2003

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Recommended Citation
Ryan, T. J., and R. D. Semlitsch. 2003. Growth and the expression of alternative life cycles in the salamander *Ambystoma talpoideum* (Caudata: Ambystomatidae). *Biological Journal of the Linnean Society* 80:639-646.

Growth and the expression of alternative life cycles in the salamander *Ambystoma talpoideum* (Caudata: Ambystomatidae)

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Abstract

Complex life cycles (CLCs) contain larval and adult phases that are morphologically and ecologically distinct. Simple life cycles (SLCs) have evolved from CLCs repeatedly in a wide variety of lineages but the processes that may underlie the transition have rarely been identified or investigated experimentally. We examined the influence of larval growth rate on the facultative expression of alternative life cycles (metamorphosis or maturation as gill-bearing adults [= paedomorphosis]) in the salamander *Ambystoma talpoideum*. We manipulated growth rates by altering the amount of food individuals received throughout larval development. The expression of alternative life cycles in *A. talpoideum* is influenced by growth via food levels, but the same growth rates at different points in the larval period elicit different responses. Individuals were more likely to metamorphose (i.e. express a CLC) when food levels and growth rates were high later in development and more likely to mature without metamorphosing (SLC) when growth rates were comparatively low during the same point in development. Growth rates at particular points in development, rather than overall larval growth rate, may be an important proximate factor in salamander life-cycle evolution.

Keywords: life-cycle evolution; life-cycle polymorphism; metamorphosis; paedogenesis; paedomorphosis

INTRODUCTION

Complex life cycles (CLCs) are characterized by at least two distinct postembryonic morphologies and associated ecologies. Understanding the evolution and maintenance of CLCs has been a vexing ecological and evolutionary problem. Istock (1967) asserted that CLCs were inherently unstable and predicted that only one stage should persist. Yet CLCs are not only phylogenetically ancient and highly conserved (Moran, 1994) but also more common among animals than are simple life cycles (SLCs). While SLCs have evolved repeatedly in a wide array of taxa (Werner, 1988), the transition from CLC to SLC is difficult to study on the proximate level. Lineages representing each life cycle are most often separated by millions of years of divergent evolution obscuring the ecological factors that shaped or forced the transition.

Among amphibians the most common SLC is direct development, the elimination of the free-living larval stage. While direct development can be achieved by simply 'moving' metamorphosis to a point prior to hatching (Ryan & Bruce, 2000), more frequently a significant reorganization of the developmental program is required (Hanken, Jennings & Olsson, 1997; Marks, 2000). Because a morphogenic rearrangement is necessary, direct development is not easily incorporated into an ecological model. The other SLC exhibited by amphibians is commonly called paedomorphosis (Gould, 1977) or perennibranchism (Rose, 1996). Paedomorphosis may result from simply delaying metamorphosis until some point after maturation (Reilly, Wiley & Meinhardt, 1997; Ryan & Bruce, 2000). Paedomorphosis is fixed in many taxa (e.g. *Siren* and *Necturus*) but is facultative in some (e.g. *Ambystoma* and *Notophthalmus*). The facultatively paedomorphic species (also referred to as paedotypic by Reilly *et al.*, 1997) make it possible to study the costs, benefits and limitations of alternative life cycles. If facultatively paedomorphic lineages give rise to obligately paedomorphic species (Shaffer, Clark & Kraus, 1991), then present day facultative species are the ideal model for investigating the ecology that underlies amphibian life-cycle evolution.

The proximate mechanism responsible for the expression of alternative life cycles in polymorphic (i.e. facultatively paedomorphic) salamanders is largely unknown. Whiteman (1994) suggested that larval growth rate may be a key determinant of life-cycle expression. According to his model, the adult phenotypes (metamorphic and paedomorphic) associated with CLCs and SLCs, respectively, vary significantly in fitness. His model regards fitness as a function of body size, which is in turn affected mainly by larval growth rate (Figs 1, 2 and table 2 in Whiteman, 1994). The validity of the relationship between body size and fitness as a causative agent in salamander life-cycle polymorphisms is not altogether clear (Ryan & Semlitsch, 1998). The role of larval growth as a determinant of life-cycle expression is theoretically sound (Whiteman, 1994) and enjoys some circumstantial support (e.g. Harris, 1987; Denoël & Poncin, 2001) but has not been rigorously tested.

The mole salamander, *Ambystoma talpoideum* (Holbrook), is the best studied of the polymorphic salamander species. Life-cycle expression in *A. talpoideum* is influenced by environmental conditions (e.g. hydroperiod and larval density; Semlitsch, 1985, 1987a, 1987b) but it also has a genetic component that is responsive to natural (Semlitsch & Gibbons, 1985; Harris *et al*., 1990; Semlitsch, Harris & Wilbur, 1990) and artificial (Semlitsch & Wilbur, 1989) selection. Both adult

phenotypes may be expressed in a single population provided that the breeding site holds water continuously throughout the year rather than drying annually (Semlitsch *et al.*, 1990; Winne & Ryan, 2001). Here we investigate the role of an ecologically mediated trait, larval growth rate, in the expression of alternative life cycles in *A. talpoideum*. We simulated a variety of growth conditions in the laboratory by controlling the food availability for individual *A. talpoideum* larvae. We ask: how does the availability of resources throughout larval development relate to growth and the expression of alternative life cycles?

METHODS

The design of this experiment was intended to eliminate behavioural and ecological differences in the ability of larvae to procure resources so that growth would depend solely on availability and assimilation of resources. We controlled resource availability experimentally but did not control natural genetic variation in assimilation. To generate different growth trajectories, we used six feeding regimes, varying the amount of food during three discrete periods of larval development using two constant food levels (low [1×] and high [4×]; symbolized as LLL and HHH, respectively) and four treatments with a shift in food levels (from high to low or vice versa) that was implemented either early (day 87; HLL and LHH) or late (day 128; HHL and LLH). This is essentially the same protocol as that used by Alford & Harris (1988) and others (e.g. with larval amphibians: Hensley, 1993; Leips & Travis, 1994; Beck, 1997; Beachy, Surges & Reyes, 1999; with insects: Bradshaw & Johnson, 1995; with marine invertebrates: Twombly, 1996) to investigate not only the role of growth in life-cycle transitions, but also the importance of ontogenetic changes in growth that occur as resource levels fluctuate with time (see Alford & Harris, 1988). The switch points used in this experiment are relevant to *A. talpoideum*, as they bracket the minimum age at which metamorphosis is known to occur (Petranka, 1998). Each treatment combination was replicated seven times in each of three spatial blocks (shelves), for a total of 126 individually reared larvae.

Procedures

We used the progeny of wild-caught metamorphic adult *A. talpoideum* from Rainbow Bay (Barnwell County, South Carolina, USA). Rainbow Bay is an ephemeral pond and thus the population tends to express only the CLC in most years. However, in experiments when water levels are held constant, as much as 85% of the population may demonstrate the SLC (e.g. Semlitsch *et al.*, 1990; Ryan & Semlitsch, 1998). On 25 February 1997, six male and six female adults were introduced into a polyethylene cattle tank 1.36 m in diameter, containing approximately 1000 L aged tap water and 2 kg leaf litter. Eggs were first observed in the breeding pond on 1 March 1997; we removed males and spent females between 1 and 9 March 1997.

Approximately 500 developing eggs were transported from the breeding pond to the laboratory on 10 March 1997. Once hatching was completed, individual larvae were randomly assigned to 5-L polyethylene containers (32 cm long × 19 cm wide × 11 cm deep) filled with 3 L carbon-filtered tap water. We randomly assigned treatments to containers. The feeding regimes were initiated on 17 April 1997 (day 1). Initially, larvae were fed freshly hatched brine shrimp (*Artemia*) daily. On day 38, we began feeding all animals newly hatched earthworms (*Lumbricus* sp.) every other day,

and fed them larger earthworms as the larvae grew. We based food levels on the amount that larvae in the HHH treatment consumed in a single feeding. The 1 : 4 ratio between low and high food levels was maintained regardless of food type. The food levels thus increased throughout the experiment, approximating mass-specific food levels (see Beck, 1997).

We changed the water in all containers every 5–7 d. We measured the size (snout–vent length [SVL]: the distance in mm from the tip of the snout to the caudal margin of the cloacal aperture) of larvae approximately every 20 d until the second food-shift (day 128), and approximately every 40 d thereafter until the termination of the experiment on day 228. All larvae were checked daily for morphological indications of metamorphosis (resorption of external gills and tail fin). Upon complete gill resorption we sacrificed and preserved each metamorph (via prolonged exposure to chlorotone solution followed by fixation in 7% formalin). On day 228, all surviving gill-bearing individuals (immature and mature, hereafter referred to collectively as branchiates) were similarly sacrificed and preserved. All animals were then measured (mm SVL) and dissected to determine reproductive status. We assigned individuals to reproductive categories based on the gross morphology of their reproductive organs. Males with enlarged testes and coiled vasa deferentia or females with enlarged ova (>1 mm diameter) and convoluted oviducts were regarded as mature (Ryan & Semlitsch, 1998; Winne & Ryan, 2001). All individuals that had completed metamorphosis were scored as expressing the CLC. Mature branchiates were scored as expressing the SLC. Individuals with undeveloped gonads and immature vasa deferentia/oviducts were considered immature juveniles, and had at that point committed to neither the CLC nor the SLC.

Analyses

For each of the switch treatments we used a two-part analysis to analyse growth rates. First, we used equivalence tests (see Dixon, 1998) to determine whether prior to a switch individuals were similar in size to individuals in the other treatments that experienced the same food levels (e.g. at the early switch, HLL vs. [HHL + HHH]). When equivalence tests indicated no growth difference prior to the switch, we used two-way analysis of variance (ANOVA; treatment and blocks as main effects) to test whether growth of switch and non-switch groups differed following the switch. We measured growth as the difference in log-transformed body size (SVL) between the switch date and 40 d thereafter. A significant result in the ANOVA indicated the food switch was responsible for generating different growth trajectories.

We used goodness-of-fit tests to determine the effect of growth on life-cycle expression. Tests determined whether a given food level (either H or L) at a particular point in the larval period (prior to the early switch, between the two switch times, or after the late switch) influenced the expression of phenotypes. Because the experimental design was not fully crossed (i.e. no treatments featured a switch back to an original food level), a series of three three-way goodness-of-fit tests were required to resolve these effects. Each treatment was therefore used in two of the three tests; we therefore reduced α to 0.025 (= 0.05/2) a priori in these tests. Finally, we used ANOVA to determine whether the immature and mature branchiates differed in size at the end of the experiment.

Distribution of phenotypes across treatments

Overall, at the end of the experiment, 48.4% of the survivors were mature branchiates, 42.7% were metamorphs, and 8.9% were immature larvae. The different growth trajectories also influenced the expression of life cycles (Fig. 2). However, only food level during the final growth period (i.e. after the second food-shift) was significant, along with an interaction between the final and earlier growth (Table 2). The food levels in the early or middle periods did not effect phenotypic expression (Table 2), although growth during each of the periods was affected by the food treatments (Table 1, Fig. 1). High food levels after the late switch resulted in a higher expression of metamorphosis regardless of the previous food and growth levels (Fig. 2). The response to late food levels was symmetrical for both of the previous food levels (Fig. 3). Thus the interaction effect (Table 2) was due to the absence of metamorphs in the LLL treatment, the only treatment in which none were produced. Mature branchiates were most common in the treatments that ended on low food levels (HLL, HHL, and LLL).

Mature branchiates (32–59 mm SVL) encompassed the size range of metamorphs (36–53 mm) and immature larvae (36–50 mm), save for one small immature individual (31 mm). Across treatments, mature branchiates were larger than immature larvae ($F_{1,76}$ = 4.63, P = 0.0346; mature: mean = 43.7 mm, SE = 0.51; immature: mean = 39.5 mm, SE = 1.68). Because we could not assay branchiates for maturation prior to the end of the experiment, we cannot describe the onset of maturation, although we have previously shown that maturation occurs in branchiates as early as 120–150 d posthatching (Ryan & Semlitsch, 1998).

Maturation among metamorphs and branchiates

Most metamorphs were immature at the completion of metamorphosis (83%), although some individuals showed early signs of maturation (15%) and one individual was sexually mature. Metamorphs <1 year posthatching are usually sexually immature in experiments (Semlitsch, 1987a,b; Jackson & Semlitsch, 1993; Ryan & Semlitsch, 1998) as well as natural populations (Semlitsch, 1985; Semlitsch, Scott & Pechmann, 1988; T. J. Ryan, unpubl. data). The mature metamorph in our experiment probably matured in the larval form and then subsequently underwent metamorphosis (Semlitsch, 1985; Winne & Ryan, 2001). This was the largest (53.5 mm SVL) and oldest (222 d) individual to metamorphose in the study.

All branchiates were above the minimum size for maturation (about 30 mm SVL (Semlitsch, 1985, 1987a,b; Ryan & Semlitsch, 1998)) and most were sexually mature (87%). The immature branchiates were from treatments with initially low food levels. The majority of immature branchiates (64%) were from the LLL treatment.

DISCUSSION

Our study is the first to rigorously test the hypothesized link between larval growth and alternative life cycles in amphibians (Whiteman, 1994). We found that food levels influenced larval growth rates regardless of the point in the larval period at which different food levels were experienced. Life-cycle expression, however, was influenced primarily by growth rate in the later portion of the larval period (and its interaction with earlier growth rate). Among branchiates, mature individuals were slightly larger on average compared with immature ones. Few metamorphs showed early

signs of maturation. The majority of immature branchiates came from the LLL treatment and all immature branchiates came from treatments in which initial growth conditions were low.

Whiteman's (1994) model for the evolution of facultative paedomorphosis presumes that there are fitness differences among immature larvae, metamorphic adults and branchiate adults. Fecundity, mating success, and survival are the life history traits most frequently investigated as sources of fitness inequality. Because these fitness components are influenced by adult body size (Salthe, 1969; Kaplan & Salthe, 1979; Verrell, 1982; Howard, 1983) and larval growth rate may be a significant determinant of adult body size in many amphibians (Wilbur, 1980) – and perhaps the greatest predictor in pond-breeding salamanders (Semlitsch *et al.*, 1988) – larval growth rate plays a key role in determining the expression of alternative life cycles in Whiteman's (1994) model.

In this regard, our results might be viewed as qualified support for Whiteman's Best-of-a-Bad Lot hypothesis. According to this hypothesis, larvae that pass a theoretical maturation threshold but do not reach a critical size for metamorphosis become branchiate adults (Whiteman, 1994). Due to their small body size these branchiate adults are expected to have lower fitness compared with metamorphic adults but higher fitness compared with immature larvae of the same age. We found that low growth rates late in development most often resulted in precocious maturation and the expression of an SLC. More robust growth rates late in the larval period led to metamorphosis. Our results clearly demonstrate a link between larval growth rate and life-cycle expression, with growth relatively late in the larval period being considerably more important than is earlier growth.

Although larval growth rate may influence life-cycle expression, there is no clear association between adult body size and life-cycle expression, as presumed in Whiteman's model. In a previous experiment (Ryan & Semlitsch, 1998) we demonstrated that maturation is asynchronous among the adult phenotypes, with branchiates maturing significantly (as early as 150 d posthatching) earlier than did metamorphs (all still immature at 240 d). Also, there were no significant differences in size among metamorphs and mature or immature branchiates of the same age (Ryan & Semlitsch, 1998). Larval growth rates averaged over the entire larval period (i.e. broadly estimated as [size at metamorphosis or maturation − size at hatching]/length of larval period) appear to be independent of life-cycle expression (e.g. *A. talpoideum*: Jackson & Semlitsch, 1993; Ryan & Semlitsch, 1998; Winne & Ryan, 2001; *A. tigrinum*: Voss, 1995; *A. gracile*: Licht, 1992). A notable exception is Harris's (1987) cattle tank experiment using *Notophthalmus viridescens*: metamorphosis was most common in high-density tanks in which growth rates were low compared with low-density (1/4×) tanks in which growth rates were higher and the SLC was more frequent. Although this may be interpreted as being support for Whiteman's Paedomorph Advantage hypothesis (in which robust larval growth rates lead to branchiate adults which are larger, and thus presumably more fit, than are metamorphic adults), overall growth rates in Harris's experiment are confounded with density and the potential associated 'social' effects (e.g. Brunkow & Collins, 1996). Furthermore, Harris's growth rates were based on population (= pond) means determined at the end of the experiment rather than on individual growth trajectories measured continuously throughout the larval period.

The primary benefits of the SLC in *A. talpoideum* is likely reduced time to first reproduction and a release from the costs and constraints of annual migrations rather than any size- or growth-based

fitness source, such as fecundity (Ryan & Semlitsch, 1998). Although individuals may be 'making the best-of-a-bad lot' by becoming mature because recent or early growth histories are insufficient to initiate metamorphosis (when the latter might be preferable in terms of body size), the benefits of early maturation and reproduction (Scott, 1993; Krenz & Sever, 1995) can offset a reduction in fecundity (Semlitsch, 1985) or mating success suffered as a result of maturing at a smaller body size. The nature and extent of the trade-off between the timing of reproduction and fitness has not been thoroughly examined, and promises to be extremely informative with regard to the evolution and ecology of this life-cycle polymorphism.

We have demonstrated that in *A. talpoideum* the SLC results from precocious maturation (Ryan & Semlitsch, 1998) and that larval growth rate during a critical period is a causal factor (this study). In most salamander families SLCs have evolved via paedomorphosis at least once (the exception being the monogeneric Rhyacotritonidae) and multiple times within the Ambystomatidae (Shaffer *et al.*, 1991), Plethodontidae (Ryan & Bruce, 2000), and Salamandridae (Griffiths, 1996). We do not suggest that larval growth rate has been causal in the evolution of SLCs in other salamander lineages. For example, Voss (1995) demonstrated that growth rates do not correlate with life-cycle expression in back-crossed lines of *A. mexicanum* and *A. tigrinum*. In this lineage, genetic factors appear to be far more important than are ecological ones (Voss & Shaffer, 1997). With perhaps as many as a dozen independent origins of SLCs within the Ambystomatidae (both fixed and facultative; Shaffer, 1993) and another dozen or more in the rest of the Caudata (T. Ryan & C. Beachy, unpubl. data), and given the general complexity of paedomorphosis on physiological and genetic scales (Tompkins, 1978; Voss, 1995; Rosenkilde & Ussing, 1996; Voss & Shaffer, 2000), it is reasonable to conclude that SLCs have probably developed via a diversity of mechanisms. In most cases it may be impossible to identify the past ecological processes that have resulted in the present evolutionary patterns. However, species exhibiting a life-cycle polymorphism – where both the ancestral and derived life cycles are expressed – provide a valuable opportunity to examine the link between evolutionary patterns and ecological processes within an experimental framework.

ACKNOWLEDGEMENTS

J. H. K. Pechmann donated the parental stock of *A. talpoideum* from the University of Georgia's Savannah River Ecology Laboratory; C. M. Bridges supplied food and, with J. R. Bodie, M. D. Boone, M. J. Parris, and A. M. Welch, fed animals when we could not; N. Mills was especially helpful in maintaining the experiment. We are grateful to C. K. Beachy, R. J. Denver, C. Galen, T. H. Holtsford, D. B. Noltie, D. N. Reznick, R. J. Wassersug, A. M. Welch, H. H. Whiteman, and a pair of anonymous reviewers for discussion of these topics and/or comments on this manuscript. This research was funded by a University Research Board Grant (to R.D.S.); a Grant-in-Aid of Research from Sigma Xi (to T.J.R.); a Summer Research Fellowship from the Division of Biological Sciences at University of Missouri (to T.J.R.); and a Doctoral Dissertation Improvement Grant from the National Science Foundation (NSF DEB 9801458 to R.D.S. and T.J.R.).

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Table 2 Summary of three-way goodness-of-fit tests

Period	*G*	d.f.	*P*
Early	9.096	4	>0.025
Middle	4.228	4	>0.025
Late	29.39	4	<0.0001
Late × [early + middle]	34.27	2	<0.0001

Tests determined whether food levels (high or low) experienced during each period of larval development (early, middle or late) influenced the expression of life-cycle phenotypes. Non-significant interactions are excluded. Early = day 1 to day 87; middle = day 88 to day 128; late = day 129 to day 228.

Table 1 Summary of ANOVAs for growth following food switches

	d.f.	Sums of squares	*F*	*P*
Early H to L				
Treatment	1	0.04625	47.19	<0.0001
Error	60	0.05881		
Total	61	0.10506		
Early L to H				
Treatment	1	0.08396	28.00	<0.0001
Error	60	0.17993		
Total	61	0.26389		
Late H to L				
Treatment	1	0.02595	28.74	<0.0001
Block	2	0.00533	3.72	0.0528
Interaction	2	0.00718	5.01	0.0244
Error	13	0.00931		
Total	18	0.03520		
Late L to H				
Treatment	1	0.05631	41.52	<0.0001
Error	38	0.05154		
Total	39	0.10785		

In each comparison, the response variable is the difference in size (log-transformed snout–vent length) between the day of the switch and ~40 d postswitch. All non-significant block and interaction effects have been omitted for brevity and are presented as one-way ANOVAs, except in the late H to L switch where there was a significant interaction and the full two-way ANOVA is presented.

Figure 1. Growth trajectories for individuals in six different feeding regimes. Each point represents the mean snout–vent length (±1 SE) for untransformed individuals remaining in the treatment. Arrows indicate the implementation of early (day 87) and late (day 128) switches in feeding regimes. Note that growth trajectories changed when feeding regimes changed; differences in size between switch and non-switch groups before switches were not significant whereas differences subsequent to switches were highly significant.

Figure 2. Distribution of phenotypes across treatments. Bars represent the proportion of survivors from each treatment that became mature branchiate (stippled), metamorphic (cross-hatched), or remained immature larvae (open).

Figure 3. Proportion of survivors metamorphosing in response to late food levels. Although response differed depending on early growth (HH- or LL-), the magnitude of change based on late food levels (-H or -L) was similar.