

Maternal investment mediates offspring life history variation with context-dependent fitness consequences

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Abstract. Maternal effects, such as per capita maternal investment, often interact with environmental conditions to strongly affect traits expressed early in ontogeny. However, their impact on adult life history traits and fitness components is relatively unknown. Theory predicts that lower per capita maternal investment will have strong fitness costs when the offspring develop in unfavorable conditions, yet few studies have experimentally manipulated per capita maternal investment and followed offspring through adulthood. We used a surgical embryonic yolk removal technique to investigate how per capita maternal investment interacted with an important ecological factor, larval density, to mediate offspring life history traits through reproductive maturity in an amphibian, *Ambystoma talpoideum*. We predicted that increased larval density would reinforce the life history variation induced by differences in per capita investment (i.e., Controls vs. Reduced Yolk), with Reduced larvae ultimately expressing traits associated with lower fitness than Controls when raised at high densities. We found that Reduced individuals were initially smaller and more developed, caught up in size to Controls within the first month of the larval stage, but were smaller at the end of the larval stage in low densities. Reduced individuals also were more likely to undergo metamorphosis at high densities and mature females invested in more eggs for their body sizes than Controls. Together, our results do not support our hypothesis, but instead indicate that Reduced individuals express traits associated with higher fitness when they develop in high-density environments, but lower fitness in low-density environments. The observed life history and fitness patterns are consistent with the “maternal match” hypothesis, which predicts that when the maternal environment (e.g., high density) results in phenotypic variation that is transmitted to the offspring (e.g., reduced per capita yolk investment), and offspring face that same environment (e.g., high larval density), the fitness of both mother and offspring is maximized.

Key words: embryonic yolk removal; life history traits; maternal effects; maternal match hypothesis; polyphenism; reproductive investment.

INTRODUCTION

Intraspecific variation in life history patterns is known to be a primary target of selection, and thus can have important consequences for fitness, population dynamics, and speciation (Stearns 1992, Roff 2002, Roff and Fairbairn 2007). One important source of this variation early in ontogeny is through maternal effects: the influence of the maternal phenotype on the phenotypes of her offspring (Mousseau and Fox 1998, Marshall and Uller 2007). However, little is known about the role maternal effects have on adult life history traits (Marshall and Uller 2007, Räsänen and Kruuk 2007, Uller 2008), despite multiple mechanisms by which they

may affect traits through reproductive maturity. For example, maternal effects may influence adult life history traits by directly mediating early ontogenetic traits, which then have carryover effects through adulthood (Kaplan 1989, Mousseau and Fox 1998, Svensson and Sinervo 2000). Additionally, “maternal programming” of offspring developmental plasticity may provide a mechanism by which maternal effects directly regulate offspring traits through adulthood (Mousseau and Fox 1998, Uller 2008, Sheriff et al. 2010, Love et al. 2013, Sheriff and Love 2013). While a strong theoretical framework implicates the long-term importance of maternal effects, studies following individuals from the embryonic stage to reproductive maturity across a range of environmental conditions are needed to assess their longitudinal consequences (Marshall and Uller 2007, Uller 2008).

One source of maternal effects, per capita investment in offspring (hereafter “maternal investment”), is highly sensitive to maternal condition, and can strongly

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influence the size and performance of offspring early in ontogeny (Kaplan 1989, Svensson and Sinervo 2000, Pfennig and Martin 2009, Martin and Pfennig 2010). In many species, a female's maternal investment depends on her physiological condition and the prevailing environmental quality (Kaplan 1987, Sinervo 1990). Additionally, the effects of maternal investment on offspring performance are often context dependent, where no single optimum investment pattern exists (Kaplan 1992, Allen et al. 2008, Segers and Taborsky 2012). In particular, lower maternal investment is predicted to result in a strong fitness cost when the offspring develop in unfavorable environments (e.g., Kruuk et al. 1999, Einum and Fleming 2000, Allen et al. 2008). The fitness consequences of maternal investment are thus expected to be a complex interaction between the maternal environment, direct investment in offspring, and the subsequent offspring environment. Several studies have closely examined the fitness consequences of maternal investment to offspring, but have primarily emphasized its implications to early life stages (Sinervo et al. 1992, Einum and Fleming 1999, MacAdam et al. 2002, Räsänen et al. 2005; but see Sinervo and Doughty 1996, Dias and Marshall 2010, Marshall and Monro 2012).

In amphibians, investment in offspring yolk stores is believed to be the primary source of maternal investment (Kaplan 1980, 1998). Many amphibians tend to exhibit low maternal investment when the environment is unfavorable and females are small or in poor condition (Semlitsch 1985, Kaplan 1987, Martin and Pfennig 2010; but see Räsänen et al. 2005). As investment often ceases at oviposition and is strongly regulated by a female's physiological state, broad insight into the fitness consequences of variation in maternal investment may be gained by investigating how it affects performance across a range of offspring environments in amphibians. However, while several studies have demonstrated an interactive effect of maternal investment and the offspring environment on early ontogenetic performance in amphibians (e.g., growth [Kaplan 1989, 1992]; age at metamorphosis [Räsänen et al. 2005]), following individuals through reproductive maturity remains necessary.

In this study, we investigated how maternal investment in offspring yolk reserves mediates offspring life history traits through reproductive maturity across a range of environmental conditions. We experimentally manipulated maternal investment using a surgical technique that removes embryonic yolk reserves (Landberg 2014, 2015), and reared individuals at varying conspecific densities to manipulate the quality of the offspring environment. We assumed that given equal amounts of resources, low-density conditions would be more favorable to offspring than high-density conditions. Following theoretical predictions, we expected that the life history and fitness consequences of variation in maternal investment would be context dependent.

Under favorable (low-density) aquatic conditions, we predicted that individuals receiving less investment would be initially smaller, but that they would rapidly compensate for size differences at hatching (e.g., Semlitsch and Gibbons 1990, Räsänen et al. 2005, Landberg 2014). Thus, we expected that these conditions would lead to minimal life history and fitness variation resulting from differences in maternal investment. Under unfavorable (high-density) aquatic environments, we predicted that individuals would be initially smaller and that differences would be reinforced through adulthood by the highly competitive conditions. Therefore, we expected that these conditions would lead to substantially smaller body sizes and reproductive outputs of individuals receiving lower maternal investment.

MATERIALS AND METHODS

Study system, collection, and breeding

The mole salamander, *Ambystoma talpoideum*, is a facultatively paedomorphic salamander endemic to the southeastern United States. This species is well suited for longitudinal life history studies, as individuals reach reproductive maturity within their first year (Petranka 1998), and express one of two discrete adult phenotypes (terrestrial metamorphs and aquatic paedomorphs), whose induction and fitness consequences are well studied (Semlitsch 1985, Whiteman 1994, 1997, Ryan and Semlitsch 1998, Denoël et al. 2005, Whiteman et al. 2012). Whiteman (1994) identified mechanisms by which each morph is produced. Under favorable aquatic conditions, all individuals tend to be large, and the largest will become paedomorphic adults, while smaller individuals will undergo metamorphosis to escape strong intraspecific competition (Paedomorph Advantage Mechanism). Under unfavorable aquatic conditions, all individuals are smaller, and the largest will metamorphose, while smaller individuals that fail to reach the critical size threshold for metamorphosis may mature as small paedomorphic adults or remain as immature larvae (Best-of-a-Bad-Lot Mechanism). Metamorphs have the capacity to disperse, but often mature at a later age; paedomorphs tend to mature earlier and do not make the potentially costly metamorphic shift, but forfeit the ability to disperse in their first year (Scott 1993, Ryan and Semlitsch 1998). In addition to the rapid maturation of both morphs, offspring of paedomorphs are very likely to experience an environment that is similar to that of their mothers, and thus the environment may be very predictable across generations, which is an important feature of maternal effects (Marshall and Uller 2007, Uller et al. 2013). Consequently, *A. talpoideum* represents a well-suited amphibian system for considering how differences in maternal investment and the offspring environment mediate adult traits and fitness.

Adult animals were collected from Ridley Pond (Graves County, Kentucky, USA) during the breeding

season in February 2012. To simulate natural breeding aggregations, two males and two females were each placed into one of eight 55-L rubber breeding tanks at the Hancock Biological Station (Calloway County, Kentucky, USA). Breeding tanks received ~0.5 kg of leaves and sticks, and then were filled with aged water. Breeding tanks were floated on the surface of the water within a 3800-L polyurethane tank for thermal buffering. Once the animals were added to the breeding tanks, the tanks were covered with mesh netting to prevent individuals from escaping. Breeding tanks were checked for clutches of embryos every night, and embryos were removed within 24 hours of their observation. Embryos produced by these captive matings were transported to a laboratory at Murray State University and stored by clutch in an incubator set to 4°C (Doyle and Whiteman 2008). Maternity for all embryos used in the experiment could be determined unambiguously, as only one female in each breeding tank lost enough mass to suggest that she had oviposited her clutch. Additionally, no more than two clutches ever came from the same breeding tank, thus limiting the potential number of individuals from separate clutches that were paternal half-siblings.

Overview of experimental design

Embryos from seven clutches were used in this study (laid between 29 February and 7 March; there were 108.00 ± 55.35 embryos per clutch [mean \pm SD]). This longitudinal study was designed as a 3×3 factorial experiment, with three Embryonic Yolk Manipulation treatments (Unmanipulated, Surgical Control, and Reduced; see *Embryonic stage and yolk investment manipulation*) and three Larval Density treatments (Low, Medium, and High; see *Larval stage and density manipulations*). Each of the nine total treatments was replicated six times in experimental ponds. The study followed individuals across four major life history stages. (1) In the embryonic stage, yolk reserves were manipulated, and the sizes and developmental stages at hatching were evaluated. (2) During the larval stage, conspecific density was manipulated, and the growth of larvae was analyzed. (3) Prior to overwintering, the sexual maturity and morphotype expression (i.e., paedomorph, metamorph, or immature) of individuals was determined. Individuals were then overwintered by morphotype, sex, and initial density. (4) During the following spring, first-year reproductive traits (egg number and egg size) were sampled from females across the range of body sizes produced in this study.

Embryonic stage and yolk investment manipulation

Once embryos had developed to between Harrison stages 28 and 34 (prior to development of axial muscle [Harrison 1969]), embryos within each clutch were haphazardly assigned to one of the three Embryonic treatments, and manipulated following methods described in Landberg (2014). In the Reduced treatment, a glass surgical needle (0.2 mm diameter opening) was

inserted through the egg membranes, ventrally into the abdominal yolk mass, and ~20% of the embryonic yolk stores were removed using gentle vacuum suctioning. This technique isolated the specific effect of variation in investment in embryonic yolk reserves, the primary source of maternal investment in most amphibians, and previous work indicates that such variation in yolk reserves falls within the range observed across several *Ambystoma* (Landberg 2014, 2015). The Surgical Control treatment followed the same procedure as the Reduced treatment, but no yolk was removed. The Unmanipulated treatment served as an additional control in which the embryos were subjected to the same handling, but were not pierced with the surgical needle. All Embryonic treatments were performed under 20 \times magnification on a stereomicroscope, using a micromanipulator to control the needle.

Following the treatment, embryos were allowed to develop by clutch and treatment in an incubator set to 4°C until hatching. Mortality during the embryonic stage was low (<12%), and not influenced by treatment or clutch (all $F < 1.4$, $P > 0.3$). Embryos from all clutches were represented across the three Embryonic treatments. Once all individuals had hatched, total length (millimeters) and Harrison developmental stage (Harrison 1969) were assessed for each individual with an ocular micrometer. Total length was the only size measurement taken, as hatchlings are too delicate for extensive handling. Individuals were then randomly assorted by Embryonic treatment into one of three conspecific Density treatments (Low, 6 larvae; Medium, 12 larvae; High, 24 larvae), chosen to reflect natural larval densities (Petranka 1998). While not all clutches were represented in every replicate, random assortment facilitated similar genetic diversity across replicates (5.02 ± 1.29 clutches per replicate [mean \pm SD]).

Larval stage and density manipulations

Fifty-four 1000-L polyurethane tanks arranged across six spatial blocks at the Hancock Biological Station served as the experimental ponds for this study. Beginning on 13 March 2012, tanks were filled with tap water by block and allowed to dechlorinate for at least 72 hours. Dechlorinated tap water was added to tanks as needed throughout the experiment to maintain approximately constant water levels. Leaf litter (4 kg) was added to each tank on 23 March. A 2-L mixture of zooplankton collected from nearby natural ponds was added to each tank on 2, 9, and 16 April, providing a self-sustaining source of natural prey for salamander larvae. Tanks were individually fitted with mesh covers that were draped over the top of the tanks, thus permitting colonization of *Chironomidae* and *Culicidae* insect larvae while preventing oviposition of tree frogs (*Hyla* spp.) and aquatic predators (e.g., *Anax* spp.). On 21 May, a mesh shade cover was erected over the entire experimental pond array. This provided ~50% shade, but allowed natural precipitation (Doyle and Whiteman

2008). When larvae had outgrown the threat of predation by aquatic macroinvertebrates (3 September, experimental day 135), individual tank covers were removed to more easily observe and retrieve metamorphic individuals.

Larvae were added to tanks by assigned treatment (Embryonic treatment \times Density treatment) on 15 April (Experimental Day 1). Tanks were sampled five times over the course of the larval growth season, with sample periods beginning on days 31, 43, 57, 86, and 108. Within each sample period, individuals from two blocks of tanks were captured over two consecutive nights using 30 person-minutes of dip-netting on the first night and unbaited minnow traps on both nights. Captured individuals were housed separately in opaque plastic cups (473 mL) and measured for mass (grams), snout-vent length (SVL, millimeters), and total length (millimeters). To avoid recapture, individuals remained in their cups until after the two-night session ended, and were then returned to their respective tanks.

Morphotype expression and overwintering

Beginning on day 113, all tanks were checked every other night for individuals beginning metamorphosis (i.e., observable gill and tail fin resorption). These individuals were allowed to complete metamorphosis at laboratory temperatures (22°–25°C) in plastic boxes filled with moist paper towels (Doyle and Whiteman 2008). Upon completion of metamorphosis (total resorption of gills and tail fin), individuals were measured for mass, SVL, and total length, uniquely marked with toe clips, and then released into one of three terrestrial habitats. By day 203, all individuals were determined to have metamorphosed, matured as paedomorphs, or begun overwintering as larvae. Tanks were emptied and remaining individuals were collected and visually checked for maturity and sex, as well as measured for mass, SVL, and total length. Individuals were uniquely toe clipped, and then released into aquatic habitats for overwintering.

Aquatic individuals were sorted by sex (i.e., male, female, immature) and initial density into nine 3800-L polyurethane tanks (overwintering mesocosm ponds) that were set up in the same manner as the experimental 1000-L tanks (e.g., leaf litter and dechlorinated water). Tanks remained uncovered to allow natural oviposition of insects. Metamorphosed individuals were assigned to one of three terrestrial overwintering habitats by initial density, irrespective of sex. These habitats were constructed in uncovered 3800-L polyurethane tanks, filled with soil and woody debris collected from the forest floor, and placed under a large tree for shade. Metamorphs were not assorted by sex because individuals require water for reproduction, and sex cannot always be determined unambiguously for recently metamorphosed individuals. Individuals were assorted to overwintering habitats by initial density, rather than randomly across treatments, to more realistically simu-

late the natural size structure environment experienced by individuals at overwintering. Spatial and material limitations prevented further assortment of individuals by Embryonic treatment.

First-year reproductive investment

A sample of mature females ($n = 43$) across the range of body sizes was haphazardly collected from the aquatic overwintering habitats between 9 February and 17 March 2013. This period corresponds with the natural breeding season (Petranka 1998). Previous work has demonstrated that eggs are generated by the end of November, which are retained by the female through the winter, and neither egg number nor egg size changes substantially between November and the end of our sampling period (Semlitsch 1985). Thus, reproductive traits assessed in this study are likely to most strongly reflect larval conditions. Aquatic females and immature larvae were captured with dip nets and minnow traps. Terrestrial females were collected by hand. Mature females were sacrificed in 1% benzocaine, fixed in 10% formalin for 24 hours, and preserved in 70% EtOH. Females were carefully dissected, and all eggs were removed from the body cavity and counted. Fifteen eggs from each clutch were measured along the longest axis to the nearest 0.01 mm using an ocular micrometer to estimate the mean egg size for each female.

STATISTICAL ANALYSIS

Embryonic stage and yolk investment manipulations

Initial Harrison developmental stage per replicate was analyzed using analysis of variance with Embryonic treatment as a factor. Mean total length at hatching for each replicate tank was analyzed using analysis of covariance with Embryonic treatment as a factor and Harrison developmental stage as a covariate. Tukey post hoc tests were used to detect pairwise statistical differences among Embryonic treatments.

Larval stage and density manipulations

Substantial mortality (63.2%) was detected shortly after releasing hatchlings into experimental ponds, and led to no tanks possessing their original density, including nine tanks with no surviving individuals (see Appendix: Table A1). All available evidence suggests that this mortality was random, as it could not be explained by any experimental treatment (generalized linear mixed effects model: all $\chi^2 < 2.7$, $P > 0.15$) or initial mean phenotypes (i.e., size, developmental stage) within treatments (all $\chi^2 < 1.9$, $P > 0.17$). To more accurately reflect the actual larval density environment, tanks were reassigned to one of three binned densities (see Appendix for binning methods; [mean \pm SD] low, 2.30 ± 1.04 individuals per tank, range 1–4 individuals; medium, 6.40 ± 1.02 individuals per tank, range 5–8 individuals; high, 13.42 ± 2.36 , range 9–16 individuals per tank; Appendix: Table A1). In all cases, the binned density models fit the observed larval responses better

than the initial density models, and better than (or in one case equal to) the continuous density models (Appendix: Table A2). Thus binned densities were used exclusively in all further analyses.

Larval growth was analyzed using a linear mixed-effects model with Time (experimental days 31, 43, 57, 86, or 108), Embryonic treatment (Reduced, Surgical Control, or Unmanipulated), and Density (Low, Medium, or High) as fixed effects. Tank nested within Block was included as a random intercept, and Time as a random slope to account for repeated measures. Tank means at each sampling period were used as the unit of replication. Analyses indicated that Time interacted with other fixed effects (see *Results*), and individual analyses were conducted for each sample period with Embryonic treatment and Density as fixed effects. Though mass, SVL, and total length were all measured at each sample period, mass measurements were taken with an electronic balance, and were considered more repeatable and precise than measurements of length, which were assessed visually using a ruler. Body mass was therefore used exclusively in growth analyses, although exploratory analyses suggested that all metrics showed qualitatively similar trends.

Morphotype expression and overwintering

The number of individuals expressing each morphotype (metamorphs, paedomorphs, immature larvae) was analyzed using multinomial logistic regression by Embryonic treatment, Density, and Block. Mass at and time of metamorphosis for each individual were analyzed using a linear mixed-effects model with Embryonic treatment and Density as fixed effects and Tank of Origin nested within Block as random effects. Very low total numbers of metamorphs (27 out of 263 overwintering animals) precluded the use of interactions in analyses because many treatment combinations produced no metamorphs. Time to metamorphosis was calculated as the experimental day an individual completed metamorphosis, and was natural log-transformed to improve normality.

First-year reproductive investment

Reproductive traits (egg number and mean egg size) were analyzed using linear mixed effects models with Embryonic treatment and an individual's body size at the end of the larval period as fixed effects and Tank of Origin nested within Block as random effects. Although mass was used as the primary response variable when analyzing larval growth, this metric potentially confounded a female's body and clutch size, and thus SVL was used as the measure of female size. Density never significantly predicted either reproductive trait beyond its effects on SVL (all $\chi^2 < 2.3$, $df = 2$, $P > 0.30$), and was not included in subsequent models. No metamorphic females were observed to be in reproductive condition, and analyses therefore reflect reproductive traits of only paedomorphic females. The egg number or

mean egg diameter of a given female was used as the unit of replication. Egg number was natural log-transformed to meet the assumptions of a linear model.

To estimate the fitness consequences of variation in maternal investment across offspring environments, first-year reproductive output was analyzed using a linear mixed-effects model with Density and Embryonic treatment as fixed effects and Tank of Origin nested within Block as random effects. First-year reproductive output was calculated as the product of a female's number of eggs and their mean diameter. As amphibian egg size is correlated with multiple factors influencing viability through the earliest ontogenetic stages (e.g., Kaplan 1980, Merilä et al. 2004, Räsänen et al. 2005, Ficetola and de Bernardi 2009), scaling egg number by mean size should provide a reasonable estimate of fitness via the number of offspring a female can produce that survive through hatching. Moreover, because this reproductive output metric is sensitive to the interaction of multiple traits affected by the experimental manipulations (i.e., body size, clutch size, mean egg size; see *Results*), it represents an integrative estimate of potential fitness differences among females (e.g., Howard 1979, Marshall and Monro 2012). For analyses, each female's reproductive output was divided by the mean reproductive output for all sampled females (i.e., relative performance [Lande and Arnold 1983]). Reproductive traits of Surgical Control and Unmanipulated (i.e., Combined Controls) females never differed, and thus were combined for this analysis to increase statistical power. Reproductive output values were natural log-transformed to improve normality.

All statistical analyses were performed using the statistical software R version 3.0.2 (R Development Core Team 2013). Linear mixed-effects models were conducted with the lme4 package (Bates et al. 2014) by first fitting the maximal model (Bolker et al. 2009). Multinomial regression was conducted using the nnet package (Venables and Ripley 2002) following procedures described in Qian et al. (2012). Significance of main effects and their interactions was assessed using likelihood ratio tests to compare nested models with and without the effect.

RESULTS

Embryonic stage and yolk manipulations

Initial Harrison developmental stage differed among Embryonic treatments ($F_{2,51} = 18.6$, $P < 0.001$; all Tukey post hoc: $P < 0.02$). Reduced individuals were on average 1.5% and 0.7% more advanced in Harrison developmental stage than Unmanipulated and Surgical Control individuals, respectively. Moreover, after controlling for the effect of developmental stage ($F_{1,50} = 10.9$, $P = 0.002$), larval body size at hatching varied among Embryonic treatments ($F_{2,50} = 9.9$, $P < 0.001$; all Tukey post hoc: $P < 0.005$). Reduced hatchlings were 10.0% and 8.0% smaller than the Unmanipulated and Surgical Control hatchlings, respectively.

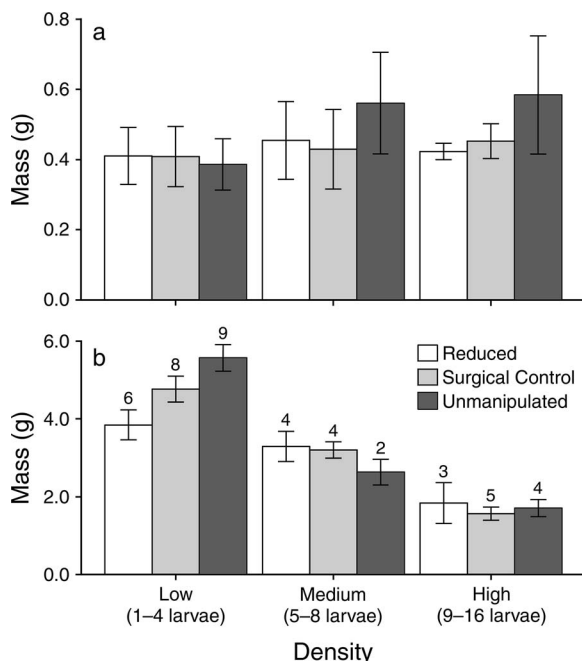


FIG. 1. Larval body mass as a function of Embryonic treatment and Density at (a) day 31, and (b) day 108. There was a significant Time \times Embryonic treatment \times Density interaction through the larval growth period of the experiment ($P < 0.001$). (a) At day 31 in the experimental ponds, there was no overall density effect ($P = 0.44$) and Reduced treatment individuals were the same size as Control treatment individuals on average ($P = 0.57$). (b) At day 108, there was a strong overall density effect and a significant Embryonic treatment \times Density effect ($P = 0.026$), where Reduced individuals were smaller than Control individuals at low densities but not at medium or high densities. Bars represent treatment means \pm SE, and numbers above bars represent number of tanks included in each treatment.

Larval stage and density manipulations

Over the course of the larval growth period, a significant Time \times Embryonic treatment \times Density interaction was detected on larval mass ($\chi^2 = 48.4$, $df = 16$, $P < 0.001$). Neither Density nor Embryonic treatment influenced larval mass prior to day 57 (all $P > 0.4$; Fig. 1a). By day 57, Density negatively affected larval body mass ($\chi^2 = 15.4$, $df = 2$, $P < 0.001$), with no main effect of Embryonic treatment ($\chi^2 = 1.2$, $df = 2$, $P = 0.55$). On days 86 and 108, there were significant effects of Density ($\chi^2 > 44.5$, $df = 2$, $P < 0.001$) and the Density \times Embryonic treatment interaction (all $\chi^2 > 11.1$, $df = 4$, $P < 0.030$). Notably, at low densities, Reduced larvae were 26% smaller than larvae from the Combined Controls, but were similarly sized within medium and high densities (Fig. 1b).

Morphotype expression

Density significantly influenced morphotype expression ($\chi^2 = 91.4$, $df = 4$, $P < 0.001$). The overall likelihood of an individual becoming paedomorphic was greater in low-density (79%) than high-density (31%) environ-

ments. Additionally, the likelihood of an individual undergoing metamorphosis was greater in low (20%) than high (4%) densities. Conversely, the likelihood of an individual remaining as an immature larva was very small at low densities ($< 0.01\%$), but relatively large at high densities (64%). While there was no significant main effect of Embryonic treatment ($\chi^2 = 2.6$, $df = 4$, $P = 0.62$), there was a significant interaction between Embryonic treatment and Density ($\chi^2 = 15.8$, $df = 8$, $P = 0.044$). In particular, Unmanipulated and Surgical Control individuals were less likely to undergo metamorphosis as density increased, while the likelihood for Reduced individuals remained approximately the same (Fig. 2). There was no difference in the mean time to metamorphosis between Embryonic treatments ($\chi^2 = 2.2$, $df = 2$, $P = 0.29$) or Densities ($\chi^2 = 1.5$, $df = 2$, $P = 0.83$). There was a strong negative effect of Density on the size at metamorphosis ($\chi^2 = 33.8$, $df = 2$, $P < 0.001$), but no effect of Embryonic treatment ($\chi^2 = 1.8$, $df = 2$, $P = 0.23$). Individuals from low densities tended to be larger than those from either medium or high densities.

First-year reproductive investment

The number of eggs produced by females increased with SVL across all Embryonic treatments ($\chi^2 = 80.9$, $df = 1$, $P < 0.001$). Additionally, Embryonic treatment influenced the number of eggs a female produced (i.e., intercept, $\chi^2 = 20.8$, $df = 2$, $P < 0.001$), but not the relationship between SVL and egg number (i.e., slope: Embryonic treatment \times SVL, $\chi^2 = 1.2$, $df = 2$, $P = 0.56$). Reduced females produced, on average, more eggs than females from either Control treatment (Unmanipulated and Surgical Control; Fig. 3a). Analyses of mean egg diameter showed that, in general, larger females produced larger eggs ($\chi^2 = 8.4$, $df = 1$, $P = 0.004$), but there was neither a main effect of Embryonic treatment ($\chi^2 = 3.9$, $df = 2$, $P = 0.14$), nor an interaction between Embryonic treatment and SVL ($\chi^2 = 3.6$, $df = 2$, $P = 0.17$). However, the relationship between SVL and mean egg diameter was not significant for the Reduced females ($\chi^2 = 0.09$, $df = 1$, $P = 0.77$; Fig. 3b).

There was a significant main effect of larval density on reproductive output ($\chi^2 = 45.9$, $df = 2$, $P < 0.001$). While no main effect of Embryonic treatment was detected ($\chi^2 = 0.1$, $df = 1$, $P = 0.72$), the interaction between Density and Embryonic treatment was significant ($\chi^2 = 10.1$, $df = 2$, $P = 0.007$). In particular, reproductive output of Reduced females was higher than that of the Combined Control females when comparing within high larval densities, but lower than that of Combined Control females when comparing within low larval densities (Fig. 4).

DISCUSSION

In this study, we examined how maternal investment in offspring yolk reserves influenced adult life history traits and important fitness components in an amphibian. We predicted that, in favorable (low-density)

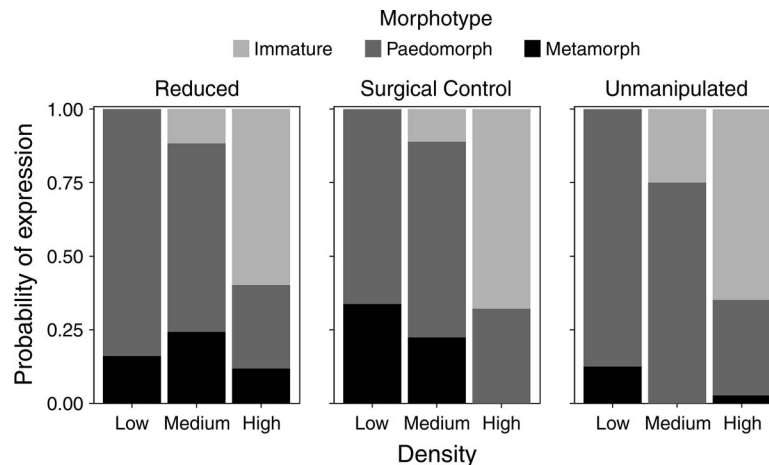


FIG. 2. Probability of larvae becoming metamorphs (black), paedomorphs (dark gray), or remaining immature (light gray) as a function of Embryonic treatment and Density. The overall likelihood of undergoing metamorphosis or paedomorphosis decreased strongly with increasing density ($P < 0.001$). There was no main effect of Embryonic treatment ($P = 0.62$), but there was a significant Density \times Embryonic treatment interaction ($P = 0.044$). The height of each bar represents the probability of an individual becoming the corresponding morph. Sample sizes are as indicated in Fig. 1b.

environments, the offspring receiving less investment would rapidly compensate and exhibit life history traits and fitness components comparable to those receiving more investment. In contrast, we predicted that unfavorable (high-density) offspring environments would reinforce differences resulting from maternal investment variation, and consequently offspring receiving less investment would express life history traits associated with lower relative fitness. We reject this hypothesis, and instead suggest that lower maternal investment in yolk stores actually promotes offspring life history traits that increase relative fitness in unfavorable environments but decrease relative fitness in favorable environments.

Maternal investment mediates offspring life history variation through adulthood

The findings of this study indicate that variation in embryonic yolk reserves, the primary source of maternal investment in amphibians without parental care, mediated offspring life history variation through adulthood. In the embryonic stage, reduced yolk reserves resulted in individuals hatching at a smaller size. This observed variation was similar to those observed naturally among females of different body sizes (e.g., Kaplan 1980, Semlitsch and Gibbons 1990). During the larval period, Reduced individuals caught up to Combined Control individuals in body size within the first month (Fig. 1a), but achieved smaller body sizes by the end of the larval stage in the low-density environments (Fig. 1b). Embryonic yolk reserves and offspring larval density interacted to influence morphotype expression (Fig. 2), whereby the Reduced individuals were more likely to undergo metamorphosis at high densities than either Surgical Control or Unmanipulated individuals. Following the overwintering period, Reduced paedomorphic females produced more eggs for their body size

than Surgical Control or Unmanipulated females (Fig. 3a). Taken together, these results indicate that differences in maternal provisioning in yolk affect a suite of offspring life history traits, and further underscore the importance of considering the longitudinal impact of maternal effects through reproductive maturity.

Research in other organisms has also indicated a broad importance of variation in yolk stores to suites of offspring traits (e.g., Lancaster et al. 2010, Landberg 2014, 2015), and the findings of our study add to the body of work suggesting an integrative role of yolk stores to offspring traits. Additionally, there was a weak tendency for the Surgical Control individuals to express trait values that were intermediate to the Reduced and Unmanipulated individuals (e.g., Fig. 1b). Use of this yolk manipulation technique in other *Ambystoma* has also indicated an effect of the Surgical Control on offspring traits (Landberg 2014, 2015), and may reflect the well-documented response of amphibians to pre-hatching environmental effects (e.g., Touchon et al. 2013) or an artifact of our sampling design. Despite these small effects, our experimental study strongly implicates an integrative relationship between yolk stores and a suite of offspring traits. However, the proximate mechanisms connecting maternal yolk investment to offspring traits in this study and others remain unknown. Recent work in the mouthbrooding cichlid, *Simochromis pleurospilus*, demonstrated that individuals with smaller yolk sacs had persistently elevated expression of somatic cell endocrine receptors (Segers et al. 2012). Given the longitudinal impact of maternal investment on offspring life history variation observed here, it is possible that reduced embryonic yolk in *A. talpoideum* has similar gene expression effects on sensitivity to endocrine factors, but future work remains

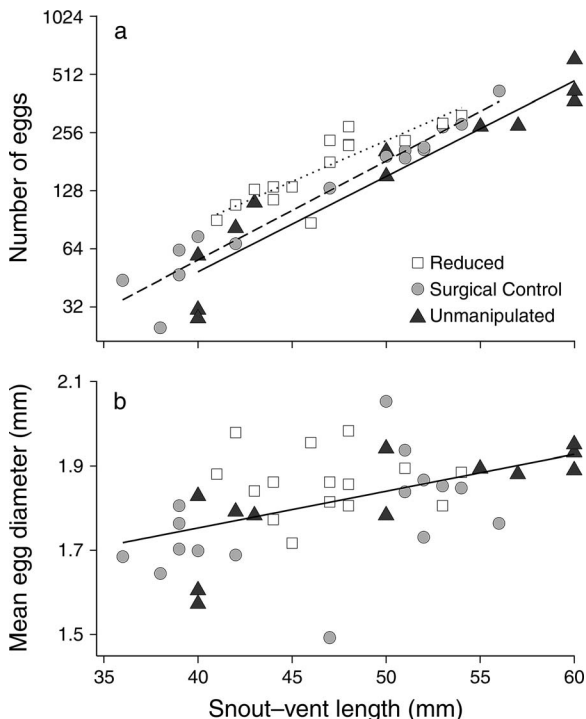


FIG. 3. Reproductive traits as a function of body size and Embryonic treatment. (a) As females increased in body size, the number of eggs they produced also increased across all treatments ($P < 0.001$). Reduced females produced more eggs for a given body size than either control treatment (Least Squares Means \pm SE: $\bar{X}_{\text{Reduced}} = 181.79 \pm 1.06$ eggs, $\bar{X}_{\text{Controls}} = 123.98 \pm 1.05$ eggs; $P < 0.001$). The y -axis is natural log-transformed, and each point represents the number of eggs produced by a female. (b) There was a strong effect of SVL on mean egg diameter ($P = 0.004$), but there were no differences among Embryonic treatments ($P > 0.14$). However, the relationship between body size and egg size was not significant for Reduced females ($P = 0.77$). Each point represents the mean value for a female.

necessary to better understand such proximate mechanisms.

Associated fitness consequences

The life history variation that was induced through differences in yolk investment was associated with important fitness components in this study. Because of the high mortality of *A. talpoideum* in the wild (Semlitsch et al. 1988, Petranka 1998), first-year reproductive output is likely a very important fitness component (e.g., Howard 1979). In favorable environments (low larval density), the Reduced paedomorphic females were observed to have lower reproductive output (Fig. 4). Despite the large clutch size advantage of Reduced paedomorphic females relative to their body sizes (Fig. 3a), the smaller maximum body sizes of the Reduced females in low-density environments prevented those females from matching the high reproductive output of their Control counterparts (Fig. 4). Moreover, the large size advantage of the Combined Control

individuals could potentially increase adult survival, as has been shown in many amphibians (e.g., Altwegg and Reyer 2003, Schmidt et al. 2012). In unfavorable environments, the clutch size advantages (Fig. 3) of the Reduced paedomorphic females at the small body sizes promoted by the high densities (Fig. 1b) facilitated higher reproductive output than the Combined Controls (Fig. 4). Additionally, in these unfavorable environments, Reduced individuals were more likely to undergo metamorphosis. While we do not know the reproductive traits of the metamorphs, the increased likelihood of metamorphosis could enable those individuals to disperse from the unfavorable environment and reproduce in a more favorable pond (Whiteman 1994, Denoël et al. 2005, Benard and McCauley 2008). As our results indicate that any offspring developing in low-density (favorable) environments are likely to have higher reproductive output than those from high-density (unfavorable) environments (Fig. 4), such a scenario would further improve the fitness of Reduced females relative to the Controls. Admittedly, our direct comparison of reproductive output among treatments is based on a small sample of only paedomorphic females. However, the general patterns underlying this synthetic estimate of an important fitness component (for example, where Reduced individuals are 26% smaller than the Combined Controls at low densities [Fig. 1b], and Reduced paedomorphic females produce 50 more eggs [Fig. 3a]), are very strong and suggest that the qualitative pattern illustrated in Fig. 4 is robust. In sum, the offspring life history variation resulting from differences in maternal investment are strongly associ-

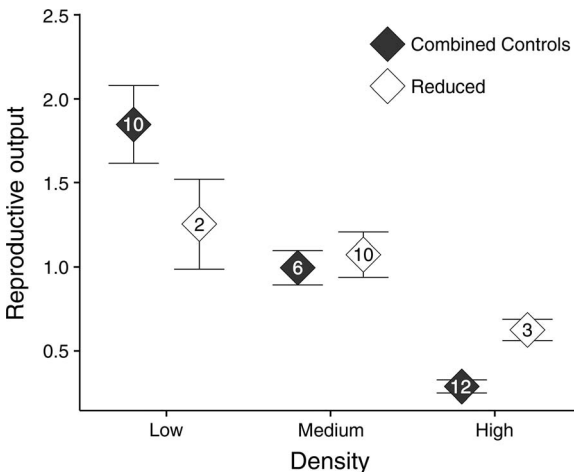


FIG. 4. Reproductive output of paedomorphic females among treatments. Reduced females demonstrated higher reproductive output in high-density environments, while Control females had higher reproductive output in low-density environments ($P = 0.007$). Control values are the combined means for Surgical Control and Unmanipulated females. Each point represents the treatment mean \pm SE, and numbers within the diamonds indicate sample sizes.

ated with fitness consequences that depend on the environment in which the offspring develop.

Maternal investment patterns and adaptive maternal effects

Our results demonstrate that variation in maternal investment interacts with the offspring environment to mediate fitness patterns. In natural populations of *A. talpoideum*, maternal investment may itself reflect multiple environmental factors (Semlitsch 1985), but is frequently associated with maternal body size in *A. talpoideum* and other ambystomatids (e.g., Kaplan and Salthe 1979, Semlitsch 1985, 1987b). Generally, small females exhibit lower maternal investment than larger females (Semlitsch 1985). This variation in maternal body size, in turn, is often associated with the larval density conditions experienced by the mother (Semlitsch 1987a, b, Scott 1994). Indeed, larval density dependence in this species is frequently the strongest ecological pressure that individuals face (Semlitsch 1987a, Semlitsch and Gibbons 1990, Anderson and Whiteman 2015a, b). It is thus possible that many females with lower maternal investment experienced high-density conditions during their larval stage. Consequently, such females may produce offspring that express traits associated with higher fitness if they develop in high-density environments. As the larval density conditions are often strongly correlated over short interannual time scales for species that mature rapidly (e.g., Meyer et al. 1998, Marsh 2001, Salvadio 2009), this overall pattern could be described as an adaptive maternal effect (Marshall and Uller 2007, Uller et al. 2013).

These findings are contrary to our prediction that low maternal investment would result in a fitness cost to offspring developing in unfavorable conditions (e.g., Kruuk et al. 1999, Einum and Fleming 2000, Allen et al. 2008). Instead, the potentially adaptive nature of lower investment improving offspring fitness in unfavorable conditions is consistent with the “maternal match” hypothesis (reviewed in Sheriff and Love 2013). This hypothesis suggests that organisms have evolved mechanisms by which the maternal physiological state is transmitted to offspring, which prepares offspring for the environment that the mothers faced. When the offspring subsequently encounter that environment, they express phenotypic values associated with higher fitness under those conditions (i.e., matching). If the offspring do not face that environment, they may express traits associated with lower fitness (i.e., mismatching). While previous work has implicated the importance of cross-generational transmission of stress hormones to offspring developmental plasticity (reviewed in Love et al. 2013, Sheriff and Love 2013), offspring yolk stores may similarly signal to offspring about their mother’s size, and therefore also her larval environment. Our results indicate, for the first time, that such differences in offspring yolk stores may also have long-term effects on offspring fitness components that are consistent with the

predictions of the maternal match hypothesis, and not with previous theoretical work on maternal investment. However, further evaluating this prediction with naturally occurring, in addition to experimentally manipulated, maternal and offspring phenotypic variation remains necessary.

The evolution of maternal investment strategies will ultimately depend on the interrelationships between the maternal environment, direct investment in offspring, and the offspring environment. Given the importance of larval density conditions to amphibian life history variation, it is likely that the evolution of the offspring response to yolk stores has been strongly mediated by its effects. However, very little is known about how other environmental variables, such as hydroperiod or predation, may affect maternal investment in amphibians (but see Räsänen et al. 2005, Landberg 2015). In particular, no studies have directly investigated adaptive adjustments of per capita investment and offspring number in response to environmental conditions for *A. talpoideum* or its close relatives. Nonetheless, studies of natural populations tend to find that individuals within a species do not trade off between offspring number and per capita investment (Kaplan and Salthe 1979, Kaplan 1980, Semlitsch 1985). This contrasts with other taxa where adaptive adjustments of per capita investment have been observed, and may enable mothers to maximize offspring performance across the range of environments in those species (e.g., Fox et al. 1997, Allen et al. 2008, Leips et al. 2009). The results of this study indicate that mechanisms to maximize maternal fitness beyond such adaptive adjustments likely exist, as lower investment actually improved offspring fitness components in unfavorable environments. For such responses to evolve, environmental conditions must be relatively predictable across generations (Marshall and Uller 2007, Uller et al. 2013), and may not be an optimal strategy for organisms with longer generation times or that can adaptively adjust offspring number and per capita investment. However, given our findings, it may be necessary to extend the existing theoretical framework of the evolution of maternal investment to include the potential for such patterns.

CONCLUSIONS

The results of this study show that maternal investment mediates life history traits with longitudinal and context-dependent fitness consequences. Contrary to our predictions, lower maternal investment actually promoted traits associated with higher fitness in high-density environments, but traits associated with lower fitness in low-density environments. Future research should continue the use of longitudinal experiments to investigate how maternal investment patterns influence offspring fitness components under alternative ecologically relevant scenarios, such as variable predation or hydroperiod. Ultimately, these findings highlight the importance of longitudinally investigating maternal

effects through adulthood, even if effects seem to disappear early in ontogeny. Furthermore, these results suggest that maternal investment and the offspring environment may exhibit more complex interactions on important fitness components than theoretical models have previously considered.

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LITERATURE CITED

- Allen, R. M., Y. M. Buckley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *American Naturalist* 171:225–237.
- Altwegg, R., and H. U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* 57:872–882.
- Anderson, T. L., and H. H. Whiteman. 2015a. Non-additive effects of intra- and interspecific competition between two larval salamanders. *Journal of Animal Ecology* 84:765–772.
- Anderson, T. L., and H. H. Whiteman. 2015b. Asymmetric effects of intra- and interspecific competition on a pond-breeding salamander. *Ecology* 96:1681–1690.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-6. <http://CRAN.R-project.org/package=lme4>
- Benard, M. F., and S. J. McCauley. 2008. Integrating across life-history stages: consequences of natal habitat effects on dispersal. *American Naturalist* 171:553–567.
- Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, H. H. Stevens, and J. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* 24:127–135.
- Denoël, M., P. Joly, and H. H. Whiteman. 2005. Evolutionary ecology of facultative paedomorphosis in newts and salamanders. *Biological Reviews* 80:663–671.
- Dias, G. M., and D. J. Marshall. 2010. Does the relationship between offspring size and performance change across the life history? *Oikos* 119:154–162.
- Doyle, J. M., and H. H. Whiteman. 2008. Paedomorphosis in *Ambystoma talpoideum*: effects of initial body size variation and density. *Oecologia* 156:87–94.
- Einum, S., and I. A. Fleming. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proceedings of the Royal Society B* 266:2095–2100.
- Einum, S., and I. A. Fleming. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature* 405:565–567.
- Ficetola, G. F., and F. de Bernardi. 2009. Offspring size and survival in the frog *Rana latastei*: from among-population to within-clutch variation. *Biological Journal of the Linnean Society* 97:845–853.
- Fox, C. W., M. S. Thakar, and T. A. Mousseau. 1997. Egg size plasticity in a seed beetle: an adaptive maternal effect. *American Naturalist* 149:149–163.
- Harrison, R. G. 1969. Harrison stages and description of the normal development of the spotted salamander, *Ambystoma punctatum* (Linn.). Pages 44–66 in R. G. Harrison, editor. *Organization and development of the embryo*. Yale University Press, New Haven, Connecticut, USA.
- Howard, R. D. 1979. Estimating reproductive success in natural populations. *American Naturalist* 114:221–231.
- Kaplan, R. H. 1980. The implications of ovum size variability for offspring fitness and clutch size within several populations of salamanders (*Ambystoma*). *Evolution* 34:51–64.
- Kaplan, R. H. 1987. Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*. *Oecologia* 71:273–279.
- Kaplan, R. H. 1989. Ovum size plasticity and maternal effects on the early development of the frog, *Bombina orientalis* Boulenger, in a field population in Korea. *Functional Ecology* 3:597–604.
- Kaplan, R. H. 1992. Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. *Ecology* 73:280–288.
- Kaplan, R. H. 1998. Maternal effects, developmental plasticity, and life history evolution: an amphibian model. Pages 244–260 in T. A. Mousseau and C. W. Fox, editors. *Maternal effects as adaptations*. Oxford University Press, Oxford, UK.
- Kaplan, R. H., and S. N. Salthe. 1979. The allometry of reproduction: an empirical view in salamanders. *American Naturalist* 113:671–689.
- Kruuk, L. E. B., T. H. Clutton-Brock, K. E. Rose, and F. E. Guinness. 1999. Early determinants of lifetime reproductive success differ between the sexes in red deer. *Proceedings of the Royal Society B* 266:1655–1661.
- Lancaster, L. T., A. G. McAdam, and B. Sinervo. 2010. Maternal adjustment of egg size organizes alternative escape behaviors, promoting adaptive phenotypic integration. *Evolution* 64:1607–1621.
- Landberg, T. 2014. Embryonic yolk removal affects a suite of hatchling salamander traits. *Journal of Experimental Zoology B* 322:45–53.
- Landberg, T. 2015. Evolution of maternal egg size effects in sister salamander species. *International Journal of Developmental Biology*, in press. <http://dx.doi.org/10.1387/ijdb.140324TL>
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Leips, J., J. M. L. Richardson, F. H. Rodd, and J. Travis. 2009. Adaptive maternal adjustments of offspring size in response to conspecific density in two populations of the least killifish, *Heterandria formosa*. *Evolution* 63:1341–1347.
- Love, O. P., P. O. McGowan, and M. J. Sheriff. 2013. Maternal adversity and ecological stressors in natural populations: the role of stress axis programming in individuals with implications for populations and communities. *Functional Ecology* 27:81–92.
- MacAdam, A. G., S. Boutin, D. Réale, and D. Berteaux. 2002. Maternal effects and the potential for evolution in a natural population of animals. *Evolution* 56:846–851.
- Marsh, D. M. 2001. Fluctuations in amphibian populations: a meta-analysis. *Biological Conservation* 101:327–335.
- Marshall, D. J., and K. Monro. 2012. Interspecific competition alters nonlinear selection on offspring size in the field. *Evolution* 67:328–337.
- Marshall, D. J., and T. Uller. 2007. When is a maternal effect adaptive? *Oikos* 116:1957–1963.
- Martin, R. A., and D. W. Pfennig. 2010. Maternal investment influences expression of resource polymorphism in amphibians: implications for the evolution of novel resource-use phenotypes. *PLoS ONE* 5:e9117.
- Merilä, J., F. Söderman, R. O'Hara, K. Räsänen, and A. Laurila. 2004. Local adaptation and genetics of acid-stress tolerance in the moor frog, *Rana arvalis*. *Conservation Genetics* 5:513–527.

- Meyer, A. H., B. R. Schmidt, and K. Grossenbacher. 1998. Analysis of three amphibian populations with quarter-century long time-series. *Proceedings of the Royal Society B* 265:523–528.
- Mousseau, T. A., and C. W. Fox. 1998. The adaptive significance of maternal effects. *Trends in Ecology and Evolution* 13:403–407.
- Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Smithsonian Institution Press, Washington, D.C., USA.
- Pfennig, D. W., and R. A. Martin. 2009. A maternal effect mediates rapid population divergence and character displacement in spadefoot toads. *Evolution* 63:898–909.
- Qian, S. S., T. F. Cuffney, and G. McMahon. 2012. Multinomial regression for analyzing macroinvertebrate assemblage composition data. *Freshwater Science* 31:681–694.
- R Development Core Team. 2013. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Räsänen, K., and L. E. B. Kruuk. 2007. Maternal effects and evolution at ecological time-scales. *Functional Ecology* 21:408–421.
- Räsänen, K., A. Laurila, and J. Merilä. 2005. Maternal investment in egg size: environment- and population-specific effects on offspring performance. *Oecologia* 142:546–553.
- Roff, D. A. 2002. *Life history evolution*. Sinauer, Sunderland, Massachusetts, USA.
- Roff, D. A., and D. J. Fairbairn. 2007. The evolution of trade-offs: Where are we? *Evolution* 20:433–447.
- Ryan, T. J., and R. D. Semlitsch. 1998. Intraspecific heterochrony and life history evolution: decoupling somatic and sexual development in a facultatively paedomorphic salamander. *Proceedings of the National Academy of Sciences USA* 95:5643–5648.
- Salvidio, S. 2009. Detecting amphibian population cycles: the importance of appropriate statistical analyses. *Biological Conservation* 142:455–461.
- Schmidt, B. R., W. Hödl, and M. Schaub. 2012. From metamorphosis to maturity in complex life cycles: equal performance of different juvenile life history pathways. *Ecology* 93:657–667.
- Scott, D. E. 1993. Timing of reproduction of paedomorphic and metamorphic *Ambystoma talpoideum*. *American Midland Naturalist* 129:397–402.
- Scott, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology* 75:1383–1396.
- Segers, F. H. I. D., G. Berishvili, and B. Taborsky. 2012. Egg size-dependent expression of growth hormone receptor accompanies compensatory growth in fish. *Proceedings of the Royal Society B* 279:592–600.
- Segers, F. H. I. D., and B. Taborsky. 2012. Competition level determines compensatory growth abilities. *Behavioral Ecology* 23:665–671.
- Semlitsch, R. D. 1985. Reproductive strategy of a facultatively paedomorphic salamander, *Ambystoma talpoideum*. *Oecologia* 65:303–313.
- Semlitsch, R. D. 1987a. Paedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. *Ecology* 68:994–1002.
- Semlitsch, R. D. 1987b. Density-dependent growth and fecundity in the paedomorphic salamander *Ambystoma talpoideum*. *Ecology* 68:1003–1008.
- Semlitsch, R. D., and J. W. Gibbons. 1990. Effects of egg size on success of larval salamanders in complex aquatic environments. *Ecology* 71:1789–1795.
- Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* 69:184–192.
- Sheriff, M. J., C. J. Krebs, and R. Boonstra. 2010. The ghosts of predators past: population cycles and the role of maternal programming under fluctuating predation risk. *Ecology* 91:2983–2994.
- Sheriff, M. J., and O. P. Love. 2013. Determining the adaptive potential of maternal stress. *Ecology Letters* 16:271–280.
- Sinervo, B. 1990. The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* 44:279–294.
- Sinervo, B., and P. Doughty. 1996. Interactive effects of offspring size and timing of reproduction: experimental maternal and quantitative genetics aspects. *Evolution* 50:1314–1327.
- Sinervo, B., P. Doughty, R. B. Huey, and K. Zamudio. 1992. Allometric engineering: a causal analysis of natural selection on offspring size. *Science* 258:885–899.
- Stearns, S. C. 1992. *The evolution of life histories*. Oxford University Press, Oxford, UK.
- Svensson, E., and B. Sinervo. 2000. Experimental excursions on adaptive landscapes: density-dependent selection on egg size. *Evolution* 54:1396–1403.
- Touchon, J. C., M. W. McCoy, J. R. Vonesh, and K. M. Warkentin. 2013. Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. *Ecology* 94:850–860.
- Uller, T. 2008. Developmental plasticity and the evolution of parental effects. *Trends in Ecology and Evolution* 23:432–438.
- Uller, T., S. Nakagawa, and S. English. 2013. Weak evidence for anticipatory parental effects in plants and animals. *Journal of Evolutionary Biology* 26:2161–2170.
- Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. Fourth edition. Springer, New York, New York, USA.
- Whiteman, H. H. 1994. Evolution of facultative paedomorphosis in salamanders. *Quarterly Review of Biology* 69:205–221.
- Whiteman, H. H., S. A. Wissinger, M. Denoël, C. J. Mecklin, N. M. Gerlanc, and J. J. Gutrich. 2012. Larval growth in polyphenic salamanders: making the best of a bad lot. *Oecologia* 168:109–118.

SUPPLEMENTAL MATERIAL

Ecological Archives

The Appendix is available online: <http://dx.doi.org/10.1890/14-1602.1.sm>