

# Determinants of clinal variation in life history of dusky salamanders (*Desmognathus ocoee*): prey abundance and ecological limits on foraging time restrict opportunities for larval growth

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## Abstract

Recent models argue that thermal environments are the major cause of ectotherm life-history clines. However, elevational clines in body size in the mountain dusky salamander *Desmognathus ocoee* (family Plethodontidae) shift from positive at hatching, to negative at metamorphosis to positive again as adults, and so are not consistent with this explanation. The clinal shift from hatching to metamorphosis was investigated by examining the clinal and seasonal feeding patterns of larval salamanders at high and low elevation sites in rockface and woodland habitats. Repeated cohort sampling was also used to examine clinal and seasonal patterns in body size and to estimate average growth rates. Larval growth in both rockface and woodland habitats was tightly correlated with feeding activity. Although temperature was found to vary between high and low elevation sites, the greatest growth occurred in a cold woodland habitat with a high elevation, and the lowest growth occurred in an adjacent rockface habitat. Because this difference in growth cannot be attributed to thermal differences, we conclude that local food resource levels are the predominant source of local differences in growth. These findings, clinal patterns of variation in other predatory salamanders, and experimental analyses in which both food and temperature are orthogonally manipulated, indicate that general models that single out temperature as the principle cause of ectotherm life-history clines should be viewed with caution.

**Key words:** *Desmognathus ocoee*, life history, amphibian, ectotherm growth, metamorphosis, clinal variation, geographical variation, temperature

## INTRODUCTION

Ectotherms often show phenotypic clines in body size with larger body size at cooler sites (high elevations or latitudes), and biologists have tried to explain these patterns for more than a century. One common idea is that clines in ectotherm body size (Berrigan & Charnov, 1994; Sibly & Atkinson, 1994; Atkinson & Sibly, 1997) and other life-history attributes such as egg size (Azevedo, French & Partridge, 1996; Yampolsky & Scheiner, 1996) and size at metamorphosis (Smith-Gill & Berven, 1979) are largely determined by thermal effects upon growth and development (reviewed by Bernardo & Reagan-Wallin, 2002). This view has seen resurgent interest, much of which stems from the analysis of rearing studies in the laboratory using controlled temperature (Atkinson, 1994, 1996; Berrigan & Charnov, 1994; Partridge *et al.*, 1994; Partridge & French, 1996; Sibly & Atkinson, 1994; Van Voorhies, 1996; Atkinson & Sibly, 1997; Robinson & Partridge 2001, and many others). An alternative idea

is that temperature is but one of many environmental variables, including predation regime, other forms of selection on growth and body size, and productivity gradients, that can produce identical phenotypic clines (reviewed by Bernardo & Reagan-Wallin, 2002). Indeed, numerous other studies that have explored the mechanistic details of body size differences between natural populations in the field find a plurality of causes for body size clines in ectotherms (reviewed by Bernardo & Reagan-Wallin, 2002).

These contrasting explanations of the underlying causes of phenotypic clines in ectotherm body size are significant because they influence the evolutionary interpretation of why such repeated patterns develop among different ectotherm species. In the temperature-determination school, such patterns are viewed as an unavoidable consequence of ectothermy, that may not be, or are not, adaptive (e.g. Smith-Gill & Berven, 1979). The contrasting view, that other factors besides temperature can lead to the same phenotypic patterns, are typically based on extensive mechanistic analyses of growth and developmental trajectories (Bernardo, 1994; Jonsson *et al.*, 2001), energy budget analyses (Dunham, 1978,

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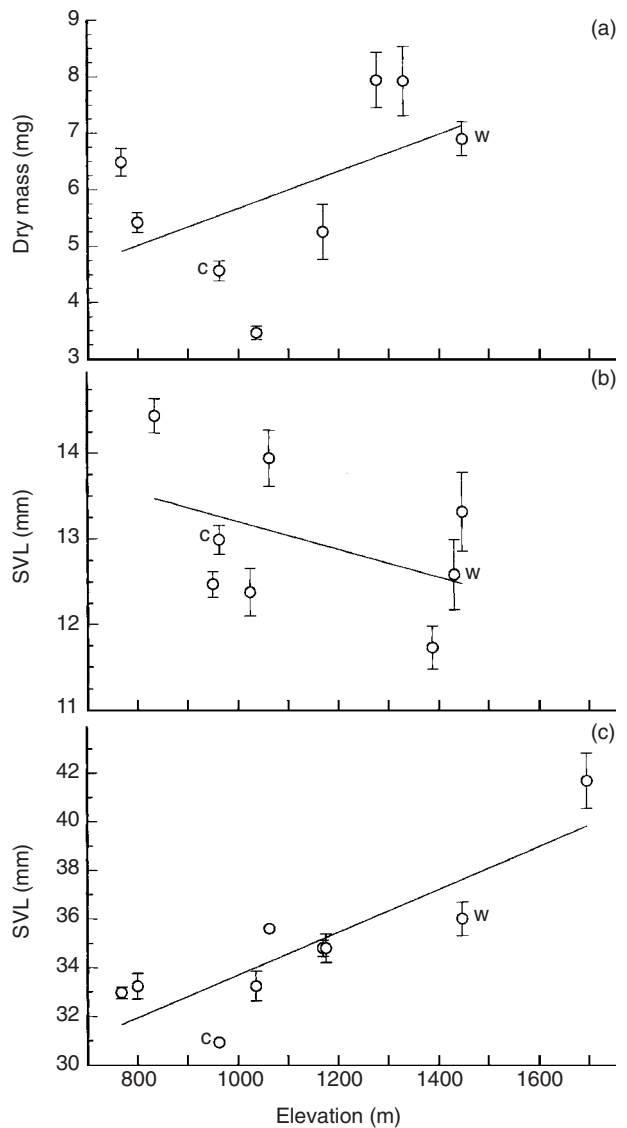
1980, 1981; Grant & Dunham, 1988, 1990; Dunham, Grant & Overall, 1989; Grant, 1990; Beaupre, Dunham & Overall, 1993a,b), sources of selection (Tilley, 1980; Reznick, 1982, 1990; Reznick & Endler, 1982; Endler, 1995), and analyses of patterns of genetic variation in responses to these selective pressures (Reznick, Bryga & Endler, 1990; Bernardo, 1994; Reznick, Butler *et al.*, 1996). Such mechanistic studies have the advantage of examining multiple potential causes of phenotypic clines, as well as the potential to evaluate the adaptive significance, if any, of a particular phenotypic pattern by examining the phenotypes in their relevant selective milieus.

Amphibians provide some oft-cited examples of body size clines that have been attributed to the effects of temperature (Berven, Gill & Smith-Gill, 1979; Smith-Gill & Berven, 1979; Berven, 1982a,b), yet many amphibians do not conform to this pattern (for a critical review see Bernardo & Reagan-Wallin, 2002). For instance, in the Ocoee dusky salamander *Desmognathus ocoee* (a lungless salamander; family Plethodontidae), field transplant experiments demonstrate that larger adult body size in high elevation populations is the result of a genetically based (Bernardo, 1994) heterochrony in maturation (Tilley, 1980), not to the effects of temperature on development rates.

Complex life cycles make the dynamics of body size clines of many amphibians even more complicated. For instance, in anuran amphibians, metamorphosis marks a dramatic morphological and ecological shift from a swimming, herbivorous tadpole to a terrestrial, saltatory predator. Because the timing of, and size at this ecological transition are thought to influence performance in the terrestrial environment, and hence, fitness (Wilbur & Collins, 1973), the sources of variation in metamorphic phenotypes within and among amphibian populations have been extensively studied from theoretical and empirical perspectives (reviewed by Bernardo & Reagan-Wallin, 2002). In lungless salamanders such as *D. ocoee*, the ecological shift at metamorphosis is no less remarkable, because feeding mode changes from aquatic gape and suck feeding to terrestrial feeding with elaborate tongue protrusion (Wake & Deban, 2000).

Moreover, in this species, the cline in body size shifts at different parts of the life cycle (Fig. 1), which is not consistent with a hypothesis that thermal effects upon development are the sole determinant of body size clines. If they were, we would expect the same (positive) phenotypic cline throughout the life cycle (Bernardo & Reagan-Wallin, 2002).

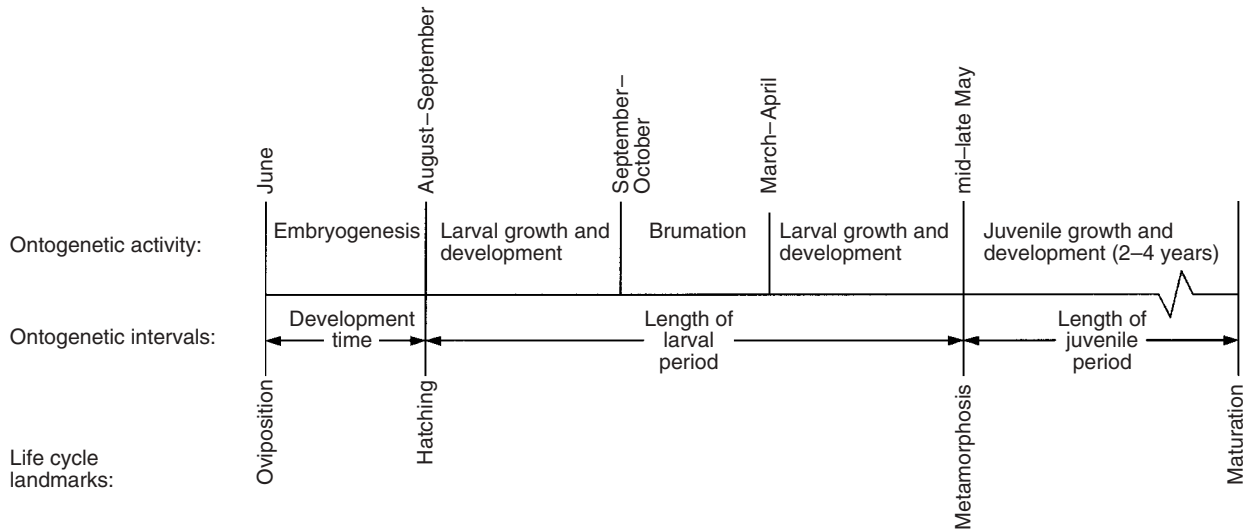
This paper is part of an ongoing mechanistic analysis of clinal patterns in body size in *D. ocoee*, a common salamander in the Southern Appalachian Mountains, U.S.A. (Tilley, 1977, 1980; Bernardo, 1994; Bernardo & Reagan-Wallin, 2002). The goal of this larger analysis of the *D. ocoee* life-history cline is to develop a full picture of the sources and relative magnitudes of selection on growth and development throughout the life cycle (Bernardo, 1993), using quantitative field data (Bernardo, 1998; Bernardo & Reagan-Wallin, 2002; this study),



**Fig. 1.** Summary of size clines in *Desmognathus ocoee* from Cowee and Nantahala Mountains, North Carolina, at three different life cycle stages. All points show mean  $\pm$  1 SE. In each panel, C and W are shown next to the points from the Cullasaja and Whiteside populations that are the subject of long-term study. Each mean represents five or more individuals (or clutches for panel (a)). (a) Egg size in relation to elevation; each datum represents the mean of at least five clutches (five females); (b) size at metamorphosis in relation to elevation; (c) adult body size of brooding females in relation to elevation.

and experimental manipulations of both environments (Bernardo, 1994, 1998) and genetic architecture of life-history traits.

Most of the prior analysis of body size clines in *D. ocoee* has centred on the terrestrial phase of the life cycle, whereas the larval life history is poorly understood. Because prior field data showed that low elevation larvae (that hatch from smaller eggs; Bernardo, 1994) are larger than their counterparts at higher elevations (that hatch from larger eggs; Bernardo, 1994) by the time they metamorphose (Fig. 1; Bernardo & Reagan-Wallin, 2002),



**Fig. 2.** Summary of developmental phenology, landmarks, and transition intervals for *Desmognathus ocoee*. Top row, natural phenology of oviposition through metamorphosis beginning in late spring (mid-April to mid-June, etc.) until following spring (mid-late May). Beyond this point, terrestrial growth of post-metamorphic juveniles varies from 2 to 4 or more years. Second row, developmental phase occurring in the specified interval (e.g. embryogenesis, etc.). Third row, names of specific intervals and their duration. Fourth row, specific landmarks in the life history which represent transitions in the life cycle. A few females may oviposit later in autumn and winter, and their eggs typically hatch in early spring as Tilley (1973) observed for *D. carolinensis*, but we have only observed three such clutches in over 15 years of field work (J. Bernardo, pers. obs).

there is a reversal in the body size cline during the larval stage. In this study, an examination was made of clinal and seasonal patterns of variation in larval growth based on repeated cohort sampling, as well as clinal and seasonal patterns in temperature, food availability, and foraging activity of larvae at a variety of sites. Our specific goals were: (1) to describe quantitatively the comparative dynamics of larval growth at high and low elevation sites between oviposition and metamorphosis; (2) to understand whether differences exist in the opportunities for larval growth in populations that inhabit different microhabitats and elevations; (3) to determine whether different feeding patterns exist between high and low elevation sites.

## MATERIALS AND METHODS

### Study organism and background

Within the Southern Appalachian region there are more than a dozen species of salamanders that have complex life cycles. Species having multi-year larval periods have been studied extensively (for reviews see Tilley & Bernardo, 1993; Ryan & Bruce, 2000), whereas those species that have brief larval periods, including *D. ocoee*, are only poorly understood (but see Bruce, 1989; Bernardo, 2000; Bernardo & Reagan-Wallin, 2002). This is surprising because *D. ocoee* is one of the most common salamanders in the region (Smith & Petranka, 2000), and it has figured prominently as a model system for many other studies (Bernardo, 2003). Although a great deal is known about oviposition behaviour, clutch size and brooding behaviour in *D. ocoee* (Tilley, 1972; Forester, 1979a,b,

1981, 1983, 1984, 1986), and much has been learned about its size at metamorphosis (Bernardo & Reagan-Wallin, 2002), juvenile growth and development during the terrestrial stage, survival and maturation (Tilley, 1977, 1980; Bernardo, 1994 and unpublished), its larval life history has been largely unstudied.

A brief summary of the generalized life cycle of *D. ocoee* (Fig. 2) aids in understanding the phenology of the larval stage and thus, the logic and goals of the sampling scheme used in the present study. Females oviposit a clutch of 6–30 eggs under moss in headwater seepage areas, springheads, and similar aquatic microsites in mid-summer (June or July) and then brood the clutches until they hatch 6–8 weeks later. Oviposition may be relatively synchronous among females at numerous sites if local droughts are followed by a rainy period (J. Bernardo, pers. obs.). Eggs hatch by late summer (August–September) into gilled larvae that live in marginal aquatic habitats such as gravel beds and seepage areas in headwater streams, or thin films of water on vertical rock faces. Larvae hatch with yolk reserves, but also may feed and grow during the autumn, overwinter, feed and grow again in the spring, and then metamorphose in early summer (May–June) at 9–10 months of age (Fig. 2).

The only quantitative data (*sensu* Bernardo, 1998) about the larval life history of *D. ocoee* is a report by Bruce (1989) who studied larval growth and development within a single population of *D. ocoee* at Wolf Creek (average elevation 950 m) in the Cowee Mountains. Data from a closely related species, *D. carolinensis* (previously considered conspecific; Tilley & Mahoney, 1996) were provided by Tilley (1973), but this species does not show the same clinal pattern in size at metamorphosis as

*D. ocoee* (Bernardo & Reagan-Wallin, 2002) and so may not be directly comparable.

### Study sites

The larval growth and development of *D. ocoee* was studied by analysing repeated cohort samples from 5 populations near the Highlands Plateau in the southern Cowee Mountains, Macon and Jackson Counties, North Carolina (Table 1). Four of the 5 populations occur in wet rockface habitats, where seepage of the water table flows over road cuts or natural rock faces. These sites in the Southern Appalachians support dense populations of *D. ocoee* and other salamanders (Huheey & Brandon, 1973; Tilley, 1973, 1980). All life stages including reproduction occur in the rockface habitats. Larvae live and forage in areas where thin films of water flow over rock surfaces, in algal ooze that accumulates on rock underhangs, and in narrow cracks and crevices.

The 2 principal sites, Cullasaja and Whiteside, have been the subject of intensive analysis of life-history variation by Tilley (1977, 1980) and Bernardo (1994). Cullasaja occurs in the Cullasaja River Gorge at an elevation of 963 m, whereas the Whiteside population occurs near the top of Whiteside Mountain, the highest site in the area, at 1448 m. Larval collections were also made at 2 additional sites, Scaly Mountain, and Blue Valley Vista, which occur along the edge of Blue Ridge on the western slopes of the Cowee Mountains. The fifth population studied also occurred at Whiteside, but within a forest seepage area at 1400 m. Forest-dwelling populations do not occur at low elevations in this region (Tilley, 1977), so it was not possible to sample a woodland site near Cullasaja.

### Sampling

Serial larval collections were made over a 2–3 day interval in late autumn (2–3 October 1992) and early spring (9–12 April 1993) at each site. Larvae were collected at night while they were active on the surfaces and were placed immediately into containers and held on ice in coolers until return to the laboratory. Salamanders were anaesthetized in 0.1% 2-Phenoxyethanol (Sigma), and species identity was confirmed. In some sites, *D. ocoee* is sympatric with the seal salamander *Desmognathus monticola*, which has a similar larva. However, the 2 species can be confidently discriminated based on several morphological features, including the number of pigmented blotches between the limb insertions, the shapes of these patches, the extent of chestnut orange dorsal coloration, and importantly, the relative head width per body length; *D. monticola* has a wider head for its body size (Bruce, 1989; J. Bernardo, pers. obs.). Only 3 of the salamanders collected could not be confidently assigned to species, and these were excluded from the study. Larvae were measured for snout–vent length (SVL) with callipers under a dissecting microscope, weighed to 0.0001 g and preserved in 10% buffered formalin. Preserved salamanders were dissected to examine gut

contents. Animals were conservatively scored as feeding if there was any trace of non-yolk material in the gut, even if residual yolk was present. Several clutches of hatchling larvae were collected in 1988 at Whiteside and Cullasaja for which SVL data were obtained, and several others in 1994 for which mass data were obtained.

At Whiteside and Cullasaja, data were collected on prey abundance by placing a decimetre square grid against the rockface and counting all visible invertebrates in the grid; 25–30 such samples were taken on each of 5 dates throughout the active season at both sites. Grids were placed haphazardly across the rock faces in an effort to sample the range of conditions on each site. Microhabitat temperature data were also recorded in the centre of each grid using a rapid reading Type T thermocouple. Additional temperature data were collected using data loggers at Whiteside and Cullasaja in late summer to late autumn (66 days) to record hourly temperature data on the rock faces.

### Analyses

Patterns of feeding activity were analysed using  $\chi^2$  or Fisher's exact tests. Patterns of variation in size (SVL) and growth ( $\Delta$ SVL) among all 5 populations were compared with *t*-tests and analysis of variance depending on the hypothesis being tested. Because changes in SVL between autumn and spring might be due only to growth fuelled by residual yolk after hatching (Bradford, 1984; Montague, 1987), a more detailed analysis of growth was conducted in the Whiteside and Cullasaja populations based on mass, for which there were more extensive data. This analysis was conducted with and without the data from the Whiteside woodland population. All analyses were conducted using JMP 4.04 (SAS Institute, 2001); all means are reported  $\pm 1$  SE.

## RESULTS

Differences in habitat characteristics of the five study sites, larval SVL and mass data, and sample sizes are summarized in Table 1. A total of 438 larvae was sampled for this study.

### Thermal environments and prey availability

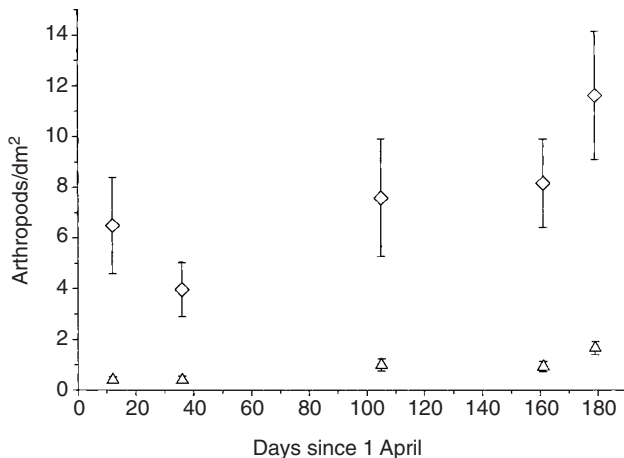
Rockface surface temperatures measured periodically from spring to autumn during the invertebrate sampling were generally higher at Cullasaja than at Whiteside (Table 1). Also, the data from the loggers recorded from mid-summer to autumn indicate that the mean maximum daily temperature at Cullasaja was significantly higher than at Whiteside ( $18.75 \pm 0.42$  °C,  $n = 66$  vs  $16.26 \pm 0.42$  °C,  $n = 66$ ;  $t = 4.151$ , d.f. = 130,  $P < 0.0001$ ); however, there was no difference between the sites in mean minimum daily temperatures (Cullasaja,  $10.93 \pm 0.51$ ,  $n = 65$ ; Whiteside,  $11.93 \pm 0.50$ ,  $n = 66$ ;  $P > 0.05$ ). Mean prey abundance was higher at Cullasaja on all dates sampled (Fig. 3), and overall, the abundance of arthropod

**Table 1.** Summary of site and population characteristics of the larval *Desmognathus ocoee* study populations

	Cullasaja	Scaly	Blue valley	Whiteside	
<b>Site characteristics</b>					
Elevation (m)	963	1024	1063	1448	1,400
Microhabitat	Rock face	Rock face	Rock face	Rock face	Woodland
Slope aspect	South-west	North-west	South-east	North-west	North-west
<b>Rockface surface temperature (°C ± SE)<sup>a</sup></b>					
Early spring	5.51 ± 0.68			4.81 ± 0.40	
Late spring	8.80 ± 0.48			8.25 ± 0.81	
Summer	14.12 ± 0.34			14.70 ± 1.36	
Early autumn	19.69 ± 0.56			17.69 ± 0.27	
Late autumn	12.56 ± 0.42			11.34 ± 0.25	
Active season length (days) <sup>a</sup>	190–210			145–170	
Arthropod abundance (no./dm <sup>2</sup> ± SE)	7.57 ± 0.89, n = 145			0.88 ± 0.10, n = 148	
<b>Population traits SVL (mm)</b>					
Hatching			7.3–7.7, n = 4	6.0–6.2, n = 9	
Autumn	9.5–11.2, n = 11	10.0–12.2, n = 14	9.7–12.1, n = 18	9.6–13.2, n = 16	9.7–13.0, n = 40
Spring	9.5–14.1, n = 43	9.7–13.7, n = 51	9.3–16.2, n = 35	9.7–12.2, n = 5	10.3–18.3, n = 57
<b>Mass (g)</b>					
Hatching	0.013–0.032, n = 43			0.014–0.032, n = 18	
Autumn	0.021–0.034, n = 13			0.019–0.032, n = 15	0.023–0.053, n = 34
Spring	0.022–0.048, n = 39			0.017–0.045, n = 5	0.023–0.147, n = 52
Metamorphosis <sup>b</sup>	0.020–0.087, n = 58			0.025–0.095, n = 14	

<sup>a</sup> Bernardo (1994).

<sup>b</sup> 1990 data.



**Fig. 3.** Comparative seasonal patterns of arthropod prey abundance in rockface habitats of larval *D. ocoee* at low elevation (Cullasaja, diamonds) and high elevation (Whiteside, triangles) sites. Symbols show the mean number of arthropods observed/dm<sup>2</sup> (± SE) based upon 30 1-dm<sup>2</sup> samples, except for two samples where n = 25 and 28.

prey was an order of magnitude higher at Cullasaja than at Whiteside (Mann-Whitney *U*-test,  $Z = 9.689, P < 0.0001$ ; Table 1).

**Comparison of feeding activity among all rockface sites between autumn and spring**

Considering all of the rockface sites, 84.3% of larvae were feeding in the autumn, but a significantly larger

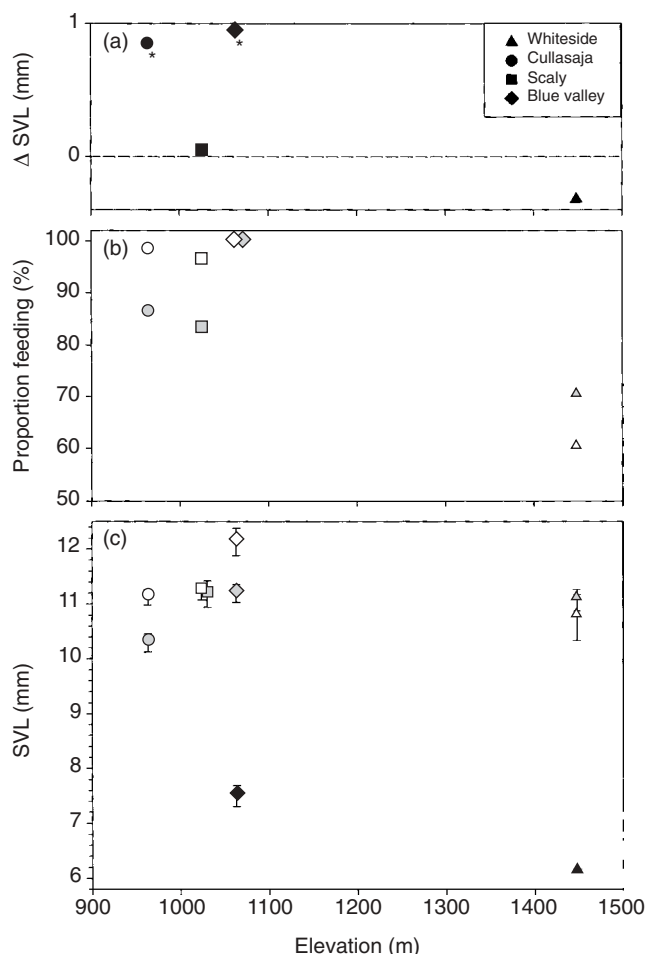
proportion, 97.6%, were feeding by the spring sample (d.f. = 185,  $\chi^2 = 6.91, P = 0.0086$ ). However, the pattern of feeding at different sites varied a great deal (Fig. 4). Moreover, a higher proportion of larvae were feeding in the autumn (91.2% vs 70.6%) and spring (97.7% vs 60.0%) at all three low elevation populations compared with the high elevation population (Fisher's exact test, two-tail, autumn: d.f. = 49,  $P = 0.0986$ ; spring: d.f. = 132,  $P = 0.0107$ ; Fig. 4).

**Comparison of size and growth between high and low elevation rockface sites**

Comparisons among rockface populations show considerable variation in mean SVL at each sampling stage and mean growth ( $\Delta$ SVL) between stages (Fig. 4). Both low elevation Cullasaja and Blue Valley larvae grew significantly from autumn to spring (Cullasaja:  $\Delta$ SVL = 0.83 mm;  $t = -2.817, d.f. = 52, P = 0.0068$ ; Blue Valley:  $\Delta$ SVL = 0.94 mm;  $t = -2.486, d.f. = 51, P = 0.0163$ ; asterisks in Fig. 4a). In contrast, little growth occurred during this same interval at one low elevation rockface (Scaly:  $t = -0.124, d.f. = 61, NS$ ) and at the high elevation rockface (Whiteside:  $t = 0.735, d.f. = 19, NS$ ).

**Comparison of feeding and growth between high elevation rockface and woodland sites**

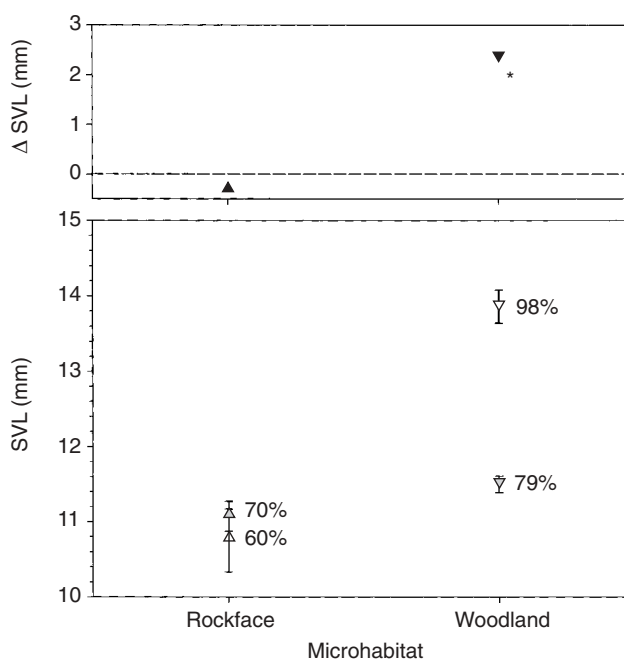
There was no difference between the Whiteside woodland and rockface populations in the proportion of larvae



**Fig. 4.** Comparison of larval *D. ocoee* from four rockface populations at different elevations: (a)  $\Delta$ SVL; (b) proportion feeding; (c) mean SVL ( $\pm$  SE). (a) Difference in mean SVL between autumn and spring; (b) and (c), larval stage symbol shade: hatching (solid); autumn (shaded); spring (open). Note that some symbols are offset by 6 m elevation purely for clarity to minimize symbol overlap. See Table 1 for sample sizes.

feeding in the autumn (70% vs 79%; Fisher’s exact test, two-tail, d.f. = 43, NS), but there was a significant difference in feeding activity between the microhabitats in the spring (60% vs 98%; Fisher’s exact test, two-tail, d.f. = 60,  $P = 0.0153$ ). This difference is solely the result of a significant increase between autumn and spring in feeding activity by the woodland larvae (79% vs 98%; Fisher’s exact test, two-tail, d.f. = 83,  $P = 0.0046$ ) compared with the rockface larvae, which showed no significant change in feeding activity between autumn and spring (70% vs 60%; Fisher’s exact test, two-tail, d.f. = 20, NS).

These patterns of feeding activity translated into parallel differences in larval growth. Comparison of larval growth ( $\Delta$ SVL) between the rockface and woodland populations at Whiteside revealed significant growth by woodland larvae between autumn and spring ( $\Delta$ SVL = 2.36;  $t = -8.596$ , d.f. = 95,  $P < 0.0001$ ; Fig. 5) but no growth



**Fig. 5.** Comparison of larval *D. ocoee* growth ( $\Delta$ SVL), in different microhabitats at a high elevation site (Whiteside Mountain). (a) Difference between mean SVL in autumn and spring; asterisk, significant difference (see text); (b) mean SVL ( $\pm$  SE) in autumn (shaded symbols) and spring (open symbols). Percentages adjacent to means indicate proportion of larvae feeding. See Table 1 for sample sizes.

in rockface larvae ( $\Delta$ SVL =  $-0.32$ ;  $t = 0.735$ , d.f. = 19, NS; Fig. 5).

**Stage-specific growth between the Cullasaja and Whiteside rockface populations**

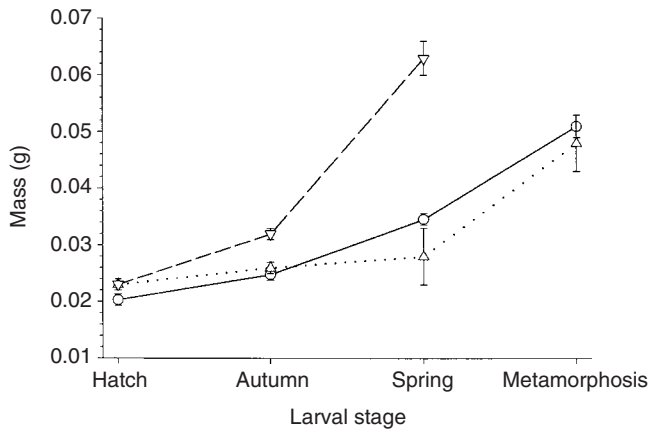
The most extensive data set on larval growth was generated for Cullasaja and Whiteside, the focal populations of the long-term study of *D. ocoee*. These data enabled us to analyse patterns of mass change as a function of larval stage (i.e. hatching, autumn, spring) at each site, both with and without the data from the Whiteside woodland population.

In both analyses, there was a significant effect of larval stage on mass (Table 2a,b), indicating that the magnitude of larval growth in both rockface and woodland habitats varies through the larval period.

There was no significant site effect on larval mass overall when considering only the rockface populations (Table 2a), but there was a significant site effect when the woodland population was included in the analysis (Table 2b). Thus, overall, the rockface populations did not differ in mass. However, in the ANOVA of the rockface populations only (Table 2a), there was a significant site  $\times$  stage interaction for mass, indicating that, although there was no overall difference between the sites, the pattern of mass variation between the sites depended upon

**Table 2.** Effects of larval stage and site on larval mass in the Cullasaja and Whiteside study populations. ANOVA: (a) comparing only the rockface populations; (b) including the Whiteside woodland population

Source	d.f.	Mass		
		Sums of squares	F-value	P
<b>(a)</b>				
Stage	2	0.0011	14.60	< 0.0001
Site	1	0.0000	0.44	0.5046
Stage × site	2	0.0003	4.28	0.0159
Error	127	0.0050		
<b>(b)</b>				
Stage	2	0.0089	27.55	< 0.0001
Site	2	0.0080	24.78	< 0.0001
Stage × site	4	0.0077	11.97	< 0.0001
Error	228	0.0367		



**Fig. 6.** Trajectories of growth in mass of three larval *D. ocoee* populations from different elevations and/or microhabitats: Cullasaja (solid line), low elevation, rockface; Whiteside (dotted line), high elevation, rockface; Whiteside (dashed line), high elevation, woodland. Note that hatchling data for the Whiteside rockface population were also used for the Whiteside woodland population (see explanation in text). Mass at metamorphosis data were collected from different cohorts and so were not included in statistical analyses. There was a significant effect of larval stage, site, and stage × site on mass (ANOVA, Table 2).

the stage at which the comparisons were made. This is clearly evident in Fig. 6. Although Whiteside rockface larvae, which hatch from larger eggs (Fig. 1; Bernardo, 1994), had an initially larger mass than Cullasaja larvae, there was no significant difference in mass by the autumn sample; this must be because of greater growth at Cullasaja. By the spring sample, Cullasaja larvae had surpassed their counterparts at Whiteside in mean mass, so the growth curves of Cullasaja and the Whiteside rockface populations crossed sometime between late autumn and early spring as a result of the continued greater growth at Cullasaja. This mass difference is preserved throughout the rest of the larval period because Cullasaja larvae

metamorphose at a larger size than the Whiteside rockface larvae do (Fig. 6; Bernardo & Reagan-Wallin, 2002).

While Cullasaja rockface larvae grew relative to Whiteside rockface larvae, Whiteside woodland larvae grew more and were much larger than both rockface populations (Fig. 6, Table 2b). In fact, Whiteside woodland larvae grew more and were larger than larvae from all other populations in autumn and spring.

## DISCUSSION

### Larval life history of *Desmognathus ocoee*

Our data shed new light on the larval life history of *D. ocoee* and the dynamics of growth in a species of plethodontid salamander with a short larval period. Before this study, little was known about the larval life history of *D. ocoee* except for Bruce's (1989) analysis of a single population at an average elevation of 950 m, and comparative data on size at metamorphosis from five populations ranging from 805 to 1448 m (Bernardo & Reagan-Wallin, 2002). This study shows that, like many other amphibian species, there is significant growth during the larval phase of the life cycle of *D. ocoee* beyond that fuelled by residual yolk. Moreover, our study shows that the magnitude and seasonal pattern of growth varies within and among populations, and that the amount and pattern of growth is associated with variation in levels of feeding activity, both within and among populations.

The larval growth patterns described in this study extend previously published data in several ways. First, Bruce (1989) sampled *D. ocoee* larvae in October/November, April/May, and June. He found mean growth (*c.* 1 mm SVL) only between the April/May and June samples and concluded that most growth occurred only during this brief period leading to metamorphosis. However, he did not examine hatchlings to determine whether his late autumn samples (October–November) showed growth relative to hatchling size. Our samples spanned the entire larval life history, from hatching, through autumn, early spring (April) and metamorphosis in early summer (Fig. 2). Although our data, like Bruce's (1989), show accelerated growth during this last part of the larval life history (Fig. 6), significant growth was also found throughout the larval stage.

Second, substantial variation among populations in the magnitude of larval growth is documented for the first time, and this itself depends upon the stage and the site at which comparisons are made. We found no growth in some populations, growth of about 1 mm SVL (comparable to that documented by Bruce, 1989) in others, and in the Whiteside woodland population, > 2 mm growth from autumn to spring (Figs 2 & 3). In terms of mass, more dramatic differences were found, ranging from a doubling in mass in some rockface populations, to a tripling in mass in the Whiteside woodland population (Fig. 6).

Third, significant variation was found in the feeding activity and growth of larvae at different elevations and in different microhabitats. A higher proportion of larval

*D. ocoee* was feeding in low elevation rockface and high elevation woodland habitats than in the high elevation rockface habitat (Figs 4, 5). The lower proportion of individuals feeding at the high elevation rock face is probably a result of the cooler environment (which both reduces activity levels (Bernardo & Reagan-Wallin, 2002: Fig. 5, path 1) and rates of yolk absorption) as well as to less food resources being available. In addition, Whiteside individuals hatch out of larger eggs than individuals from low elevations, providing them with more yolk to sustain them for a longer period before feeding. With the exception of Scaly larvae, greater feeding activity corresponded with greater growth in terms of SVL (Figs 4 & 5) and mass (Fig. 6).

### Causes of variation in larval growth and the role of temperature

Although the widely accepted model of the life history of amphibian larvae (Smith-Gill & Berven, 1979) argues that the effect of temperature on ectotherm development is the principal cause of clinal variation in their growth, data from several species of plethodontid salamanders are inconsistent with this model (Bernardo & Reagan-Wallin, 2002). Temperature affects ectotherm life histories through multiple avenues (influencing activity patterns, active season length, and metabolic expenditures) and larval growth is also affected by a variety of factors in addition to temperature (Newman, 1998; Bernardo & Reagan-Wallin, 2002). Moreover, experiments with other amphibians in which multiple factors are simultaneously manipulated suggest that food abundance, rather than temperature, has the most significant effect on larval growth (Newman, 1998). Note that no larval growth was observed in one *D. ocoee* population at a low elevation (Scaly, Fig. 4), suggesting that factors other than temperature or food abundance (e.g. larval density; Newman, 1998) influence larval growth.

In this study, arthropod prey availability (Fig. 3) and maximum daily temperature co-varied in the rockface populations studied, with both variables being higher at the representative low elevation site compared with the high elevation site. Thus, there is a longer seasonal opportunity for larval activity as well as higher resources available to larvae at low, compared with high sites. Also, Cullasaja larvae hatch from smaller eggs (Fig. 2) in this warmer environment. Both of these factors speed yolk resorption (Montague, 1979, 1987; Bradford, 1984, 1990) meaning that low elevation larvae also initiate feeding sooner than high elevation larvae. The combination of initiating feeding activity sooner, having a longer active season into the autumn and earlier in the spring, and having higher food availability, all permit significant larval growth at Cullasaja compared with the Whiteside rockface population. Indeed, Cullasaja larvae surpassed Whiteside larvae in body size by metamorphosis (Figs 2 & 6), thus producing the shift in the size clines between life cycle stages shown in Fig. 2.

However, our data show that larval growth in any particular population depends on the peculiarities of local resource levels. Both the relative scarcity of prey and limited active season at the Whiteside rockface site could explain the poor larval growth there, but the rapid growth in the adjacent woodland site, which must experience a nearly identical active season and biophysical environment (compared with the low elevation sites), implicates local prey abundance rather than local thermal environments as the dominant factor influencing larval growth. Tilley (1974) compared the growth of terrestrial juveniles of two *D. carolinensis* (a closely related species; Tilley & Mahoney, 1996) populations from rockface and woodland habitats and observed greater growth in the animals from the woodland. He postulated that greater juvenile growth rates in woodland habitats reflected more extensive and dependable food resources in leaf litter than on rockfaces. Bernardo (1994) experimentally supplemented prey for juvenile *D. ocoee* and observed remarkable acceleration in growth, indicating that their growth is very sensitive to local food levels. Although food and temperature levels were not manipulated in this study, our comparative quantitative field data (*sensu* Bernardo, 1998) show clearly that other factors besides temperature can profoundly influence patterns of amphibian larval growth. In the light of Tilley's (1974) and Bernardo's (1994) data on juvenile growth as well as the correlation between feeding activity and larval growth reported here, the extraordinary larval growth observed in the Whiteside woodland population compared with all rockface populations (regardless of elevation), and especially compared with that of the adjacent rockface population in the same biophysical environment, strongly implicate the direct role of local food availability in determining larval growth rates (Figs 5 & 6).

### Implications of larval growth dynamics for life cycle evolution in *D. ocoee*

Our quantitative field data on feeding and growth patterns of various populations have provided insights into sources of selection on the life cycle in *D. ocoee*. In particular, the limited opportunity for larval growth at the rockface site at high elevation could select for increased maternal investment, an adaptive maternal effect (Bernardo, 1996a,b) as suggested by Bernardo (1994).

When larvae hatch in late summer, opportunities for growth are greater at lower than at higher elevations because the active season is longer (Bernardo, 1994), and food availability is ten times higher than at high elevations. The quantitative field data on cohort growth patterns reported here show that larvae from the Whiteside rockface have grown less than larvae from low sites by the following spring (Figs 4 & 6), and by metamorphosis in early summer (Fig. 2), larvae from low elevations have surpassed the body size of their counterparts at the Whiteside rockface (Figs 1 & 6).

If there is a minimum threshold for size at metamorphosis (Wilbur & Collins, 1973), perhaps related to

the allometry of prey capture in the terrestrial habitat and below which a metamorph would have low fitness, selection would be expected to adjust metamorphic size towards that threshold, e.g. by extending larval period or accelerating growth. However, if larvae cannot achieve sufficient growth because of extrinsic resource limitation and limited opportunities for growth at high elevation sites, females may have to compensate for the lack of larval growth by increasing their *per capita* investment to produce offspring with a viable metamorphic size (Bernardo, 1996a,b).

*Desmognathus* larvae hatch with substantial yolk reserves (Orr & Maple, 1978; Bradford, 1984; Montague, 1987). In *D. ochrophaeus* (a similar-sized, closely related species; Tilley & Mahoney, 1996) incubated at constant temperatures of 10–15°C, these reserves last for 70 to 148 days after hatching (Orr & Maple, 1978; Montague, 1979; Bradford, 1984). It is clear that *D. ocoee* invest far more in an egg than is required to produce a viable hatchling, and that these reserves are greater the larger the eggs. It is possible, therefore, that the later-maturity and average larger body sizes attained by higher elevation females (Tilley, 1980) may be an adaptation to produce larger offspring at metamorphosis by influencing the starting size of a larva, or by insuring adequate hatchling growth by provision of post-hatching yolk reserves (Bradford, 1984). Thus, it would seem that the elevational cline in egg size in *D. ocoee* reflects a gradient in selection for increasing maternal compensation for lack of larval growth in the form of larger eggs (Fig. 1). Ongoing experimental analyses of how egg size and local environments influence larval growth and survival (J. Bernardo & S. J. Arnold, pers. obs.) will disentangle how habitats with distinct elevations have moulded the evolution of lifecycle in *D. ocoee*.

The detailed analysis of growth variation within and among natural populations occurring in a variety of habitats and elevations show that the dynamics of ectotherm growth are complexly related to multiple variables, and consequently, that the ultimate size attained by an ectotherm is not predictable by consideration of a single variable. In laboratory studies of ectotherm growth and development, in which food level is not usually manipulated, temperature has been found to influence body size (e.g. Smith-Gill & Berven, 1979; Partridge *et al.*, 1994; Partridge & French, 1996; Van Voorhies, 1996; Robinson & Partridge, 2001 and others; see discussion in Bernardo & Reagan-Wallin, 2002). In contrast, where both temperature and food level have been manipulated orthogonally, the overwhelming impact upon growth rates is found to be food level (e.g. Bernardo, 1994; Newman, 1998). If a variable is not permitted to vary in an experiment, it cannot explain any of the variance observed in the response variable (Bernardo, 1998). Moreover available data suggest that in natural ectotherm populations, local resource environments are far more closely related to differences in ectotherm growth and body size than is temperature (e.g. Bernardo, 1994; Newman, 1998; Jonsson *et al.*, 2001; Bernardo & Reagan-Wallin, 2002; this study), or that the two variables

complexly determine clinal body size patterns (e.g. Dunham *et al.*, 1989). Future laboratory analyses aimed at understanding the dynamics of ectotherm growth and size along clines should consider these findings and should adopt factorial designs in which food and temperature are manipulated orthogonally (as in Bernardo, 1994 or Newman, 1998). It will be interesting to see whether such studies will support the sweeping generalizations that have been made about ectotherm size clines and temperature (Atkinson, 1994, 1996; Berrigan & Charnov, 1994; Sibly & Atkinson, 1994; Atkinson & Sibly, 1997).

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