Evol Ecol
https://doi.org/10.1007/s10682-018-9941-8

ORIGINAL PAPER

Are direct density cues, not resource competition, driving life history trajectories in a polyphenic salamander?

Jason R. Bohenek[1] · **William J. Resetarits Jr.**[1]

Received: 12 February 2018 / Accepted: 18 May 2018

Abstract Polyphenisms, where multiple, discrete, environmentally-cued phenotypes can arise from a single genotype, are extreme forms of phenotypic plasticity. Cue acquisition and interpretation are vital for matching phenotypes to varying environments, but can be difficult if cues are unreliable indicators or if multiple cues are present simultaneously. Facultative paedomorphosis, where juvenile traits are retained at sexual maturity, is a density-dependent polyphenism exhibited by many salamanders. Favorable conditions such as low larval densities and stable hydroperiod delay metamorphosis and promote a paedomorphic strategy. We investigated proximate cues affecting facultative paedomorphosis in order to understand how larval newts (*Notophthalmus viridescens louisianensis*) assess conspecific density. To isolate the effects of density cues from the effects of resources and agonistic behavior, we caged larval newts in mesocosms in a 2×2 factorial design that manipulated both background larval newt densities (high or low) and food levels (ambient or supplemented). We found strong effects of both food and density on caged individuals. Under high densities, caged larvae were more likely to become efts, a long-lasting juvenile terrestrial stage, across both food levels, while paedomorphs were more common under low densities. Though food levels increased growth rates, density had strong independent effects on metamorphic timing and phenotype. Competition for food and space are classical density-dependent processes, but density cues themselves may be a mediator of density-dependent effects on polyphenisms and life history responses.

Keywords Crowding · Eft · Metamorphosis · Mesocosm · Neoteny · Paedomorphosis

✉ Jason R. Bohenek
jason.bohenek@gmail.com

William J. Resetarits Jr.
wresetar@olemiss.edu

[1] Department of Biology and Center for Water and Wetland Resources and Center for Biodiversity and Conservation Research, The University of Mississippi, University, MS 38677, USA

Published online: 25 May 2018

Introduction

In variable environments, a singular, inflexible phenotype may not be optimal compared to polyphenisms, which are a type of phenotypic plasticity where multiple, environmentally-cued phenotypes arise from a single genotype (West-Eberhard 1989). Polyphenisms are potentially adaptive in that each alternative phenotype maximizes survival and reproductive output under particular environmental conditions (Moran 1992). The environment bombards organisms with cues and parsing informative, reliable cues from uninformative, unreliable cues is necessary to optimize responses. Phenotype mismatching, due to either poor cue acquisition or integration, can result in potentially severe fitness consequences, thus, we should expect strong selection on acquisition and evaluation of reliable environmental cues (Getty 1996).

A variety of biotic and abiotic cues influence polyphenisms across a range of environments and taxa (Grunt and Bayly 1981; Pfennig 1990; McCollum and Van Buskirk 1996; Moczek 1998; Michimae and Wakahara 2002; Nijhout 2003; Laforsch et al. 2009; Maher et al. 2013). Many polyphenisms are density-dependent, where crowding elicits alternative phenotypes such as dispersal phenotypes in insects (Uvarov 1921; Nijhout 2003; Pener and Simpson 2009), and terrestrial (Harris 1987b; Grayson and Wilbur 2009) and cannibalistic phenotypes in salamanders (Collins and Cheek 1983). Density-dependence is an important driver of competition, population size and community structure (Verhulst 1838; Pearl and Reed 1920; Volterra 1926; Lotka 1932; Gause 1934; Brook and Bradshaw 2006). Through a variety of mechanisms, density-dependence affects fitness components such as survival, growth rate, fecundity, and parasite load (Brockelman 1969; Wilbur and Collins 1973; Hassell 1975; Anderson and Gordon 1982; Petranka 1989b; Turchin 1999). Organisms can respond to negative effects of high population density via phenotypic plasticity, initiating changes in morphology (Hoffman and Pfennig 1999), developing dispersal phenotypes (Harrison 1980; Applebaum and Heifetz 1999; Cisse et al. 2015), or initiating ontogenetic niche shifts (Collins and Cheek 1983; Harris 1987b; Semlitsch 1987; Pfennig 1992; Newman 1994; Hoffman and Pfennig 1999).

Many amphibians are explosive breeders, resulting in the potential for extreme crowding and density-dependence in the larval stage (Wilbur and Collins 1973; Petranka 1989a; Van Buskirk and Smith 1991; Wildy et al. 2001). Thus, larval amphibians possess phenotypic plasticity in development rate through metamorphosis that can be modulated to either escape deteriorating conditions or exploit favorable conditions (Wilbur and Collins 1973; Werner and Gilliam 1984; Newman 1992; Denver et al. 1998). High conspecific density can restrict growth via exploitative competition to the point where organisms cannot reach minimum size required for metamorphosis (Newman 1987; Scott 1990). However, density-dependent effects may also arise from alternative mechanisms (Richter et al. 2009). Stress, as a result of agonistic behavior (Walls and Jaeger 1987; Petranka 1989a; Semlitsch and Reichling 1989; Wildy et al. 2001; Glennemeier and Denver 2002), tactile and visual cues (Rot-Nikcevic et al. 2005, 2006) or chemical cues, has been implicated in increasing amphibian development rates through metamorphosis (Glennemeier and Denver 2002). Thus, larval growth and survival patterns typically attributed to exploitative competition may actually be a result of stress from a variety of cue sources, which can scale with conspecific density and influence development rates (Wildy et al. 2001; Rot-Nikcevic et al. 2005, 2006; Richter et al. 2009). Understanding the effects of density cues (tactile, visual, and chemical), independent of

the effects of competition and injury from agonistic behavior, is problematic, as they are confounded in nature (Petranka 1989a; Kuzmin 1995; Richter et al. 2009).

Some salamanders are polyphenic and can delay or prevent metamorphosis in favor of paedomorphosis (Fig. 1), which is broadly defined as the retention of aquatic juvenile characteristics at sexual maturity (Gould 1977). Paedomorphosis in salamanders is either *obligate*, where the ability to metamorphose has been lost, or *facultative*, which is a polyphenism where either metamorphic or paedomorphic adult phenotypes are possible (Harris 1987b; Semlitsch 1987; Whiteman 1994; Denoël et al. 2005; Denoël and Ficetola 2014). Delaying or preventing metamorphosis can be a viable strategy if aquatic conditions are favorable (Wilbur and Collins 1973; Werner and Gilliam 1984; Whiteman 1994), and such conditions have been

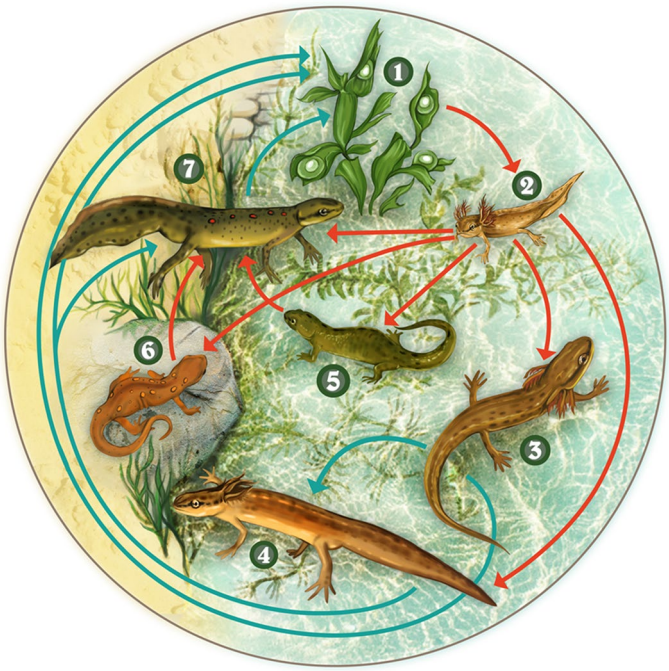

Fig. 1 The complex life cycle of *Notophthalmus viridescens*. (1) Eggs are laid singly in aquatic vegetation and hatch into (2) aquatic larvae. Larval development is plastic with four distinct post-larval phenotypes (Reilly 1987; Petranka 1998). Paedomorphs (3, 4) are sexually mature individuals that retain gills, compressed tail fins and aquatic lifestyles. However, the transition between the paedomorphic and metamorphic morphologies is a continuous reaction norm and may be arrested at any stage in the transition. Thus, paedomorph morphology can range from (3) retaining a full larval morphology with gill slits, large fleshy gills and large tail fins to (4) having no gill slits and partially resorbed gills and tail fins (Reilly 1987). Metamorphosed phenotypes include (5) aquatic juveniles, (6) terrestrial efts and (7) semi-aquatic adults. (5) Aquatic juveniles are metamorphosed individuals that are sexually immature and have not made the transition to terrestrial habitats. They resemble small metamorphosed adults with smooth skin and slightly compressed tailfins. (6) Terrestrial efts are metamorphosed individuals that are sexually immature and have made the transition to terrestrial habitats. They have dry, hydrophobic and often brightly colored skin and tubular tails. While (5) aquatic juveniles can reach sexual maturity as soon as the next breeding season, (6) efts can take up to 8 years to reach sexual maturity (Healy 1974). (7) Semi-aquatic adults are the sexually mature, metamorphosed adult life stage that seasonally migrates between terrestrial and aquatic habitats. Although seasonal migration is typical, both semi-aquatic adults and aquatic juveniles can forgeo migration and overwinter in ponds. Illustration by Tatiana Tushyna

shown to increase the frequency of paedomorphosis ("paedomorph advantage" hypothesis) (Wilbur and Collins 1973; Harris 1987b; Semlitsch 1987; Whiteman 1994; Denoël and Ficetola 2014), but other conditions may as well (see "best of a bad lot" scenario, Whiteman 1994; Whiteman et al. 2012). Relevant environmental factors that affect the expression of paedomorphosis include pond drying (Semlitsch 1987; Semlitsch et al. 1990), conspecific density (Harris 1987b; Semlitsch 1987), temperature (Sprules 1974), and food availability (Sprules 1974; Ryan and Semlitsch 2003). Under poor aquatic conditions, metamorphosis may be a better alternative; however, initiating metamorphosis in a productive aquatic habitat precludes substantial growth opportunities that can lead to greater fecundity and offspring opportunities (Denoël et al. 2005). Maximizing growth in the aquatic stage is optimal because size at metamorphosis is a strong correlate of fitness (Semlitsch et al. 1988). Additionally, favorable conditions may allow larvae to skip juvenile stages and develop directly into paedomorphs or metamorphosed adults, thus decreasing age at maturity, which has strong fitness effects (Cole 1954; Stearns and Koella 1986). Environmental cues affecting timing of metamorphosis, feeding rates and/or onset of sexual maturity have a large impact because opportunities in the larval stage set the trajectory of future fitness.

Plentiful food should be essential for "favorable conditions" and should have obvious effects on growth. However, the interaction between food and facultative paedomorphosis are complex, conflicting and unresolved. For example, Semlitsch (1987) found no effect of food levels on the expression of paedomorphosis in *Ambystoma talpoideum* Holbrook. In contrast, Denoël and Poncin (2001) found that captive paedomorphic newts (*Ichthyosaura alpestris* Laurenti) metamorphosed later with ad libitum food and metamorphosed earlier with low food levels. The effect of food on the expression of paedomorphosis may interact with the various stages of larval development. Ryan and Semlitsch (2003) found that high food levels later in development promote metamorphosis, while low food levels late in development promote paedomorphosis. Food levels should theoretically interact with larval density (but see Petranka (1989a)), but Semlitsch (1987) crossed these factors and found only density effects, suggesting that larvae directly assess density. However, Semlitsch (1987) still confounded food, density, and physical interactions and it is unknown whether larvae assess density via growth effects arising from competition and stress from agonistic behavior or via alternative density cues like tactile, visual and chemical cues.

An experiment was conducted using central newts (*Notophthalmus viridescens louisianensis* Rafinesque) (Fig. 1) to determine the cues used by larval newts to assess density. We utilized a fully factorial design with two levels of conspecific density (high and low) crossed with two levels of food (supplemented and ambient). Our response individuals were individually caged in each mesocosm to prevent physical interaction, agonistic behavior and environmental exploration, but still allowed access to water-borne cues, which we hypothesized as the most informative and reliable indicator of conspecific density (Dettner and Liepert 1994) and, thus, habitat quality. We expected that cues indicating high density (i.e. waterborne cues) can independently promote terrestrial life history strategies, increase development rate and decrease length of larval periods and body size at metamorphosis.

Methods

Study system

The study was conducted in an old field at Tyson Research Center (38.5259°N, −90.5617°W) of Washington University (Resetarits and Silberbush 2016). Eastern newts (*Notophthalmus viridescens*) are a polyphenic, keystone predator (Morin 1981) that possess a complex life history subject to considerable environmental influence (Fig. 1, see early debates in Pope 1921; 1924, 1928; Noble 1926, 1929). Newt metamorphosis is density-dependent and previous studies have shown that their life history polyphenism complies with the "paedomorph advantage" hypothesis, where high initial larval density decreases the proportion of paedomorphs compared to efts; however, it was not determined if this response is mediated through resource competition or agonistic behavior (Harris 1987b).

Breeding Mesocosms

On 5 April 2013, metamorphosed adult newts were collected from ponds at the Tyson Research Center. Twelve cattle tanks (breeding mesocosms) were filled with 1 kg leaf litter and 1200L of well water. For oviposition substrate, four sprigs of either *Egeria densa* (Planch) and/or *Elodea Canadensis* (Michx.) were planted in plastic pots and added to each mesocosm. On 7 April, one male and one female newt were added to each of nine mesocosms and on 7 May, a pair of newts was added to two more breeding mesocosms. On 26 May, seven male and three female newts were added to the final breeding mesocosm—all offspring from this mesocosm were used only as background density (see below) due to multiple females. Eggs were collected every few days starting 6 May by searching through the water plants. The eggs were transferred to the lab and placed into individually marked hatching containers filled with aged tap water (5.68 L, 34.3 × 21.0 × 12.1 cm), separating the eggs by consanguinity. The larvae were fed a mixture of bloodworms (San Francisco Bay Brand, Inc., Newark, CA & Hikari BIO-PURE Blood Worms, Hikari Sales USA Inc., Hayward, CA) ad libitum until they reached a minimum total length of 10 mm, which was large enough to prevent them from passing through the 1.3 × 1.13 mm cage mesh.

Experimental Mesocosms

Sixteen cylindrical, 1200L plastic mesocosms (1.8 m diameter, 50 cm depth; ARM-10138, Ace Roto-Mold, Hospers, Iowa, USA) were constructed at Tyson from 9 to 10 May, filled with well-water and allowed to age for ~ 50 days. Each mesocosm had 0.5 kg of dry leaf litter added on 13 June, and were covered with fiberglass screen lids (1.3 × 1.13 mm mesh) to prevent colonization and oviposition by other organisms. The experiment was a randomized complete block factorial design: two levels of density (low density [LD] = 8 and high density [HD] = 40 larval newts), similar to those found in natural ponds (Harris 1987b; Harris et al. 1988), crossed with two levels of food (ambient [AF] and supplemented food [SF]). Mesocosms were assigned into blocks based on date of larval addition (see below). Each treatment was represented once in each of the four blocks. Overall, the design consisted of four distinct treatment combinations (k = 4) replicated across four blocks (n = 4) for a total of 16 mesocosms (N = 16).

Each tank held eight cages consisting of a large, black, plastic plant pot (28 cm height × 32 cm diameter) with an open cylindrical mesh top (1.3 × 1.13 mm mesh, 35.5 cm h × 32 cm d) extending halfway through the water column and above the water level, supported and propped open by four wooden dowels. Cages were tall enough to rest on the bottom (stabilized with a randomly selected rock) and extend out of the water so individuals that developed lungs could gulp air and metamorphosed efts could crawl out of the water. The bottom half of the cages were opaque except for 1.3 × 1.13 mm screen covering five ~ 1 cm diameter holes. LD treatments contained 8 total individuals (6 larval newts/m^3) that were all separately caged and HD treatments contained 40 individuals (32 larval newts/m^3), 8 caged and 32 not caged. The caged individuals were "response" individuals, since they were subject only to density cues. LD individuals are only exposed to cues from other caged individuals while HD individuals are exposed to cues from both caged and background individuals. Thus, HD response individuals should be exposed to greater density cues than LD response individuals.

Larvae were assigned one at a time to each mesocosm by first randomly selecting a source breeding mesocosm, and then randomly selecting an individual hatchling. This randomized process was performed for all individuals in all mesocosms, ensuring that each mesocosm received randomly selected individuals from a randomly selected breeding mesocosm. Since newts oviposit single eggs over multiple weeks, larvae were introduced one block at a time, between the dates of 27 June and 13 July, once sufficient numbers were accumulated. Each cage was marked with the caged individual's source breeding mesocosm so that genetic differences could be accounted for in statistical analyses. Only caged response individuals could be tracked as background individuals were randomized, but not individually marked. Of the eleven total females that contributed to mesocosm cages, each experimental mesocosm received input from 4 to 6 different breeding mesocosms (mean = 5.3), providing a relatively balanced contribution across the experimental array.

To parse the effects of resource competition from density effects, supplemental food was added to SF treatments once per week beginning 7 July until 6 October. Supplemental food consisted of 0.5 g/individual/week of frozen bloodworms (Omega One Whole Frozen Bloodworms, Omega Sea Ltd., Sitka, AK, 6.3% min. crude protein, 0.8% min. crude fat, 0.3% max. crude fiber, 91.2% max. crude moisture), with amount based on the ad libitum quantities consumed in hatching containers. Individual rations were greater than individual larval body mass (> 100%) for nearly the entire experiment across all treatments. HDSF tanks received 20 g/wk of bloodworms (4 g evenly distributed among the 8 cages and 16 g outside the cages) and LDSF received 4 g/wk (distributed evenly among the cages). All caged individuals were briefly removed (< 3 min) and photographed for measurements of snout-vent length (SVL) on 27 July (Day 30) and 17 August (Day 51) in order to track growth rates. The open cage tops were closed from 31 August—3 September to prevent climbing efts from escaping and mixing with background individuals.

The larval period ended with larvae either becoming paedomorphic or metamorphosing into efts, aquatic juveniles or semi-aquatic adults. At the end of the larval period, newts were removed, massed, and photographed. Newts began to metamorphose in August and continued into mid-October. Mesocosms were checked every other night for emerging metamorphs beginning 15 August. Caged individuals were checked weekly for signs of paedomorphosis or metamorphosis. The experiment ended on 26 October and all remaining background and caged individuals were removed, massed and photographed. All caged individuals were euthanized with Tricaine-S (MS-222; Ferndale, WA), fixed with 10% neutral buffered formalin solution (Sigma-Aldrich Corporation, LLC., St. Louis, MO) and then preserved in 70% ethanol. To

properly evaluate phenotypes, a total of ten representative individuals (5 efts, 3 aquatic juveniles and 2 paedomorphs) representing all blocks and treatments were selected from the preserved specimens and had their hyobranchial apparati cleared and double-stained (Hanken and Wassersug 1981). Staining was done to ensure gilled individuals were actually paedomorphs and not simply large larvae, as the two phenotypes can be ambiguous before secondary sexual characteristics develop during the breeding season. Stained individuals were evaluated for the presence of larval ceratobranchials, which are a key skeletal trait in the head that distinguishes larval newts from post-larval phenotypes. Any gilled individuals lacking larval ceratobranchials were considered paedomorphs (Reilly 1987).

Data analysis

Final SVL was determined from photographs using a standard 1×1 mm grid background and Image-J v1.49 (Schneider et al. 2012). We used linear mixed effects models (LMMs) for variables that followed a normal distribution and generalized linear mixed effects models (GLMMs) for binomial variables. Models were developed using the lme4 v1.1.13 package (Bates et al. 2015) in R v3.4.0 (R Core Team 2017). Significance for LMMs was tested with lmerTest v2.0.33 Approximate F Tests (Type III Satterthwaite denominator degrees of freedom approximation) (Kuznetsova et al. 2015), while fixed effects of GLMMs were analyzed using z-test statistics from lme4 summary output (Bolker et al. 2009). AIC_c was calculated with MuMIn v1.40.4 (Barton 2018). All analyses used $\alpha = 0.05$, and all figures were made using raw data with ggplot2 v2.2.1 (Wickham 2009) and sciplot v1.1.1 (Morales and R Core Team 2012).

Survival was modeled as a logistic GLMM (Warton and Hui 2011) on data aggregated by mesocosm (counts per mesocosm). Phenotype proportions were also modeled as a logistic GLMM, but with non-aggregated data. Since newt post-larval phenotypes are not binomial, two phenotype analyses were conducted: efts versus non-efts and paedomorphs versus non-paedomorphs. These analyses were chosen to contrast the terrestrial life history choice (eft) with aquatic life history choices (aquatic juveniles, paedomorphs) and to contrast metamorphosed individuals (efts, aquatic juveniles) with paedomorphs. However, due to complete separation, paedomorphs versus non-paedomorphs was analyzed as a LMM using proportional data aggregated by mesocosm. Length of the larval period (days), SVL, growth rate (SVL/day) and body condition were analyzed using LMMs. Body condition (size independent mass) was analyzed in two ways: first by analyzing mass with SVL as a fixed covariate in a LMM (Garcia-Berthou 2001), then by mean-scaling masses of caged individuals to decouple variance from the measurement scale and means, regressing against SVL, and modeling the residuals (Berner 2011). The base statistical model for all analyses included density, food and their interaction as fixed effects and mesocosm nested with block as a random effect. AIC_c was used to compare the base model with those that also included any combination of overall larval survival per mesocosm as a fixed covariate and source breeding mesocosm (to account for genetic effects) as a random effect (Appendix, Table 2). Using the same approach to alternative models as above, repeated measures was conducted on SVL by including time as a fixed effect crossed with density and food to explore patterns over time and individual as a random effect to control for pseudoreplication (Appendix, Table 2).

Results

One LDSF mesocosm was excluded from all analyses because of failed cages. Of the 376 individuals in the study, 74.7% survived until the end of the experiment, with 83.3% of the 120 focal, caged individuals surviving and 66.4% of the 256 background individuals surviving. Survival was not different across densities, food levels, or the density × food interaction (Table 1).

Phenotypic proportions

Individuals started metamorphosing in late August, which eventually slowed in early October and then stopped in mid October when temperatures began to drop, which directly mirrored patterns seen by Harris (1987b). Newts were categorized as efts, aquatic juveniles or paedomorphs. All ten individuals (5 efts, 3 aquatic juveniles and 2 paedomorphs) that were cleared and stained were clearly non-larval in structure (Reilly 1987). All paedomorphs were partially metamorphosed with closed gill slits and partially reduced gills, as is typical for *Notophthalmus* (Reilly 1987). Due to logistical constraints, the experiment was not carried out long enough into their breeding season to properly assess sexual maturity. All metamorphosed newts that remained in the water until the end of the experiment were categorized as aquatic juveniles because they retained smooth skin, remained in the water for extended periods while metamorphosed and showed no attempts at dispersal. Besides differences in size, aquatic juveniles are anatomically indistinguishable from metamorphosed adults until the following breeding season when any adults will show visible secondary sexual characteristics. Efts were easily identified as they had dry, rough, hydrophobic skin and attempted to disperse from the mesocosms.

Efts were the most common phenotype among caged individuals at 79%, while aquatic juveniles and paedomorphs represented 15 and 6%, respectively (Fig. 2). The proportion of efts versus non–efts varied across density, but not food levels or the density × food interaction (Fig. 2) (Table 1). Paedomorphs were uncommon, but were marginally more common in low density with no difference across food levels or the density × food interaction (Fig. 2) (Table 1).

Life history traits

Larvae had fast development times in high density as evidenced by their shorter larval periods. Larval period was also shorter with supplemented food compared to ambient food, but there was no significant density × food interaction or any effect of survival (Fig. 3a) (Table 1). Shorter larval periods resulted in reduced SVL (Fig. 3b) (Table 1), which incurs a fitness cost to individuals because SVL has a positive relationship with fitness (Semlitsch et al. 1988). Unlike with larval period, food levels did not have the same strong effect on SVL, and similarly there was no density × food interaction or survival effect (Fig. 3b) (Table 1). Supplemental food increased growth rates but there was no difference across densities and no density × food interaction, or survival effect (Fig. 3c) (Table 1). These patterns suggest that supplemental food shortened larval period via accelerated growth rates that allowed larvae to reach minimum size required for metamorphosis earlier (Fig. 3a). However, the timing of metamorphosis and likelihood of its onset was more strongly predicted by density than food levels (Fig. 3a). The two body condition analyses produced

Table 1 Significance tests for GLMMs and analyses of variance table with Type III Satterthwaite denominator degrees of freedom approximations for LMMs

Survival (binomial GLMM)†		Model: Y ~ Density*Food + (1|Block/Mesocosm)†				
Source	*z*	*p* (> |z|)				
Density	0.51	0.610				
Food	1.32	0.187				
Density × Food	0.63	0.528				
Efts versus non-Efts (binomial GLMM)		Model: 3	AIC_c: 101.1	Δ_i AIC_c: 0.92		
Source	*z*	*p* (> |z|)				
Density	3.24	**0.001**				
Food	0.25	0.803				
Density × Food	0.47	0.638				
Paedomorphs versus non-Paedomorphs		Model: 2	AIC_c: 12.6	Δ_i AIC_c: 8.41		
Source	Num. *df*	Den. *df*	SS	MS	*F*	*p*
Density	1	11	0.03	0.03	3.34	0.095
Food	1	11	0.00	0.00	0.25	0.629
Density × Food	1	11	0.00	0.00	0.12	0.732
Larval period (days)		Model: 1	AIC_c: 852.0	Δ_i AIC_c: 3.38		
Source	Num. *df*	Den. *df*	SS	MS	*F*	*p*
Density	1	91.25	4472.10	4472.10	16.63	**< 0.001**
Food	1	89.13	1871.20	1871.20	6.96	**0.010**
Survival	1	90.05	263.90	263.90	0.98	0.324
Density × Food	1	94.20	39.50	39.50	0.15	0.702

Table 1 (continued)

SVL (mm)		Model: 1	AIC_c: 442.8	Δ_i AIC_c: 2.27		
Source	Num. *df*	Den. *df*	SS	MS	*F*	*p*
Density	1	7.52	40.24	40.24	12.14	**0.009**
Food	1	7.23	6.89	6.89	2.08	0.191
Survival	1	8.12	1.65	1.65	0.50	0.501
Density × Food	1	7.43	4.99	4.99	1.51	0.257
Growth rate (mm SVL/day)		Model: 1	AIC_c: 615.3	Δ_i AIC_c: 0.44		
Source	Num. *df*	Den. *df*	SS	MS	*F*	*p*
Density	1	12.80	31.30	31.30	1.25	0.284
Food	1	10.67	480.36	480.36	19.36	**0.001**
Survival	1	12.93	38.26	38.26	1.54	0.236
Density × Food	1	12.10	34.62	34.62	1.40	0.260
Residuals body condition approach		Model: 2	AIC_c: 877.2	Δ_i AIC_c: 2.42		
Source	Num. *df*	Den. *df*	SS	MS	*F*	*p*
Density	1	14.75	169.06	169.06	0.39	0.542
Food	1	10.11	0.53	0.53	0.00	0.973
Survival	1	63.97	0.73	0.73	0.00	0.967
Density × Food	1	9.87	514.38	514.38	1.18	0.303
Covariate body condition approach		Model: 2	AIC_c: 676.0	Δ_i AIC_c: 2.48		
Source	Num. *df*	Den. *df*	SS	MS	*F*	*p*
Density	1	13.18	32.20	32.20	0.65	0.434
Food	1	9.60	4.70	4.70	0.10	0.764
SVL	1	64.25	9644.20	9644.20	195.09	**< 0.001**
Survival	1	10.22	22.70	22.70	0.46	0.513
Density × Food	1	9.16	67.30	67.30	9.16	0.273

Table 1 (continued)

SVL (mm) repeated measures		Model: 2	AIC_c: 1305.1	Δ_i AIC_c: 0.86		
Source	Num. *df*	Den. *df*	SS	MS	*F*	*p*
Density	1	6.69	18.81	18.81	4.34	0.077
Food	1	6.49	0.12	0.12	0.03	0.871
Time	2	178.60	3113.39	1556.70	359.53	**< 0.001**
Survival	1	6.59	0.11	0.11	0.02	0.880
Density × Food	1	6.48	3.77	3.77	0.87	0.384
Density × Time	2	178.66	133.17	66.58	15.38	**< 0.001**
Food × Time	2	178.60	37.61	18.81	4.34	**0.014**
Density × Food × Time	2	178.53	0.06	0.03	0.01	0.993

Δ_i = Difference in AIC_c from second lowest model

Bold = Significant

[†]Survival was modeled on aggregated data using the methods of Warton and Hui (2011)

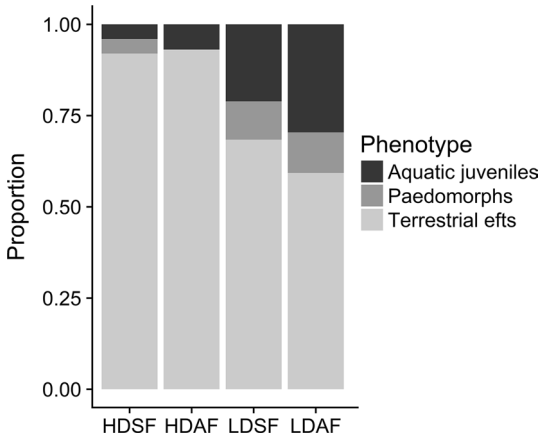

Fig. 2 Phenotype proportions of response (caged) individuals across the four treatments. There was a significant density effect on proportion of efts ($p = 0.001$) and a marginal effect on proportion of paedomorphs ($p = 0.095$). HD = high density, LD = low density, SF = supplemented food and AF = ambient food

nearly identical results: there was no difference in body condition across density, food level or their interaction, but SVL strongly predicted mass in the covariate approach (Table 1). We present the residual index for visualization (Fig. 3d).

Body size differences between treatments were gradually accumulated over time. Repeated measures revealed a significant density × time interaction as SVL was initially indistinguishable among treatments, but started diverging by the second sampling period (Day 51) and showed the greatest differences in SVL at the end of the larval period (Fig. 4). Repeated measures also showed main effects of time and a food × time interaction and marginal density main effect. There was no effect of food level, the density × food interaction, the density × food × time interaction or survival (Table 1). See Appendix: Tables 2 and 3 for complete statistical models and result summaries.

Discussion

Cues indicating density come in a variety of forms and via different sensory modalities, from internal cues like stress, hunger or growth rate, to external cues like visual or tactile cues when encountering conspecifics. Here, the effects of food and density appear to be mostly additive due to their non-interactive, parallel effects (Fig. 3a–c). Food levels play an obvious role in promoting growth (Fig. 3c), but our results suggest that cues indicating high density, unrelated to resource competition or agonistics behavior, are an independent driver of metamorphosis timing (Fig. 3a) and thus phenotype (Fig. 2) in developing larval newts. It has long been assumed that increased competition for food was the main consequence of high conspecific density (Wilbur and Collins 1973), but the mechanism of density-dependent effects is confounded with a multitude of factors. Past studies have suggested that these plastic growth and developmental responses are unrelated to resource competition (Semlitsch 1987; Petranka 1989a; Semlitsch and Reichling 1989) and others have related density effects to an integrated stress response (Glennemeier and Denver 2002).

In the Wilbur-Collins model (1973), metamorphosis optimally occurs once growth in the aquatic environment diminishes below some threshold, while the Werner-Gilliam model (1984). predicts that ontogenetic niche shifts (metamorphosis, in this case) should occur when the ratio of mortality (μ) to growth (g) of the occupied habitat (e.g. aquatic

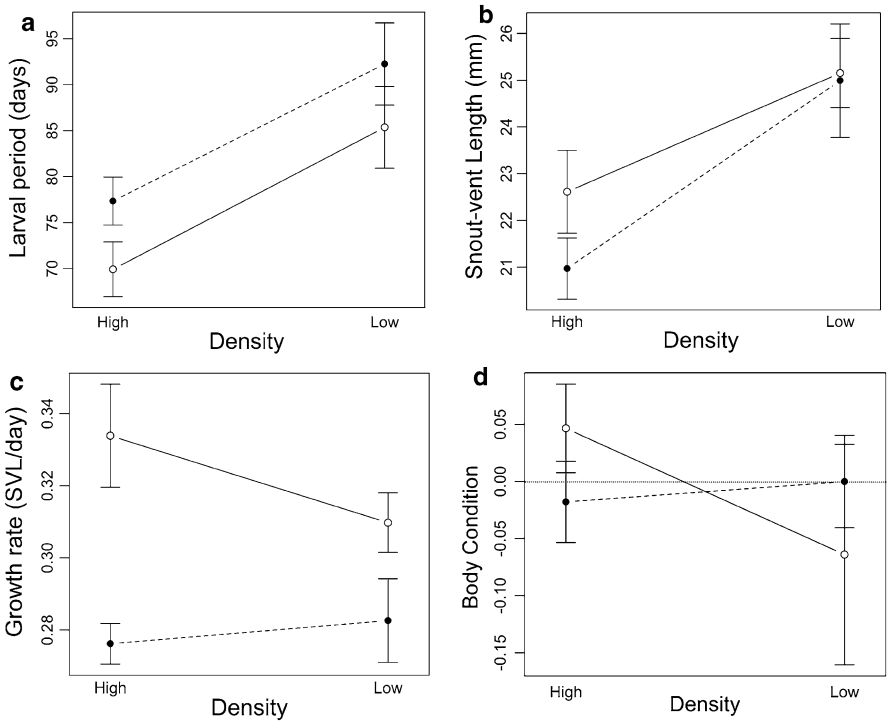

Fig. 3 **a** The larval period of response (caged) individuals measured as days to metamorphosis (mean ± SE). There were significant density ($p < 0.001$) and food level ($p = 0.010$) effects, but no density × food interaction ($p = 0.702$) or survival effect ($p = 0.324$). **b** Final snout-vent length (mm) (mean ± SE) of response (caged) individuals. There was a significant density effect ($p = 0.009$), but no food level effect ($P = 0.191$) or density × food interaction ($p = 0.257$) or survival effect ($p = 0.501$). **c** Growth rate (SVL/day) (mean ± SE) of response (caged) individuals. There was a significant food ($p = 0.001$) effect, but no density effect ($p = 0.284$) or density × food interaction ($p = 0.260$) or survival effect ($p = 0.236$). **d** Body condition (mean ± SE) of response (caged) individuals was not different between density ($p = 0.542$) or food levels ($p = 0.973$) and there was no density × food interaction ($p = 0.303$) or effect of survival ($p = 0.967$). Ambient food is represented by closed circles and supplemented food by open circles. Raw data is used in all graphs except body condition, which is a derived index

environment) outweighs that of another habitat (e.g. terrestrial environment). In a natural setting, high larval density combined with an unproductive environment (e.g. low food) should exhaust growth opportunities quickly due to competition and the associated stress of crowding. These "unfavorable" environments should also have high perceived μ/g ratio throughout the experiment relative to other treatments due to the outcomes of competition and agonistic behavior, as well as cannibalism, which is common in larval newts (Harris 1987a). However, despite the HDAF treatment having perceived conditions that are theoretically "unfavorable", larvae from HDAF metamorphosed later than those from the "favorable" HDSF treatment. These results mirror previous studies that found exceptions to the Wilbur-Collins model (reviewed in Morey and Reznick 2000) and likely occurred because larvae in HDAF grew more slowly than larvae in HDSF thereby taking longer to reach the minimum body size required for metamorphosis (Wilbur and Collins 1973; Semlitsch 1987). Our results suggest that the optimal body size for metamorphosis is inversely related to conspecific density, as larvae from HDSF larvae grew rapidly (since growth

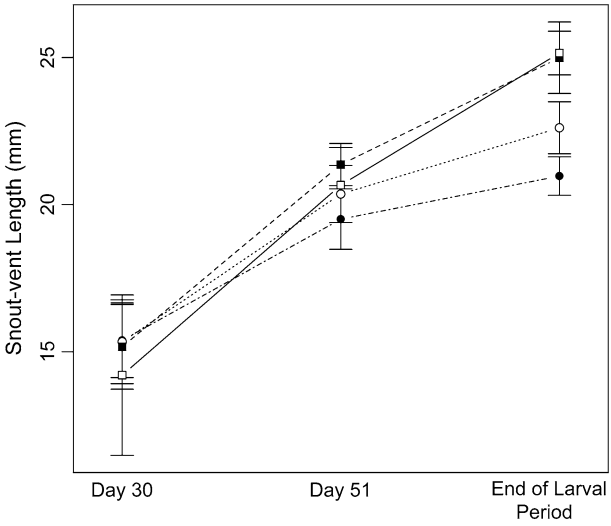

Fig. 4 Mean snout-vent length (mm) (mean ± SE) of response individuals of each treatment over time. SVL measurements were taken 30 (27 July) and 51 days (17 August) after larvae were introduced into the first block and the third measurement was taken at the end of the larval period, which varied between individuals. There was a significant density × time interaction ($p < 0.001$) and food × time interaction ($p = 0.014$) (Table 1). LDSF = open squares, LDAF = closed squares, HDSF = open circles, HDAF = closed circles

was unrestricted due to supplemented food) (Fig. 3c), but traded additional SVL growth (Fig. 3b) for an earlier onset of metamorphosis (Fig. 3a).

Our primary goal was to investigate the effects of density cues on post-larval phenotype. The general pattern across densities was as predicted—high densities resulted in more efts and fewer aquatic phenotypes (Fig. 2), while "favorable" conditions in the form of supplemented food had no effect on phenotype. All individuals from HDSF became efts, despite relatively high body condition and growth rates that would predict extended larval periods, suggesting that density cues are the primary indicator of habitat quality (Fig. 2). High density seemed to truncate SVL growth via an early onset of metamorphosis and thus canalize the ontogenetic response resulting in efts. Due to earlier metamorphosis, individuals from HDSF only attained body sizes typical of efts, suggesting that larval density can affect population dynamics, since efts take multiple years (2 years in North Carolina, USA (Harris 1987b) and 3–8 years in Massachusetts, USA populations (Healy 1974)) to reach sexual maturity (Cole 1954; Stearns and Koella 1986). Additionally, the potential for mortality during the lengthy eft stage would reduce fitness to zero, thereby increasing the risk associated with that phenotype. Nevertheless, the eft stage has its obvious benefits as an alternative strategy. Efts are chemically protected and mobile, which allows individuals to disperse and colonize other, potentially more suitable, ponds (Gill 1978). Since efts are terrestrial and adults and paedomorphs are aquatic, habitat (and therefore resource) partitioning may be occurring (Lejeune et al. 2018), where smaller, less competitive individuals (efts) are avoiding habitats with larger, competitively superior individuals (adults or paedomorphs) (Denoël et al. 2005).

Maintenance of polyphenisms, especially those involving more than two phenotypes, is a fascinating question in evolutionary biology (West-Eberhard 1989; Moran 1992; Whiteman 1994; Getty 1996). Imposing discrete phenotypic traits onto an environmental gradient (e.g. conspecific density, food level) is problematic because developmental thresholds have no clear environmental correlates. Under a continuous environmental gradient, a generalist phenotypic strategy or continuous phenotypic plasticity should be favored. However, if phenotype-environment matching is accurate and the fitness advantages are large, then polyphenisms can be maintained (West-Eberhard 1989; Moran 1992). Polyphenisms have naturally selected thresholds creating reaction norms, but individuals must still accurately

assess and respond to environmental conditions. Therefore, reliable cues are of utmost importance because they can be the difference between a fitness-increasing phenotype match or a fitness-reducing mismatch. Since density-dependence is of considerable importance in a variety of systems, we expect strong selection on an organism's ability to assess density. Encounter rates can be a useful indicator of conspecific density, but only if organisms can distinguish between repeated encounters with the same individuals and encounters with different individuals. Visual cues may provide this information for a variety of organisms, but they are less reliable in aquatic environments (Dettner and Liepert 1994).

Adult newts are capable of utilizing pheromonal chemical cues to assess conspecific density, at least while breeding (Park and Propper 2001; Rohr et al. 2005).Our results suggest larvae may also be able to assess conspecific density via chemical cues, which ultimately translates as a proxy of long-term environmental quality. The nature of these cues is unknown, but possible origins are conspecific diet cues, secondary metabolites, prey alarm cues, exogenous hormones, cannibalism or conspecific mortality. Since background individuals occupied the same mesocosm, the possibility of visual or tactile cues affecting response individuals is not entirely eliminated. However, visual or tactile cues seem unlikely to transmit across the cage mesh, because (1) background larvae take refuge in leaf litter except at night, when visual cues are limited or absent, (2) caged larvae spent most of their time in the cage bottoms, which were opaque, (3) visual cues are less reliable in aquatic environments relative to chemical cues (Dettner and Liepert 1994; Wisenden 2000), (4) larval salamanders have sluggish behavior that would not likely transmit tactile cues well, and (5) visual and tactile cues transmitted from predators, competitors and prey are likely indistinguishable (especially at night) and thus uninformative.

Polyphenism reaction norms vary between species and populations (Semlitsch and Gibbons 1985; Semlitsch et al. 1990; Takahashi and Parris 2008; Takahashi et al. 2011).Though a weaker density-dependent response was observed here compared to some other studies (Harris 1987b; Semlitsch 1987), we found results for this subspecies similar to those of Takahashi and Parris (2008). The design of Takahashi and Parris (2008) allowed full interactions between individuals (competition, agonistic behavior, visual, tactile and chemical cues) and our design only permitted chemical cues (and potentially visual and tactile cues), yet there were similar phenotype proportions for both studies. This comparison suggests that physical interactions may not be necessary to elicit effects on polyphenisms in this system.

The importance of chemical cues in aquatic systems (Dettner and Liepert 1994; Wisenden 2000) is widely recognized (Chivers and Smith 1998; Kats and Dill 1998; Brönmark and Hansson 2000; Wisenden 2000; Ferrari et al. 2010). They are known to elicit many alternative phenotypes, principally in the form of inducible defenses (Grunt and Bayly 1981; Harvell 1990; McCollum and Van Buskirk 1996; Gilbert 1999; Kuhlmann et al. 1999; Laforsch et al. 2009; van Donk et al. 2011). Chemical cues have advantages over other cues because of their specificity, which can reveal not only the presence of competitors and predators, but also their identity through species-specific signatures, density through cue concentration, and preferred prey through dietary cues, all of which can be integrated into a threat level. In the case of newts, cannibalism is a real predation threat that simulatenouly scales and is confounded with intraspecific competition, thus chemical cues can provide information about both potential predation and competition. We minimized the potential for cannibalism in our background populations by introducing similar-sized individuals, however percieved threat of cannibalism may be inherent in cues indicating high density.

We provide evidence that larval salamanders utilize chemical density cues to modulate developmental trajectories and assess habitat quality. Conspecific density itself may be a more reliable, and more comprehensive, indicator of long term habitat quality than food resources, or at least be predictive of future food resources. Understanding the modality

and relative importance of different cues will allow us to decipher complex life history decisions in polyphenic organisms, and understand the contributions of those decisions to population dynamics and evolutionary maintenance of alternative phenotypes.

Acknowledgements We would like to thank Tyson Research Center and Washington University in St. Louis, especially K. Smith and B. Schall, for allowing us to use their facilities and providing a stimulating environment. Also, B. Biro helped substantially with collecting, L. Eveland & M. Pintar contributed to the study in many ways, J. Hoeksema provided advice on mixed models, and L. Fuller and B. Mikah provided encouragement. Also, thanks to the National Science Foundation (DEB-0516298), Texas Tech University, The University of Mississippi, and the Henry L. and Grace Doherty Foundation. This research conformed to institutional guidelines (IACUC protocol 14–028) and all state and federal regulations (MDC permit no. 15680).

Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

Appendix

See Tables 2 and 3.

Table 2 Summary of alternative models in lme4 notation

Model ID	Model
	Efts versus non-efts (binomial GLMM)[†]
1	Y ~ Density * Food + Survival + (1|Block/Mesocosm) + (1|BreedingMesocosm)
2	Y ~ Density * Food + Survival + (1|Block/Mesocosm)
3	Y ~ Density * Food + (1|Block/Mesocosm)
	Paedomorphs versus non-Paedomorphs (LMM)[‡]
1	Y ~ Density * Food + Survival + (1|Block)
2	Y ~ Density * Food + (1|Block)
	Larval period, growth rate, SVL and body condition
1	Y ~ Density * Food + Survival + (1|Block/Mesocosm) + (1|BreedingMesocosm)
2	Y ~ Density * Food + Survival + (1|Block/Mesocosm)
3	Y ~ Density * Food + (1|Block/Mesocosm) + (1| BreedingMesocosm)
4	Y ~ Density * Food + (1|Block/Mesocosm)
	SVL repeated measures
1	Y ~ Density * Food * Time + Survival + (1|Block/Mesocosm) + (1|ID) + (1|BreedingMesocosm)
2	Y ~ Density * Food * Time + Survival + (1|Block/Mesocosm) + (1|ID)
3	Y ~ Density * Food * Time + (1|Block/Mesocosm) + (1|ID) + (1|BreedingMesocosm)
4	Y ~ Density * Food * Time + (1|Block/Mesocosm) + (1|ID)

Only the factors in parentheses are random effects

[†]Failure to converge of model "Y ~ Density * Food + (1|Block/Mesocosm) + (1| BreedingMesocosm)," which was excluded

[‡]This analysis was conducted on aggregated data (proportions) and necessarily excluded Mesocosm and Breeding Mesocosm terms

Table 3 Summary of complete model parameters

Survival (binomial GLMM)	Model: Y ~ Density * Food + (1|Block/Mesocosm)[†]				
Source	Estimate	SE		*z*	*p* (>|z|)
Fixed effects					
Intercept	1.686	0.487		3.46	**< 0.001**
Density	0.582	0.778		0.75	0.454
Food	− 0.351	0.700		− 0.50	0.616
Density × Food	− 0.644	1.020		− 0.63	0.528
Random effects	Variance	SE			
Mesocosm × Block	0.000	0.000			
Block	0.000	0.000			
Efts versus non-Efts (binomial GLMM)	Model: 3	AIC_c: 101.1		Δ_i AIC_c: 0.92	
Source	Estimate	SE		*z*	*p* (>|z|)
Fixed effects					
Intercept	− 0.355	0.454		− 0.78	0.434
Density	− 2.321	0.850		− 2.73	**0.006**
Food	− 0.444	0.657		− 0.68	0.499
Density × Food	0.579	1.230		0.47	0.638
Random effects	Variance	SE			
Mesocosm × Block	~ 0.000	~ 0.000			
Block	0.183	0.427			
Paedomorphs versus non-paedomorphs (LMM)	Model: 2	AIC_c: 12.6		Δ_i AIC_c: 8.41	
Source	Estimate	SE	*df*	*t*	*p* (>|t|)
Fixed effects					
Intercept	0.107	0.047	11	2.27	**0.044**
Density	− 0.107	0.067	11	− 1.60	0.137
Food	− 0.007	0.072	11	0.10	0.923
Density × Food	0.035	0.098	11	0.35	0.732
Random effects	Variance	SE			
Block	0.000	0.000			
Residuals	0.009	0.094			
Larval period (days)	Model: 1	AIC_c: 852.0		Δ_i AIC_c: 3.38	
Source	Estimate	SE	*df*	*t*	*p* (>|t|)
Fixed effects					
Intercept	107.138	15.007	86.56	7.14	**< 0.001**
Density	− 18.019	4.791	88.44	− 3.76	**< 0.001**
Food	− 11.344	5.303	91.82	− 2.14	**0.035**
Survival	− 16.404	16.557	90.05	− 0.99	0.324
Density × Food	2.797	7.296	94.20	0.38	0.702
Random effects	Variance	SE			
Mesocosm × Block	~ 0.000	~ 0.000			
Breeding Mesocosm	48.010	6.929			

Table 3 (continued)

Larval period (days)	Model: 1	AIC_c: 852.0		Δ_i AIC_c: 3.38	
Source	Estimate	SE	*df*	*t*	*p* (> |*t*|)
Block	22.370	4.730			
Residuals	268.890	16.400			
SVL (mm)	Model: 1	AIC_c: 442.8		Δ_i AIC_c: 2.27	
Source	Estimate	SE	*df*	*t*	*p* (> |*t*|)
Fixed effects					
Intercept	23.027	3.050	8.81	7.55	**< 0.001**
Density	− 3.926	1.038	6.90	− 3.78	**0.007**
Food	0.229	1.125	7.51	0.20	0.844
Survival	2.430	3.448	8.12	0.71	0.501
Density × Food	1.886	1.537	7.43	1.23	0.257
Random effects	Variance	SE			
Mesocosm × Block	1.482	1.217			
Breeding Mesocosm	0.612	0.782			
Block	0.632	0.795			
Residuals	3.316	1.821			
Growth rate (mm SVL/day)	Model: 1	AIC_c: 615.3		Δ_i AIC_c: 0.44‡	
Source	Estimate	SE	*df*	*t*	*p* (> |*t*|)
Fixed effects					
Intercept	23.250	4.384	14.26	5.30	**< 0.001**
Density	0.081	1.474	10.49	0.06	0.957
Food	3.719	1.622	12.14	2.29	**0.041**
Survival	6.082	4.898	12.93	1.24	0.236
Density × Food	2.629	2.226	12.10	1.18	0.260
Random effects	Variance	SE			
Mesocosm × Block	0.165	0.406			
Breeding Mesocosm	3.371	1.836			
Block	0.000	0.000			
Residuals	24.817	4.982			
Residuals body condition approach	Model: 2	AIC_c: 877.2		Δ_i AIC_c: 2.42‡	
Source	Estimate	SE	*df*	*t*	*p* (> |*t*|)
Fixed effects					
Intercept	− 13.596	21.183	11.85	− 0.64	0.533
Density	− 0.292	7.270	9.55	− 0.04	0.969
Food	− 4.610	7.886	10.34	− 0.59	0.571
Survival	16.211	23.978	11.22	0.68	0.513
Density × Food	12.499	10.612	9.66	1.18	0.267
Random effects	Variance	SE			
Mesocosm × Block	34.440	5.869			
Block	0.000	0.000			
Residuals	430.910	20.758			

Table 3 (continued)

Covariate body condition approach‡	Model: 2	AIC_c: 676.0		Δ_i AIC_c: 2.48	
Source	Estimate	SE	*df*	*t*	*p* (> |*t*|)
Fixed effects					
Intercept	−77.742	10.450	26.53	−7.44	**< 0.001**
Density	−0.313	2.826	12.33	−0.11	0.914
Food	−1.551	2.735	9.47	−0.57	0.584
SVL	4.587	0.328	64.25	13.97	**< 0.001**
Survival	5.659	8.350	10.22	0.68	0.513
Density × Food	4.349	3.727	9.16	1.17	0.273
Random effects	Variance	SE			
Mesocosm × Block	4.500	2.120			
Block	0.000	0.000			
Residuals	49.440	7.031			
SVL (mm) repeated measures	Model: 2	AIC_c: 1305.1		Δ_i AIC_c: 0.86	
Source	Estimate	SE	*df*	*t*	*p* (> |*t*|)
Fixed effects					
Intercept	16.418	2.945	8.25	5.58	**< 0.001**
Density	−0.699	1.098	11.16	−0.64	0.537
Food	−1.478	1.175	10.91	−1.26	0.235
Time–Day 51	5.415	0.668	171.90	8.10	**< 0.001**
Time–Final	0.155	0.653	183.16	14.02	**< 0.001**
Survival	−0.501	3.192	6.59	−0.16	0.880
Density × Food	1.256	1.572	10.27	0.80	0.442
Density × Time–Day 51	−1.306	0.871	172.14	−1.50	0.135
Density × Time–Final	−3.476	0.867	180.74	−4.01	**< 0.001**
Food × Time–Day 51	1.014	0.967	174.27	1.05	0.296
Food × Time–Final	1.843	0.970	187.96	1.90	0.059
Density × Food × Time–Day 51	0.131	1.253	173.87	0.11	0.917
Density × Food × Time–Final	0.014	1.264	183.06	0.01	0.991
Random effects	Variance	SE			
ID	0.914	0.956			
Mesocosm × Block	1.131	1.145			
Block	3.226	1.796			
Residuals	4.330	2.081			

Models used default treatment contrasts, which sets the first level of factors (Low Density, Ambient Food and Day 30) as the reference level and then compares with the additional factor levels (High Density, Supplemented Food, Time–Day 51 and Time–Final) of the listed fixed effects

Δ_i = difference in AIC_c from second lowest model

SE = standard error

Bold = Significant

†Survival was modeled on aggregated data using the methods of Warton and Hui (2011)

‡× 100 to reduce variance decimal places

References

Anderson RM, Gordon DM (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. Parasitology 85:373–398

Applebaum SW, Heifetz Y (1999) Density-dependent physiological phase in insects. Annu Rev Entomol 44:317–341

Barton K (2018) MuMIn: multi-model inference. R package version 1.40.4

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using {lme4}. J Stat Softw 67:1–48

Berner D (2011) Size correction in biology: how reliable are approaches based on (common) principal component analysis? Oecologia 166:961–971

Bolker BM, Brooks ME, Clark CJ et al (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135

Brockelman WY (1969) An analysis of density effects and predation in *Bufo americanus* tadpoles. Ecology 50:632–644

Brönmark C, Hansson L (2000) Chemical communication in aquatic systems: an introduction. Oikos 88:1–7

Brook BW, Bradshaw CJA (2006) Strength of evidence for density dependence in abundance time series of 1198 species. Ecology 87:1445–1451

Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. Ecoscience 5:338–352

Cisse S, Ghaout S, Mazih A et al (2015) Estimation of density threshold of gregarization of desert locust hoppers from field sampling in Mauritania. Entomol Exp Appl 156:136–148

Cole LC (1954) The population consequences of life history phenomena. Q Rev Biol 29:103–137

Collins JP, Cheek JE (1983) Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. Integr Comp Biol 23:77–84

Denoël M, Ficetola GF (2014) Heterochrony in a complex world: disentangling environmental processes of facultative paedomorphosis in an amphibian. J Anim Ecol 83:606–615

Denoël M, Poncin P (2001) The effect of food on growth and metamorphosis of paedomorphs in *Triturus alpestris apuanus*. Arch fur Hydrobiol 152:661–670

Denoël M, Joly P, Whiteman HH (2005) Evolutionary ecology of facultative paedomorphosis in newts and salamanders. Biol Rev Camb Philos Soc 80:663–671

Denver RJ, Mirhadi N, Phillips M (1998) Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology 79:1859–1872

Dettner K, Liepert C (1994) Chemical mimicry and camouflage. Annu Rev Entomol 39:129–154

Ferrari MCO, Wisenden BD, Chivers DP (2010) Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. Can J Zool 88:698–724

Garcia-Berthou E (2001) On the misuse of residuals in ecology: testing regression residual versus the analysis of covariance. J Anim Ecol 70:708–711

Gause G (1934) The struggle for existence. Williams and Wilkins, Baltimore

Getty T (1996) The maintenance of phenotypic plasticity as a signal detection problem. Am Nat 148:378–385

Gilbert JJ (1999) Kairomone-induced morphological defenses in rotifers. In: Tollrian R, Harvell CD (eds) Ecology and evolution of inducible defenses. Princeton University Press, Princeton, pp 127–141

Gill DE (1978) The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). Ecol Monogr 48:145–166

Glennemeier KA, Denver RJ (2002) Role for corticoids in mediating the response of *Rana pipiens* tadpoles to intraspecific competition. J Exp Zool 292:32–40

Gould SJ (1977) Ontogeny and phylogeny. Harvard University Press, Cambridge

Grayson KL, Wilbur HM (2009) Sex- and context-dependent migration in a pond-breeding amphibian. Ecology 90:306–312

Grunt JWG, Bayly I (1981) Predator induction of crests in morphs of the *Daphnia carinata* King complex. Limnol Oceanogr 26:201–218

Hanken J, Wassersug RJ (1981) The visible skeleton. Funct Photogr 16(22–26):44

Harris RN (1987a) An experimental study of population regulation in the salamander, *Notophthalmus viridescens dorsalis* (Urodela: Salamandridae). Oecologia 71:280–285

Harris RN (1987b) Density-dependent paedomorphosis in the salamander *Notophthalmus viridescens dorsalis*. Ecology 68:705–712

Harris RN, Alford RA, Wilbur HM (1988) Density and phenology of *Notophthalmus viridescens dorsalis* in a natural pond. Herpetologica 44:234–242

Harrison RG (1980) Dispersal polymorphisms in insects. Annu Rev Ecol Syst 11:95–118

Harvell CD (1990) The ecology and evolution of inducible defenses. Q Rev Biol 65:323–340

Hassell MP (1975) Density-dependence in single-species populations. J Anim Ecol 44:283
Healy W (1974) Population consequences of alternative life histories in *Notophthalmus v. viridescens*. Copeia 1974:221–229
Hoffman EA, Pfennig DW (1999) Proximate causes of cannibalistic polyphenism in larval tiger salamanders. Ecology 80:1076–1080
Kats LB, Dill LM (1998) The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience 5:361–394
Kuhlmann H-W, Kusch J, Heckman K (1999) Predator-induced defenses in ciliated protozoa. In: Tollrian R, Harvell CD (eds) Ecology and evolution of inducible defenses. Princeton University Press, Princeton, pp 142–159
Kuzmin SL (1995) The problem of food competition in amphibians. Herpetol J 5:252–256
Kuznetsova A, Brockhoff PB, Christensen RHB (2015) Package "lmerTest"
Laforsch C, Tollrian R, Gene E (2009) Cyclomorphosis and phenotypic changes. In: Likens GE (ed) Encyclopedia of inland waters. Academic Press, Oxford, pp 1159–1166
Lejeune B, Sturaro N, Lepoint G, Denoël M (2018) Facultative paedomorphosis as a mechanism promoting intraspecific niche differentiation. Oikos 127:427–439
Lotka AJ (1932) The growth of mixed populations: two species competing for a food supply. J Washingt Acad Sci 22:461–469
Maher JJM, Werner EE, Denver RJ (2013) Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. Proc R Soc B Biol Sci 280:1–9
McCollum SA, Van Buskirk J (1996) Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. Evolution 50:583–593
Michimae H, Wakahara M (2002) A tadpole-induced polyphenism in the salamander *Hynobius retardatus*. Evolution 56:2029–2038
Moczek AP (1998) Horn polyphenism in the beetle *Onthophagus taurus*: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. Behav Ecol 9:636–641
Morales M, Core Team R (2012) Sciplot: scientific graphing functions for factorial designs. R Foundation for Statistical Computing, Vienna
Moran NA (1992) The evolutionary maintenance of alternative phenotypes. Am Nat 139:971–989
Morey S, Reznick D (2000) A comparative analysis of plasticity in larval development in three species of spadefoot toads. Ecology 81:1736
Morin PJ (1981) Predatory salamanders reverse the outcome of competition among three species of anuran tadpoles. Science 80(212):1284–1286
Newman RA (1987) Effects of density and predation on *Scaphiopus couchi* tadpoles in desert ponds. Oecologia 71:301–307
Newman RA (1992) Adaptive plasticity in amphibian metamorphosis. Bioscience 42:671–678
Newman RA (1994) Effects of changing density and food level on metamorphosis of a desert amphibian, *Scaphiopus couchii*. Ecology 75:1085–1096
Nijhout HF (2003) Development and evolution of adaptive polyphenisms. Evol Dev 5:9–18
Noble G (1926) The Long Island newt: a contribution to the life history of Triturus viridescens. Am Museum Novit 228:1–11
Noble GK (1929) Further observations on the life-history of the newt, *Triturus viridescens*. Am Museum Novit 348:1–22
Park D, Propper CR (2001) Repellent function of male pheromones in the red-spotted newt. J Exp Zool 289:404–408
Pearl R, Reed LJ (1920) On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proc Natl Acad Sci 6:275–288
Pener MP, Simpson SJ (2009) Locust phase polyphenism: an update. Adv Insect Physiol 36(36):1–272
Petranka JW (1989a) Density-dependent growth and survival of larval ambystoma: evidence from whole-pond manipulations. Ecology 70:1752–1767
Petranka JW (1989b) Chemical interference competition in tadpoles: Does it occur outside laboratory aquaria? Copeia 1989:921–930
Petranka JW (1998) Salamanders of the United States and Canada. Smithsonian Books, Washington
Pfennig D (1990) The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. Oecologia 85:101–107
Pfennig DW (1992) Proximate and functional causes of polyphenism in an anuran tadpole. Funct Ecol 6:167–174
Pope PH (1921) Some doubtful points in the life-history of *Notophthalmus viridescens*. Copeia 91:14–15
Pope PH (1924) The life-history of the common water-newt (*Notophthalmus viridescens*), together with observations on the sense of smell. Ann Carnegie Museum 15:305–368

Pope PH (1928) The life-history of *Triturus viridescens*—some further notes. Copeia 168:61–73
R Core Team (2017) R: A language and environment for statistical computing
Reilly SM (1987) Ontogeny of the hyobranchial apparatus in the salamanders *Ambystoma talpoideum* (Ambystomatidae) and *Notophthalmus viridescens* (Salamandridae): the ecological morphology. J Morphol 214:205–214
Resetarits WJ Jr, Silberbush A (2016) Local contagion and regional compression: habitat selection drives spatially explicit, multiscale dynamics of colonisation in experimental metacommunities. Ecol Lett 19:191–200
Richter JAR, Martin L, Beachy CK (2009) Increased larval density induces accelerated metamorphosis independently of growth rate in the frog *Rana sphenocephala*. J Herpetol 43:551–554
Rohr JR, Park D, Sullivan AM et al (2005) Operational sex ratio in newts: field responses and characterization of a constituent chemical cue. Behav Ecol 16:286–293
Rot-Nikcevic I, Denver RJ, Wassersug RJ (2005) The influence of visual and tactile stimulation on growth and metamorphosis in anuran larvae. Funct Ecol 19:1008–1016
Rot-Nikcevic I, Taylor CN, Wassersug RJ (2006) The role of images of conspecifics as visual cues in the development and behavior of larval anurans. Behav Ecol Sociobiol 60:19–25
Ryan TJ, Semlitsch RD (2003) Growth and the expression of alternative life cycles in the salamander *Ambystoma talpoideum* (Caudata: Ambystomatidae). Biol J Linn 80:639–646
Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675
Scott DE (1990) Effects of larval density in *Ambystoma opacum*: an experiment in large-scale field enclosures. Ecology 71:296–306
Semlitsch RD (1987) Paedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. Ecology 68:994–1002
Semlitsch RD, Gibbons JW (1985) Phenotypic variation in metamorphosis and paedomorphosis in the salamander *Ambystoma talpoideum*. Ecology 66:1123–1130
Semlitsch RD, Reichling SB (1989) Density-dependent injury in larval salamanders. Oecologia 81:100–103
Semlitsch RD, Scott DE, Pechmann HK (1988) Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192
Semlitsch RD, Harris RN, Wilbur HM (1990) Paedomorphosis in *Ambystoma talpoideum*: maintenance of population variation and alternative life-history pathways. Evolution 44:1604–1613
Sprules WG (1974) The adaptive significance of paedogenesis in North American species of *Ambystoma* (Amphibia: Caudata): an hypothesis. Can J Zool 52:393–400
Stearns SC, Koella JC (1986) The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40:893
Takahashi MK, Parris MJ (2008) Life cycle polyphenism as a factor affecting ecological divergence within *Notophthalmus viridescens*. Oecologia 158:23–34
Takahashi MK, Takahashi YY, Parris MJ (2011) Rapid change in life-cycle polyphenism across a subspecies boundary of the Eastern Newt, *Notophthalmus viridescens*. J Herpetol 45:379–384
Turchin P (1999) Population regulation: a synthetic view. Oikos 84:153–159
Uvarov BP (1921) A revision of the genus *Locusta*, L. (=*Pachytylus*, Fieb.), with a new theory as to the periodicity and migrations of locusts. Bull Entomol Res 12:135–163
Van Buskirk J, Smith DC (1991) Density-dependent population regulation in a salamander. Ecology 72:1747–1756
van Donk E, Ianora A, Vos M (2011) Induced defences in marine and freshwater phytoplankton: a review. Hydrobiologia 668:3–19
Verhulst PF (1838) Notice sur la loi que la population suit dans son accroissement. Corresp Math Phys 10:113–121
Volterra V (1926) Fluctuations in the abundance of a species considered mathematically. Nature 118:558–560
Walls SC, Jaeger RG (1987) Aggression and exploitation as mechanisms of competition in larval salamanders. Can J Zool 65:2938–2944
Warton DI, Hui FKC (2011) The arcsine is asinie: the analysis of porportions in ecology. Ecology 92:3–10
Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. Annu Rev Ecol Syst 15:393–425
West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. Annu Rev Ecol Syst 20:249–278
Whiteman HH (1994) Evolution of facultative paedomorphosis in salamanders. Q Rev Biol 69:205–221
Whiteman HH, Wissinger SA, Denoel M et al (2012) Larval growth in polyphenic salamanders: making the best of a bad lot. Oecologia 168:109–118

Wickham H (2009) ggplot2: Elegant graphics for data analysis. Springer, New York
Wilbur HM, Collins JP (1973) Ecological aspects of amphibian metamorphosis. Science 182:1305–1314
Wildy EL, Chivers DP, Kiesecker JM, Blaustein AR (2001) The effects of food level and conspecific density on biting and cannibalism in larval long-toed salamanders, *Ambystoma macrodactylum*. Oecologia 128:202–209
Wisenden BD (2000) Olfactory assessment of predation risk in the aquatic environment. Philos Trans R Soc B Biol Sci 355:1205–1208