Determinants of Metamorphic Timing in the Black-bellied Salamander, *Desmognathus quadramaculatus*

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Abstract - We used two experiments to test the hypothesis that variation in growth rate, temperature, and thyroid hormone exposure will induce variation in metamorphic timing in the Black-bellied Salamander, *Desmognathus quadramaculatus* (Holbrook). In one experiment, second-year larvae (i.e., those approaching a metamorphic summer) were treated with high or low food and exposed to high or low temperature. Low temperature resulted in delayed metamorphosis, while food regime had no effect on metamorphic timing. In a second experiment, first-year larvae (i.e., those not expected to undergo natural metamorphosis during the experiment) were grown at two temperatures and treated with thyroid hormones or control supplements. Larvae at low temperature grew more slowly. Larvae treated with thyroid hormone failed to show any sign of metamorphosis compared to control larvae.

Introduction

Stream-dwelling salamanders of the family Plethodontidae have extremely long larval periods (8–60 months), and many are found in eastern North America in association with the cool, upland streams of Appalachia (Beachy and Bruce 1992, Dunn 1926, Petranka 1998, Wilder and Dunn 1920). Within-stream variation in both food and temperature regimes is less than in ephemeral, high-productivity pools and ponds where other kinds of larval amphibians develop. However, among-stream variation in temperature and productivity regimes can affect duration of larval period (Juterbock 1990, Voss 1993). Stream-dwelling plethodontids exhibit interspecific and intraspecific geographic variation in duration of the larval period, and these differences contribute to the extreme variation in adult body size within the family (Bruce 1988, Camp et al. 2000, Ryan and Bruce 2000).

Two environmental sources of population differences in metamorphic timing are stream productivity and stream temperature. Geographic variation in productivity can result in population-specific growth rates with possible effects on metamorphic timing. In general, larval amphibians of similar genetic background that grow at differ-

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ent rates will metamorphose at different times (Alford and Harris 1988; Beachy 2001; Beachy et al. 1999; Hensley 1993; Newman 1994, 1998; Ryan 1998). Plethodontids, however, may represent an exception to this rule; in three experimental studies of growth, larvae of the Ocoee Salamander, *Desmognathus ocoee* Nicholls (Beachy 1995a), Blue Ridge Two-lined Salamander, *Eurycea wilderae* Dunn (Beachy 1997), and Four-toed Salamander, *Hemidactylium scutatum* (Schlegel) (O'Laughlin and Harris 2000), grown at different rates failed to metamorphose at different times.

Temperature can also affect metamorphic timing. Individuals that experience colder temperatures have longer larval periods and consequently metamorphose at larger sizes (Beachy 1995a, Leips and Travis 1994, Uhlenhuth 1919). Thus, geographic variation in temperature can result in population differences in metamorphic timing. For example, tadpoles of the Green Frog, *Rana clamitans melanota* (Rafinesque), from ponds at higher elevations have longer larval periods than conspecifics from lower elevations that are warmer (Berven et al. 1979), and larvae of the salamander *Eurycea wilderae* from higher order streams have longer larval periods than those from headwater streams (Voss 1993). In both of these species, animals that metamorphose later also do so at larger sizes because they grow for an additional year.

A likely intrinsic organismal source of population difference in metamorphic parameters is the timing of thyroid activity. From an endocrine perspective, metamorphosis is under control of the thyroid hormones, thyroxine and triiodothyronine (Etkin 1968). Thyroid secretion of these hormones is, in turn, regulated by the hypothalamus-pituitary axis (Denver et al. 2002, Rosenkilde and Ussing 1996). Three observations suggest that intraspecific variation in larval period could be mediated by geographic variation in timing of thyroid hormone secretion. First, paedomorphic Northwestern Salamanders, *Ambystoma gracile* (Baird), from a high elevation lake had weakly-developed median eminences (the vascular connection between the hypothalamus and pituitary), suggesting that tissues that could otherwise have undergone metamorphosis were not exposed to thyroid hormones (Eagleson 1976; Eagleson and McKeown 1978a,b). Second, it is likely that all salamanders (including paedomorphic species) make the nuclear receptors for thyroid hormones (Safi et al. 1997a, 1997b, 2004; Voss et al. 2000). Third, Rose (1995a,b) has shown that even young larval *Eurycea bislineata* (Green) can be induced to partial metamorphosis by immersion in thyroid hormones. The possibility that geographic variation in larval period is due to variation in timing of thyroid hormone secretion has not been explored.

Using two experiments, we tested the hypothesis that variation in growth rate, temperature regime, and thyroxine exposure will induce variation in metamorphic timing in the Black-bellied Salamander, *Desmognathus quadramaculatus* (Holbrook), a plethodontid with a long larval period. This species is found in the Appalachian Mountains from southern West Virginia, and south of the Tennessee Valley Divide in the Allegheny Mountains in Virginia, southward to northern Georgia. The species lives in cool, rapidly flowing water, at elevations from 490–1700 m (Hairston 1949, Organ 1961, Petranka 1998). Throughout its range, *D. quadramaculatus* varies in timing of metamorphosis (range 24–48 months), and this variation may, in part, explain geographic variation in adult body size (Beachy and Bruce 2003, Bruce 1988, Bruce et al. 2002, Camp et al. 2000). Furthermore, the genus *Desmognathus* is remarkable because species differences in body size are correlated with length of larval period: *D. quadramaculatus* has the longest larval periods and is the largest member of the genus; in contrast, the miniaturized *D. wrighti* King and *D. aeneus* Brown and Bishop lack free-living larvae (Dunn 1926, Organ 1961, Petranka 1998).

Methods

Larval *D. quadramaculatus* (range 13–29 mm length from tip of the snout to the posterior margin of the cloacal vent [SVL]) were collected from an elevation of 730–790 m in October 1998 from Gott Farm Creek, a tributary of Shelton Laurel Creek. At Gott Farm Creek, *D. quadramaculatus* metamorphose at either 24 or 36 months (Beachy and Bruce 2003). The site is located in the Bald Mountains, NC (White Rock Quadrangle, 35°58'05"N, 82°41'40"W). The animals were immediately shipped from the collection site to the laboratory at Minot State University, Minot, ND. Upon receipt of animals, individuals were partitioned into two experiments based on body size: larger larvae (≥ 20 mm SVL) were used for Experiment 1 and smaller larvae (< 20 mm SVL) were used for Experiment 2 (see explanation below).

Experiment 1

We tested the effects of two levels of larval growth rate and temperature on metamorphic size and metamorphic timing in a 2 X 2 factorial design. Eight larvae were randomly assigned to each of four treatments. Larvae were exposed to 7 °C or 11 °C and fed four tubificid worms once each week (the high food groups) or once every other week (the low food groups). This experiment was conducted using large larvae (20–29 mm SVL) because larger larvae are generally older and these larvae were likely to be approaching a metamorphic summer (Beachy and

Bruce 2003). Initial size of larvae was not significantly different across treatments ($F = 0.66$; df = 3,28; $P = 0.59$).

On 13 November 1998, each salamander was placed in an individual plastic box (24 x 24 x 6 cm LWH) with a snap-on lid. Each box contained 400 g of a sand/gravel substrate and 450 ml of distilled water. Water was replaced each week. The salamanders were placed in one of two environmental chambers (7 °C and 11 °C) with ambient light exposure. Beginning 1 April 1999, temperatures in the environmental chambers were raised to simulate typical rising stream temperatures. The temperatures were raised 1 °C every two days until chamber temperatures were 11 °C and 15 °C.

All animals were weighed to the nearest mg at the beginning of the experiment and every 30 days thereafter. The salamanders were also weighed upon completion of metamorphosis. Metamorphosis was defined as the completion of gill resorption. Data for initial mass and metamorphic mass were log-transformed, and data for metamorphic timing (number of days from the beginning of the experiment to metamorphosis) were inverse-transformed (Alford and Harris 1988) to reduce heteroscedasticity.

A two-factor multivariate analysis of covariance (MANCOVA) was used to evaluate the effects of temperature and growth rate on metamorphic date and metamorphic mass. Initial mass was used as a covariate in order to compensate for individual variation in size at the beginning of the experiment. Subsequent univariate analysis of covariance (ANCOVA) on metamorphic date and mass was performed only when MANCOVA indicated a significant treatment effect. In all analyses, the significance criterion was set at $\alpha = 0.05$, and Wilks' lambda was chosen as the test statistic. Where significant treatment effects were detected, we compared treatment means using Scheffe's a posteriori group contrasts (Sokal and Rohlf 1995).

Experiment 2

On 13 November, 32 first-year larvae (range 13–19 mm SVL [Beachy and Bruce 2003]) were weighed and placed individually in plastic boxes (8 x 3 x 4 cm LWH) with 110 g of sand, 100 g of gravel, and 250 ml distilled water. Larvae were randomly assigned to one of the four treatment groups. Initial size did not differ signicantly among treatment groups ($F = 0.89$; df = 3,28; $P = 0.47$). One-half of the animals were placed in one environmental chamber at 7 °C, and the rest were placed in another environmental chamber at 11 °C. Animals were fed brine shrimp nauplii and tubificid worms ad libitum. On 9 April 1999, temperature increases were administered (see Experiment 1). On this same date, one-half of the animals were immersed in a 1.2 nM thyroxine solution. The thyoxine treatment was

administered weekly (at the same time as water changes occurred) until the termination of the experiment. The concentration of the thyroxine solution was doubled every second week until final thyroxine concentration was 4.8 nM. These exogenous thyroxine concentrations were chosen so that only a small supplement would be provided. Our exogenous thyroxine concentration is between those found endogenously in premetamorphic salamanders and salamander larvae undergoing metamorphosis (Alberch et al. 1986, Larras-Regard et al. 1981). Rose (1995a, 1995b) found that immersion with 5 nM thyroxine is sufficient to accelerate metamorphosis in premetamorphic *Eurycea bislineata*.

This experiment was conducted on smaller larvae in their first year, which would not be approaching metamorphosis naturally (i.e., premetamorphic larvae). Our intent was to determine if metamorphosis can be accelerated by hormonal treatment, to test the hypothesis that populations may differ in metamorphic timing simply because thyroid hormones are being released at different times in life.

We weighed the animals every 30 days and at metamorphosis or upon termination of the experiment on 10 June 1999. At metamorphosis or termination of the experiment, each animal was killed by prolonged immersion in 1% MS-222 solution, preserved in 10% formalin, and stored in 70% ethanol. After preservation, each animal was measured for snout-vent length (SVL) and scored for metamorphic progress: 1 = no metamorpic characters evident, 2 = developing nasolabial groove evident and labial fold still visible, 3 = labial fold absent, and 4 = gill loss and gill slit closure complete (i.e., metamorphosis). Data were analyzed using a two-way multivariate analysis of covariance (MANCOVA) with two levels of each factor (high vs. low temperature, thryoxine supplement vs. control supplement). Initial mass of each animal was included as a covariate in the MANCOVA. All variables were log-transformed for analysis. In all analyses, the significance criterion was set at $\alpha = 0.05$, and Wilks' lambda was chosen as the test statistic. Where significant treatment effects were detected, we compared treatment means using Scheffe's a posteriori group contrasts (Sokal and Rohlf 1995).

Results

Inspection of the treatment group growth profiles confirmed that feeding treatments had expected results, i.e., larval *D. quadramaculatus* in high food groups grew faster (Fig. 1). The distinction between food treatment groups was more pronounced at cooler temperature. In addition, larvae at lower temperatures also

grew more slowly (Figs. 1 and 2).

Experiment 1

All larvae survived to the termination of the experiment on day 283, which was 13 August 1999. On this date, all but four larvae had metamorphosed. These remaining larvae were from the cold temperature treatment, showed no metamorphic progress, and were not used in the analysis.

MANCOVA indicated a significant effect of food and temperature on the metamorphic mass/date response vector (Table 1). Larval *D. quadramaculatus* in cooler temperatures had longer larval periods (Figs. 1 and 3). Larval periods ranged from 148 to 281 d in cold temperature treatments and from 120 to 212 d in high temperature. While temperature did not produce significant variation in metamorphic size, there is a suggestion that the effect is complex and contingent upon growth rate (i.e., Temperature X Food, Table 1). In the high food treatment, animals at low temperature metamorphosed larger than those at high temperature. In contrast, in the low food treatment, animals at low temperature metamor-

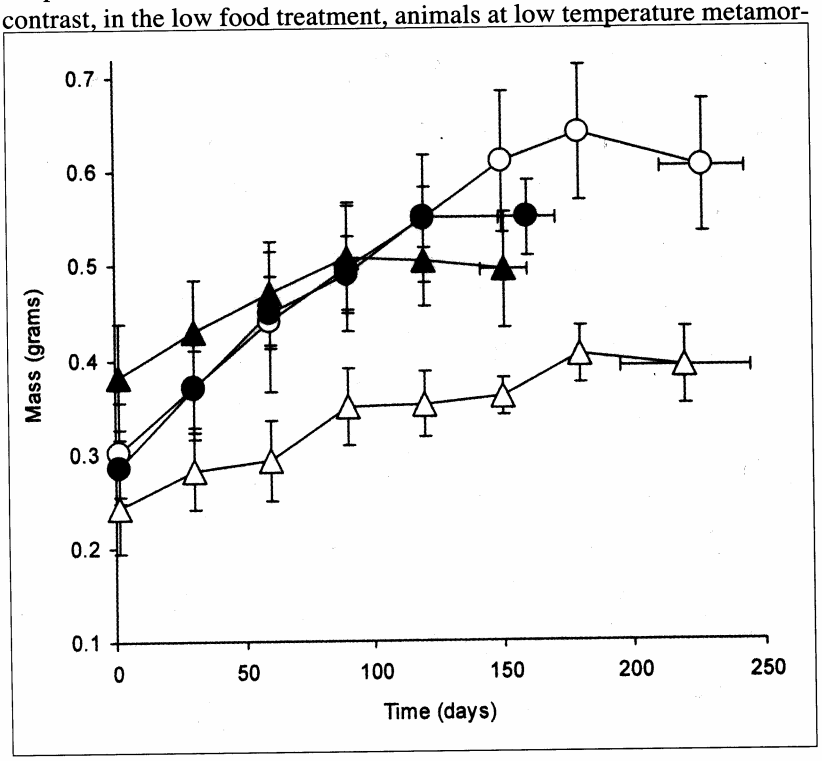

Figure 1. Growth profiles of larval *Desmognathus quadramaculatus* in Experiment 1 (see text for details). Profiles terminate at treatment means for metamorphic timing and size. Closed circles = 11–15 °C and high food; open circles = 7–11 °C and high food; closed triangles = 11–15 °C with low food; and open triangles = 7–11 °C with low food. Symbols indicate means "+" or "-" 1 SE.

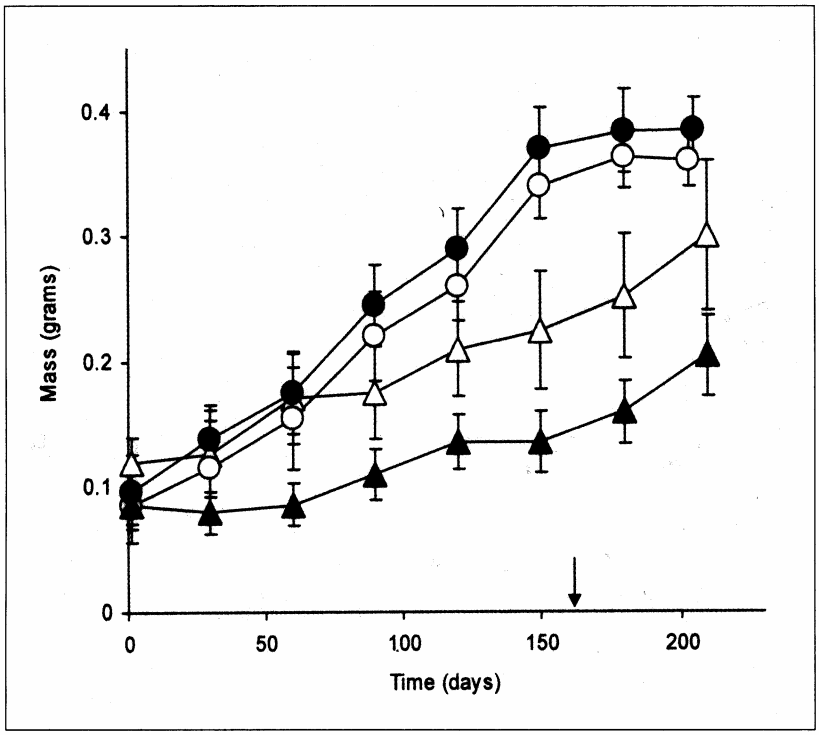

Figure 2. Growth profiles of larval *Desmognathus quadramaculatus* in Experiment 2 (see text for details). Early termination of high temperature trajectories indicates metamorphosis by some larvae; no low temperature larvae metamorphosed. Closed circles = 11–15 °C; open circles = 11–15 °C with thyroxine supplement; closed triangles = 7–11 °C; and open triangles = 7–11 °C with thyroxine supplement. Symbols indicate means "+" or "-" 1 SE. Arrow symbol indicates date of initial application of thyroxine.

Table 1. Summary of MANCOVA and ANCOVA of inverse-transformed metamorphic date and log-transformed initial and metamorphic (or final) mass of *Desmognathus quadramaculatus* using initial mass as covariate. Mean squares are x 10^{-3} for the metamorphic date and x 10^{-2} for metamorphic mass, and degrees of freedom are 2,21 for the multivariate analyses and 1,22 for the univariate analyses.

				Univariate analysis					
				Metamorphic date			Metamorphic mass		
	Multivariate analysis			Mean			Mean		
Source	Wilks' λ	*F*	*P*	square	*F*	*P*	square	*F*	*P*
Temperature	0.342	20.21	< 0.001	1.66	41.50	< 0.001	0.17	1.55	0.230
Food	0.159	55.49	< 0.001	0.01	0.25	0.621	12.90	117.27	< 0.001
Temp. x food	0.823	2.26	0.129	0.06	1.50	0.219	0.34	3.09	0.095
Initial mass	0.101	92.95	< 0.001	1.61	40.25	< 0.001	17.60	160.00	< 0.001
Residual				0.04			0.11		

phosed at smaller sizes than animals at high temperature (Figs. 1 and 3).

As expected, salamanders on high food treatment were larger at metamorphosis. However, food treatment had no effect on larval period (Table 1). Salamanders in the high food, high temperature treatment did not have different larval periods than salamanders in the low food, high temperature treatment. This effect is the same in low temperature groups: those in the high food treatment did not metamorphose at different times than those in the low food treatment (Figs. 1 and 3).

Initial mass had significant effects on both variables (Table 1). Regression of larval period and metamorphic mass on initial mass indicated that larval *D. quadramaculatus* that were larger at the beginning of the experiment metamorphosed earlier and at larger sizes (metamorphic date: $r^2 = 0.43$, df = 26, $P < 0.001$; metamorphic mass: $r^2 = 0.51$, df = 26, $P < 0.001$).

Experiment 2

Twenty-three animals survived to the termination of this experiment, and 11 animals from warm temperature treatments metamorphosed (= developmental score of 4) before the termination of the experiment. MANCOVA indicated a significant effect of temperature on the mass/SVL/score response vector (Table 2). Animals at low temperatures grew more slowly and few animals in low temperature treatments showed any metamorphic progress (Fig. 4). Thyroxine treatment caused no significant variation in any variables (Table 2).

Initial mass had significant effects on mass and SVL, but not on

Table 2. Summary of MANCOVA and ANCOVA on log-transformed size (SVL), initial mass, ending mass, and developmental score in Experiment 2. Mean squares are x 10^{-2} for SVL, x $10^{-1}$for mass, and x 10^{-1} for developmental score. Degrees of freedom are 3,16 for multivariate analyses, and 1,18 for the univariate analyses.

Multivariate analysis

Source	Wilks' λ	*F*	*P*
Temperature	0.19	21.83	< 0.001
Thyroxine	0.86	0.88	0.474
Temperature x Thyroxine	0.91	0.50	0.685
Initial mass	0.46	6.32	0.005

Univariate analysis

	SVL			Mass			Developmental score		
Source	Mean square	*F*	*P*	Mean square	*F*	*P*	Mean square	*F*	*P*
Temperature	2.67	38.14	< 0.001	23.70	37.03	< 0.001	10.40	34.67	< 0.001
Thyroxine	0.09	1.29	0.266	0.18	0.28	0.605	0.25	0.83	0.370
Temp. x thyr.	0.10	1.43	0.246	0.79	1.23	0.279	0.08	0.27	0.603
Initial mass	1.26	18.00	< 0.001	13.20	20.63	< 0.001	0.04	0.13	0.725
Residual	0.07			0.64			0.30		

developmental score (Table 2). Regression of final mass and SVL on initial mass indicated that larvae that were larger at the beginning of the experiment were larger at the termination of the experiment (mass: r^2 = 0.25, df = 21, P = 0.016; SVL: r^2 = 0.22, df = 21, P = 0.023).

Discussion

For amphibian species that have a larval stage, metamorphosis is a critical event that can affect the time and size of the animal at first reproduction; e.g., an animal that is large at metamorphosis can reach maturity faster and at a larger size than conspecifics that metamorphose at smaller size (Semlitsch et al. 1988, Smith 1987). While an increase in

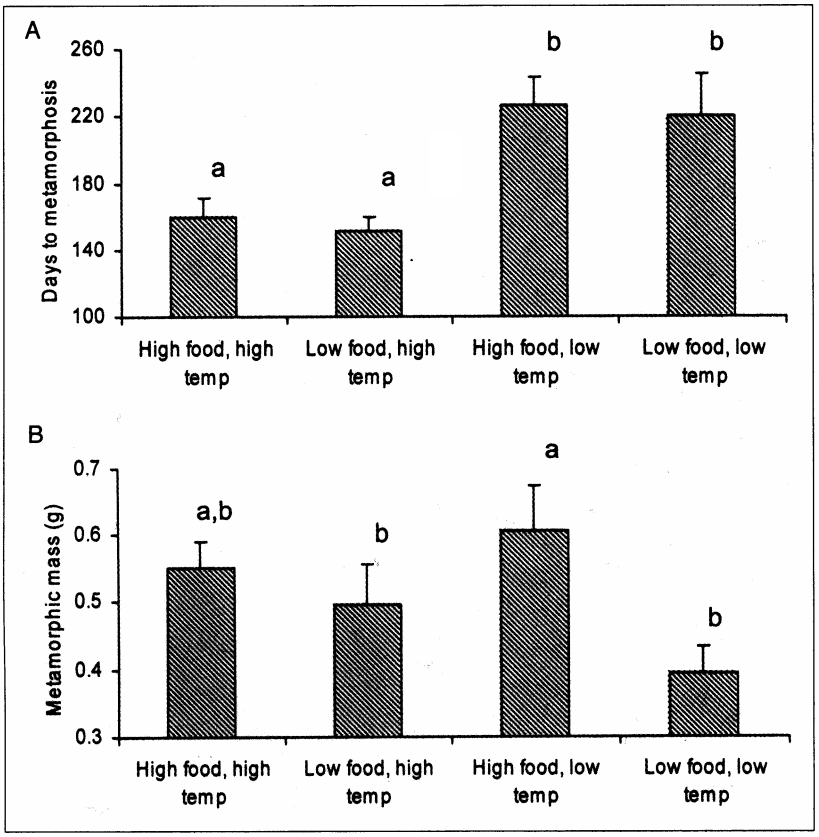

Figure 3. Summary of responses of larval *Desmognathus quadramaculatus* to treatments in Experiment 1 (see text for details). (A) Days from beginning of treatment until metamorphosis is completed (assayed as complete resorption of gills). (B) Mass at metamorphosis. Bars represent means + 1 SE. Letters above bars indicate treatment group means not significantly different using Scheffe's a posteriori contrasts.

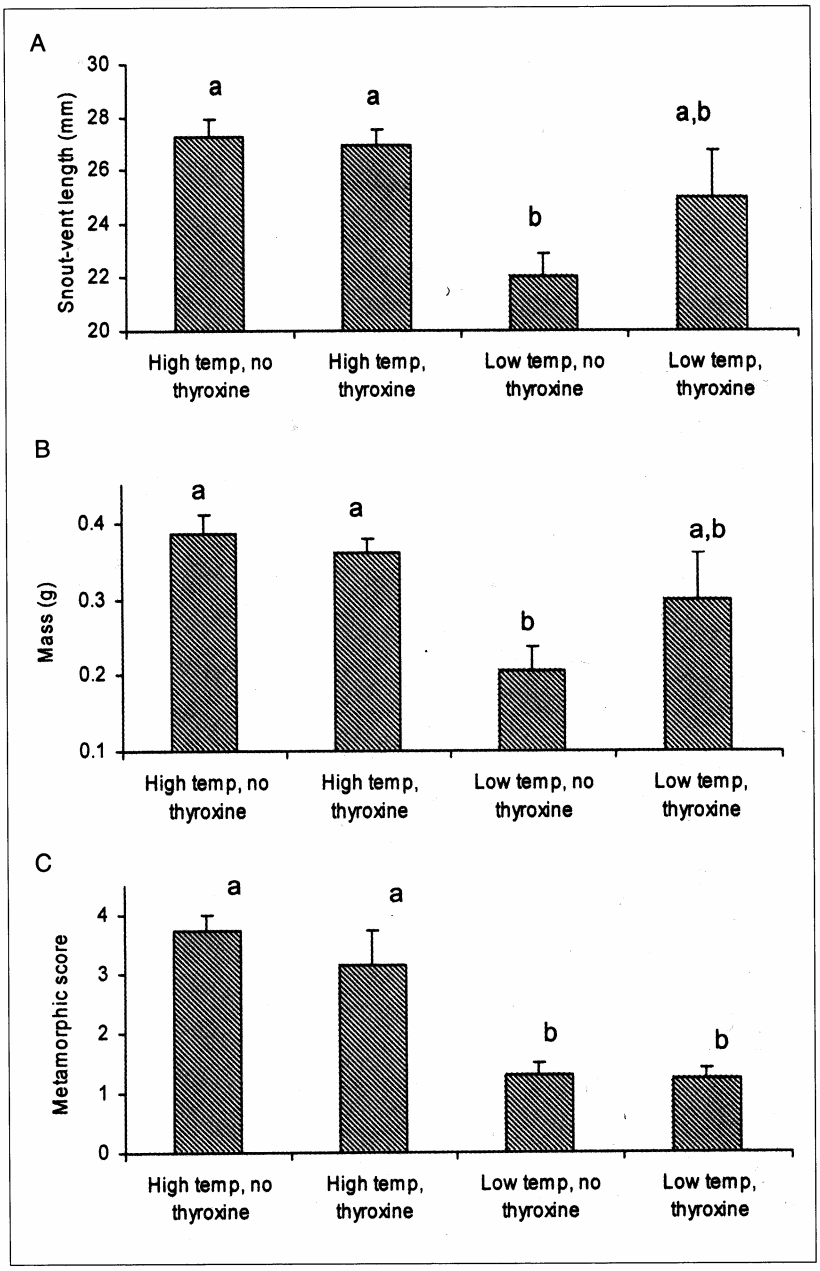

Figure 4. Summary of responses of larval *Desmognathus quadramaculatus* to treatments in Experiment 2 (see text for details). (A) Snout-vent length, (B) mass, and (C) metamorphic score (see text) of animals at termination of the experiment. Bars represent means + 1 SE. Letters above bars indicate treatment group means not significantly different using Scheffe's a posteriori contrasts.

growth has the predictable result of increasing size at metamorphosis, three novel ideas concerning determinants of metamorphosis emerge from our experiments: food variation does not result in variation in metamorphic timing in *D. quadramaculatus*; lower temperatures delay metamorphosis; and immersion in thyroxine fails to induce metamorphosis in young (premetamorphic) *D. quadramaculatus* larvae. Further, these data suggest that the interaction of temperature and food could produce significant variation in metamorphic mass.

Variation in growth rate

Variation in food abundance in laboratory manipulations of growth rate in many larval amphians results in variation in metamorphic timing (Alford and Harris 1988; Beachy et al. 1999; Hensley 1993; Leips and Travis 1994; Newman 1994, 1998; Ryan 2000). However, this study corroborates an emerging pattern: metamorphic timing in plethodontid larvae is unaffected by growth rate (Beachy 1995a, 1997; O'Laughlin and Harris 2000).

Wilbur and Collins (1973) proposed that variation in growth rate should result in variation in metamorphic timing. After competence to metamorphose is attained (e.g., minimal size threshold), metamorphosis should be initiated upon a reduction in mass-specific growth rate. This decrease in growth is suggested to act as a transducer of deteriorating environmental quality (e.g., an increase in predator-threat or competition, and/or a drying pond). In contrast, larvae that grow at consistent rates should continue to grow until a maximal size threshold is attained whereupon metamorphosis is obligatory (Wilbur and Collins 1973).

This model predicts that three kinds of metamorphosing larvae may exist at a single locality: larvae that metamorphose early and at small size (due to a reduction in growth rate), larvae that metamorphose at the maximal size threshold (due to consistent and rapid growth), and those that metamorphose later and at the maximal size threshold (due to consistently slow growth). We found that larval *D. quadramaculatus* with different growth rates metamorphose at the same time (at different sizes). While other models may make different predictions concerning timing and size at metamorphosis (e.g., Day and Rowe 2002, Rowe and Ludwig 1991, Smith-Gill and Berven 1979, Werner 1986), our results are not consistent with any theoretical model.

The models of amphibian metamorphosis cited above were developed to explain within-population variation in larval period in amphibians using highly productive yet ephemeral ponds that experience higher temperatures than the streams occupied by larval plethodontids. Several authors (e.g., Bernardo and Reagan-Wallin 2002) have suggested that

the failure of general ectotherm life history models to predict metamorphic timing and size may be related to this habitat dichotomy. The phenotypic plasticity in metamorphic timing and size so often inferred to have adaptive value in ephemeral, high-productivity habitats may entail costs that are not economical in the permanent, low-productivity larval environments experienced by most plethodontid larvae (Beachy 1995a, 1997; Bernardo and Reagan-Wallin 2002).

Lower temperature delays metamorphosis

The impact of temperature on metamorphic timing was similar to the temperature effects seen in other plethodontid larvae. Lower temperature results in later oviposition (Bruce 1982, Voss 1993), and slower growth and development (Beachy 1995a, this study). The consequence is that larvae that experience cooler larval habitats metamorphose later, a pattern seen in other amphibians that utilize more productive and more ephemeral habitats (e.g., Berven 1982, Berven et al. 1979, Bizer 1978, Sexton and Bizer 1978, Smith-Gill and Berven 1979). In a review of temperature-based models of life history variation, Bernardo and Reagan-Wallin (2002) noted that the pattern of later metamorphosis at larger sizes in pond-breeding amphibians (e.g., *Rana* and *Ambystoma*) at high latitudes or elevations is contradicted by studies on larval plethodontids. Some species of the plethontid genera *Desmognathus*, *Pseudotriton*, and *Eurycea* metamorphose at larger sizes at lower latitudes and elevations (Bernardo and Reagan-Wallin [2002] did not discuss age at metamorphosis). Such a pattern is not thought to conform to "general rules" of ectothermic vertebrates (e.g., Smith-Gill and Berven 1979), and calls into question the utility of such temperature-based models. Our results suggest that a consideration of how temperature regimes and growth rate may interact could be instructive. At high food levels, larval *D. quadramaculatus* at low temperature metamorphosed later and at larger sizes than larvae at high temperature, consistent with the predictions of Smith-Gill and Berven (1979). In contrast, at low food levels, larval *D. quadramaculatus* metamorphosed at smaller sizes in the low temperature treatments compared to the high temperature treatments, in agreement with the observations for plethodontid larvae noted by Bernardo and Reagan-Wallin (2002).

Given Bernardo and Reagan-Wallin's (2002) critique of these "general rules" of ectothermic development, it seems essential to have data for the specific stream temperature regimes experienced by plethodontid larvae. Generally, the assumption is that a negative correlation exists between elevation (or latitude) and stream temperature (e.g., Bernardo and Reagan-Wallin 2002, Camp et al. 2000). However, Voss (1993) has shown that streams at the *same* elevation can have

different temperature regimes and that it is the thermal regimes due to stream order (rather than elevation) that results in variation in metamorphic age in *E. wilderae*: first order streams are cooler in summer but warmer in winter than higher-order streams (Voss 1993).

Furthermore, how variation in temperature regime interacts with variable rates of growth and development is not well understood (Leips and Travis 1994). In the stream-dwelling *Desmognathus ocoee* Nicholls, the complex interaction of different food levels and different temperatures results in a more dramatic cold-induced delay of metamorphosis at high food levels than at low food levels (Beachy 1995a). Coupled with the results on metamorphic size suggested in our results, we suggest that the pattern of larger size at higher elevations may be less apparent in stream-dwelling plethodontids than in amphibians that utilize warmer and more productive larval habitats. While this does not refute the idea of differing patterns of allocation among environments that differ in productivity, a consideration of the complex interaction of food and temperature may be sufficient to explain patterns of variation in age and size of metamorphosing amphibians.

Effect of thyroxine immersion

There was no effect of thyroxine on metamorphic score. Usually, application of thyroxine to a metamorphic species (including the stream-dwelling larval plethodontid *E. bislineata*) causes (at least) partial metamorphosis (Brown 1997; Etkin 1968; Rose 1995 a,b; Rosenkilde and Ussing 1996). The lack of induced metamorphosis in these premetamorphic *D. quadramaculatus* is difficult to explain. However, it is similar to results seen with the paedomorphic salamanders *Necturus maculosus* (Rafinesque) and *Proteus anguinus* Laurenti, which are insensitive to immersion in thyroid hormones despite the production of nuclear receptors for thyroid hormones (Safi et al.1997b). Assuming that larval *D. quadramaculatus* express thyroid hormone receptors, the lack of inducible metamorphosis is due to an interruption of the signal transduction chain prior to thyroid hormone reception at the nucleus. Thus it appears unlikely that differences in timing among populations of *D. quadramaculatus* are due to differential timing of thyroid hormone secretion.

Plethodontid salamanders exhibit a greater range of life history and body size variation than any other comparable taxon of tetrapods. Because age at metamorphosis, age at maturation, size at metamorphosis, and adult body size are positively covarying, e.g., larger species have longer larval periods and are larger at metamorphosis (Beachy 1995b, Ryan and Bruce 2000, Tilley and Bernardo 1993), it is important to identify the causal mechanisms involved in covariation of these traits. The genus *Desmognathus* is of special

interest because it includes species with extremely long larval periods (and large body size, e.g., *D. quadramaculatus*) and those with direct-development (*D. wrighti* and *D. aeneus*, which are among the smallest tetrapods). At the Bald Mountains (the locality from which our animals were drawn), *D. quadmaculatus* are smaller than anywhere else in their range because metamorphosis and maturation occur earlier here than in other populations (Beachy and Bruce 2003). Our results indicate that variation in metamorphic timing at this locality is due to variation in thermal regime, and is not influenced by food/growth rate.

Local variation in metamorphic timing is probably due to spatial and temporal thermal heterogeneity experienced by these larvae. Can thermal heterogeneity explain geographic variation in larval periods in *D. quadramaculatus*? Camp et al. (2000) performed an analysis of life history features from throughout the range of this species and found that elevation and its complex relationship with local precipitation were the principal determinants of larval period. This is consistent with our results and suggests that thermal variation can play a significant role in generating geographic patterns in larval period. Because metamorphic timing can have direct impacts on fitness (Semlitsch et al. 1988, Smith 1987), geographic variation in temperatures of larval environments may significantly influence adult body size.

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Literature Cited

Alberch, P., E.A. Gale, and P.R. Larsen. 1986. Plasma T_4 and T_3 levels in naturally metamorphosing *Eurycea bislineata*. General and Comparative Endocrinology 61:153–163.

Alford, R.A., and R.N. Harris. 1988. Effects of larval growth history on anuran metamorphosis. American Naturalist 131:91–106.

Beachy, C.K. 1995a. Effects of larval growth history on metamorphosis in a stream-dwelling salamander (*Desmognathus ochrophaeus*). Journal of Herpetology 29:375–382.

Beachy, C.K. 1995b. Age at maturation, body size, and life-history evolution in the salamander family Plethodontidae. Herpetological Review 26:179–181.

Beachy, C.K. 1997. Effects of predatory larval *Desmognathus quadramaculatus* on growth, survival, and metamorphosis of larval *Eurycea wilderae*. Copeia 1997:131–137.

Beachy, C.K. 2001. Effects of growth history and exogenous thyroxine on size and age at metamorphosis in the toad *Bufo americanus*. Copeia 2001:829–834.

Beachy, C.K., and R.C. Bruce. 1992. Lunglessness in plethodontid salamanders is consistent with the hypothesis of a mountain stream origin: A response to Ruben and Boucot. American Naturalist 139:839–847.

Beachy, C.K., and R.C. Bruce. 2003. Life history of a small form of the plethodontid salamander *Desmognathus quadramaculatus*. Amphibia-Reptilia 24:13–26.

Beachy, C.K., T.H. Surges, and M. Reyes. 1999. Effects of developmental and growth history on metamorphosis in the Gray Treefrog, *Hyla versicolor*. Journal of Experimental Zoology 283:522–530.

Bernardo, J., and N.L. Reagan-Wallin. 2002. Plethodontid salamanders do not conform to "general rules" for ectotherm life histories: Insights from allocation models about why simple models do not make accurate predictions. Oikos 97:398–414.

Berven, K.A. 1982. The genetic basis of altitudinal variation in the Wood Frog *Rana sylvatica*. II. An experimental analysis of larval development. Oecologia 52:360–369.

Berven, K.A., D.E. Gill, and S.J. Smith-Gill. 1979. Countergradient selection in the Green Frog, *Rana clamitans*. Evolution 33:609–623.

Bizer, J.R. 1978. Growth rates and size at metamorphosis of high elevation populations of *Ambystoma tigrinum*. Oecologia 34:175–184.

Brown, D.D. 1997. The role of thyroid hormone in zebrafish and axolotl development. Proceedings of the National Academy of Sciences (USA) 94:13011–13016.

Bruce, R.C. 1982. Egg-laying, larval periods, and metamorphosis of *Eurycea bislineata* and *E. junaluska* at Santeetlah Creek, North Carolina. Copeia 1982:755–762.

Bruce, R.C. 1988. Life history variation in the salamander *Desmognathus quadramaculatus*. Herpetologica 44:218–227.

Bruce, R.C., J. Castenet, and H. Francillon-Vieillot. 2002. Skeletochronological analysis of variation in age structure, body size, and life history in three species of desmognathine salamanders. Herpetologica 58:181–193.

Camp, C.D., J.L. Marshall, and R.M. Austin, Jr. 2000. The evolution of adult body size in Black-bellied Salamanders (*Desmognathus quadramaculatus* complex). Canadian Journal of Zoology 78:1712–1722.

Day, T., and L. Rowe. 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. American Naturalist 159:338–350.

Denver, R.J., K.A. Glennemeier, and G.C. Boorse. 2002. Endocrinology of complex life cycles: Amphibians. Hormones, Brain, and Behavior 2:469–513.

Dunn, E.R. 1926. The Salamanders of the Family Plethodontidae. Smith College, Northampton, MA.

Eagleson, G.W. 1976. A comparison of the life histories and growth patterns of populations of the salamander *Ambystoma gracile* (Baird) from permanent low-altitude and montane lakes. Canadian Journal of Zoology 54:2098–2111.

Eagleson, G.W., and B.A. McKeown. 1978a. Changes in thyroid activity of *Ambystoma gracile* (Baird) during different larval, transforming, and postmetamorphic phases. Canadian Journal of Zoology 56:1377–1381.

Eagleson, G.W., and B.A. McKeown. 1978b. Localization of the pituitary lactotropes and thyrotropes within *Ambystoma gracile* by histochemical and immunochemical methods. A developmental study of two populations. Cell and Tissue Research 189:53–66.

Etkin, W. 1968. Hormonal control of amphibian metamorphosis. Pp. 427–468, *In* W. Etkin and L.I. Gilbert (Eds.). Metamorphosis: A Problem in Developmental Biology. Appleton-Century-Crofts, New York, NY. 459 pp.

Hairston, N.G. 1949. The local distribution and ecology of the plethodontid salamanders of the southern Appalachians. Ecological Monographs 19:49–73.

Hensley, F.A. 1993. Ontogenetic loss of phenotypic plasticity of age at metamorphosis in tadpoles. Ecology 74:2405–2412.

Juterbock, J.E. 1990. Variation in larval growth and metamorphosis in the salamander *Desmognathus fuscus*. Herpetologica 46:291–303.

Larras-Regard, E., A. Taurog, and M. Dorris. 1981. Plasma thyroxine and triiodothyronine levels in *Ambystoma tigrinum* at various stages of metamorphosis. General and Comparative Endocrinology 43:443–450.

Leips, J., and J. Travis. 1994. Metamorphic responses to changing food levels in two species of hylid frogs. Ecology 75:1345–1356.

Newman, R.A. 1994. Effects of changing density and food level on metamorphosis of a desert amphibian, *Scaphiopus couchii*. Ecology 75:1085–1096.

Newman, R.A. 1998. Ecological constraints on amphibian metamorphosis: Interactions of temperature and larval density with responses to changing food level. Oecologia 115:9–16.

O'Laughlin, B.E., and R.N. Harris. 2000. Models of metamorphic timing: An experimental evaluation with the pond-dwelling salamander *Hemidactylium scutatum* (Caudata: Plethodontidae). Oecologia 124:343–350.

Organ, J.A. 1961. Studies of the local distribution, life history, and population dynamics of the salamander genus *Desmognathus* in Virginia. Ecological Monographs 31:189–220.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC. 587 pp.

Rose, C.S. 1995a. Skeletal morphogenesis in the urodele skull: II. Effect of developmental stage in thyroid hormone-induced remodeling. Journal of Morphology 223:149–166.

Rose, C.S. 1995b. Skeletal morphogenesis in the urodele skull: III. Effect of hormone dosage in TH-induced remodeling. Journal of Morphology 223:243–261.

Rosenkilde, P., and A.P. Ussing. 1996. What mechanisms control neoteny and regulate induced metamorphosis in urodeles? International Journal of Developmental Biology 40:665–673.

Rowe, L., and D. Ludwig. 1991. Size and timing of metamorphosis in complex life cycles: Time constraints and variation. Ecology 72:413–427.

Ryan, T.J. 1998. Larval life history and abundance of a rare salamander, *Eurycea junaluska*. Journal of Herpetology 32:10–17.

Ryan, T.J. 2000. Expression of a life cycle polymorphism: Facultative paedomorphosis in *Ambystoma talpoideum*. Ph.D. Dissertation, University of Missouri, Columbia, MO. 129 pp.

Ryan, T.J., and R.C. Bruce. 2000. Life history evolution and adaptive radiation of hemidactyliine salamanders. Pp. 303–326, *In* R.C. Bruce, R.G. Jaeger, and L.D. Houck (Eds.). The Biology of Plethodontid Salamanders. Kluwer Academic/Plenum, New York, NY. 485 pp.

Safi, R., A. Begue, C. Hanni, D. Stehelin, J.R. Tata, and V. Laudet. 1997a. Thyroid hormone receptor genes of neotenic amphibians. Journal of Molecular Evolution 44:595–604.

Safi, R., S. Bertrand, O. Marchand, M. Duffraisse, A. de Luze, J. Vanacker, M. Maraninchi, A. Magotat, B. Demeneix, and V. Laudet. 2004. The Axolotl (*Ambystoma mexicanum*), a neotenic amphibian, expresses functional thyroid hormone receptors. Endocrinology 145:760–772.

Safi, R., A. Deprez, and V. Laudet. 1997b. Thyroid hormone receptors in perennibranchiate amphibians. International Journal of Developmental Biology 41:533–535.

Semlitsch, R.D., D.E. Scott, and J.H.K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.

Sexton, O.J., and J.R. Bizer. 1978. Life history patterns of *Ambystoma tigrinum* in montane Colorado. American Midland Naturalist 99:101–118.

Smith, D.C. 1987. Adult recruitment in Chorus Frogs: Effects of size and date at metamorphosis. Ecology 68:344–350.

Smith-Gill, S.J., and K.A. Berven. 1979. Predicting amphibian metamorphosis. American Naturalist 113:563–585.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry, 3rd Edition. W.H. Freeman, New York, NY. 887 pp.

Tilley, S.G., and J. Bernardo. 1993. Life history evolution in plethodontid salamanders. Herpetologica 49:154–163.

Uhlenhuth, E. 1919. Relation between metamorphosis and other developmental phenomena in amphibians. Journal of General Physiology 1:525–544.

Voss, S.R. 1993. Relationship between stream order and length of larval period in the salamander *Eurycea wilderae*. Copeia 1993:736–742.

Voss, S.R., H.B. Shaffer, J. Taylor, R. Safi, and V. Laudet. 2000. Candidate gene analysis of thyroid hormone receptors in metamorphosing vs. nonmetamorphosing salamanders. Heredity 84:107–114.

Werner, E.E. 1986. Amphibian metamorphosis: Growth rate, predation risk, and the optimal size at transformation. American Naturalist 128:319–341.

Wilbur, H.M., and J.P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305–1314.

Wilder, I.W., and E.R. Dunn. 1920. The correlation of lunglessness in salamanders with a mountain brook habitat. Copeia 84:63–68.