POPULATION ECOLOGY - ORIGINAL PAPER

Paedomorphosis in *Ambystoma talpoideum*: effects of initial body size variation and density

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Abstract Facultative paedomorphosis is the ability of a salamander to either metamorphose into a terrestrial, metamorphic adult or retain a larval morphology to become a sexually mature paedomorphic adult. It has been hypothesized that density and initial body size variation within populations are instrumental in cueing metamorphosis or paedomorphosis in salamanders, yet few studies have adequately tested these hypotheses in long-term experiments. Beginning in the spring of 2004, 36 experimental ponds were used to manipulate three body size variation levels (low, medium, high) and two density levels (low, high) of *Ambystoma talpoideum* larvae. Larvae were individually marked using visible implant elastomers and collected every 2 weeks in order to measure snout–vent length and mass. Bi-nightly sampling was used to collect new metamorphs as they appeared. Analysis revealed significant effects of density, size variation and morph on body size of individuals during the summer. Individuals that metamorphosed during the fall and following spring were significantly larger as larvae than those becoming paedomorphic across all treatments. These results support the Best-of-a-Bad-Lot hypothesis, which proposes that the largest larvae

metamorphose in order to escape unfavorable aquatic habitats. Large larvae may metamorphose to leave aquatic habitats, regardless of treatment, due to the colder climate and lower productivity found in Kentucky, which is in the northern-most part of *A. talpoideum's* range. By maintaining a long-term experiment, we have provided evidence for the transition of both larvae and paedomorphs into metamorphs during fall and spring metamorphosis events. Furthermore, the production of similar morphs under different environmental conditions observed in this research suggests that the ecological mechanisms maintaining polyphenisms may be more diverse that first suspected.

Introduction

Understanding the evolution of phenotypic plasticity is one of the most fundamental questions in biology (Scheiner 1993; Schlichting and Pigliucci 1998; West-Eberhard 2003). Polyphenisms are discrete examples of plasticity in which two or more phenotypes are produced in response to varying environmental conditions (West-Eberhard 1986; Moran 1992). Each alternative phenotype's fitness is dependent on its match to the prevailing environment, so that the evolution of polyphenisms is most frequently seen in environments that vary temporarily and spatially. In such variable environments, trade-offs occur in the fitness payoffs to each morph (Roff 1996; Smith and Skulason 1996; Schlichting and Pigliucci 1998). Polyphenisms are therefore maintained by selection, and studies may help clarify the role of the environment in the production and maintenance of plasticity (Caswell 1983; Smith-Gill 1983;

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West-Eberhard 1986, 2003). Unfortunately, our knowledge of the mechanisms that induce alternative morphs is still poor for many polyphenic species (Whiteman 1994; Roff 1996; Smith and Skulason 1996; West-Eberhard 2003; Denoel et al. 2005). An understanding of these mechanisms is especially important given the critical role that polyphenisms are thought to play in population divergence, speciation and life history evolution (West-Eberhard 1986,1989,2003; Via 2001).

Facultative paedomorphosis, a polyphenism in several species of salamanders, is the ability to either metamorphose into a terrestrial, metamorphic adult or retain a larval morphology to become a sexually mature paedomorphic adult (Whiteman 1994). The salamander's adult morphology is a response to the combined factors of its genotype and the environmental effects experienced as a larva (Wilbur and Collins 1973; Whiteman 1994). In species such as *Ambystoma tigrinum nebulosum*, *A. talpoideum* and *Triturus alpestris*, both sexes display the polymorphism, and interbreeding occurs between morphs (Whiteman et al. 1999; Denoel et al. 2001; Krenz and Verrell 2002).

Several hypotheses have been proposed for the production and maintenance of facultative paedomorphosis (Whiteman 1994; Denoel et al. 2005). The Paedomorph Advantage (PA) hypothesis suggests that larvae with an initial growth advantage and subsequent competitive advantage should become paedomorphic, while slower growing larvae should metamorphose to escape competition with paedomorphic adults. The Best-of-a-Bad-Lot (BOBL) hypothesis, in contrast, suggests that larvae that metamorphose should be larger than those that become paedomorphic. In this hypothesis, paedomorphic individuals are unable to obtain the critical size for metamorphosis (Wilbur and Collins 1973; Wilbur 1980), and the advantages of paedomorphosis at a small size outweigh delaying reproduction to attain larger size and subsequent metamorphosis. The Dimorphic Paedomorph hypothesis combines these two hypotheses and argues that the largest larvae in a pond should become paedomorphic because they are capable of out-competing smaller individuals, medium sized larvae metamorphose to escape competition and the smallest larvae become paedomorphic rather than delaying maturity in favor of metamorphosis (Whiteman 1994). It is possible that the different mechanisms may each occur under different environmental conditions.

A variety of observational and experimental studies have supported either the PA hypothesis or the BOBL hypothesis (reviewed in Whiteman 1994; Denoel et al. 2005). However, no current research has provided evidence for the Dimorphic Paedomorph hypothesis. This discrepancy may be due to the general lack of long-term studies on amphibians (Marsh 2001; Whiteman and Wissinger 2005). For example, several experimental studies of the facultatively

paedomorphic salamander, *A. talpoideum*, were completed while a large proportion of the animals were still larval and had yet to express their adult morphology (Semlitsch 1987a; Jackson and Semlitsch 1993; Ryan and Semlitsch 1998). These studies were initiated in February or March and terminated 8–9 months later. In these studies, favorable conditions prompted large individuals to become paedomorphic while smaller individuals metamorphosed, or a larger proportion of larvae matured as paedomorphs, both of which support the PA hypothesis (Whiteman 1994). The results and length of the experiment, however, suggest that the slowest growing larvae, if given the opportunity, may also have become paedomorphic, thus supporting the Dimorphic Paedomorph hypothesis. Field data from natural populations of *A. talpoideum* also support this idea, as small paedomorphs (presumably young of the year) have been observed breeding in the late winter (Whiteman, unpublished data), 4–5 months after the above concurrent experiments (Semlitsch 1987a,b; Jackson and Semlitsch 1993; Ryan and Semlitsch 1998) would have ended. In addition, while there is evidence that individuals can mature as paedomorphs in the fall, and subsequently metamorphose in the spring (Patterson 1978; Whiteman personal communication), no prior experiments have lasted long enough to provide experimental support.

To date, no studies have directly manipulated one of the most important variables underlying these hypotheses: larval body size variation. Instead, previous studies indirectly manipulated this variation using density or food level treatments. The results may, therefore, be in response to variables other than larval body size variation. Thus, the effect of larval body size variation on facultative paedomorphosis has never been directly tested, although such variation has been shown to have an effect on other salamander polyphenisms (Maret and Collins 1994). Finally, past studies have focused on the average size of individuals within a tank (tank means: Semlitsch 1987a,b; Jackson and Semlitsch 1993) instead of following individuals. The single study that has followed individuals maintained larvae in individual microcosms (Ryan and Semlitsch 2003) and thus cannot address competitive effects on phenotypic plasticity.

In the study reported here, we simultaneously manipulated larval body size variation and density using an experimental design which allowed a greater opportunity for individuals to become sexually mature (15 months) than has been the case in previous studies. We predicted that the high density treatment would produce smaller larvae and, therefore, smaller adults than the low density treatment due to competition and that there would be differences in variance, but not mean body size, among size variation treatments. We predicted that the BOBL mechanism would be found in the high density treatment, while the low density treatment would be more likely to support the PA

mechanism. Furthermore, we predicted that high body size variation treatments would produce results in accordance with the Dimorphic Paedomorph hypothesis, while low body size variation treatments would support either the BOBL or PA mechanisms, depending on initial density. Finally, we marked individuals within our experimental populations to provide a more accurate picture of the responses of larvae to variation in the environment.

Methods

Study organism

Ambystoma talpoideum, the mole salamander, is widespread across the southeastern USA. Metamorphic adults migrate into breeding ponds between September and March (Gibbons and Semlitsch 1991; Whiteman and Semlitsch 2005). Larvae can mature and begin metamorphosing by the following summer, and those that do not metamorphose mature as paedomorphs (Semlitsch 1987a,b; Gibbons and Semlitsch 1991). Individuals of both morphotypes may breed in the following winter (Semlitsch 1987b), and there is evidence from the field that some animals that matured as paedomorphs in the fall can metamorphose in the following spring (Patterson 1978).

Experimental design

The experimental design included six treatments, with two density levels and three levels of size variation. Thirty-six 1000-l cattle tanks were used to implement the six treatments, each with six replicates, separated into six blocks. Ponds were filled with tap water (11–19 January 2004), and leaf litter (primarily Eastern Black Oak, Southern Red Oak and Shagbark Hickory) was added to each artificial pond (19 February). After aging for 2 weeks, zooplankton were added to each pond to create a prey base for developing larvae. Zooplankton, primarily copepods and daphnids, were collected from Fenton ponds 1–3 found within the Land Between the Lakes National Recreation Area, KY, USA using a plankton net. After removing potential predators, the resulting concentrated solution was added to each tank in 500-ml aliquots approximately every other day from 25 February through 15 March.

Ambystoma talpoideum eggs were collected from Ridley Pond in Calloway County, KY on 10 February 2004 [Electronic Supplementary Material (ESM)]. Embryos were raised at one of three temperatures (9, 12.5 and 16°C) within an incubator in order to create variation in initial development. When the incubation ended, embryos that developed at warmer temperatures were in later Harrison stages of development than those raised at cooler temperatures. Specifically, embryos raised at 9, 12.5, and 16°C were at Harrison stages 26–28, 35–37 and 44–46, respectively (Harrison 1969). After introduction into an aquatic environment, we assumed that individuals raised at higher temperatures would hatch earlier, thereby giving them an initial growth advantage and subsequent greater size in comparison to individuals raised at cooler temperatures. Therefore, after being incubated for 2 weeks, unhatched embryos were randomly distributed on 1 March into treatments that included high, medium, and low body size variation and high and low density (Table 1). Size variation treatments were created by varying the ratios of individuals raised at different temperatures, which consequently altered the amount of size variation within the different treatment populations.

On 21 May 2004, a custom-made mesh covering by Xtreme Shelters (Murray, KY) was constructed over the experimental pond array; this provided 50% shade and allowed natural precipitation to reach the tanks. Between 7 June and 1 September 2004 mesh lids were used to keep out predators (e.g. odonates) during the day and removed at night to allow natural colonization by potential prey (e.g. chironomids). The use of tank covers was discontinued when the majority of larvae had reached a size larger than that of their potential predators (e.g. dragonfly larvae, etc.). Tap water was added to the tanks periodically so that water level would remain constant.

Beginning 18 May 2004, we began collecting larvae with minnow traps twice a month in order to measure snout-to-vent length (SVL) and total length with a metric ruler (nearest mm) and mass with a PB303 Delta Range balance (nearest mg), and to assess sexual maturity. By 1 July 2004, larvae were large enough to be marked individually (>17 mm; Doyle, unpublished data) using visible implant elastomer tags from Northwest Marine Technologies (Tumwater, WA). Despite all efforts, not all animals were captured and marked successfully (at the termination of the experiment, the percentage of unmarked individuals from each tank ranged from 0 to 33%, with one extreme

Table 1 Experimental design including the distribution of *Ambystoma talpoideum* embryos raised at different temperatures amongst the six treatments (two levels of density, three levels of size variation)

Variation classes	Low density	High density
Low variation	Ten larvae/12.5°C	Twenty larvae/12.5°C
Medium variation	Two larvae/9.0°C	Four larvae/9.0°C
	Six larvae/12.5°C	Twelve larvae/12.5°C
	Two larvae/16.0°C	Four larvae/16.0°C
High variation	Four larvae/9.0°C	Eight larvae/9.0°C
	Two larvae/12.5°C	Four larvae/12.5°C
	Four larvae/16.0°C	Eight larvae/16.0°C

value of 75%). By 14 July, animals had begun to reach the minimum size for metamorphosis (35 mm; Semlitsch 1987a), and tanks were checked by headlamp every other night for metamorphosing individuals. The fall metamorphosis event continued until early November, and individuals began metamorphosing again in March 2005, continuing until May (ESM). Metamorphosing individuals from both periods were immediately measured (SVL, mass) and then reared to maturity in individual sweater boxes by feeding 0.20 g of California black worms per day per individual. During 14–18 May 2005, all metamorphs, paedomorphs and larvae were sacrificed in 0.04% benzocaine solution, dissected to determine reproductive condition, fixed in 10% formalin for 24 h and preserved in 70% EtOH.

Analysis

All statistical analyses were conducted using STATVIEW (StatView 1999). The body condition of each animal was calculated by dividing the mass (mg) by SVL^3 (mm). Body condition is a more accurate reflection of the health and energetic reserves of the individual than either mass or SVL alone, and it is more closely associated with environmental quality than mass or SVL in larvae of the congener *Ambystoma tigrinum* (Denoel et al. 2002; Schulte-Hostedde et al. 2005; Whiteman et al., unpublished data). Mass and body condition were utilized for each individual prior to the beginning of each (fall and spring) metamorphosis event when data for that individual were available. The proportion of larvae that became metamorphs or paedomorphs or which remained larval was calculated by dividing the number of each type of individual produced by the total number of individuals that survived to the end of the experiment. The proportion total survival was calculated by dividing the total number of individuals collected during the experiment or at the termination of the experiment by the total number of embryos initially introduced to each tank. All analyses were conducted using two- or three-way ANOVA or repeated-measures ANOVA, depending on the comparison being made. Data were log or arcsine-square root transformed when it was clear that assumptions of homogeneity and/or normality were not met. Post hoc testing was done using Fisher Least Significant Difference (LSD).

Results

Initial treatment effects on larval body condition

Throughout the summer sampling period (May through July) and before the first metamorphosis event, body condition across all treatments increased significantly with time

($F_{2, 50} = 13.9$, $P < 0.0001$). There were no effects of size variation, density or their interaction on body condition (all $F < 4.3$, all P values > 0.05). Mean mass across tanks was affected by a significant interaction among time, density and size variation ($F_{4, 50} = 6.4$, $P = 0.003$; Fig. 1), and it increased more over time in low density tanks than in high density tanks. Additionally, mass increased substantially more in medium variation, low density tanks than in any other size variation and density combination. These general patterns, with larvae from the low density treatment weighing more than those from the high density treatment and larvae from the medium size variation treatment weighing more than those from low or high size variation treatments, continued throughout much of the remainder of the experiment whenever density and size variation had an effect on mass. For example, at the time of the first metamorphosis event, there were significant effects of density ($F_{1, 146} = 120.0$, $P < 0.0001$) and size variation ($F_{2, 146} = 14.3$, $P < 0.0001$) on mean mass of individuals within tanks, with the low density treatment having a greater mass than the high density treatment.

Metamorphosis and paedomorphosis

35 and 45 metamorphs were collected during the first (June–October of 2004) and second (April–May of 2005) metamorphosis events, respectively. Due to the small number of metamorphs collected during both events, the proportions of metamorphs, paedomorphs and larvae were calculated across the entire length of the experiment. There was a significant interaction between density and morph on the proportion of individuals ($F_{2,102} = 32.9$, $P < 0.0001$; Fig. 2), but there was no effect of size variation or an interaction between the three variables ($F < 0.3$, all $P > 0.05$). Low density tanks produced significantly higher proportions of mature animals (metamorphs and paedomorphs) than high density tanks, and significantly more animals matured as paedomorphs than as metamorphs (Fisher's LSD $P < 0.0001$; Fig. 2). The mean mass of metamorphs from low density tanks was significantly greater than that of metamorphs from high density tanks ($F_{1, 29} = 10.38$, $P < 0.004$; low density: 4.07 ± 0.37; high density: 2.62 ± 0.14). The mean body condition of metamorphs was not significantly affected by size variation or the interaction term (both $F < 0.71$, both $P > 0.05$).

First metamorphosis event—morph choice with body condition and treatment

Across all treatments, individual larvae that became metamorphic were significantly larger than those that became paedomorphic, and individuals from both groups were
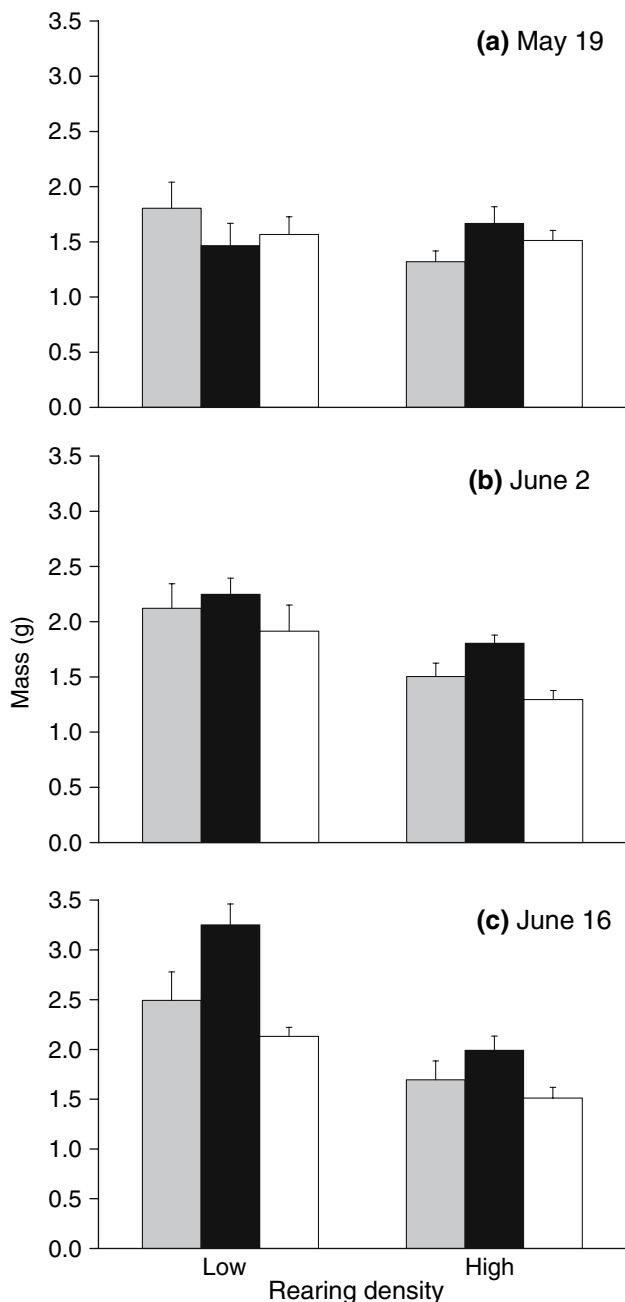
Fig. 1 Mass (mean + SE) of *Ambystoma talpoideum* salamander larvae in the low (*grey bars*), medium (*black bars*) or high (*white bars*) body size variation treatments when reared at low or high density: **a** May 19, **b** June 2, **c** June 16. *n* = 6 for each treatment

Fig. 2 Effects of morphotype (larvae, paedomorphs, metamorphs) and low (*gray bars*) or high (*black bars*) density on the proportion of individuals (mean + SE) at the conclusion of the experiment. *n* = 18 for each density treatment

Fig. 3 Mass (mean + SE) of larvae in June/July 2004 that would remain larval (*n* = 22), become paedomorphic (*n* = 109) and become and metamorphic (*n* = 21) during the fall metamorphosis event. *Different letters* represent Fisher's LSD *P* < 0.05

Second metamorphosis event—morph choice with body condition and treatment

There were no significant interactions between final morph in the spring and initial density ($F_{2, 114} = 1.7$, $P = 0.19$); however, there was a significant interaction between final morphotype and size variation, where metamorphs from medium variation tanks had significantly greater mass and body condition than metamorphs from low or high density tanks (all $F > 3.8$, all $P < 0.006$). There were significant differences in fall mass among the possible fates of individuals from fall to spring ($F_{4, 115} = 37.8$, $P < 0.0001$). Across all treatments, paedomorphs which had not metamorphosed in the fall but did in the spring had significantly greater mean mass prior to the spring metamorphosis event than those that remained paedomorphic in the spring (Fisher's

larger than those that remained larval ($F_{2, 149} = 12.9$, $P < 0.0001$, all Fisher's LSD < 0.005; Fig. 3). There were no significant interactions between fall morphotype and size variation or between fall morphotype and density on mass (both $F < 1.2$, both $P > 0.30$). There were no effects of morph, size variation, density or their interaction on body condition (all $F < 0.9$, all $P > 0.40$).
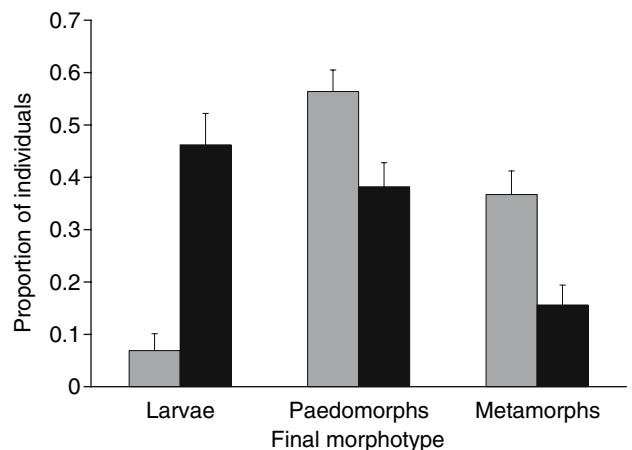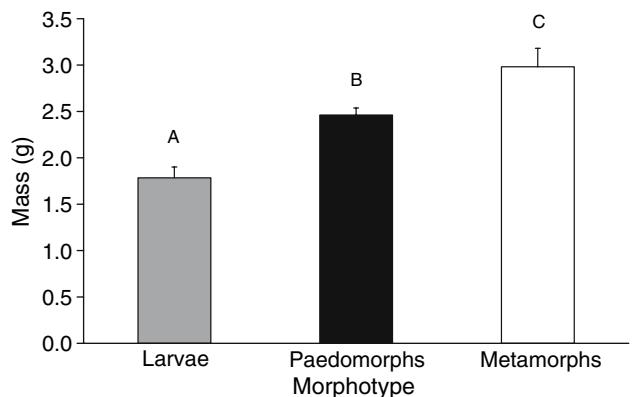
LSD $P < 0.0001$; Fig. 4a). Animals that were larval in the fall and became metamorphic, paedomorphic or remained larval were not significantly different in size (Fisher's LSD $P > 0.05$; Fig. 4a). All animals that were larval in the fall, however, were significantly smaller than those that had been paedomorphic in the fall (Fisher's LSD $P < 0.0001$; Fig. 4a). A similar pattern existed for body condition and possible fates of individuals from fall to spring ($F_{4,115} = 6.5$, $P < 0.0001$, all Fisher's LSD < 0.05; Fig. 4b).

Survival

Survival across tanks ranged from 30 to 100%, although there was one extreme low value of 10% survival. There was no effect of density, size variation or their interaction on the survival of larvae throughout the experiment (density: $F_{1, 30} = 0.8$, $P = 0.38$; size variation: $F_{2, 30} = 2.2$, $P = 0.12$; interaction: $F_{2, 30} = 0.8$, $P = 0.47$).
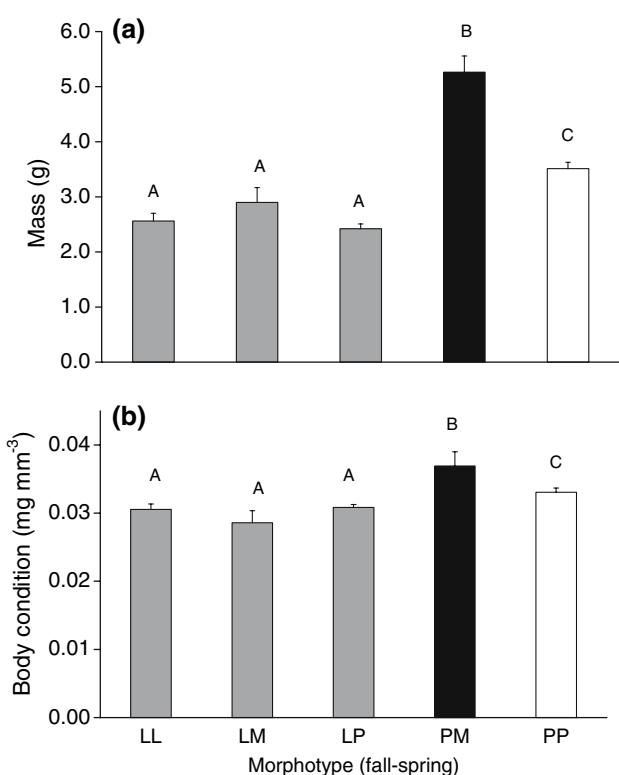

Fig. 4 Mass (**a**) and body condition (**b**) (+1 SE) of larvae and pae-domorphs that would remain larval, become metamorphic and become paedomorphic during the spring metamorphosis event. *LL, LM, LP* Animals that were larvae during the fall and later became larvae, meta-morphs and paedomorphs, respectively. *PM, PP* Animals that were sexually mature paedomorphs during the fall and later became meta-morphs and paedomorphs, respectively. Sample sizes: LL = 20, LM = 5, LP = 28, PM = 14, PP = 53. *Different letters* represent $P < 0.05$ for Fisher's LSD

Discussion

The aim of our study was to examine the effects of density and initial size variation on larval growth and subsequent morphology. Larvae from high density treatments had sig-nificantly reduced mass in comparison to those from low density treatments, most likely due to increased competi-tion for food. Larvae are unable to metamorphose until reaching a critical size (35 mm; Semlitsch 1987a); there-fore, the low proportion of metamorphs relative to pae-domorphs in high density treatments may be due to the smaller size of the larvae. In support of this hypothesis, the larvae that metamorphosed from high density treatments weighed less than those from low density treatments. Initial body size variation affected mass, although not in the ways that we had initially predicted. The experiment was designed with the expectation that different proportions of larvae of different sizes would allow the variance in mass, rather than the mean, to differ among treatments. However, interactions between the density and size variation treat-ments influenced mean mass in each tank. For example, medium variation, low density tanks had significantly greater mean mass than other treatment combinations. Although unexpected, these mean differences provided sub-stantial size variation across treatments, allowing us to evaluate our alternative hypotheses.

Our results support the BOBL hypothesis, irrespective of the effects of size variation and density. Across all treat-ments, larvae that metamorphosed had greater mass than those that became paedomorphic. Of the animals that were sexually mature paedomorphs in the fall, those that meta-morphosed in the spring weighed more and had a better body condition than those that remained paedomorphic. It is notable that significant differences in body condition between treatments were not apparent until the second metamorphosis event, in the spring of 2005. This suggests that resources were likely directed toward body length growth during the summer and fall months, with increases in mass relative to length (i.e. energetic reserves) accumu-lating only in the latter part of the year.

Singular support for the BOBL mechanism contrasts with our expectations for the project and similar experi-ments on this species performed in the past, which primar-ily supported the PA hypothesis (Semlitsch 1987a,b; Jackson and Semlitsch 1993; Whiteman 1994; Ryan and Semlitsch 1998). It is possible that densities were too high to allow the growth rates necessary for expression of the PA mechanism; however, past research has included densi-ties of up to 36 larvae per 1000-l cattle tank (compared to our high density of 20 larvae per 1000 l), and the results still supported the PA mechanism (Semlitsch 1987a,b). It is readily apparent that our treatments produced effects on mass consistent with favorable and unfavorable environments.

However, animals from all treatments responded as if they were exposed to only an unfavorable environment.

Our study site in western Kentucky is at the northern edge of *A. talpoideum's* range, and thus it is colder and has a shorter growing season than habitats where animals for previous experiments were collected (South Carolina). Whiteman (1994) predicted that shorter growing seasons and subsequent lower productivity, such as at higher latitudes and elevations, would produce poor aquatic environments and paedomorphosis through the BOBL mechanism, which is consistent with our findings. Because the embryos used in this experiment came from a pond that was semi-permanent, it is also possible that the population has been under strong selection for metamorphosis (Whiteman 1994). Most previous studies were conducted with individuals from more permanent habitats (Semlitsch 1987a,b; Semlitsch et al. 1990; Jackson and Semlitsch 1993; Voss et al. 2003). Thus, it is possible that the latitude and hydroperiod of our study site may have selected for individuals that were genetically predisposed toward metamorphosis, while larvae that became paedomorphic did so because they were unable to reach the critical size for metamorphosis.

Although the vast majority of our results support the BOBL, the PA mechanism cannot be completely dismissed. Analysis of frequency distributions revealed a notable number of paedomorphs whose size, when compared to the fates of individuals with similarly greater size, would have suggested that they would become metamorphic. Thus, although evidence overwhelmingly supports the BOBL mechanism in this experiment, contrasting interpretations due to different analyses (tank means vs. individual data) may signal the influence of the differing treatment pressures that were not evident from the individual data. Evidence for this idea is also apparent in the effects of treatment on the proportion of paedomorphs and metamorphs (Fig. 2), as traditional methodology would have interpreted high numbers of paedomorphs as evidence of the PA mechanism. Although all treatments producing high numbers of mature individuals show the majority of them becoming paedomorphic (Fig. 2), these animals are consistently smaller than those that became metamorphic, ultimately supporting the BOBL mechanism. This is an excellent example of how individual marking of animals can provide a more accurate evaluation of the mechanisms operating within a population than analyses of tank means.

Although different analyses of the same data may support the predictions of both the PA and BOBL mechanisms, these combined results should not be interpreted as direct evidence for the Dimorphic Paedomorph mechanism. With this latter mechanism, we would have predicted that the variation in the size of the paedomorphs would be greater than that of the metamorphs, reflecting the presence of both very large and very small paedomorphs. We found no such trend (Figs. 3,4), despite the influence of a very few large paedomorphs noted in the body condition frequency distribution. Therefore, results from neither the tank analyses nor the individual analyses correlate adequately with the predictions of the Dimorphic Paedomorph hypothesis.

One complication in comparing our results to those of previous researchers, and another argument in favor of marking individuals in these experiments, is that previous studies have only been able to measure individuals after they metamorphosed, rather than before (Semlitsch 1987a,b; Jackson and Semlitsch 1993; Ryan and Semlitsch 2003). Amphibians are typically smaller in terms of SVL and mass after transformation (Duellman and Trueb 1986). It is therefore possible that, in studies where the difference between the mean size of metamorphs and paedomorphs is small (Jackson and Semlitsch 1993), metamorphosing larvae that were larger than others in the population prior to metamorphosis appeared to be smaller because of the timing of the measurements. Future studies should focus on measurements before metamorphosis/paedomorphosis "decisions" have been made, rather than afterwards, to gain a better understanding of how larval body size affects life history.

By maintaining a long-term experiment, we have obtained valuable information not only on facultative paedomorphosis but also on the natural history of *A. talpoideum*. Our study documented two metamorphosis events (fall and spring), while other studies have only focused on activity during the first fall after hatching. Our long-term data have provided unique insights into the transition of both larvae and paedomorphs to metamorphic adults and have provided experimental confirmation that adults of this species that were paedomorphic in the fall of their first year are capable of metamorphosing during the following spring (see also Patterson 1978).

Our research supports the BOBL mechanism, while other studies on this species are more consistent with the PA mechanism, suggesting that alternative ecological mechanisms can produce the same adaptive polyphenism within a species (Whiteman 1994; Denoel et al. 2005). The production of similar morphs under different environmental conditions suggests that the ecological mechanisms producing other types of plasticity, discrete or otherwise, are more diverse than first suspected and that multiple mechanisms account for the variation present in natural systems (see also West-Eberhard 2003). Given the importance of polyphenisms to macroevolutionary change (West-Eberhard 1986,1989; Via 2001), such alternative mechanisms may also provide different avenues for population divergence and speciation. However, whether multiple mechanisms can operate within a single population or experiment could not be conclusively addressed by this study. Future long-term studies on this and other polyphenisms, particu-

larly those utilizing natural and experimentally-induced size variation, may provide further insight into these questions.

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