Habitat traits and species interactions differentially affect abundance and body size in pond-breeding amphibians

Brittany H. Ousterhout[1]*, Thomas L. Anderson[1], Dana L. Drake[1], William E. Peterman[1,2] and Raymond D. Semlitsch[1]

[1]*Division of Biological Sciences, University of Missouri, 110 Tucker Hall, Columbia, MO 65211, USA; and* [2]*Illinois Natural History Survey, Prairie Research Institute, University of Illinois, 1916 S. Oak Street, Champaign, IL 61820, USA*

Summary

1. In recent studies, habitat traits have emerged as stronger predictors of species occupancy, abundance, richness and diversity than competition. However, in many cases, it remains unclear whether habitat also mediates processes more subtle than competitive exclusion, such as growth, or whether intra- and interspecific interactions among individuals of different species may be better predictors of size.

2. To test whether habitat traits are a stronger predictor of abundance and body size than intra- and interspecific interactions, we measured the density and body size of three species of larval salamanders in 192 ponds across a landscape.

3. We found that the density of larvae was best predicted by models that included habitat features, while models incorporating interactions among individuals of different species best explained the body size of larvae. Additionally, we found a positive relationship between focal species density and congener density, while focal species body size was negatively related to congener density.

4. We posit that salamander larvae may not experience competitive exclusion and thus reduced densities, but instead compensate for increased competition behaviourally (e.g. reduced foraging), resulting in decreased growth. The discrepancy between larval density and body size, a strong predictor of fitness in this system, also highlights a potential shortcoming in using density or abundance as a metric of habitat quality or population health.

Key-words: *Ambystoma*, competition, density, habitat features, marbled salamander, ringed salamander, size, spotted salamander

Introduction

Community composition is fundamentally shaped by the interactions between individuals of different species and habitat features (Chesson 2000). While competition has long been thought to be one of the major processes structuring communities (Gurevitch *et al.* 1992), landscape characteristics and habitat filtering through processes such as phylogenetic niche conservatism (Losos 2008; Wiens *et al.* 2010) have emerged in recent studies as strong predictors of occupancy, abundance, richness and diversity (Mazerolle & Villard 1999). Habitat traits and competition can also interact, resulting in variation in competitive

outcomes in different habitat types (Amarasekare 2003). Thus, investigations of both habitat traits and intra- and interspecific interactions among individuals are necessary to disentangle which processes drive community structure.

Pond communities in particular are structured through combinations of biotic interactions and habitat gradients, both of which generally affect abundance and patterns of species co-occurrence (Wellborn, Skelly & Werner 1996). While early research emphasized the role of competitive and predatory interactions in structuring pond communities (Brooks & Dodson 1965; Stenhouse, Hairston & Cobey 1983; Wilbur 1997), in recent years models of species occurrence and abundance which incorporate local and landscape features have been supported over those with competition, particularly in studies of pond-breeding

amphibians (Skelly, Werner & Cortwright 1999; Van Buskirk 2005; Werner *et al.* 2007a,b). These findings indicate that variation among site characteristics may be more important than competition for mediating the abundance of species.

While explaining patterns of co-occurrence is important for understanding species' distributions, variation in other traits of populations, such as individual body size, may be equally important to determine the dynamics of a population or community. Body size is commonly measured in experiments with larval amphibians because it is a strong predictor of metamorph size (Wilbur & Collins 1973; Scott 1990) and individual fitness (Semlitsch, Scott & Pechmann 1988; Scott 1994), yet most field studies of pond amphibian communities have quantified competition only in terms of density or abundance (but see Urban 2008; Van Buskirk 2009, 2011). This emphasis on occupancy and abundance data, but not fitness consequences of competition, in observational studies occurs in most vertebrate taxa (Martin & Martin 2001; but see Nunn, Tewson & Cowx 2012 and references therein). To our knowledge, no observational study has examined the relative influence of intra- and interspecific interactions among individuals and habitat traits on body size in natural ponds. Data from field studies are necessary to evaluate whether intra- and interspecific interactions are more important in determining the dynamics of pond-breeding amphibian populations, or whether, like abundance, site characteristics account for most of the variation in body size.

To understand whether body size, like abundance, is affected more strongly by site characteristics or the density of competitors, we measured larval salamander density and body size in 192 ponds across a landscape. We tested for effects of intraspecific interactions, interspecific interactions, and habitat on density and body size in three species of *Ambystoma*: ringed salamanders (*Ambystoma annulatum*), marbled salamanders (*A. opacum*) and spotted salamanders (*A. maculatum*). Although multiple studies have found antagonistic interactions between these congeners (Stenhouse, Hairston & Cobey 1983; Urban 2007b; Anderson & Semlitsch 2014), as would be predicted by phylogenetic niche conservatism, it is unknown whether fitness varies with the density of competitors or is mediated by habitat features. Here we report that the size of larvae, a strong predictor of fitness, is affected by different processes than the abundance of larvae, and discuss the impact on the determination of habitat quality and identification of source populations.

Materials and Methods

STUDY SITE

Our study was conducted at Fort Leonard Wood (FLW), Missouri (37·92°N, 92·17°W) (Fig. 1). FLW is an active military training facility encompassing 24,852 ha in the northern Ozark Highland. Eighty per cent of FLW is forested, characterized by oak-hickory forests (*Quercus* spp., *Q. stellata*, *Carya* spp. and *C. texana* canopy; *Rhus aromatic* and *Cornus florida* understorey) or short-leaf pine plantations (*Pinus echinata*). There are over 500 ponds at FLW which are either constructed or unintentional water bodies (e.g. tire ruts). These ponds vary in size from 1 to 42 549 m^2. While most of the ponds are small (<0·04 ha), fishless, constructed wildlife ponds, the area also includes several large ponds and small lakes (>1 ha) stocked with game fish. We sampled 192 representative ponds in a 7140 ha area in west central portion of FLW (hereafter focal area). The focal area encompasses the range of pond sizes, including both small ponds and stocked lakes, and dominant land cover types (i.e. deciduous and coniferous forests, open fields and improved areas).

STUDY SPECIES

In this study, we focused on the three sympatric pond-breeding salamanders in the family Ambystomatidae which occur at FLW: *A. annulatum*, *A. opacum* and *A. maculatum*. *Ambystoma annulatum* are endemic to the Ozark Plateau and Ouachita Mountains of Missouri, Arkansas and Oklahoma. *Ambystoma opacum* and *A. maculatum* can be found throughout much of the eastern United States (Petranka 1998). These three species vary in the timing of their breeding and fecundity. Both *A. annulatum* and *A. opacum* breed in the fall, while *A. maculatum* breeds in early spring (Hocking *et al.* 2008). Unlike *A. annulatum* and *A. maculatum* that deposit eggs on submerged substrates (Semlitsch *et al.* 2014), *A. opacum* females deposit eggs in nests in dried or partially dried pond beds and eggs hatch when they are inundated. The timing of fall rains and nest inundation thus mediates size differences in the larvae. On average, *A. annulatum* and *A. maculatum* are more fecund than *A. opacum* (144–390 eggs/female versus 95–150 eggs/female; Petranka 1998 and references therein). The adults of all three species are associated with hardwood and mixed hardwood–pine forests (Petranka 1998).

FIELD SAMPLING METHODS

To estimate the density of larval salamanders, we conducted repeated surveys of ponds within the focal area as described in Peterman *et al.* (2014). We sampled all focal area ponds using dip net sweeps and funnel traps standardized to pond surface area (Shulse *et al.* 2010) during the second third of the larval period for each species (18 February–12 March 2012 for *A. annulatum* and *A. opacum*; 11 May–28 May 2012 for *A. maculatum*). At each site, we conducted three independent dip net surveys, and typically three trapping sessions ($N = 168$ ponds), although some sites were precluded due to their location (e.g. roadbed ditch) or water depth ($N = 24$ ponds). A previous analysis with these data found detection did not vary by sampling method (Peterman *et al.* 2014). We therefore included all sites surveyed in analyses regardless of whether funnel traps were set. All captured salamander larvae were identified (Trauth, Robinson & Plummer 2004), counted and returned to the pond. We assumed the first 20 larvae of each species captured at a pond constituted a random sample of larvae occupying the pond, and these individuals were photographed and then measured for total length (TL), snout-vent length (SVL), girth and head width using ImageJ 1·45s (Rasband 2013). We used TL for analyses instead of SVL, because it had far fewer missing values based on image analysis (TL: 3% missing, SVL: 50% missing). Although tail truncation
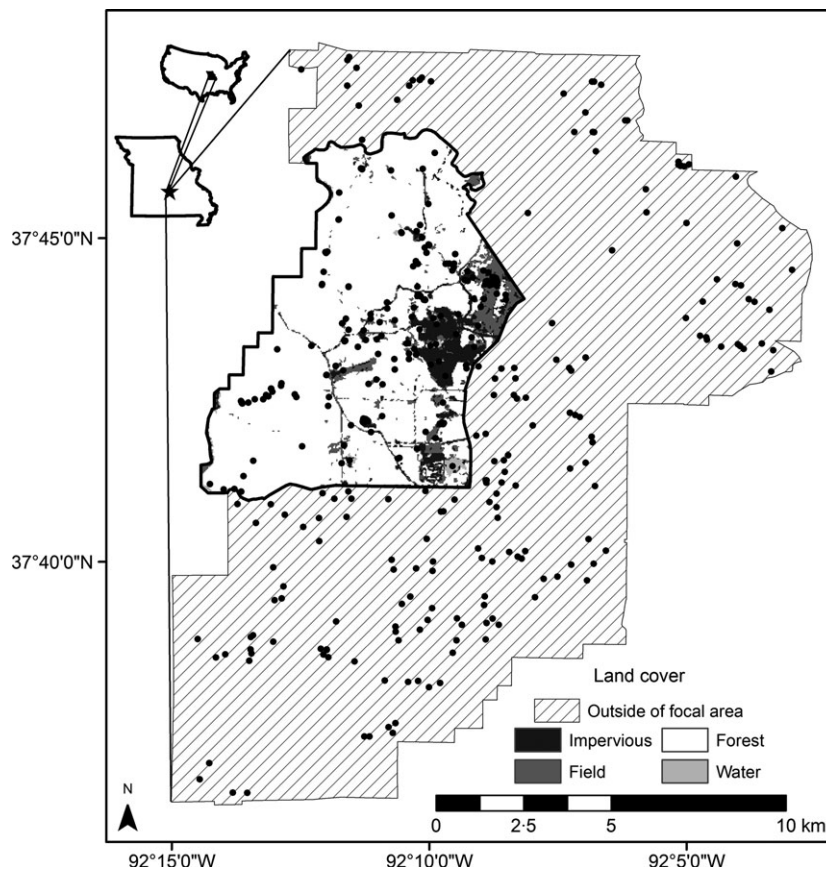
Fig. 1. Map of Fort Leonard Wood, Missouri, with land cover in the focal area. All known ponds (black dot) within the focal area were sampled in this study.

from predation can lead to head width being a better proxy for SVL, in our data, TL had a greater correlation with SVL (TL: $r^2 = 0.92$; head width: $r^2 = 0.86$). We also measured pond characteristics that may have affected larval density and interactions (Peterman *et al.* 2014). We measured pond area assuming all ponds were ellipses. We categorized pond hydroperiod based on expert knowledge and data from temperature loggers (Anderson *et al.* in review) into the following categories: ephemeral: dries multiple times a year; seasonal: dries during the summer; and permanent: may dry during extreme droughts. We derived the percentage of forest within 300 m of a pond using 2005 land use land cover layer (http://misdis.missouri.edu) using ARC GIS 9.3 (ESRI, Redlands, CA, USA). Finally, we identified ponds occupied by fish on the basis of whether fish were captured while we sampled for salamander larvae.

ANALYSIS

We calculated the density of larvae as total number of larvae of a species captured at a pond divided by the total sampling effort (sum of dip net sweeps and traps surveyed) during that sample period. A different analysis with these data found no difference in detection between dip net sweeps and funnel traps (Peterman *et al.* 2014). We estimated that each dip net sweep sampled an area of 1 m^2 and assumed that each funnel trap sampled a comparable area. We elected to use the mean number of larvae captured per area sampled rather than abundance estimates, because detection of salamander larvae did not differ in relation to covariates (Peterman *et al.* 2014). Additionally, abundance estimates made using N-mixture models were imprecise; larval counts between days and sites were highly variable, necessitating

modelling approaches that improved model fit at the cost of precision of abundance estimates (Peterman *et al.* 2014). As such, we used mean density estimates for all ponds in the focal area for our analyses. We constructed generalized linear models using Program R (R Core Team 2012) to test for the effects on two response variables: body size (Gaussian error structure) and density (negative binomial error structure) of a focal salamander species. If there was an effect of competition or predation, a species may experience elevated mortality and thus be found in lower densities in ponds with more competitors or intraguild predators. Alternatively, larvae could respond to increased competitor or predator density with compensatory behaviour (e.g. reduce foraging), resulting in smaller individuals.

We compared two sets of models to test the relative influence of habitat characteristics and competition on body size and density of larval salamanders. In the larval interaction models, we included the presence or density of salamander larvae which could have co-occurred when ponds were surveyed as independent variables. If the presence of a species strongly affected density in a nonlinear manner, a binary of presence/absence would best capture the relationship. Alternatively, if the presence of a species affected density in a continuous manner, the density of the independent variable would provide the best fit. The density of larvae of *A. maculatum* was not included in the *A. opacum* or *A. annulatum* models, as *A. maculatum* had not yet oviposited at the time *A. opacum* and *A. annulatum* surveys were conducted. When using body size as a response variable, we also included the density of the focal species as an independent variable to account for intraspecific competition. All continuous predictor variables were scaled and centred to facilitate comparisons of effect sizes. We ran the full model set for both response variables for each species (Tables 1 and 2).

The habitat models tested for the effects of four factors commonly found to affect larval *Ambystoma* salamander density: presence of fish, hydroperiod, percentage of habitat within a 300-m buffer that was forested and pond area (Tables 1 and 2; R.D. Semlitsch, W.E. Peterman, T.L. Anderson, D.L. Drake & B.H. Ousterhout, in review; Peterman *et al.* 2014). We treated hydroperiod as an ordinal variable in all analyses. Habitat models of *A. maculatum* did not include fish because we did not capture enough larvae in occupied ponds to model their relationship. We also included a random intercept model and expected it to be best supported if habitat features and competition did not influence the density or body size of larvae. For each species, we compared the model sets (larval interactions and habitat) for each response variable with an information theoretic approach using small sample Akaike Information Criteria (AIC$_c$). We considered all models within the 95% confidence set as assessed by relative likelihood to have substantial support (Burnham & Anderson 2002).

We tested for spatial autocorrelation using a semi-variogram (package 'ncf', Bjornstad 2013) and found ponds within 500 m were autocorrelated. To account for spatial autocorrelation within our data, parameter estimates for each top model were derived from a nonparametric bootstrap analysis. We randomly generated 30,000 data sets, iteratively selecting ponds such that no two sites were within 500 m. While this approach removed autocorrelation from residuals, each bootstrap included approximately 50 sites, a 73% reduction in our data set. This highly conservative approach constrained our ability to draw inferences from our overdispersed data. We present the conservative mean parameter estimates from this analysis; however, we focus on the results from the models with the full data which do not account for autocorrelation.

Results

AMBYSTOMA DETECTED

We sampled 192 ponds and detected larvae of *A. annulatum* in 41% of ponds (N = 78), *A. opacum* in 25% of ponds (N = 48) and *A. maculatum* in 28% of ponds (N = 54). We identified 46 ponds with a single *Ambystoma* species (24 *A. annulatum* ponds, 7 *A. opacum* ponds and 15 *A. maculatum* ponds) and detected no *Ambystoma* in 88 ponds. Larvae of *A. annulatum* and *A. opacum* co-occurred in 18% of ponds (N = 35). Larvae of *A. maculatum* co-occurred with *A. annulatum* in 18% of ponds (N = 35), *A. opacum* in 11% of ponds (N = 22) and with both of these fall-breeding species in 8% of ponds (N = 16). We captured 1317 *A. annulatum*, 436 *A. opacum* and 838 *A. maculatum* larvae. Across all ponds in which larvae were detected, the density of larvae of *A. annulatum* was 1.7 ± 1.81 larvae per m^2 (mean $\pm$ 1 S.D.), the density of *A. opacum* was 0.9 ± 1.25 larvae per m^2, and the density of *A. maculatum* was 1.6 ± 2.62 larvae per m^2.

HABITAT TRAITS

Of the 104 ponds where larvae of at least one species were detected, 43% were ephemeral (N = 45), 14% were seasonal (N = 15), and 41% were permanent (N = 43). The habitat in the focal area was on average 71% $\pm$ 31 forested within 300 m of ponds in which we detected *Ambystoma* larvae. We sampled for salamanders in 26 ponds that contained fish and detected larval salamanders in four of them (*A. maculatum* and *A. annulatum*: N = 1; *A. opacum*: N = 3). Pond area ranged from less than 1 m^2 to 20,341 m^2 (mean $\pm$ 1 S.D.: 794.5 $\pm$ 2472 m^2).

DENSITY OF LARVAE

There was a positive relationship between the density of larvae of fall-breeding species and between the larvae of *A. annulatum* and *A. maculatum* (Spearman's rank

Table 1. Summary of AIC$_c$ model selection results for the density of larvae. Best supported models are in bold face. K is the number of parameters estimated in the model including the intercept. Akaike weight (w_i) can be interpreted as the probability that a model is the best approximating model in the set

Species	Model	AIC$_c$	K	ΔAIC$_c$	Weight
A. maculatum	**Hydroperiod + Percentage forest + Pond area**	**471·1**	**4**	**0·0**	**1·000**
	A. annulatum density+ *A. opacum* presence	505·2	3	34·1	<0·001
	A. opacum presence	508·6	2	37·5	<0·001
	A. annulatum presence	510·1	2	39·0	<0·001
	A. opacum density + *A. anulatum* presence	510·1	3	39·0	<0·001
	A. annulatum density	511·0	2	39·9	<0·001
	A. opacum density + *A. annulatum* density	511·5	3	40·4	<0·001
	Intercept	512·8	1	41·7	<0·001
	A. opacum density	514·6	2	43·5	<0·001
A. annulatum	**Fish + Hydroperiod + Percentage forest + Pond area**	**808·4**	**5**	**0·0**	**1·000**
	Intercept	835·3	1	26·9	<0·001
	A. opacum presence	836·3	2	27·9	<0·001
	A. opacum density	837·1	2	28·7	<0·001
A. opacum	**Fish + Hydroperiod + Percentage forest + Pond area**	**423·9**	**5**	**0·0**	**1·000**
	A. annulatum presence	443·7	2	19·8	<0·001
	Intercept	444·3	1	20·4	<0·001
	A. annulatum density	446·0	2	22·1	<0·001

Table 2. Parameter estimates, standard deviation (SD) and 95% confidence intervals for generalized linear models (GLM) and bootstrap analysis of ambystomatid salamanders at Fort Leonard Wood, MO. Bolded values did not overlap zero and were significant for that parameter–species combination. Bias is the difference between the mean parameter estimates of the two analyses. Relative bias is the ratio of the mean parameter estimate of the GLM analysis to the mean parameter estimate of the bootstrap analysis. A relative bias of 1 would indicate both analyses had the same mean parameter estimate

		GLM analysis				Bootstrap analysis				Bias	Relative bias
		Mean	SD	2·5%	97·5%	Mean	SD	2·5%	97·5%		
Density models											
A. annulatum	Intercept	**1·73**	**3·55**	**0·68**	**2·78**	1·36	1·33	−1·65	4·02	−0·37	0·79
	Hydroperiod	0·18	1·41	−0·23	0·60	0·24	0·45	−0·68	1·25	0·06	1·31
	Fish	**−5·18**	**8·19**	**−7·60**	**−2·75**	**−13·78**	**16·96**	**−56·96**	**−3·74**	−8·61	2·66
	Percentage forest	0·14	1·20	−0·21	0·49	**0·70**	**0·40**	**0·10**	**1·76**	0·56	4·96
	Pond area	−0·57	5·85	−2·30	1·16	0·08	1·38	−1·54	3·96	0·65	−0·14
A. opacum	Intercept	**1·59**	**4·12**	**0·37**	**2·80**	2·15	1·14	−0·47	4·09	0·57	1·36
	Hydroperiod	**−0·74**	**1·98**	**−1·32**	**−0·16**	**−1·11**	**0·49**	**−2·17**	**−0·18**	−0·37	1·50
	Fish	−1·32	6·85	−3·34	0·70	−1·01	3·69	−4·09	1·95	0·31	0·77
	Percentage forest	**1·83**	**2·28**	**1·16**	**2·51**	**1·22**	**0·54**	**0·29**	**2·38**	−0·62	0·66
	Pond area	0·22	3·14	−0·71	1·15	−0·33	1·15	−1·81	1·80	−0·55	−1·47
A. maculatum	Intercept	**−2·98**	**5·93**	**−4·73**	**−1·22**	−2·75	2·20	−6·57	0·21	0·23	0·92
	Hydroperiod	**1·06**	**1·93**	**0·50**	**1·63**	0·67	0·74	−0·39	1·89	−0·39	0·63
	Percentage forest	**1·92**	**2·34**	**1·23**	**2·61**	**2·31**	**1·00**	**1·16**	**5·12**	0·39	1·20
	Pond area	**−4·49**	**11·52**	**−7·89**	**−1·09**	**−3·58**	**1·40**	**−5·99**	**−1·57**	0·91	0·80
Body size models											
A. annulatum	Intercept	**43·93**	**10·01**	**40·97**	**46·89**	**42·50**	**2·93**	**37·34**	**48·76**	−1·43	0·97
	A. opacum density	**−3·09**	**8·39**	**−5·57**	**−0·61**	−3·75	3·33	−10·44	0·72	−0·67	1·22
	A. annulatum density	−0·14	7·34	−2·31	2·03	−0·07	1·68	−3·69	3·13	0·07	0·50
A. maculatum	Intercept	**31·73**	**5·88**	**29·99**	**33·47**	**31·37**	**3·22**	**29·26**	**33·67**	−0·36	0·10
	A. maculatum density	−2·43	10·35	−5·49	0·63	−1·13	1·19	−3·40	1·34	1·30	−0·49
	A. opacum density	**−1·68**	**4·18**	**−2·92**	**−0·45**	0·27	10·29	−3·47	10·81	1·96	−6·13

correlation: *A. annulatum* and *A. opacum*: ρ = 0·24, P = 0·002; *A. maculatum* and *A. annulatum*: ρ = 0·31, P < 0·001; *A. maculatum* and *A. opacum*: ρ = 0·12, P = 0·117). However, for all species, the density of larvae was best explained by the habitat models (Table 1). We found *A. annulatum* in greater densities in permanent ponds without fish (Fig. 2). The effect of fish was ten times that of any other habitat covariate (Table 2). In the bootstrap analysis, the confidence intervals of percentage forest also did not overlap zero; however, its effect size was 5% of the effect of fish (Fig. 2, Table 2). *Ambystoma opacum* densities were greatest in fishless ponds with more surrounding forest and an ephemeral hydroperiod (Fig. 2). The amount of surrounding forest had twice the effect size of hydroperiod (Table 2). The same parameters were significant in the bootstrap analysis, and both tests had similar effect sizes (Table 2). We captured only one larva of *A. maculatum* in a pond occupied by fish. The density of larvae of *A. maculatum* was greater in ponds with a longer hydroperiod (Fig. 2), smaller area (Fig. 2) and a greater percentage of forest within 300 m (Fig. 2). The effect size of pond area was twice that of percentage surrounding forest and over four times greater than the effect size of hydroperiod (Table 2). In the bootstrap analysis, the confidence intervals of percentage forest and pond area did not overlap zero and the difference in the effect size between percentage surrounding forest and pond area was reduced (Table 2).

BODY SIZE

Body size of *A. annulatum* and *A. maculatum* was best explained by larval interaction models (Table 3). The best supported models of *A. annulatum* body size included *A. opacum* density; larger *A. annulatum* were found in ponds with fewer *A. opacum* (Table 3; Fig. 3). Although there was some support for a model including intraspecific density, the effect size of *A. annulatum* density was 4% of the effect size of *A. opacum* density (Table 2; Fig. 3). In contrast to *A. annulatum*, *A. maculatum* body size was best explained by models including intraspecific and *A. opacum* density (Table 3). *Ambystoma maculatum* were larger in ponds with fewer *A. opacum* (Fig. 3). The most supported models included intraspecific density, and the effect size of intraspecific density was 44% greater than the effect size of *A. opacum* density in the top model (Fig. 3; Table 2). However, in this model, the confidence intervals of the density of *A. maculatum* overlapped zero (Table 2). While the density of larvae of *A. annulatum* was in a highly supported model, the effect size of *A. annulatum* was 60% that of intraspecific density and 30% of *A.*
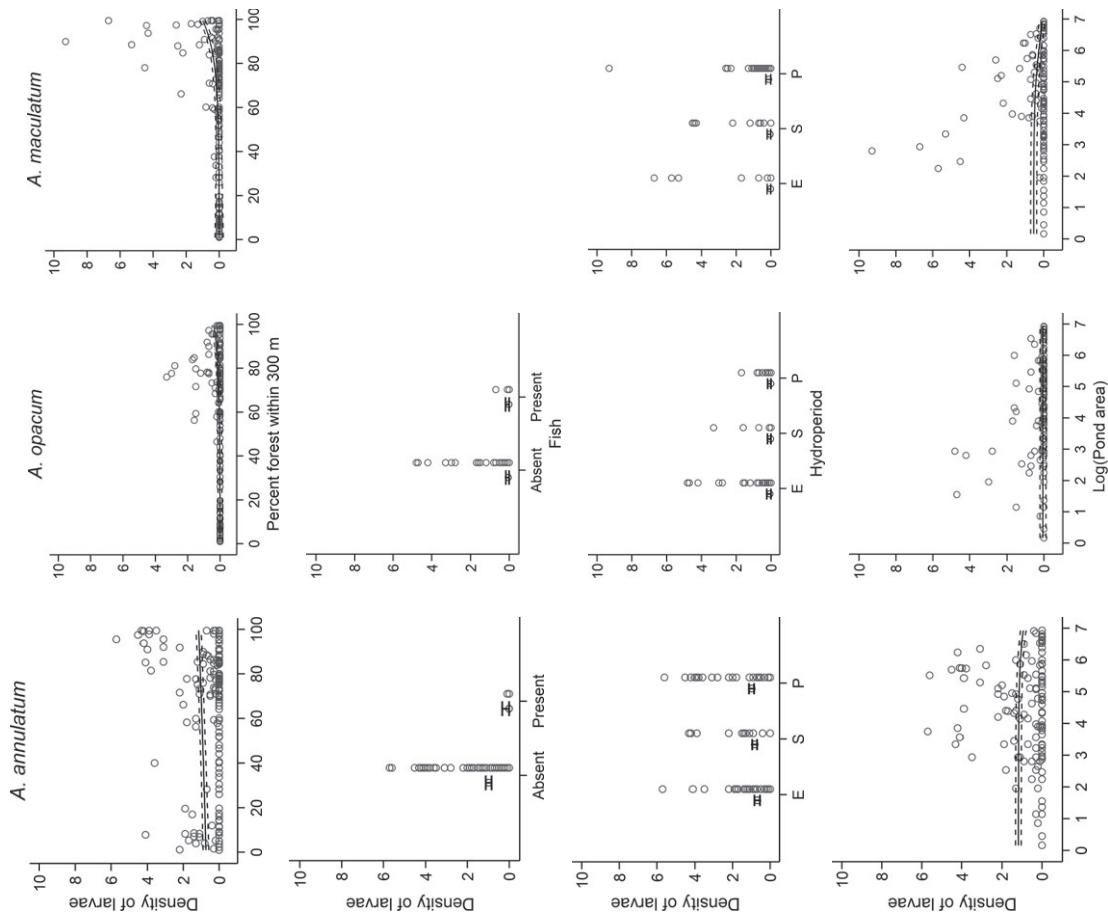
Fig. 2. Estimated effects of percentage forest within 300 m, fish presence/absence, hydroperiod and pond area on the density of larvae of *A. annulatum*, *A. opacum* and *A. maculatum* (larvae/m²), respectively. Open grey circles represent observed values, open black circles or dotted lines are predicted values, and error bars or dotted lines indicate ± 1 SE. Percentage forest within 300 m was evaluated under fish absence, permanent hydroperiod and mean pond area. Fish presence/absence was evaluated under mean percentage forest, permanent hydroperiod and mean pond area. Hydroperiod was evaluated under mean percentage forest, fish absence and mean pond area (E = ephemeral, S= seasonal and P = permanent). Pond area was evaluated with mean percentage forest, permanent hydroperiod and fish absence.

Table 3. Summary of AIC$_c$ model selection results for the body size of larvae. Models with ΔAICc < 4 are presented. K is the number of parameters estimated in the model including the intercept. Akaike weight (w$_i$) can be interpreted as the probability that a model is the best approximating model in the set

Species	Model	AICc	*K*	ΔAICc	Weight
A. maculatum	*A. maculatum* density + *A. opacum* density	266·1	3	0·0	0·231
	A. maculatum density + *A. opacum* density + *A. annulatum* density	266·3	4	0·2	0·210
	A. maculatum density	266·5	2	0·4	0·187
	A. maculatum density + *A. opacum* presence	267·5	3	1·4	0·116
	A. maculatum density + *A. annulatum* density	267·6	2	1·5	0·110
	A. maculatum density + *A. annulatum* presence	268·4	3	2·3	0·073
A. annulatum	*A. opacum* density	597·3	2	0·0	0·622
	A. annulatum density + *A. opacum* density	599·5	3	2·2	0·206
	Intercept	601·2	1	3·9	0·088
A. opacum	Intercept	279·3	1	0·0	0·214
	A. opacum density	279·6	2	0·3	0·183
	Fish + Hydroperiod + Percentage Forest + Pond Area	279·8	5	0·5	0·167
	A. annulatum density	279·9	2	0·6	0·162
	A. annuhtum presence	280·8	2	1·5	0·100
	A. annulatum density + *A. opacum* density	280·9	3	1·6	0·098
	A. opacum density + *A. annulatum* presence	281·4	3	2·1	0·075

opacum density (Fig. 3). No model of the body size of larvae of *A. opacum* received more support than the null model (Table 3).

Discussion

The habitat features model best explained the larval density data for all three species, indicating that density of *Ambystoma* larvae is better explained by habitat features than intraguild interactions. While this result could have been driven by the different extent and precision at which our habitat predictor variables, biotic interaction predictors and our response variables were measured (Clark *et al.* 2011), we think this is unlikely. If scale was biasing our results, we would expect habitat models to consistently outperform interaction models. However, we found clear and unambiguous support for larval interactions best predicting larval size, suggesting the scale and precision with which we measured different variables did not unduly influence our results. Larvae were more abundant in the absence of fish and in ponds surrounded by more forest habitat. Larvae of *A. maculatum* were also more abundant in ponds with longer hydroperiods. Although such ponds have a greater number of invertebrate predators and are more able to sustain fish populations (Shulse, Semlitsch & Trauth 2013), the longer hydroperiods allow more individuals to metamorphose prior to pond drying. This may have been particularly important for *A. maculatum* during a period of regional drought in the summer of 2012 (Anderson *et al.* in press).

Although we found larvae of *A. maculatum* in higher densities in ponds with more *A. annulatum* and in the presence of *A. opacum*, the size of *A. maculatum* was negatively related to the density of larvae of *A. opacum* and conspecifics. We speculate that competition among larval salamanders may be more likely to result in behavioural responses rather than competitive exclusion. While several studies have observed that salamander larvae are more abundant in ponds with high amphibian densities (Pechmann *et al.* 1991; Semlitsch *et al.* 1996; Werner *et al.* 2007b), rarely has the relationship between competitor density and size been tested in natural ponds (Van Buskirk & Smith 1991). Our data indicate that although larvae that hatch in ponds earlier are not negatively impacting densities of later hatching species, and thus survival of larvae, increased density of larvae of fall-breeding species is associated with smaller *A. maculatum* larvae with potential implications for decreased fitness of *A. maculatum* (Semlitsch, Scott & Pechmann 1988). This association could be explained by breeding phenology. Eggs of fall-breeding species hatch in ponds five to six months before the spring breeding *A. maculatum* (Hocking *et al.* 2008; Semlitsch *et al.* 2014), resulting in a size advantage for overwintering larvae. Although our density data show that interactions between larvae of *A. maculatum* and fall-breeding species are not strong enough to substantially reduce the density of *A. maculatum*, competition could be affecting foraging behaviour and therefore growth and body size. Previous experimental studies have reported that larvae of *A. annulatum* and *A. opacum* negatively impact the growth and survival of larvae of *A. maculatum*, and hypothesized that the shift from predator to competitor is the result of gape limitations (Stenhouse, Hairston & Cobey 1983; Anderson & Semlitsch 2014). These findings support the hypothesis that fall-breeding salamanders are negatively affecting *A. maculatum*. Because we did not observe a decrease in *A. maculatum* density in ponds which had larvae of fall-breeding species, this also indicates that the effects of competition are stronger than predation for this species in natural ponds.

Larvae of *A. annulatum* were smaller in ponds with more *A. opacum*. However, this relationship was not
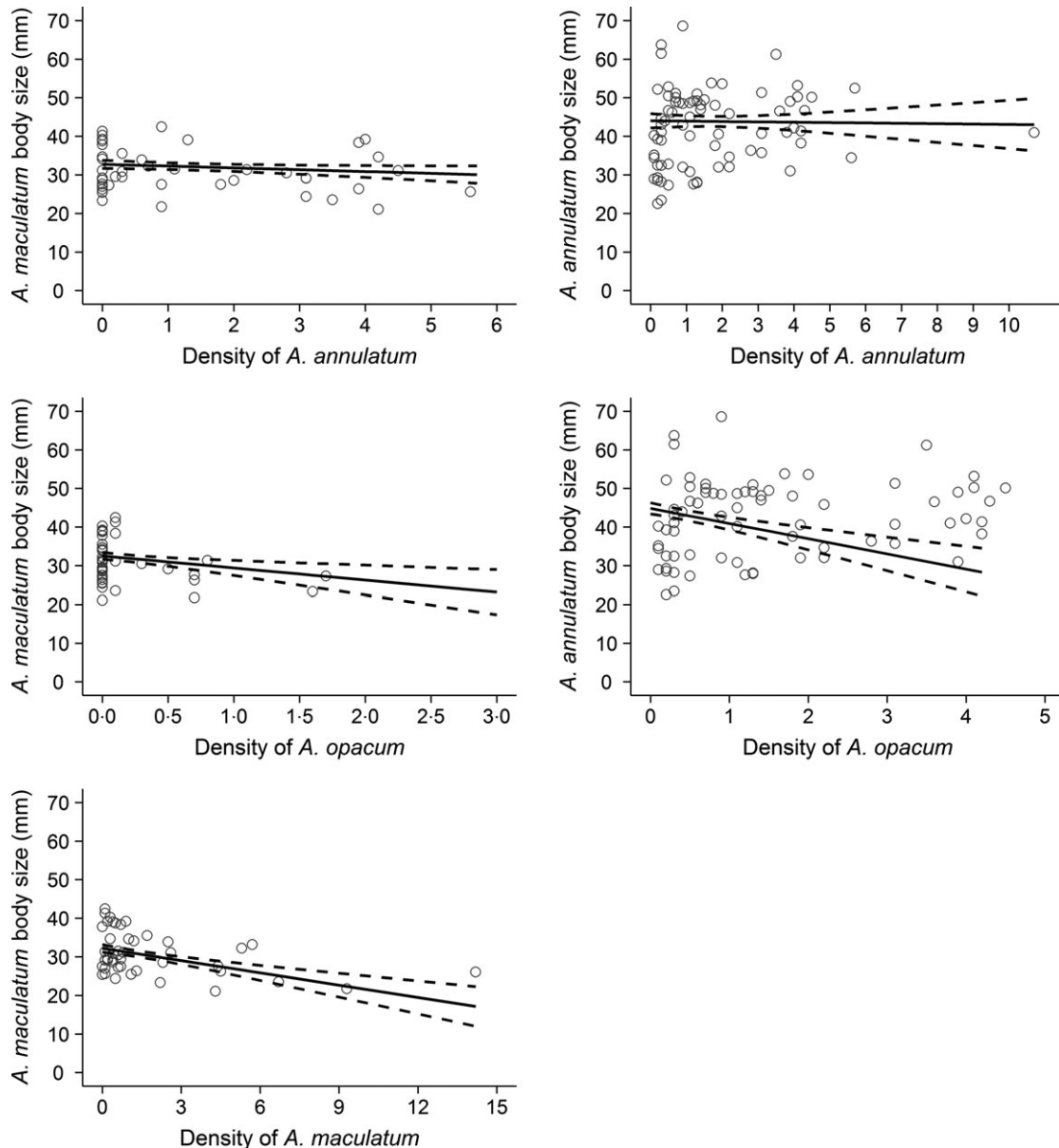
Fig. 3. Estimated effects of intra- and interspecific density on the body size (mm) of larvae of *A. maculatum* and *A. annulatum*. Open grey circles represent observed values, solid lines are predicted values, and dotted lines are ± 1 SE. *Ambystoma Maculatum* body size was predicted from the *A. maculatum* density + *A. opacum* density + *A. annulatum* density model. Each model was evaluated under mean densities of the other two species. *Ambystoma annulatum* body sizes are predicted (± 1 SE) from the *A. annulatum* density + *A. opacum* density model. *Ambystoma annulatum* density was evaluated under the mean density of *A. opacum,* and *A. opacum* presence was evaluated under the mean density of *A. annulatum.*

symmetrical: there was no effect of *A. annulatum* density on *A. opacum* body size. These results indicate that *A. opacum* may be a stronger larval competitor than *A. annulatum*. Previous studies with other species of *Ambystoma* have found differences in resource exploitation and aggression between species (Walls 1996; Brodman 1999). Mott & Maret (2011) observed high levels of aggression in larvae of *A. opacum* which rarely resulted in predation; however, experimental work is required to determine whether *A. opacum* is a superior competitor to *A. annulatum* or whether there are other factors not accounted for in our study.

POSITIVE ABUNDANCE ASSOCIATIONS INFLUENCED BY HABITAT TRAITS

We found models with habitat traits to be the best predictors of amphibian densities. This supports previous studies that have found amphibian abundance to be strongly influenced by habitat characteristics (Van Buskirk 2005; Werner *et al.* 2007a), and indicates that the benefits of habitat traits in a good pond are stronger than the negative effects of competition. This hypothesis has also been supported by previous amphibian observational studies that have found a positive correlation between species

density and congener density. Pechmann *et al.* (1991) monitored the number of emigrating juveniles of four amphibian species, including three *Ambystoma* species, over twelve years at an ephemeral Carolina bay in South Carolina. The number of recruits per female among species was significantly positively correlated for four of six pairwise comparisons. Werner *et al.* (2007a) found a similar pattern in the richness of amphibians over seven years in a study of 37 ponds in Michigan. In all cases, hydroperiod was hypothesized to determine species abundance (Pechmann *et al.* 1991; Semlitsch *et al.* 1996; Werner *et al.* 2007a). Similarly, model selection in our study strongly supported the effects of habitat features on larval *Ambystoma* density. While we found hydroperiod to only be a predictor of *A. maculatum* density, this may reflect the single year of data we report as opposed to the longitudinal studies reported above.

The positive association we found between competitors has been reported more frequently in field studies in recent years, with the abundance of a focal species increasing even at high congener densities (Bertness & Callaway 1994; Stachowicz 2001; Bruno, Stachowicz & Bertness 2003). This pattern is particularly common in harsh environments, with benefits increasing as stress increases (Bertness & Callaway 1994), and has been found in plants and sessile animals (Bertness & Leonard 1997; Forsman, Seppänen & Mönkkönen 2002), and to a lesser extent with vagile animal taxa (Pechmann *et al.* 1991; Forsman, Seppänen & Mönkkönen 2002; Hay *et al.* 2004; Werner *et al.* 2007a). Despite the importance of positive associations in structuring aquatic and terrestrial communities, such as facilitation and mutualisms, their mechanism remains little understood. This is particularly striking when comparing theory of positive associations to that of negative interactions among individuals, including competition and predation, which have received substantially more attention over the last 50 years (Bruno, Stachowicz & Bertness 2003). Hypotheses to explain positive associations between competitors require testing and include release from a shared predator, honest signals of good habitat (Forsman, Seppänen & Mönkkönen 2002) and complimentary hunting strategies (Hay *et al.* 2004).

COMPENSATORY GROWTH REDUCTION

Although we found no support for density-dependent reduction in the number of larvae, we did find smaller larvae in ponds with a greater density of species that may have occupied the pond longer. These results may indicate that individuals are compensating for competition or intraguild predation with altered behaviour to reduce risk, resulting in lower growth (Verdolin 2006 and references therein; Urban 2007). While growth reduction may allow larvae to avoid immediate mortality, small individuals have lower fitness than larger larvae. Small larvae are more susceptible to gape-limited predators and may not achieve a minimum size to metamorphose before a pond

dries (Wilbur & Collins 1973). If the small larvae survive and metamorphose, they will often metamorphose later, be smaller as juveniles (Petranka 1989; Scott 1990), and have an increased risk of terrestrial desiccation, depressed immune function, lower lipid levels and survival, later age of first reproduction and lower fecundity (Semlitsch, Scott & Pechmann 1988; Scott 1994; Davis & Maerz 2009; Peterman, Locke & Semlitsch 2013).

Our data contradict Urban (2007a,b), who suggested that when predators are gape-limited, like larval salamanders, prey may increase foraging to rapidly grow into a body size refuge despite temporarily elevated mortality risks. This disparity may reflect the importance of habitat structure and refuge in the behavioural response of prey to gape-limited predators (Verdolin 2006; Urban 2007a, 2008). Alternately, these differences may reflect the ontological periods sampled. While our study focused on the middle to late stage of the larval period, Urban (2007a) sampled early in the larval period in the five weeks following hatching. *Ambystoma maculatum* could increase foraging immediately following hatching, allowing them to escape direct predation by narrowing the window in which they are susceptible to gape-limited predators. However, once a body size is reached that protects them from direct predation, individuals in ponds with larger *Ambystoma* larvae may still be subject to non-lethal aggression (Semlitsch & Reichling 1989). Differences in foraging ability could also lead to body size asymmetries if individuals of one species are more efficient than individuals of other species (Walls 1996).

Due species specific body size responses to congener density and the asymmetrical nature of these responses, we speculate that the mechanism of reduced growth may be decreased foraging behaviour in the presence of heterospecific *Ambystoma* larvae. Foraging/predation trade-offs have been well documented in aquatic and terrestrial systems (Verdolin 2006), with many experimental and field studies demonstrating predator-induced changes in morphology, growth rates and fecundity of prey as costs of behavioural avoidance (Semlitsch 1987; Skelly 1992; Walls 1995; Werner & Anholt 1996; Davenport & Chalcraft 2012, 2014; Ingley *et al.* 2014). Although reduced foraging behaviour by prey is generally predicted under a wide range of situations, in some conditions increased foraging may also be expected (Rowe & Ludwig 1991; Abrams & Rowe 1996).

CONCLUSIONS

Here we present evidence of a compensatory response to increased density – larvae compensated for increased competition with decreased growth rather than decreased density (i.e. increased mortality). This discrepancy between larval density and fitness highlights a potential shortcoming in using density or abundance as a metric of habitat quality or population health. While a site may appear to be a source population numerically, variation in body size

and the negative effects of reduced body size on fitness may result in individuals having lower cumulative fitness than individuals at a site with fewer but larger metamorphosing juveniles. It is unknown whether productivity of a pond is maximized by the production of many individuals with low survival, or few individuals with high survival. This predicament is not limited to amphibian ecology – observational data of the effects of competition on fitness-related traits are lacking for many vertebrate taxa (Martin & Martin 2001). Therefore, field studies, particularly long-term investigations of juvenile fitness, are required to determine how to best identify source populations.

Acknowledgements

Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.s25d3 (Ousterhout *et al.* 2015).

References

Abrams, P.A. & Rowe, L. (1996) The effects of predation on the age and size of maturity of prey. *Evolution*, **50**, 1052–1061.

Amarasekare, P. (2003) Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters*, **6**, 1109–1122.

Anderson, T.L. & Semlitsch, R.D. (2014) High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations. *Population Ecology*, **56**, 265–273.

Anderson, T.L., Ousterhout, B.H., Peterman, W.E., Drake, D.L. & Semlitsch, R.D. (in press) Life history differences influence the impacts of drought on aquatic survival and occupancy of two pond-breeding salamanders. *Ecological Applications*, doi: 10.1890/14-2096.1.

Bertness, M.D. & Callaway, R. (1994) Positive interactions in communities. *Trends in Ecology & Evolution*, **9**, 191–193.

Bertness, M.D. & Leonard, G.H. (1997) The role of positive interactions in communities: lessons from intertidal habitats. *Ecology*, **78**, 1976–1989.

Bjornstad, O.N. (2013) ncf: Spatial Nonparametric Covariance Functions.

Brodman, R. (1999) Food and space dependent effects during the interactions of two species of larval salamanders. *Journal of Freshwater Ecology*, **14**, 431–437.

Brooks, J.L. & Dodson, S.I. (1965) Predation, body size, and composition of plankton. *Science*, **150**, 28–35.

Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, **18**, 119–125.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York, New York, USA.

Chesson, P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, **31**, 343–366.

Clark, J.S., Bell, D.M., Hersh, M.H., Kwit, M.C., Moran, E., Salk, C. *et al.* (2011) Individual-scale variation, species-scale differences: inference needed to understand diversity. *Ecology Letters*, **14**, 1273–1287.

Davenport, J.M. & Chalcraft, D.R. (2012) Evaluating the effects of trophic complexity on a keystone predator by disassembling a partial intraguild predation food web. *Journal of Animal Ecology*, **81**, 242–250.

Davenport, J.M. & Chalcraft, D.R. (2014) Increasing conspecific density weakens the ability of intermediate predators to develop induced morphological defences to top predators. *Freshwater Biology*, **59**, 87–99.

Davis, A.K. & Maerz, J.C. (2009) Effects of larval density on hematological stress indices in salamanders. *Journal of Experimental Zoology*, **311A**, 697–704.

Forsman, J.T., Seppänen, J.-T. & Mönkkönen, M. (2002) Positive fitness consequences of interspecific interaction with a potential competitor. *Proceedings. Biological Sciences/The Royal Society*, **269**, 1619–1623.

Gurevitch, J., Morrow, L.L., Wallace, A. & Walsh, J.S. (1992) A meta-analysis of competition in field experiments. *The American Naturalist*, **140**, 539–572.

Hay, M.E., Parker, J.D., Burkepile, D.E., Caudill, C.C., Wilson, A.E., Hallinan, Z.P. *et al.* (2004) Mutualisms and aquatic community structure: the enemy of my enemy is my friend. *Annual Review of Ecology and Systematics*, **35**, 175–197.

Hocking, D.J., Rittenhouse, T.A.G., Rothermel, B.B., Johnson, J.R., Conner, C.A., Harper, E.B. *et al.* (2008) Breeding and recruitment phenology of amphibians in Missouri oak-hickory forests. *American Midland Naturalist*, **160**, 41–60.

Ingley, S.J., Billman, E.J., Belk, M.C. & Johnson, J.B. (2014) Morphological divergence driven by predation environment within and between species of *Brachyrhaphis* fishes. *PLoS ONE*, **9**, e90274.

Losos, J.B. (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*, **11**, 995–1003.

Martin, P.R. & Martin, T.E. (2001) Ecological and fitness consequences of species coexistence: a removal experiment with wood warblers. *Ecology*, **82**, 189–206.

Mazerolle, M.J. & Villard, M. (1999) Patch characteristics and landscape context as predictors of species presence and abundance: a review. *Ecoscience*, **6**, 117–124.

Mott, C.L. & Maret, T.J. (2011) Species-specific patterns of agonistic behavior among larvae of three syntopic species of Ambystomatid salamanders. *Copeia*, **2011**, 9–17.

Nunn, A.D., Tewson, L.H. & Cowx, I.G. (2012) The foraging ecology of larval and juvenile fishes. *Reviews in Fish Biology and Fisheries*, **22**, 377–408.

Ousterhout, B.H., Anderson, T.L., Drake, D.L., Peterman, W.E. & Semlitsch, R.D. (2015) Data from: habitat traits and species interactions differentially affect abundance and body size in pond-breeding amphibians. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.s25d3.

Pechmann, J.H.K., Scott, D.E., Semlitsch, R.D., Caldwell, J.P., Vitt, L.J. & Gibbons, J.W. (1991) Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science*, **253**, 892–895.

Peterman, W.E., Locke, J.L. & Semlitsch, R.D. (2013) Spatial and temporal patterns of water loss in heterogeneous landscapes: using plaster models as amphibian analogues. *Canadian Journal of Zoology*, **140**, 135–140.

Peterman, W.E., Anderson, T.L., Drake, D.L., Ousterhout, B.H. & Semlitsch, R.D. (2014) Maximizing pond biodiversity across the landscape: a case study of larval ambystomatid salamanders. *Animal Conservation*, **2014**, 275–285.

Petranka, J.W. (1989) Density-dependent growth and survival of larval *Ambystoma*: evidence from whole-pond manipulations. *Ecology*, **70**, 1752–1767.

Petranka, J.W. (1998) *Salamanders of the United States and Canada*. Smithsonian Institution Press, Washington, DC, USA.

R Core Team. (2012) R: A language and environment for statistical computing.

Rasband, W. (2013) ImageJ.

Rowe, L. & Ludwig, D. (1991) Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology*, **72**, 413–427.

Scott, D.E. (1990) Effects of larval density in *Ambystoma opacum*: an experiment in large-scale field enclosures. *Ecology*, **71**, 296–306.

Scott, D.E. (1994) The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology*, **75**, 1383–1396.

Semlitsch, R.D. (1987) Interactions between fish and salamander larvae. Costs of predator avoidance or competition? *Oecologia*, **72**, 481–486.

Semlitsch, R.D. & Reichling, S.B. (1989) Density-dependent injury in larval salamanders. *Oecologia*, **81**, 100–103.

Semlitsch, R.D., Scott, D.E. & Pechmann, J.H.K. (1988) Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology*, **69**, 184–192.

Semlitsch, R.D., Scott, D.E., Pechmann, J.H.K. & Gibbons, J.W. (1996) Structure and dynamics of an amphibian community: evidence from a 16-year study of a natural pond. *Long-Term Studies of Vertebrate Communities* (eds M.L. Cody & J.A. Smallwood), pp. 217–248. Academic Press Inc, San Diego, California, USA.

Semlitsch, R.D., Anderson, T.L., Osbourn, M.S. & Ousterhout, B.H. (2014) Structure and dynamics of ringed salamander (*Ambystoma annulatum*) populations in Missouri. *Herpetologica*, **7**, 14–22.

Shulse, C.D., Semlitsch, R.D. & Trauth, K.M. (2013) Mosquitofish dominate amphibian and invertebrate community development in experimental wetlands (ed M Cadotte). *Journal of Applied Ecology*, **50**, 1244–1256.

Shulse, C.D., Semlitsch, R.D., Trauth, K.M. & Williams, A.D. (2010) Influences of design and landscape placement parameters on amphibian abundance in constructed wetlands. *Wetlands*, **30**, 915–928.

Skelly, D.K. (1992) Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology*, **73**, 704–708.

Skelly, D.K., Werner, E.E. & Cortwright, S.A. (1999) Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology*, **80**, 2326–2337.

Stachowicz, J.J. (2001) Mutualism, facilitation, and the structure of ecological communities. *BioScience*, **51**, 235–246.

Stenhouse, S.L., Hairston, N.G. & Cobey, A.E. (1983) Predation and competition in *Ambystoma* larvae: field and laboratory experiments. *Journal of Herpetology*, **17**, 210–220.

Trauth, S.E., Robinson, H.W. & Plummer, M.V. (2004) *The Amphibians and Reptiles of Arkansas*. University of Arkansas Press, Fayetteville, Arkansas, USA.

Urban, M.C. (2007a) Risky prey behavior evolves in risky habitats. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 14377–14382.

Urban, M.C. (2007b) Predator size and phenology shape prey survival in temporary ponds. *Oecologia*, **154**, 571–580.

Urban, M.C. (2008) Salamander evolution across a latitudinal cline in gape-limited predation risk. *Oikos*, **117**, 1037–1049.

Van Buskirk, J. (2005) Local and landscape influence on amphibian occurrence and abundance. *Ecology*, **86**, 1936–1947.

Van Buskirk, J. (2009) Natural variation in morphology of larval amphibians: phenotypic plasticity in nature? *Ecological Monographs*, **79**, 681–705.

Van Buskirk, J. (2011) Amphibian phenotypic variation along a gradient in canopy cover: species differences and plasticity. *Oikos*, **120**, 906–914.

Van Buskirk, J. & Smith, D.C. (1991) Density-dependent population regulation in a salamander. *Ecology*, **72**, 1747–1756.

Verdolin, J.L. (2006) Meta-analysis of foraging and predation risk trade-offs in terrestrial systems. *Behavioral Ecology and Sociobiology*, **60**, 457–464.

Walls, S.C. (1995) Differential vulnerability to predation and refuge use in competing larval salamanders. *Oecologia*, **101**, 86–93.

Walls, S.C. (1996) Differences in foraging behaviour explain interspecific growth inhibition in competing salamanders. *Animal Behaviour*, **52**, 1157–1162.

Wellborn, G.A., Skelly, D.K. & Werner, E.E. (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, **27**, 337–363.

Werner, E.E. & Anholt, B.R. (1996) Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. *Ecology*, **77**, 157–169.

Werner, E.E., Skelly, D.K., Relyea, R.A. & Yurewicz, K.L. (2007a) Amphibian species richness across environmental gradients. *Oikos*, **116**, 1697–1712.

Werner, E.E., Yurewicz, K.L., Skelly, D.K. & Relyea, R.A. (2007b) Turnover in an amphibian metacommunity: the role of local and regional factors. *Oikos*, **116**, 1713–1725.

Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V. *et al.* (2010) Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters*, **13**, 1310–1324.

Wilbur, H.M. (1997) Experimental ecology of food webs: complex systems in temporary ponds. *Ecology*, **78**, 2279–2302.

Wilbur, H.M. & Collins, J.P. (1973) Ecological aspects of amphibian metamorphosis: nonnormal distributions of competitive ability reflect selection for facultative metamorphosis. *Science*, **182**, 1305–1314.