Journal of Herpetology, Vol. 29, No. 3, pp. 375–382, 1995

Effects of Larval Growth History on Metamorphosis in a Stream-dwelling Salamander (*Desmognathus ochrophaeus*)

CHRISTOPHER KING BEACHY[1]

Department of Biology, University of Southwestern Louisiana, Lafayette, Louisiana 70504-2451, USA

ABSTRACT. – **Length of the larval period is a component of fitness for most amphibians. Some theoretical models predict that length of the larval period is a function of growth history; however, these models have been tested using species that inhabit temporary, productive habitats. I tested the hypothesis that length of the larval period in permanent, low productivity habitats is also a function of growth history. I exposed larvae of the stream-dwelling salamander *Desmognathus ochrophaeus* to different temperature, food, and water change regimes. Profile analysis of growth demonstrated significant effects of food and temperature on larval growth rates. Larvae that experienced high temperature and/or high food regimes grew faster than larvae on low temperature and low food regimes. Food and temperature interacted in a complex way, with food regimes affecting growth more at high temperature. Larvae at high temperatures metamorphosed earlier. Larvae at low temperature metamorphosed later at a larger size. High food regimes increased the metamorphic size of larvae at both temperatures. However, variation in food regimes did not affect length of the larval period.**

Many species of amphibians have biphasic life cycles with a larval stage that can take advantage of growth opportunities in transient, productive habitats (Wassersug, 1975; Wilbur, 1980). Because metamorphosis is a switchpoint that allows individuals to maximize the probability of rapid growth and high survival throughout the life cycle, optimal size and/or time at metamorphosis should be strongly favored (Wilbur and Collins, 1973; Werner, 1986). Furthermore, variation in metamorphic timing and size in amphibians can result in variation in age and size at maturity (Smith, 1987; Semlitsch et al., 1988), traits that directly affect fitness.

In temporary ponds, the quality of habitat varies both spatially and temporally (Semlitsch and Gibbons, 1985). A larva with a fixed time of metamorphosis may have lower fitness than one that can alter time of metamorphosis. Wilbur and Collins (1973) hypothesized that a larva that exceeds a minimum size should only metamorphose when its size-specific growth rate drops below some threshold. In contrast, a fast-growing larva should delay metamorphosis and continue exploiting opportunity for rapid growth. In theory and in nature, larval growth history appears to play a crucial role in the timing and/or size at metamorphosis (Wilbur and Collins, 1973; Collins, 1979; Travis, 1984; Werner, 1986; Alford and Harris, 1988; Newman, 1989; Hensley, 1993).

Temperature can also affect metamorphic parameters of larvae (Uhlenhuth, 1919). Cooler temperatures result in longer larval periods and larger metamorphic sizes (Bizer, 1978; Sexton and Bizer, 1978; Smith-Gill and Berven, 1979). Smith-Gill and Berven (1979) provided a predictive model of metamorphosis based on development rates, not rates of growth. They noted that temperature affects development more than growth, and that differences in temperature can change the relationship between growth and development. Thus, temperature regimes that differ spatially and temporally can affect the predicted length of the larval period.

Many amphibian species do not use habitats that are ephemeral. For example, the salamanders of the family Plethodontidae are ancestrally adapted to the mountain streams in eastern North America (Wilder and Dunn, 1920; Dunn, 1926; Wake, 1966). These habitats are relatively permanent sources of water, and have low productivity and constant temperature regimes relative to the ponds and temporary ponds used as larval habitat by other amphibians (Hynes, 1970; Wetzel, 1983). Stream-dwelling larval plethodontids have long larval periods (≥9 mo) and grow slowly (Beachy and Bruce, 1992), possibly in response to low temperature and/or low food regimes.

My study was designed to evaluate the effects of food and temperature on the growth-history and metamorphosis of a larval plethodontid, *Desmognathus ochrophaeus*. I also evaluated effects of water quality around the larva. In particular, I explored the hypothesis that models that predict time and size at metamorphosis of amphibians in temporary ponds have predic-

[1] Present Address: Department of Biology, P.O. Box 1522, Clarke College, 1550 Clarke Drive, Dubuque, Iowa 52001-3198, USA.

tive value for stream-dwelling salamander larvae.

Materials and Methods

Animals.—The mountain dusky salamander (*D. ochrophaeus*) is found throughout mountainous regions of eastern North America. This species was chosen for study because its larval period is short (8–10 mo) for stream-dwelling plethodontids, yet long relative to other families of salamanders (Bruce, 1989; Beachy and Bruce, 1992).

I collected 24 brooding *D. ochrophaeus* and their clutches (mean clutch size = 13.8) on 2 August 1991 at Buck Creek in the Nantahala National Forest, Macon County, North Carolina at 1240 msl. Mean developmental stage varied among clutches. I placed each clutch and its attendant female in a separate container and stored them in a walk-in environmental chamber at 13 C and 90% relative humidity.

Experimental Protocol.—Using a multifactorial design, I tested whether different levels of temperature, food, and water change affect the growth and metamorphosis of larval *D. ochrophaeus*. These factors were chosen because they vary spatially and temporally in streams. Water change was an attempt to mimic the water chemistry variation that results from variation in stream flow. Treatments consisted of two levels each of a temperature factor (11 C and 15 C; 11 C is the average summer temperature of the headwater seeps at Buck Creek), a food factor (a larva fed a *Tubifex* worm once per week versus twice per week; low food treatment resulted in growth rates that have been observed in natural populations of *D. ochrophaeus* [Bruce, 1989]), and a water change factor (water changed once or twice per week), for a total of eight treatment groups ($2 \times 2 \times 2 = 8$). There were 30 eggs in each treatment.

I removed 10 eggs from each clutch (for a total of 240 eggs). I placed each egg in 50 ml of stream water in an individual plastic cup and assigned each a number that identified the egg. I placed the 120 eggs in each temperature treatment on three shelves in each of two environmental chambers. Each shelf could hold 40 cups, or 10 spatial blocks of the four within-temperature treatments. Thus, there was a total of 60 blocks of four within-temperature treatments. I assigned eggs randomly to a treatment, then to a block using a random numbers table. The experiment was initiated on 15 August 1991.

While I began temperature treatments on 15 August, I did not administer the food and water change treatments until an individual hatched. I changed water once per week until hatching. Food treatments consisted of feeding one live *Tubifex* worm either twice or once weekly. Water change treatments involved washing each cup and adding 50 ml of fresh streamwater. I kept all treatments at 90% relative humidity to slow evaporation.

Cups were checked daily for hatchlings until all eggs had either hatched or died. The first hatchlings emerged on 17 August. These animals were removed from cups, blotted on a paper towel to remove excess water, and weighed to the nearest mg. Fifty ml of fresh stream water were replaced in each cup, and larvae were returned to the environmental chambers. Larvae were weighed every 30 d thereafter. For larvae hatching after 17 August, hatching date was recorded as the number of days after 17 August.

Metamorphosis occurred April–June 1992. During this time, cups were covered with glass lids to prevent escape of the metamorphs (i.e., individuals with complete resorption of gills as determined by dorsal viewing through a dissecting microscope). At the completion of metamorphosis, individuals were weighed and returned to the collection site. Metamorphic date was defined as number of days since hatching date 0 (17 August 1991). Larval period was recorded as (metamorphic date − hatching date).

Beginning on 15 October 1991, I lowered temperatures in the environmental chambers, by 1 C every 2 d so that by 1 November 1991 temperatures were reduced to 7 C and 11 C. On 1 November, food treatments were reduced by 50%, i.e., larvae were either fed once per week or once every two weeks. On 9 March 1992, temperatures were raised by 1 C every other day until temperatures on 24 March 1992 were at original levels. At this time, food treatments were increased to original levels. These manipulations were intended to simulate seasonal changes in temperature and food availability.

Statistical Analyses.—Growth data met the assumptions for analysis of variance. Analyses of growth and metamorphosis were performed using SYSTAT, version 3.2 (Wilkinson, 1989). Where applicable, I report type III mean squares. The significance criterion was set as $\alpha = 0.05$, and Wilks' lambda was used as the multivariate test statistic.

I analyzed data on growth and metamorphosis in two ways. First, I performed a profile analysis (Morrison, 1976; Simms and Burdick, 1988) to evaluate the effects of treatments on the growth trajectories that were recorded August 1991—June 1992. The profile analysis tested three hypotheses: (1) growth profiles differed among treatments in height of the growth trajectory; (2) individuals grew over time; and (3) treatment growth profiles were not parallel (Morrison, 1976). The first analysis was accomplished by an analysis of covariance (ANCOVA) on the sums of the masses measured for each

TABLE 1. Hatching date, hatching size, and metamorphic responses of larval *Desmognathus ochrophaeus* to experimental treatments. Sample size (N) is shown in parentheses, 1 standard error is shown on second line. Sample sizes for hatching size and larval period are the same as for hatching date and mass at metamorphosis, respectively. Treatment codes refer to temperature : food : water change treatments (H = high, L = low).

Treatment	Hatching date	Hatching size (mg)	Mass at metamorphosis (mg)	Larval period (d)
HHH	6.86 (28)	31	93 (21)	238.000
	1.58	1.89	3.27	1.99
HHL	11.50 (28)	29	83 (22)	233.409
	2.14	1.13	1.92	3.16
HLH	6.84 (30)	31	61 (26)	241.654
	1.63	1.46	1.77	2.10
HLL	10.80 (30)	29	61 (30)	242.233
	2.05	1.46	1.64	2.21
LHH	20.10 (29)	32	87 (25)	250.600
	4.05	1.51	7.60	3.66
LHL	18.14 (28)	29	94 (24)	251.083
	2.83	1.13	3.47	3.23
LLH	21.32 (28)	29	71 (18)	251.000
	3.39	1.13	3.54	3.79
LLL	18.30 (27)	28	64 (24)	250.417
	2.69	1.92	2.24	2.59

larva on the first eight weighings. The second two tests involved a multivariate ANCOVA (MANCOVA) on a seven-value response vector that consisted of differences between weighings on adjacent dates. If significant effects of treatment were found on the slopes of growth profiles, I performed univariate contrasts between adjacent sampling dates to determine when the growth trajectories diverged.

Second, a MANCOVA was conducted testing the treatment effects on metamorphic size and length of larval period. This analysis ignores growth history and examines only the final data set. A multivariate analysis was used because both metamorphic time and size can be the result of recent growth history, and thus may be correlated (Wilbur and Collins, 1973; Alford and Harris, 1988). Univariate results were analyzed only if MANCOVA indicated significant differences in response vectors among treatments.

To compensate for variation among treatments induced by maternal effects (Travis, 1981; Kaplan, 1985; Walls and Altig, 1986) three variables were used as covariates in the statistical analyses: clutch from which an egg was drawn, hatching size, and hatching date.

RESULTS

The experiment was terminated after all individuals had either died or metamorphosed.

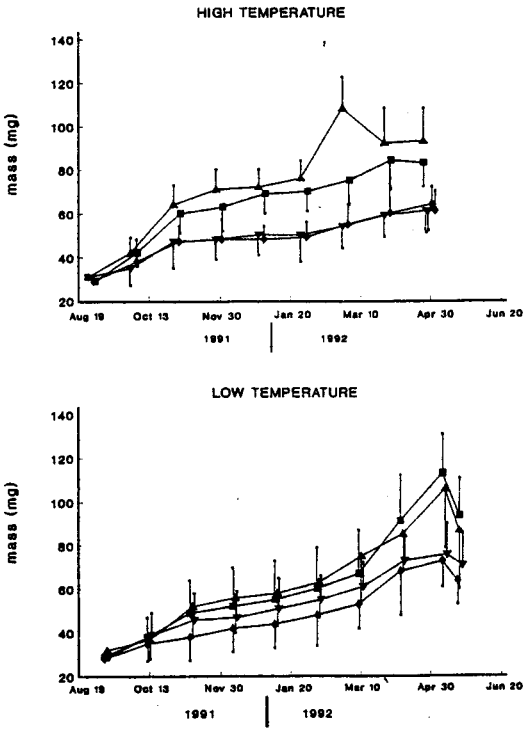

FIG. 1. Mean mass at each weighing and at metamorphosis for each treatment. Each line terminates at the mean mass at metamorphosis and the mean larval period for each treatment. Bars are + or −1 standard deviation. Treatment groups are as follows: ▲ = high food and high water change; ■ = high food and low water change; ▼ = low food and high water change; and ◆ = low food and low water change.

Unequal sample sizes in Table 1 reflect mortality before hatching or before metamorphosis. Because spatial blocks had insignificant effects, I analyzed the experiment as a fully randomized design. Heterogeneity of slope analyses were not significant.

Mean values of hatching and metamorphic variables are shown in Table 1. Growth trajectories are shown in Fig. 1. Overall survival to hatching was 95% and to metamorphosis was 80% (Table 1). Hatching was asynchronous, occurring from 19 August to 23 October. Hatching date was influenced by temperature treatment ($\bar{x}_{high} = 9.0$, $\bar{x}_{low} = 19.47$).

Profile Analysis of Growth

The profile analysis on larval growth data is summarized in Table 2. ANCOVA statistics refer to trajectory height, while MANCOVA statistics refer to slope of trajectories. If multivariate results were significant, I performed univariate contrasts on each 30 d interval to determine when growth profiles diverged (Table 3).

TABLE 2. Summary of profile analysis of growth of larval *Desmognathus ochrophaeus* through day 210 of the larval period. The ANCOVA was performed on the sums of weights and the MANCOVA was performed on the differences in weights between dates. Covariates are clutch, hatching size, and hatching date. Degrees of freedom are 1,94 and 7,88 for the ANCOVA and MANCOVA, respectively. For the ANCOVA, the residual mean square equals 0.00072.

ANCOVA ("trajectory height")		MANCOVA ("trajectory slope")	
Source	*P*	Source	*P*
		Date	<0.0001
Temp	0.126	Date × temp	0.016
Food	<0.0001	Date × food	<0.0001
Water change	0.741	Date × water	0.270
Temp × food	0.055	Date × temp × food	0.286
Temp × water	0.513	Date × temp × water	0.311
Food × water	0.432	Date × food × water	0.241
Three-way term	0.204	Date × three-way	0.564
Clutch	0.068	Date × clutch	0.869
Hatching size	0.661	Date × hatching size	0.591
Hatching date	0.0008	Date × hatching date	<0.0001

Date.—The significant date effect is trivial; it simply confirms that all larvae grew.

Temperature.—The significant Date × Temperature result indicates that larval growth trajectories differed in slope between high and low temperatures. Univariate contrasts show that the slopes were significantly different at contrasts 1 and 2 (Day 0–Day 60; Table 3). This suggests that temperature effects generated differences in growth early in the larval period of *D. ochrophaeus*, but did not continue to do so later in the larval period.

Food.—Food treatments were very effective in generating different trajectory heights (Food effect) and different trajectory slopes (Date × Food effect). Except during the first month of the larval period and as larvae approached metamorphic climax, it was usual for larvae to consume all food given to them. This suggests that the larvae were not satiated. It also suggests that larvae are capable of greater growth than exhibited under natural conditions.

Temperature × Food.—There was a nearly significant effect of this interaction on height (Temp × Food) of the growth trajectories, suggesting that the effects of temperature and food on growth are not additive.

No significant effects on height or slope of trajectories occured for Water Change, the interactions Temperature × Water, Food × Water, or the three way term.

The Covariates.—Of the three covariates, only hatching date contributed significantly to the overall variation. Hatching date influenced both the height (Hatching date) and slope (Date × Hatching date) of the growth curves (Table 2). There were more significant univariate contrasts for hatching date than for any other term in the model (Table 3). This suggests that time of oviposition and its consequent effect on hatching date is an important trait in natural populations. Eggs that hatched early produced larvae that fed early, grew early, and metamorphosed early. As a result this important effect can produce juveniles that have greater opportunity to grow prior to first reproduction.

Hatching size did not affect any aspect of the growth curves. Clutch was nearly significant in affecting height of the trajectories. However, because clutch and hatching date are correlated

TABLE 3. Univariate contrast for significant multivariate results of Table 2. Each contrast refers to growth during 30 day intervals beginning with contrast 1 (growth between day 0 [hatching date] and day 30) and ending with contrast 7 (growth between day 180 and day 210).

		Mean squares for contrast ($\times 10^{-3}$)						
Source	df	1	2	3	4	5	6	7
Date	1	6.79***	0.18	0.61***	0.07	0.04	0.00	0.03
Date × temperature	1	2.02***	0.62**	0.07	0.04	0.00	0.00	0.00
Date × food	1	9.63***	0.25	1.52***	0.03	0.17*	0.05	0.04
Date × hatching date	1	0.69*	0.13	2.40***	1.12***	1.17***	0.13*	0.00
Residual	94	0.15	0.07	0.05	0.07	0.04	0.03	0.02

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 4. Summary of MANCOVA of larval period and metamorphic size of *Desmognathus ochrophaeus*. For the multivariate statistics, df = 2,177. The univariate statistics report *P*-values. For the univariate statistics, df = 1,178. Univariate statistics are reported only where multivariate statistics are significant. For larval period, the residual mean square = 95.011; for metamorphic mass, the residual mean square = 0.00017. Clutch, hatching size, and hatching date are the covariates.

Source	Multivariate statistic			Univariate statistic	
	Wilks' λ	*F*	*P*	Larval period, *P*	Mass, *P*
Temperature	0.479	96.142	<0.0001	<0.0001	0.002
Food	0.500	88.351	<0.0001	0.151	<0.0001
Water change	0.992	0.677	0.509		
Temp × food	0.961	3.613	0.029	0.009	0.242
Temp × water	0.987	1.144	0.321		
Food × water	0.998	0.209	0.812		
Three-way term	0.941	5.567	0.005	0.575	0.003
Clutch	0.994	0.531	0.589		
Hatching size	0.999	0.079	0.924		
Hatching date	0.480	95.715	<0.0001	<0.0001	0.007

(i.e., eggs within clutches were at similar developmental states when the experiment was begun), I regard the low *P*-value for the clutch term as not suggestive.

MANCOVA of Larval Period and Metamorphic Size

This analysis considered the effects of treatment on metamorphic size and larval period. The analysis is summarized in Table 4.

Temperature.—The multivariate test for temperature effects was significant; univariate tests showed that both variables were significantly affected by temperature treatment. Lower temperatures resulted in longer larval periods and larger body sizes (Fig. 1, Table 1).

Food.—After a significant multivariate result, univariate tests indicated that high food treatment resulted in a significant increase in metamorphic size, but larval period was not affected. This means that different growth history did not result in changes of metamorphic timing. Despite different growth histories, larval *D. ochrophaeus* that experienced the same environment (i.e., temperature) metamorphosed at the same time.

Temperature × Food.—MANCOVA indicated a significant effect; univariate tests show that larval period was significantly affected by this interaction. Metamorphic size was not. This result indicates that the relationship between larval period and food changed as temperature varied. For example, the effect of exposing a population of larval *D. ochrophaeus* to a higher temperature and higher food regime resulted in a more than additive effect on larval period, i.e., exposure to higher temperatures reduced larval period, and adding food reduced it even more.

Three-way Term.—MANCOVA indicated a significant effect of this interaction; univariate tests show that metamorphic mass was significantly affected. Larval period was not. More frequent water change increased the effect of the high food treatment when temperature was high, but decreased the food effect at low temperature. This complex term is difficult to interpret, but suggests that variation in water turnover, when coupled with variation in food and temperature regimes, affects metamorphic mass.

No significant effects of Water Change or of the interactions Temperature × Water and Food × Water were observed.

Covariates.—Of the three covariates, only hatching date is significant. This confirms the earlier suggestion that the low *P*-value for clutch (Table 2) is a correlated effect of hatching date. In this analysis, clutch is clearly a non-significant effect. The analysis also re-emphasizes the important effect of hatching date. Treatment effects aside, individuals that hatched early metamorphosed early. Late hatchers spent more time as larvae and attained greater metamorphic sizes.

DISCUSSION

This experiment was designed to test the effects of temperature, food, and water change on age and size at metamorphosis in a stream-dwelling salamander. Implicitly, this study evaluated the value and/or precision of prevailing models of amphibian metamorphosis for predicting metamorphosis in stream-dwelling salamanders.

These results show that food, temperature, and food × temperature interactions are effective in generating different growth trajectories of larval *Desmognathus ochrophaeus*, and subse-

quently can result in differences in larval period and/or metamorphic size.

High temperature increased growth rate early in larval life, and decreased length of larval period and metamorphic mass. That these larval *D. ochrophaeus* exhibited variation in metamorphic timing is interesting because Bernardo (1994) found that thermal variation (his GARDEN effect) had no effect on maturation rate in juvenile *D. ochrophaeus*. This suggests that a tight link may not exist between metamorphic and maturation parameters in this salamander.

High food levels increased growth rates and mass at metamorphosis. Bernardo (1994) found that juvenile *D. ochrophaeus* responded to supplemental prey levels by increasing allocation to both growth and maturation. While the larval *D. ochrophaeus* in high food treatments did increase allocation to growth, no variation in metamorphic timing resulted.

The interaction between these factors (Temperature × Food) resulted in non-additive effects on height of the growth trajectory and larval period. The differences between food growth curves are more distinct between high temperature than low temperature treatments; this is not expected under an additive model. Assimilation efficiency is negatively correlated with temperature in two species of *Plethodon* (Bobka et al., 1981). Thus, this interaction may be due primarily to the lowered assimilation efficiency of larvae in the low food/high temperature treatment relative to larvae in the low food/low temperature treatment. This suggests that as habitats increase in temperature, food becomes a stronger influence on growth.

The third factor, water change, did not directly influence any larval life history parameters. However, the significant three-way interaction of these factors indicated that some component (water quality, flow regime?) of the immediate environment surrounding the larva interacts in a complex way with changing food and temperature to affect metamorphic mass. For example, at high temperature, an increase in mass due to high food was more pronounced in the high water treatment. High oxygen content and/or low local metabolic waste may have enhanced growth in the high water treatment. This effect is most pronounced between the water change treatments in the high temperature : high food groups.

Models of Metamorphosis

Temperature-mediated Models.—Sexton and Bizer (1978) provided a temperature-based model that predicted longer larval periods and larger metamorphic sizes in cooler environments. While their model is supported for both pond-dwelling salamanders (Bizer, 1978) and frogs (Berven et al., 1979; Berven and Gill, 1983), Juterbock (1990) noted that the model does not conform to geographic patterns in the stream-dwelling plethodontids *Eurycea wilderae* (Bruce, 1985), *Desmognathus quadramaculatus* (Bruce, 1988), or *Desmognathus fuscus* (Juterbock, 1990). These plethodontids do not clearly exhibit longer larval periods with decreasing temperature, and metamorphic size is smaller in cooler habitats. Juterbock (1990) suggested that a different paradigm be devised to explain patterns of metamorphosis in plethodontids.

My study directly evaluated the influence of temperature on larval growth, metamorphic size, and larval period. Temperature treatment did not influence overall height of the growth trajectory, but growth rates early in the larval life were significantly higher in high temperature treatments. As predicted by Sexton and Bizer (1978), high temperature significantly decreased larval period and metamorphic size.

Growth-based Models.—The Wilbur and Collins (1973) model predicted that recent growth history influences the timing of metamorphosis. While this prediction holds for other amphibians (Alford and Harris, 1988), it does not for larval *D. ochrophaeus*. Differences in growth history were caused by food treatments at both high and low temperatures. However, these differences in growth history did not result in differences in larval period within temperature treatments. Clearly, the Wilbur-Collins model proved insufficient to predict metamorphosis in this stream-dwelling salamander. The mortality risk/growth opportunity model of Werner (1986) gives predictions that are qualitatively similar to the Wilbur-Collins model, and thus also does not hold in this case. In a related field study, the presence of a predator (larval *Desmognathus quadramaculatus*) had significant effects on growth history and mortality risk of another stream-dwelling plethodontid (*Eurycea wilderae*) yet no variation in metamorphic timing resulted (Beachy, 1992).

Alford and Harris (1988) suggested that a conclusive test of the Wilbur-Collins hypothesis consists of exposing larvae to changes in food regime during the larval period. The food regimes experienced by the larval *D. ochrophaeus* were constant, and it remains a possibility that a decrease in food level during the larval period could trigger metamorphosis. Thus, while the results presented herein strongly suggest that the Wilbur-Collins model is inappropriate for this stream-dwelling salamander, they are not conclusive.

Food and Temperature Effects in Natural Populations of Stream-dwelling Larval Plethodontids

The significant interaction of food and temperature on larval period suggests that complex

relationships between food and temperature can obscure a clear link between temperature and metamorphosis in studies of natural populations of plethodontids. Seasonal differences result in temperature and food regimes that vary among populations. Thus, it is of interest to evaluate the direction of the non-additive effect of Food × Temperature on larval period. Ignoring water change effects, the mean larval periods (in days) for temperature and food treatments were: LL = 250.67, LH = 250.84, HL = 241.96, and HH = 235.65 (Table 1). The difference in larval period between LL and HH treatments was greater than that expected under an additive model (expected difference = 8.53, observed difference = 15.02). This means that as temperature and food are increased, the larval period is reduced more than expected if the two factors were acting independently. This observation can explain the discrepency between the predictions of Sexton and Bizer (1978) and the observations of Juterbock (1990). In warmer larval habitats, food regimes are likely also to be higher, thus a shorter larval period and larger metamorphic size (e.g., LL vs. HH: Table 1).

Food-mediated models of metamorphosis were developed to predict metamorphosis in amphibians utilizing transient growth opportunities (Wilbur, 1980). The failure of these models to predict the metamorphosis of the stream-dwelling *D. ochrophaeus* may be related to the low productivity regimes of cool forest streams. Plethodontids retain the ability to respond to high temperature and high food conditions by growing at significantly increased rates in a manner similar to pond-dwelling salamanders. Indeed, several plethodontids have invaded warm, high productivity swampy pools and ponds, and these species exhibit characteristic high larval growth (e.g., *Eurycea guttolineata* [Bruce, 1970], *Eurycea quadridigitata* [Semlitsch, 1980], *Hemidactylium scutatum* [Blanchard, 1923] and *Pseudotriton montanus* [Bruce, 1978]).

Have stream-dwelling plethodontids lost the ability to respond to a growth opportunity by delaying metamorphosis? Plethodontids have undergone long selection in the permanent aquatic habitat of streams (Wake, 1966). The advantage of phenotypic plasticity in larval period is less in environments that are permanent (Levins, 1968). As a result, the timing of metamorphosis may be canalized in a stable larval habitat. With little selection for ability to detect a deteriorating habitat, it appears that growth history and timing of metamorphosis in stream-dwelling plethodontids have been decoupled.

Acknowledgments.—Valuable support during this study was provided by C. L. Ory. H. M. Wilbur exposed me to profile analysis, and J. Bernardo suggested the simulated winter. The facilities to carry out this experiment were provided by the Highlands Biological Station. Versions of this manuscript were improved by R. N. Harris, R. G. Jaeger, J. F. Jackson, J. E. Neigel, J. W. Petranka, D. Simberloff, D. Smith, R. R. Twilley, M. S. Zavada and four anonymous reviewers. This study was supported by Louisiana Board of Regents Doctoral Fellowship LEQSF-(1988-94)-GF-15 to R. G. Jaeger, and a Highlands Biological Station grant-in-aid.

Literature Cited

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

Beachy, C. K. 1992. Community ecology of larval plethodontid salamanders: the effect of predation on age and size at metamorphosis. Unpubl. Ph.D. Diss., Univ. Southwestern Louisiana, Lafayette.

———, 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. Amer. Natur. 139: 839–847.

Bernardo, J. 1994. Experimental analysis of allocation in two divergent, natural salamander populations. Amer. Natur. 143:14–38.

Berven, K. A., and D. E. Gill. 1983. Intepreting geographic variation in life-history traits. Amer. Zool. 23:85–97.

———, ———, 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 (Berlin) 34:175–184.

Blanchard, F. N. 1923. The life history of the four-toed salamander. Amer. Natur. 57:262–268.

Bobka, M. S., R. G. Jaeger, and D. C. McNaught. 1981. Temperature dependent assimilation efficiences of two species of terrestrial salamanders. Copeia 1981:417–421.

Bruce, R. C. 1970. The larval life of the three-lined salamander, *Eurycea longicauda guttolineata*. Copeia 1970:776–779.

———. 1978. A comparison of the larval periods of Blue Ridge and Piedmont mud salamanders (*Pseudotriton montanus*). Herpetologica 34:325–332.

———. 1985. Larval period and metamorphosis in the salamander *Eurycea bislineata*. Herpetologica 41: 19–28.

———. 1988. Life history variation in the salamander *Desmognathus quadramaculatus*. Herpetologica 44:218–227.

———. 1989. Life history of the salamander *Desmognathus monticola*, with a comparison of the larval periods of *D. monticola* and *D. ochrophaeus*. Herpetologica 45:144–155.

Collins, J. P. 1979. Intrapopulation variation in the body size at metamorphosis and timing of metamorphosis in the bullfrog, *Rana catesbeiana*. Ecology 60:738–749.

Dunn, E. R. 1926. The salamanders of the family Plethodontidae. Smith College Press, Northampton, Massachusetts.

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

HYNES, H. B. N. 1970. The Ecology of Running Waters. Liverpool University Press, Liverpool, U.K.

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

KAPLAN, R. H. 1985. Maternal influences on offspring in the California newt, *Taricha torosa*. Copeia 1985:1028–1035.

LEVINS, R. 1968. Evolution in Changing Environments. Princeton University Press, Princeton, New Jersey.

MORRISON, D. F. 1976. Multivariate Statistical Methods. McGraw-Hill Publishers, New York.

NEWMAN, R. A. 1989. Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. Ecology 70:1775–1787.

SEMLITSCH, R. D. 1980. Growth and metamorphosis of larval dwarf salamanders (*Eurycea quadridigitata*). Herpetologica 36:138–140.

———, AND J. W. GIBBONS. 1985. Phenotypic variation in metamorphosis and paedomorphosis in the salamander *Ambystoma talpoideum*. Ecology 66: 1123–1130.

———, 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. Amer. Midl. Natur. 99:101–118.

SIMMS, E. L., AND D. S. BURDICK. 1988. Profile analysis of variance as a tool for analyzing correlated responses in experimental ecology. Biom. J. 30: 229–242.

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. BERVEN. 1979. Predicting amphibian metamorphosis. Amer. Natur. 113:563–585.

TRAVIS, J. 1981. Control of larval growth variation in a population of *Pseudacris triseriata* (Anura: Hylidae). Evolution 35:423–432.

———. 1984. Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. Ecology 65:1155–1160.

UHLENHUTH, E. 1919. Relation between metamorphosis and other developmental phenomena in amphibians. J. Gen. Phys. 1:525–544.

WAKE, D. B. 1966. Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem. S. California Acad. Sci. 4:1–111.

WALLS, S. C., AND R. ALTIG. 1986. Female reproductive biology and larval life history of *Ambystoma* salamanders: a comparison of egg size, hatchling size, and larval growth. Herpetologica 42:334–345.

WASSERSUG, R. J. 1975. The adaptive significance of the tadpole stage with comments on the maintenance of complex life cycles in anurans. Amer. Zool. 15:405–417.

WERNER, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. Amer. Natur. 128:319–341.

WETZEL, R. G. 1983. Limnology, 2nd edition. CBS College Publishing, New York.

WILBUR, H. M. 1980. Complex life cycles. Ann. Rev. Ecol. Syst. 11:67–93.

———, 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.

WILKINSON, L. 1989. SYSTAT: The System for Statistics. SYSTAT, Inc., Evanston, Illinois.

Accepted: 16 April 1995.