaining taxon differences may relate to endocrine physiology. Hormonal mechanisms that can alter the larval period within a taxon (Dodd and Dodd, 1976) could differ between taxa. Another possibility is that developmental events not related to hormonal control may underlie differences between taxa, such as development rate prior to differentiation of the thyroid gland. To date, no experiments have shown a physiological or developmental mechanism to explain larval differences between any pair of anuran taxa. The differences in time to and size at metamorphosis suggest that these taxa may have independently evolved different larval strategies for surviving in deserts. Pond laying ecology may relate to larval period length in a syndrome of traits working together, including pond size preference, interspecific competition, and predator avoidance.

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