

Determinants of size at metamorphosis in an endangered amphibian and their projected effects on population stability

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Roughly 80% of animal species have complex life cycles spanning a major habitat shift, and delayed life history effects play an important role in their population dynamics. Through their effect on size at metamorphosis, factors in the pre-metamorphic environment often have profound effects upon survival and fecundity in the post-metamorphic environment. Here, we adopted a combined experimental and field observational approach to investigate the factors that determine size at metamorphosis in pond-breeding amphibians, and to predict some of their downstream effects on population stability. We set up ecologically realistic mesocosm communities for the endangered California tiger salamander *Ambystoma californiense* to test the effects of larval density, prey density and hydroperiod on mean size at metamorphosis. We found significant effects for all three factors, with mean size at metamorphosis negatively correlated with larval density and positively correlated with prey density and hydroperiod. We also used six years of field survey data to identify the most informative model explaining mean size at metamorphosis and thus validate our mesocosm results. The optimal three-term model identified terms that were roughly analogous to each of the mesocosm treatments and with similar effect sizes, providing strong field confirmation of our experimental results. The field data also provide correlations between each factor and the number of metamorphs recruited to the population, allowing us to predict the effect of each factor on population stability. Finally, we show that these populations of the endangered *A. californiense* are strongly resource limited, which has important implications for their management and recovery as an endangered taxon.

Amphibians are known for their naturally fluctuating population sizes and the difficulty this causes in making long-term population projections (Blaustein et al. 1994, Pechmann and Wilbur 1994, Salvidio 2009). Given the striking declines of many amphibian populations and species, it is critical to develop accurate models of natural population dynamics that can help guide the interpretation of long term field studies, including the differentiation of normal population fluctuations versus long-term population declines (Taylor and Scott 1997). Since many amphibians have complex life cycles with a metamorphic transition between aquatic and terrestrial environments, accurate population models should include the relationship between fecundity as a (usually terrestrial) adult and survival and growth in both the terrestrial and aquatic environments (Wilbur 1980). It is particularly important to include any delayed life history effects, including those that span the aquatic–terrestrial interface, because they are known to create damping oscillations that can become entrained by environmental stochasticity (Leslie 1959, Beckerman et al. 2002, Searcy et al. 2014). As both ecologists and conservation biologists, we need to determine whether the population fluctuations that characterize many long-term field studies have the same period and

magnitude as those predicted by delayed life history effects, or whether they represent long-term declines that will ultimately lead to population collapse.

Although metamorphosis and its associated life-history effects are ubiquitous across metazoans, here we focus on amphibians with biphasic life cycles. Previous work has shown that larger amphibian metamorphs have higher survival in the terrestrial environment, reach sexual maturity faster, reach a larger adult size, and as a consequence have a larger clutch size (Berven 1990, 2009, Scott 1994, but see Schmidt et al. 2012 for a counter example). Since metamorph size is determined by factors in the larval aquatic environment, delayed life history effects must be ubiquitous in the life cycle of many amphibian species. This begs the question of which ecological factors of the aquatic environment are most important in determining metamorph size and the relative contributions of those factors. Petranka (1984) suggested that the four most important factors affecting larval growth rate are genetic variation, temperature, prey density and larval density; in combination with length of the larval period, these five factors presumably control size at metamorphosis. While we acknowledge that genetic variation is an important factor in determining metamorph size (Johnson et al.

2010), here we focus on the other four factors, since they act through proximate ecological effects on phenotypic plasticity. Such effects must be very large, since average metamorph mass routinely varies two-fold or more between consecutive years at the same breeding pond, and genetic composition of the population would not be expected to vary dramatically in long-lived amphibians on this time scale (Searcy et al. 2014). Larval period is positively correlated with pond hydroperiod (Tejedo and Reques 1994). Temperature is also correlated with hydroperiod, since shallower water bodies have less thermal inertia and thus experience both higher average temperatures and greater temperature fluctuations during the diurnal cycle (Tejedo and Reques 1994). We thus chose to focus on the effects of larval density, prey density and hydroperiod (which effects both larval period and temperature simultaneously) to build a realistic model of the environmental factors affecting mass at metamorphosis.

Few studies have examined all three of these factors simultaneously in any amphibian system. Smith (1983) found that anuran larval survival and size at metamorphosis are negatively correlated with larval density and positively correlated with food density, and that larval survival is also positively correlated with pond hydroperiod. The last of these inferences (on hydroperiod) was derived from observations of natural ponds, while the rest were from experimental manipulations of small breeding ponds. Semlitsch (1987) and Tejedo and Reques (1994) both used factorial mesocosm experiments to examine these factors and their interactions. They found that size at metamorphosis was negatively correlated with larval density and positively correlated with prey/resource density and hydroperiod, and that larval survival was negatively correlated with larval density. These results match very well with the traditional models for optimal size at metamorphosis (Wilbur and Collins 1973, Werner 1986).

Our study focuses on the California tiger salamander *Ambystoma californiense* (CTS), a state and federally-listed species across its range. Given their rarity, it is often difficult to develop accurate population models of endangered taxa, although such models are critical in assessing whether current land management practices are facilitating stable population dynamics. Our previous long-term field studies have shown that larger metamorphs have higher terrestrial survival, and that there is substantial natural variation in size at metamorphosis (5.7-fold variation among individual salamanders and 2.8-fold variation among cohort averages from the same site; Searcy et al. 2014). Here, we quantify the factors that generate this variation to provide a critical piece in models of population dynamics for both CTS and other amphibians with biphasic life histories. We used a two-pronged approach to attack this problem. First, we replicated the factorial mesocosm experiment used by Semlitsch (1987) in an ecologically very divergent congener to quantify the simultaneous effects of larval density, prey density, and hydroperiod on size at metamorphosis in CTS. As a part of our experimental design, we conducted extensive field surveys to quantify the natural community composition of CTS breeding ponds, allowing us to mimic these communities and make our experimental mesocosms as ecologically realistic as possible. Second, we used six years of field survey data to determine whether the factors that we tested

in the mesocosm experiment are those most important in determining size at metamorphosis in natural populations, and whether their effect sizes were similar in mesocosms and natural ponds. Finally, we used our field data to associate these factors to population recruitment. By simultaneously examining the effects of these key environmental factors on both quantity and quality of recruits, we can qualitatively predict their effects on population stability.

Methods

Field and experimental methods

Our mesocosm experiment was a $2 \times 2 \times 2$ complete factorial design with a total of eight treatment combinations. Each treatment combination was replicated five times for a total of 40 experimental units. Each unit was a different 1136-l cattle watering tank. The three treatments were: 1) larval density, 2) prey density, and 3) drying regime.

To maintain the biological realism of our experiments, levels of the density treatments were based on survey data collected in 2011. Five CTS breeding ponds were surveyed at each of: 1) Jepson Prairie Preserve (Solano County), 2) Ohlone Wilderness and Sunol Regional Wilderness (Alameda County), and 3) Virginia Smith Trust (Merced County). Surveys occurred 29 March – 7 May when larvae averaged 17–47 mm snout–vent length (SVL). Using a 5 m seine (4 mm mesh size), we seined each pond four times, measured the area covered, counted the number of CTS larvae and all macroscopic vertebrate and invertebrate species collected, and calculated the observed number of each species in an area of 2.48 m², equal to the footprint of our experimental mesocosms. We then used an empirical correction based on our observation that a first pass seine haul through a section of pond captures on average 51% of the animals that are actually present. (This correction factor is based on 58 instances when we exhaustively sampled a 25 m² section of pond for a total of 13 different species, Searcy unpubl.). We stocked our mesocosms with all potential prey species that naturally occurred at a density such that at least one individual would be found per 2.48 m² in at least half of our sampled sites. Based on this cutoff, we stocked water boatmen *Corisella* sp., California clam shrimp *Cyzicus californicus*, snails (Gastropoda sp.) and Pacific chorus frogs *Pseudacris regilla*. The only predator taxa that occur at a high enough density that they could have been stocked are backswimmers *Notonecta* sp. and predaceous diving beetle larvae (Dytiscidae), which we did not stock in our mesocosms. Based on a subsequent experiment in which we did stock these taxa at their natural densities and the rate of successful metamorphosis only decreased to 86% (down from 91% in this study), we do not feel that their presence would have greatly impacted the results of this study.

For the low density prey treatment, we stocked each species at its mean density across the survey ponds in which it was found. For the high density prey treatment, we stocked each species at its mean density across the three ponds where it was most abundant. We excluded one water boatmen density as an outlier, because it was an order of magnitude

higher than the next highest value. Based on these data, our stocking rates were: low density treatment (6 *Corisella*, 8 *C. californicus*, 3 Gastropoda and 34 *P. regilla*) and high density treatment (21 *Corisella*, 18 *C. californicus*, 6 Gastropoda and 102 *P. regilla*). Using the same calculations, we added four CTS larvae in the low density treatment and eight in the high density treatment. All tanks received an equal number of larvae from two crosses (Supplementary material Appendix 1).

At every stage of the experiment, we attempted to keep the mesocosms temporally synchronized with our study population of wild CTS at Jepson Prairie. The breeding ponds at Jepson Prairie first held water on 21 January and the tanks were filled on the 27 and 28. The first mating pairs entering the ponds at Jepson Prairie were detected on 23 January and the captive matings took place on 6 and 7 February (the first rain event following that on 23 January). We detected the first egg deposition at Jepson Prairie on 6 February and the captive eggs were produced on the 10 and 11. When the larvae were moved to the cattle tanks on 8 April at a mean size of 23 mm SVL, wild larvae measured the next day had a mean SVL of 29 mm. Finally, the first metamorphs were detected in the tanks on 24 May and the first metamorphs at Jepson Prairie were detected on 31 May. Thus, throughout the season the development of the captive larvae remained within about one week of their wild counterparts.

Our drying treatment had two levels: 1) constant water level and 2) the mean drying rate of CTS breeding ponds. We calculated this mean drying rate based on pond depth data collected over a five year period from 23 CTS breeding ponds (data provided by H. T. Harvey et al.). We modeled mean drying rate using a quadratic regression with mean drying rate per day as the response variable and Julian date as the predictor variable, treating each year as an independent observation. Based on this model, we calculated the number of days that it should take a natural pond to dry down by 2.54 cm (this interval shortened over the course of the spring as evaporation increased and rainfall decreased). At these intervals we measured the depth of every cattle tank and either 1) filled it back to 50 cm if it was in the constant water level treatment or 2) lowered the water level by the appropriate amount if it was in the drying treatment.

Results from the mesocosm experiment were compared to a model created using field data collected at the Jepson Prairie Preserve. We seined the two largest breeding pools once or twice per year (between 20 March and 29 May) from 2008-2013 to determine densities of both CTS and their primary large prey item, the endangered vernal pool tadpole shrimp *Lepidurus packardii*. Stomach flushing indicates that *L. packardii* is the predominant prey item of larval CTS at the Jepson Prairie Preserve (Searcy unpubl.). Pitfall traps adjacent to both breeding ponds were checked during the entire period of salamander surface activity for all six years (Searcy et al. 2014). For each pond-year combination, the pitfall capture data were used to calculate the: 1) number of breeding females, 2) number of emerging metamorphs, 3) mean date of metamorph emergence, 4) mean mass of emerging metamorphs, and 5) mean length of the larval period. We also recorded the yearly drying date of each pond. Annual rainfall and mean air temperature for the larval period were

collected from a local weather station (12.6 km from the Preserve in Vacaville, Solano County).

Analysis

All analyses of the mesocosm data were based on tank means and used a three-way factorial ANOVA where the response variable was modeled as a function of the three main effects (CTS density, prey density, drying regime) and their two and three-way interactions. We removed any terms for which $p > 0.2$ provided that they were not included in any interaction terms for which $p < 0.2$, and used these simplified models for our final analyses. Size measurements were log-transformed to facilitate comparisons with other taxa described in the literature.

Analysis of the field data used model selection based on AIC_c . The dependent variable was mean mass of emerging metamorphs for each pond-year combination, and the ten independent variables were: 1) number of breeding females, 2) number of emerging metamorphs, 3) mean date of metamorph emergence, 4) mean length of larval period, 5) pond drying date, 6) annual rainfall, 7) mean air temperature during the larval period, 8) density of CTS larvae, 9) prey (*L. packardii*) density, and 10) pond. All possible models were built, and the three-factor model with the lowest AIC_c was selected for further analysis. We also used a series of ANCOVAs to quantify the relationship between the number of emerging metamorphs and each of the other variables. Each ANCOVA included pond as a fixed effect, one continuous covariate, and their interaction. All analyses were performed using JMP 10 (SAS Inst.).

Results

Out of the 240 salamanders included in the experiment, 219 metamorphosed or were close to metamorphosis when the experiment concluded on 20 June. The remaining salamanders had either not reached metamorphosis yet ($n = 18$) or had died ($n = 3$). Mean metamorph mass was 7.43 g, which is below the mean from our field studies (8.87 g). However, if the wild caught salamanders are ranked by cohort mean mass, the experiment falls in the 39th percentile (Searcy et al. 2014), indicating that the larvae in the experiment metamorphosed well within typical sizes for CTS.

Size at metamorphosis

All three treatments had highly significant effects ($p < 0.001$) on metamorph mass and length (Table 1). Larval density had the largest effect size, 94% and 96% larger than water level for mass and SVL, respectively, and 143% and 129% larger than prey density for mass and SVL, respectively. On average, metamorphs were larger in the low larval density, high prey density, and high water level treatments. Metamorphs in the low larval density treatments averaged 26% larger by mass and 7% larger by length than those in the high larval density treatments. There was also a marginally significant interaction in the effect of larval and prey density on length at metamorphosis, with metamorphs from the high larval density, low prey density treatment combination being particularly small.

Table 1. Response of California tiger salamander (CTS) metrics to the three mesocosm treatments. Interaction terms were only included in the models if their associated p-value was less than 0.2. The number in each cell represents the effect size of the high level treatment relative to the grand mean followed by the p-value of an *F* statistic in parenthesis. Note that effect sizes are comparable across columns, but not across rows.

Response variable	Treatment			
	Larval density	Prey density	Water level	Larval density × prey density
CTS date of metamorphosis	1.0 (0.045)	−1.6 (0.002)	1.7 (0.002)	−1.0 (0.046)
CTS mass at metamorphosis	−0.13 (p < 0.001)	0.053 (p < 0.001)	0.067 (p < 0.001)	
CTS snout-vent length at metamorphosis	−0.036 (p < 0.001)	0.016 (p < 0.001)	0.019 (p < 0.001)	0.0058 (p = 0.060)

The mesocosm model for mean metamorph mass matched very closely with the top-ranked three-factor model from the field data (Table 2), which had a lower AIC_c than all two-factor and four-factor models. The top three factors in the field model were mean date of emergence, prey (*L. packardii*) density, and larval density, which are closely linked to the three mesocosm treatments: pool depth, prey density, and larval density, respectively. For the field data, mean mass at metamorphosis increased with mean date of metamorphosis (p = 0.02), which was generally a reflection of how long the breeding ponds held water (R² = 0.74 for the correlation between mean date of metamorphosis and drying date). Mean mass at metamorphosis also increased with prey (*L. packardii*) density (p = 0.04) and decreased with density of CTS larvae (p = 0.01).

The effect sizes between the two models were also strikingly similar (Fig. 1). Since larval density was measured on a natural log scale in the field model, the scale corrected effect size of CTS is $-0.27/e = -0.10$ (95% CI: $-0.16 - -0.040$). There was a two-fold difference in larval density between the low and high density levels in the mesocosm experiment, so the scale corrected effect size of CTS was $-0.26/2 = -0.13$ (95% CI: $-0.15 - -0.10$). A similar calculation with prey density revealed that the scale corrected effect of *L. packardii* density was $0.15/e = 0.056$ (95% CI: $0.014-0.098$). There was a three-fold difference in *P. regilla* density between the low and high prey density levels in the mesocosm experiment, and *P. regilla* is the primary large prey item of larval CTS when present (Searcy unpubl.). Thus, the scale corrected effect of *P. regilla* density was $0.11/3 = 0.035$ (95% CI: $0.019-0.052$). Salamanders in the high water level treatment metamorphosed a mean of 3.3 days later than salamanders in the low water level treatment, so the scale corrected effect size in the mesocosm model was $0.13/3.3 = 0.040$ (95% CI: $0.025-0.055$), compared with an effect size (no scale

correction needed) of 0.010 (95% CI: $0.0040-0.017$) in the field model. Thus, the only effect size that was significantly different between the two models was the per day effect size (p = 0.0002).

Number of metamorphs

In addition to quantifying predictors of size at metamorphosis, we also examined variables predicting the number of emerging metamorphs in the field (Table 3). The best predictor was prey (*L. packardii*) density (p = 0.01), although the strength of the effect varied significantly between the two breeding ponds (p = 0.02). Drying date (p = 0.03) and annual rainfall (p = 0.07) were also positively correlated with number of emerging metamorphs. None of these correlations were significant after Bonferroni correction for multiple comparisons, but given the small sample size in the field data (n = 11), we believe that a Bonferroni correction may be overly conservative.

Table 2. Best three factor model for mean mass at emergence based on field data (no other model was within 2 AIC_c units). Mean date of emergence mimics the effect of water level, since a higher water level allows metamorphs to emerge later. Larval density based on surveys mimics the larval density treatment. *L. packardii* density mimics the prey density treatment, since *L. packardii* is the chief prey item for large CTS larvae at Jepson Prairie, where the field data was collected. All variables are log-transformed except for Julian dates.

Predictor variables	Effect size	SE	p-value
Mean date of emergence	0.010	0.0033	0.02
Larval density	−0.27	0.083	0.01
<i>L. packardii</i> density	0.15	0.058	0.04

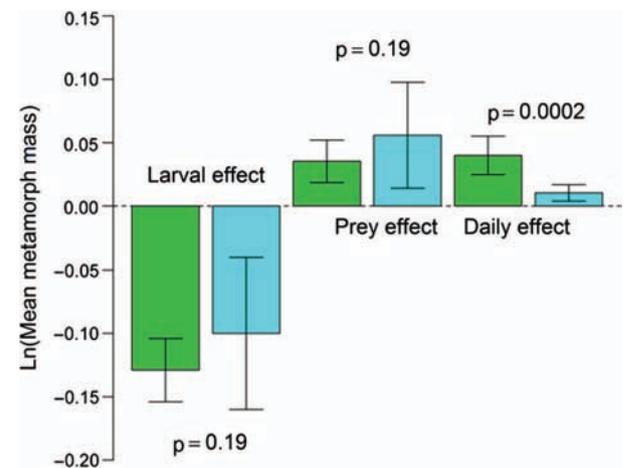


Figure 1. Comparison of effect sizes between mesocosm and field models. Green bars are mesocosm effect sizes and blue bars are field effect sizes. The per capita effect sizes of larvae and prey represent the change in average metamorph size resulting from a 50% increase in the density of larvae or prey, respectively. The daily effect size represents the change in average metamorph size resulting from spending one extra day in the mesocosm/breeding pond. The effect sizes of larvae and prey are not statistically distinguishable between the mesocosm and field models. The daily effect size in the mesocosm model is significantly larger than in the field model, perhaps because conditions in the natural breeding pond are deteriorating toward the end of the season and thus the extra growth achieved from each additional day in the pond is decreasing.

Table 3. Field measured variables presented in rank order of their ability to predict number of emerging metamorphs. Each row is the result of a separate ANCOVA with pond as the fixed effect, the continuous predictor as the covariate, and their interaction. The number in each cell is the effect size followed by the associated p-value of an *F* statistic in parenthesis. The effect size for the pond variable is the difference between the expected value at Olcott Lake and the overall mean. The effect size for the interaction term is the difference between the expected slope at Olcott Lake and the overall mean slope. All variables are log-transformed except for Julian dates and temperatures.

Continuous predictors	Effect of continuous predictor	Pond effect	Interaction effect
<i>L. packardii</i> density	2.1 (0.01)	-0.77 (0.13)	1.7 (0.02)
Pond drying date	0.048 (0.03)	0.48 (0.20)	0.027 (0.17)
Annual rainfall	3.2 (0.07)	0.44 (0.26)	2.2 (0.19)
Larval density	1.4 (0.12)	0.27 (0.56)	-0.12 (0.89)
Mean length of larval period	5.1 (0.14)	0.70 (0.17)	1.7 (0.60)
Mean date of metamorph emergence	0.034 (0.38)	0.51 (0.33)	0.0094 (0.80)
Mean temperature during the larval period	-0.33 (0.59)	0.49 (0.33)	-0.66 (0.30)
No. of breeding females	-0.13 (0.89)	0.57 (0.59)	0.24 (0.80)
Mean mass at metamorphosis	0.15 (0.94)	0.46 (0.40)	0.033 (0.99)

Timing of metamorphosis

Mesocosm treatments also had a significant effect on timing of metamorphosis. On average, salamanders in the low water level treatment metamorphosed 3.3 days earlier ($p = 0.002$), salamanders in the low larval density treatment metamorphosed 2.0 days earlier ($p = 0.045$), and salamanders in the low prey density treatment metamorphosed 3.3 days later ($p = 0.002$). There was also a significant interaction between prey density and larval density, with salamanders in the low prey density and high larval density combination metamorphosing particularly late.

Discussion

Our results provide a clear model of the factors influencing size at metamorphosis in the California tiger salamander (CTS), and in so doing contribute to a more general understanding of the role of extrinsic factors mediating metamorphosis in amphibians. Larval density, prey density, and hydroperiod all had significant effects on mass and SVL at metamorphosis in our mesocosm experiment, and they (or closely related variables) were selected as the most important in our field model. Finally, the effect sizes of larval density and prey density were very similar between the mesocosm and field models. This congruence between mesocosm and field models indicates that amphibian community ecologists (Petranka 1984) are identifying the primary factors influencing timing and size at metamorphosis, and suggests that semi-natural mesocosm experiments can capture the complexities of natural populations, particularly if care is taken to accurately mimic natural conditions.

Size at metamorphosis

From at least the time of Wilbur and Collins (1973), size at metamorphosis has been one of the key variables in models of the evolutionary ecology of amphibian metamorphosis. We found that size at metamorphosis is negatively correlated with larval density (Tejedo and Reques 1994, Boone and Semlitsch 2001, Rudolf and Rödel 2007; but see Smith and Burgett 2012), positively correlated with prey density (Denver et al. 1998, Rohr et al. 2004, Rudolf and Rödel

2007), and positively correlated with hydroperiod (Tejedo and Reques 1994, Denver et al. 1998, Rohr et al. 2004; but see Berven 1990). These results match most previous studies, as well as theoretical models of the drivers of amphibian metamorphosis (Wilbur and Collins 1973, Werner 1986).

Although the significance and directions of effects are important, effect sizes provide the additional benefit of allowing a quantitative comparison of the relative influence of these effects across studies of different species. Semlitsch (1987), Tejedo and Reques (1994), and the current study all tested the same suite of factors, allowing us to make meaningful cross-species and habitat comparisons. Since all three studies implemented their treatments in slightly different ways, we make the simplifying assumption that all responses are linear in order to facilitate these cross-study comparisons. Semlitsch (1987) studied another ambystomatid salamander (*A. talpoideum*) from the eastern US, while Tejedo and Reques (1994) investigated the European anuran *Bufo calamita*. As Table 4 shows, all three studies found equivalent effect sizes for hydroperiod, even though Tejedo and Reques (1994) only increased hydroperiod by 15 days between their low and high water level treatments, whereas both Semlitsch (1987) and our study mimicked a permanent water body in the high water level treatment.

Ambystoma talpoideum exhibited a much larger response to larval density than the other two species. This may reflect the very high larval density treatment used by Semlitsch (1987), biological differences in the amphibians and their food resources, or both. In particular, the high concentration of larvae in terms of biomass m^{-2} in Semlitsch (1987) may have led to a greater level of intraspecific competition, and subsequently a greater response in metamorph size.

Even after correcting for the variation in treatment strength used in the different studies, the anuran *B. calamita* showed a significantly larger response to prey/resource density than either salamander species. This may reflect differences in resource availability in the experimental systems, predatory versus herbivorous diet, or both. The more meaningful comparison between the two predatory, congeneric salamanders indicates that, after correcting for variation in treatment strength, *A. talpoideum* had a significantly weaker response to prey density than CTS. Again, this may reflect the ecological reality of their different habitats, or it may be an experimental artifact. Compared to our study, Semlitsch

Table 4. Comparison of effect sizes between Semlitsch (1987), Tejedo and Reques (1994), and our study. Treatment represents the difference between the low and high levels of the relevant factor. The percentages provided are the observed responses to these differences. For our study, point estimates are followed in parentheses by 95% CI.

		Semlitsch (1987)	Tejedo and Reques (1994)	This study
Larval density	treatment	6 × increase	5 × increase	2 × increase
	Δ in mass		−49%	−26% (−31%– −21%)
	Δ in SVL	−30%		−7.3% (−8.4%– −6.1%)
Prey density	treatment	6 × increase	5 × increase	3 × increase
	Δ in mass		45%	11% (5.6%–16%)
	Δ in SVL	2.9%		3.2% (2.0%–4.3%)
Hydroperiod	treatment	constant vs drying	15 day increase	constant vs drying
	Δ in mass		16%	13% (8.3%–18%)
	Δ in SVL	4.8%		3.7% (2.5%–4.9%)

(1987) provided an average of nine times as many tadpoles (with only slightly greater salamander biomass). If these prey treatment levels reflect natural prey densities in *A. talpoideum* ponds (as ours do for CTS), then this suggests a very large difference in prey availability between their respective environments, which may in turn explain why CTS are more resource limited.

These large differences in effect sizes emphasize the tremendous variation that may be present among species and experimental systems, and highlight the importance of conducting species and system-specific experiments to generate quantitatively accurate results. While the same general patterns in size at metamorphosis seem to be common across amphibian species, the strength of those patterns can differ dramatically between species and ecosystems. We need many more analyses, with experimental conditions accurately parameterized to reflect natural variation, to continue to fine-tune quantitative models explaining natural variation in size at, and time to, metamorphosis.

Predicted effects on population stability

By providing correlations between population recruitment and each of the three factors influencing mean size at metamorphosis, our field data provide the additional opportunity of predicting whether each of these factors will tend to increase or decrease population stability when incorporated into models of population dynamics. In so doing, we recognize that incorporating delayed life history effects through the influence of size at metamorphosis on post-metamorphic survival will initially be destabilizing, since it involves a time lag (Wilbur 1996). However, given that delayed life history effects are an intrinsic part of amphibian demographic models, we feel that there is value in predicting the additional changes in population stability resulting from which factors of the aquatic environment are modeled as determining size at metamorphosis. The rationale underlying these predictions is that negative feedback loops are stabilizing, while positive feedback loops are destabilizing (Robertson 1991, Brose 2008). Since higher quality (i.e. larger) recruits have a higher probability of surviving to maturity (Searcy et al. 2014), factors that increase the average mass of recruits also increase the rate of production of reproductive adults. Thus, factors that increase the rate of production of reproductive adults both directly (by increasing the number of metamorphs that have the potential to become adults), and

indirectly (by increasing the mean size at metamorphosis and thus the probability of each individual metamorph surviving to maturity), are driving a positive feedback loop, which is destabilizing. Alternatively, factors that increase either the number of metamorphs or their mean size while decreasing the other constitute a negative feedback, and will thus increase stability when incorporated into models of population dynamics.

First, consider the effect of larval density. Larval density is certainly the most important of our three factors. It has the largest effect on size at metamorphosis in both mesocosm and field models, and it directly represents the number of larvae that have the potential to become reproductive adults. Since the directions of its effects on number of metamorphs and mean size at metamorphosis are opposing, it constitutes a form of negative density-dependence, which will tend to stabilize the population. Based on our field-derived model of mass-dependent terrestrial survival (Searcy et al. 2014), the average metamorph from the low larval density treatment has 4.0 (95% CI: 1.7–6.8) times the probability of reaching maturity than does the average metamorph from the high larval density treatment. (Preliminary data from an integral projection model reduces the point estimate from 4.0 to 2.7, Searcy unpubl.). Thus, the increased survival probability of animals raised in the low density treatment probably balances out the doubling in the number of animals in the high density treatment, such that a large cohort of smaller animals and a small cohort of larger animals would contribute roughly equal numbers of breeding adults to the population. The fact that the two factors come close to cancelling each other out suggests that CTS populations may be more stable than previously thought, since a ‘bust’ year in which fewer metamorphs are recruited to the population should also produce larger individuals that are more likely to survive in the terrestrial environment. Of course, these are ‘all else being equal’ calculations, and since number and size of metamorphs are correlated with both prey density and hydroperiod, the overall effect may negate the feedback loop based on larval density.

Prey density is likely to have a destabilizing effect on amphibian populations. Prey density is the variable most strongly correlated with number of metamorphs recruited to the population (Table 3) and it also increases mean mass at metamorphosis in both the mesocosm and field models, thus constituting a form of positive density dependence. The effect of hydroperiod on population stability is more difficult

to predict. Mean date of metamorph emergence is positively correlated with number of metamorphs, but the correlation is much weaker than that with prey density (Table 3). The other measure of hydroperiod (pond drying date) and its cause (annual rainfall) both have much stronger positive correlations with number of metamorphs (Table 3). Thus, the overall pattern is probably for wet years to have a later mean date of metamorph emergence (and thus larger average size at metamorphosis) and a larger number of metamorphs, which together will tend to destabilize the population. How the stabilizing effect of larval density and the destabilizing effects of prey density and hydroperiod ultimately interact is a complex problem. The net effect may be close to zero, illustrated by the fact that the correlation between number of metamorphs and mean mass at metamorphosis is the weakest among the variables we tested (Table 3).

It is important to keep in mind that all of these predictions are only on the direction of the effects (either toward or away from stability) and not on their magnitudes. These magnitudes may be muted by the presence of an extended terrestrial phase with high annual survival rates (Taylor et al. 2006). Species with this type of life history will be more sensitive to variation in average terrestrial survivorship than to variation in reproductive parameters such as those we investigated (Trenham and Shaffer 2005, Harper et al. 2008). However, limited evidence suggests that terrestrial survivorship may be quite constant (Pechmann 1995), whereas the factors in the aquatic environment that we looked at are highly variable in natural populations (coefficients of variation: 1.4 for larval density, 0.76 for *Lepidurus* density, 0.10 for mean date of metamorphosis), and are therefore more likely to have measurable effects on population stability. To determine the magnitude of these effects and their relative importance compared to terrestrial survivorship, it will be necessary to develop a complete population dynamics model. Fortunately, the robust quantitative data generated by our combined mesocosm and field approach have brought us much closer to parameterizing such a model.

Resource limitation

While work on other amphibian systems has shown effects of both larval density and hydroperiod on mean size at metamorphosis in natural populations (Semlitsch et al. 1988, Van Buskirk and Smith 1991, Rudolf and Rödel 2007), no previous studies have demonstrated a correlation between prey density and mean size at metamorphosis in the wild. While most studies have simply lacked the data to test for this effect, Van Buskirk and Smith (1991) tested for this correlation in the blue-spotted salamander *Ambystoma laterale* and failed to find such an effect. The situation in our study is clearly quite different. We found an effect of *L. packardii* prey density on both mean size at metamorphosis and number of emerging metamorphs, indicating that CTS at Jepson Prairie are strongly resource limited. In general, the mesic woodlands inhabited by most amphibians may be more productive than the dry grassland vernal pools favored by CTS (Searcy et al. 2013) and other members of the tiger salamander complex. This raises the possibility that CTS population sizes could be increased by increasing their prey base. This finding has important conservation implications for management of this

endangered species, particularly in anthropogenically modified or created ponds with few natural prey. An important line of applied research for this and other amphibian species is the management of breeding sites to optimize the natural prey communities on which they depend.

Experimental venue

One of the most important contributions of this study is its demonstration that mesocosms can capture many of the life history patterns governing variation in metamorphosis among wild amphibian populations. Our mesocosms yielded not only metamorphs of the same average size as comparable field populations, but also showed similar responses to larval and prey density treatments. This matches the observations of Van Buskirk (2009) and arguments made by Chalcraft et al. (2005) that mesocosms can capture natural dynamics when efforts are made to accurately mimic as many characteristics of natural ponds as possible (e.g. hydroperiod). Other studies have found greater differences between mesocosms and field populations (Skelly and Kiesecker 2001, Skelly 2002), particularly with respect to interspecific interactions among competing species. We did not have the opportunity to examine such interactions as there are no other salamander species native to our ponds. It is also interesting that we obtained our results without including predators in our mesocosms, suggesting that lack of predators may not be as important in observed differences between mesocosm and field dynamics as has recently been suggested (Melvin and Houlihan 2012). The Melvin and Houlihan (2012) meta-analysis proposed that predation is a powerful homogenizing force regulating anuran larval size in natural ponds and argued that this effect should be included in mesocosm studies. However, the variation in metamorph size reported in that study for both mesocosm and field studies are much lower than those we observe in natural populations of CTS (coefficient of variation: 0.36). Perhaps predation is less of a homogenizing force on salamander larvae than on anuran larvae, since salamander occupy a higher trophic level. In any case, the strong concordance in effect sizes between our mesocosm and field populations demonstrate that it is possible to obtain realistic dynamics in mesocosms, and that all attempts should be made to mimic natural densities and phenologies of concurrently studied wild populations.

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Supplementary material (available online as Appendix oik.01775 at <www.oikosjournal.org/readers/appendix>). Appendix 1. Methodological details relating to the chronology of the mesocosm experiment, sources of the experimental animals, and statistical analyses. Data A1. Data deposited in the Dryad repository.