



Direct effects influence larval salamander size and density more than indirect effects

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Abstract

Direct and indirect effects both influence population and community dynamics. The relative strengths of these pathways are often compared using experimental approaches, but their evaluation in situ has been less frequent. We examined how individual and aggregate impacts of direct and indirect effects of species densities, proxies for competition and predation pressure, and habitat variables influenced patterns of larval density and body size of ringed (*Ambystoma annulatum*) and spotted salamanders (*A. maculatum*). We surveyed > 150 ponds in Missouri, USA, from 2012 to 2014 to measure the density and body size of each focal species, the density of co-occurring pond food web members, and select habitat features. We used structural equation modeling to quantify the relative importance of direct and indirect pathways on both body size and larval density. Overall, both responses were explained through a combination of direct and indirect effects. However, the magnitudes of direct effects were often greater than indirect effects. Some of the direct and indirect relationships with larval salamander size and density were also consistent with results from experimental studies. Finally, total direct and indirect effects were often weaker due to habitat and density variables negating each other's impacts. Overall, our study shows that direct effects were equivalent to, or more important than, indirect effects. We also demonstrate that the effects stemming from individual relationships can sum to produce net patterns that are negligible in magnitude. Further work on direct and indirect effects with observational data are needed to examine their magnitudes in natural communities.

Keywords Competition · Interaction chain · Mediation · Pond · Predation · Structural equation modeling

Introduction

Most communities are structured by both direct and indirect effects of biotic and abiotic factors. Direct effects are conceptually straightforward, as they typically involve interactions between only two species (e.g., consumer-resource dynamics), or a single environmental stressor limiting demographic rates of an organism. In contrast, indirect effects involve relationships between three or more different components of ecological systems (Strauss 1991; Wootton 1994a). Such effects may be prevalent in many ecosystems but are often overlooked or underestimated when only direct effects are assumed to operate (Werner and Peacor 2003). Therefore, investigations of both indirect and direct effects are needed to better understand the factors that structure ecological communities.

Indirect effects of species interactions influence community structure through two primary pathways, each of which involve multiple (> 2) species. First, interaction chains occur

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when the abundance of one species that is engaged in pairwise interactions with a second species is altered by the presence of a third species (Wootton 1993, 1994b; Fig. 1a); trophic cascades and apparent competition (Holt 1977) are classic examples of interaction chains. Second, interaction modifications occur when one species modifies the behavior or morphology of a second species, rather than its abundance, that results in changes in interaction strength with a third species (Schmitz et al. 2004; Wootton 1994a). For

example, predators sometimes reduce foraging rates of prey, a behavioral response that increases basal resources without decreasing prey abundance. The combined influence of these indirect effects can sometimes have equivalent or greater impacts on communities than direct effects (Abrams 1995; Strauss 1991; Werner and Peacor 2003; Wootton 1994c).

Abiotic factors (e.g., climate or habitat heterogeneity) layer additional complexities onto evaluating direct and indirect species interactions because they can also alter

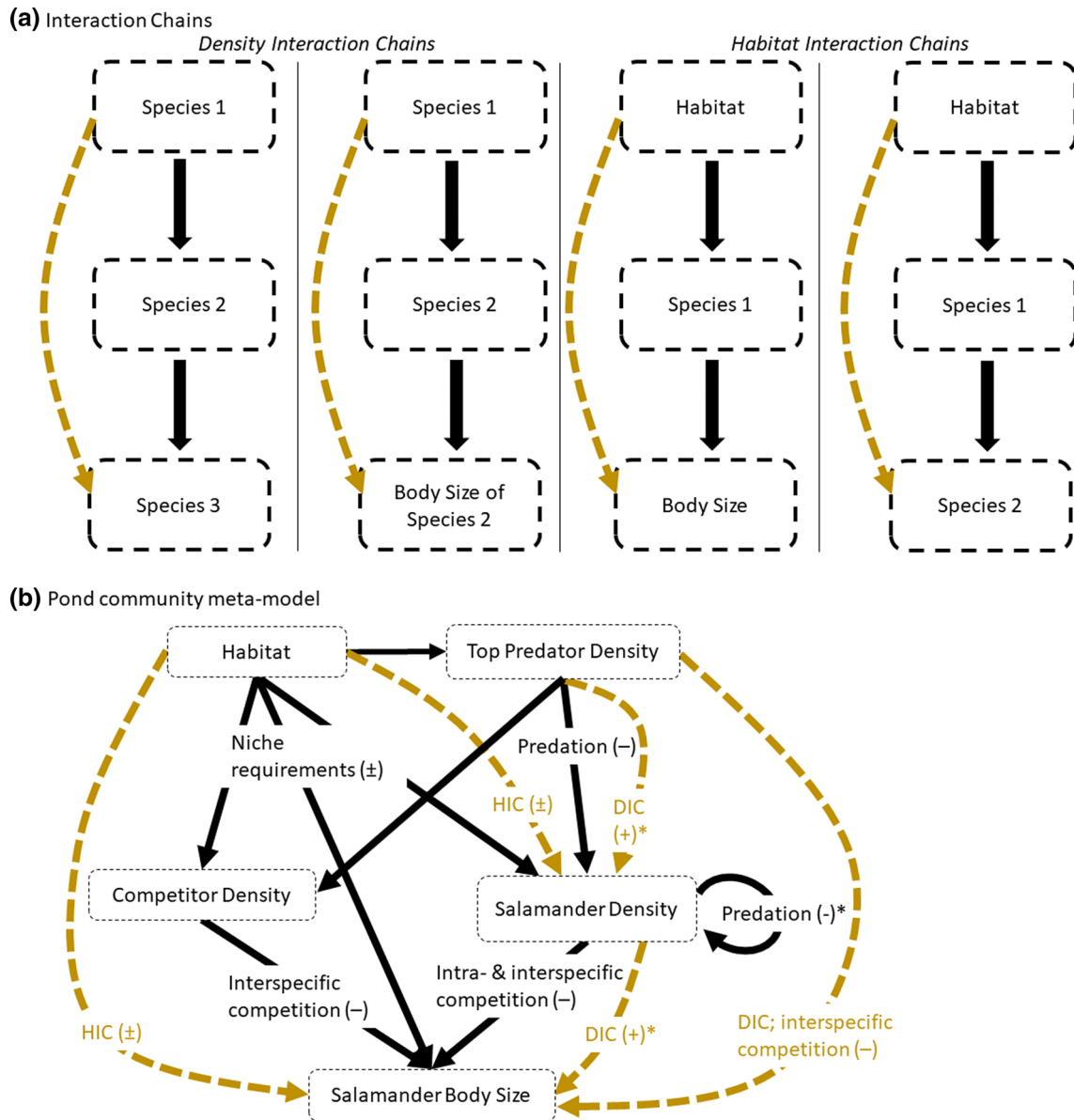


Fig. 1 **a** Visual depiction of density interaction chains and habitat interaction chains operating through variables onto body size and density in our system. **b** Meta-model of hypotheses for larval body size (head width) and density. Variables are grouped by generic categories for simplicity, but in actual analyses, individual variables are used; no latent or composite variables were used. In both panels, gold dashed lines indicate indirect effects, and black solid lines indicate

direct effects. In (b), +, -, and ± indicate the direction of the effect. * indicates pathways only possible for *A. maculatum*. Total direct effects are the sum of the solid black pathways on size or density. Total indirect effects are the sum of the gold dashed lines on salamander body size or density. Text on lines indicates the hypothesized processes that are operating

interaction strengths (Alsterberg et al. 2013; Lensing and Wise 2006; Orrock et al. 2013; Trussell et al. 2006). For instance, Trussell et al. (2006) found that the strength of indirect effects of predators varied based on the level of riskiness in a given habitat. Furthermore, habitat and climatic factors can inflict strong direct effects on species abundances and traits (Bukovinszky et al. 2008; Menéndez et al. 2007; Ogilvie et al. 2017; Ousterhout et al. 2015), creating interaction chains among abiotic and biotic variables (Fig. 1a), independent of their effects on species interactions. Thus, comparing how environmentally mediated interaction chains and modifications (Wootton 2002) affect food webs, relative to the direct effects of the environment on species abundances, may help us understand how context-dependent outcomes of species interactions contribute to community structure (Agrawal et al. 2007).

Experiments have been the standard approach for evaluating the relative magnitude of direct and indirect effects because each pathway can be isolated (e.g., Peacor and Werner 1997; Schmitz 1998). However, such methods often simplify food web dynamics and homogenize environmental/abiotic parameters—necessities that have generated important insights but limit understanding of how and whether direct and indirect effects operate similarly in natural systems. Consequently, in situ studies that compare the magnitude of direct and indirect effects have been performed less frequently (e.g., Cariveau et al. 2004; Menéndez et al. 2007; Ogilvie et al. 2017; Sargeant et al. 2011). Yet, in situ studies of direct and indirect effects are also valuable, as they circumvent the necessary limitations of controlled experiments outlined above and better encapsulate natural heterogeneity in species assemblages and environmental conditions. At the same time, inferring biotic interactions from observational data can be challenging, as numerous processes can generate patterns that appear to have resulted from species interactions (Dormann et al. 2018). For indirect effects, this may be more challenging given the numerous factors that could contribute to or affect indirect pathways in complex, natural environments. Despite this limitation, investigations of direct and indirect pathways in natural communities are needed to determine whether their relative magnitudes correspond to experimental results.

Pond communities are a model system for evaluating the relative importance of direct and indirect effects. For example, predators and hydroperiod duration (i.e., the length of time that a pond remains inundated) strongly influence the abundance and recruitment of aquatic stages of many organisms (Semlitsch et al. 1996; Semlitsch et al. 2015; Wellborn et al. 1996; Werner et al. 2009); insufficient hydroperiod lengths can inhibit metamorphosis, while predators (typically fish) can induce strong consumptive effects in permanent ponds. Hydroperiod therefore can indirectly affect amphibians by eliminating certain predator taxa. The

impacts of fish and other predators (e.g., macroinvertebrates) on amphibians can also indirectly affect other trophic levels (i.e., trophic cascades) (Huang and Sih 1990, 1991; Nyström and Åbjörnsson 2000; Peacor and Werner 1997), which sometimes depend on abiotic factors like water temperature (Kratina et al. 2012).

Density-dependent competition (both intra- and interspecific) has been well studied in its effects on demographic rates (Ousterhout and Semlitsch 2016; Scott 1994; Semlitsch and Caldwell 1982) and can be either a direct or indirect effect, depending on its form (exploitative = indirect; interference = direct). Competition is also often modified by abiotic factors like water pH (Warner et al. 1993) or predators (Morin 1981). Nearly all studies of indirect effects in pond communities have used experimental approaches, whereas evaluation of direct effects has regularly occurred in both experimental (e.g., Semlitsch 1987; Semlitsch and Wilbur 1988) and natural systems (e.g., Van Buskirk 2005; Werner et al. 2009). These well documented aspects of how pond ecosystems function makes them a good system to examine the confluence of abiotic factors, species interactions, and direct and indirect effects.

The objective of this study was to examine how direct and indirect effects of species interactions and habitat features influence demography. We assessed this objective using data from natural populations of ringed (*Ambystoma annulatum*) and spotted salamanders (*A. maculatum*), and structural equation modeling (SEM) to determine the relative magnitude of interaction chains for this system (Fig. 1). Using this analytical framework, previous knowledge on pond community dynamics, and a large spatiotemporal data set, we were able to assess the strength of direct and indirect effects on two responses, larval abundance and body size. We focused on these two response variables because abundance provides information on the relative frequency of each species across the landscape, and often is affected by habitat-related niche factors (Van Buskirk 2005; Werner et al. 2009). Larval body size can predict metamorph body size, a proxy for adult fitness (Semlitsch et al. 1988; Wilbur and Collins 1973), and is affected by species interactions and abiotic variables (Van Buskirk 2011).

We specifically focused on testing the relative importance of interaction chains that are derived from species densities (a standard interaction chain (IC); here, a density IC) and habitat variables (habitat IC) mediated by competitor or predator densities (Fig. 1a). We examined how both individual variables affected each response, as well as aggregations of direct and indirect effects stemming from habitat and density variables. We made six specific comparisons comparing the different individual or aggregate pathways: (1) direct and indirect effects of individual variables; (2) direct density vs density ICs; (3) direct habitat vs habitat ICs; (4) direct density vs habitat; (5) density ICs vs habitat ICs; and

(6) total direct vs total indirect. We predicted the Aeshnidae would be the individual variable to have the largest impact on both species densities' and size, mainly through direct effects, based on experimental evidence demonstrating its consumptive and non-consumptive predatory abilities (Anderson and Semlitsch 2016; Peacor and Werner 1997; Relyea and Yurewicz 2002). We predicted the direct effect of habitat variables would be most important for predicting density, whereas body size would be more affected by species densities (Ousterhout et al. 2015). Because of this, we expected habitat ICs to be more important than density ICs (because of the direct effect habitat has on density). In sum, these predictions would lead to the expectation that overall direct effects are more important than indirect effects. Comparison among these different types of general processes should provide novel insight that is helpful to better understand direct and indirect effects across systems and species.

Materials and methods

Study species and area: *Ambystoma annulatum* and *A. maculatum* co-occur in fishless ponds in Missouri, Arkansas, and Oklahoma, USA (Lannoo 2005), but have different life history strategies. Breeding occurs in the fall for *A. annulatum* and larvae overwinter (Semlitsch et al. 2014), whereas *A. maculatum* breed in the spring (Semlitsch and Anderson 2016). This generally makes larval *A. annulatum* a potential predator of larval *A. maculatum*, as the former attain much larger body sizes by the time the latter enters ponds (Anderson et al. 2016a). Larval abundance is often determined by habitat features such as hydroperiod, the amount of forested area surrounding ponds that determines suitable adult habitat, or the amount of canopy cover over ponds (Ousterhout et al. 2015; Peterman et al. 2014). Further, these species co-occur within pond food webs that encompass numerous invertebrates and other amphibians, which they interact with as competitors, predators, or food resources to also drive abundance or body size patterns (Ousterhout et al. 2015; Semlitsch et al. 2015).

Our study occurred within a 7140 ha area of Fort Leonard Wood (FLW), Missouri, where we monitored 201 pond communities from 2012–2014 (Anderson et al. 2015, 2016b; Ousterhout et al. 2015; Peterman et al. 2014). Here, we report on data from all fishless ponds (158–169, depending on the year) at FLW that were sampled during the larval period for each species (spring [February–March] for *A. annulatum*; summer [May–June] for *A. maculatum*). Across FLW ponds, *A. maculatum* is more common than *A. annulatum* (Table A1).

Sampling methodology: We typically sampled each pond over a 3-d period using both dip nets and funnel traps, garnering three samples with each technique. The number of

dip net and funnel traps samples was standardized to pond surface area (Shulse et al. 2010). We identified and counted all captured amphibians to life stage and species (when possible) and aquatic invertebrates to family or order, counted their abundances, and returned them to their pond unharmed. We converted abundance data (total number captured per species-pond-sampling season-year) to catch-per-unit effort (CPUE) density estimates for each taxa by dividing abundance by the total sampling effort (total number of dip net sweeps and traps).

We dorsally photographed up to 20 larvae of each focal salamander species at each site to obtain body size measurements (Anderson et al. 2016b) prior to returning to them to their pond. For this study, we used head width (hereafter, body size), which had fewer missing values than snout-vent length or total length; all three were highly correlated. We averaged body size for each species-pond-year combination across the 3 days of capture, though we acknowledge that this could have resulted in repeat measurements of some individuals. We excluded ponds from our analysis in which we measured only one individual, resulting in the following pond-year combinations: *A. annulatum*, N=202; *A. maculatum*, N=221. We also conducted a supplemental analysis that used individual salamander measurements rather than averages (see below).

We recorded the abundance of other food web members that were known a priori to strongly affect amphibian demography. We considered predators to be larval Aeshnidae dragonflies (Anderson and Semlitsch 2016), leeches (Hirudinae; Wilbur 1972), adult red-spotted newts (Drake et al. 2014), and backswimmers (Notonectidae; Stretz et al. 2019). We considered *A. annulatum* and *A. opacum*, another fall-breeding salamander species, to be predators of *A. maculatum* (Anderson et al. 2016a). We also included non-Aeshnidae larval dragonflies and larval damselflies (Zygoptera), both of which we expected to compete with larval salamanders for prey resources (Corbet 1999). Overwintering Ranidae tadpoles (*Rana clamitans* and *R. sphenoccephala*) were also included, but their exact role was difficult to predict a priori as they are sometimes omnivores (Schiesari et al. 2009). It is possible there are other species that influence direct and indirect effects in these systems, which would be subsumed into the direct effects and upwardly bias their magnitude relative to indirect effects. However, it is unlikely we missed species that imparted substantial effects given the considerable literature on which taxa interact strongly in pond communities and because we included all species that were present at high abundances at our study site.

Habitat Sampling: The habitat features we considered were canopy cover, hydroperiod, and within-pond structural complexity, all of which affect salamander abundance (Peterman et al. 2014; Van Buskirk 2005; Werner et al. 2009) or body size (Van Buskirk 2009). We assigned ponds

to one of four hydroperiod categories—ephemeral (dries multiple times every year), summer (dries once a year in the summer), semi-permanent (dries during extreme droughts), and permanent (never dries)—based on visits to all ponds that occurred approximately every other month, and from historical knowledge of the area (K. Lohraff, personal communication). Hydroperiod was treated as an ordinal variable in all models. We estimated canopy cover as the average of four measurements taken with a spherical densiometer at the pond edge during full leaf-out in the summer of 2012. We quantified structural complexity by visually assessing the percent of area within a 1 m² quadrat that had vegetation (hereafter, structure) and had benthic organic material (e.g., leaf litter; hereafter, substrate). We placed quadrats within 2 m of the shoreline in the summer of 2012 and scaled the number quadrats to pond area. We assume values for these variables are representative for each pond across the 3 years of the study.

Pond Community Meta-Model: We developed a structural equation meta-model to understand how direct and indirect effects operate in pond communities (Fig. 1b; Grace et al. 2010). Our meta-model had five main conceptual parts: habitat features, predator abundance, abundance of other food web members (e.g., invertebrate competitors), and abundance and size of salamanders (Fig. 1b). Because of the inclusion of body size, our interactions chains operated through variables, rather than just species (Fig. 1a). Based on previous work (Ousterhout et al. 2015; Van Buskirk 2005, 2009; Werner et al. 2009), habitat features predicted abundance and body size of all organisms. These pathways are labeled as having ‘±’ effects because of species-specific responses to habitat variables (Fig. 1b). These can lead to habitat ICs if, for example, habitat increased top predator abundance, leading to decreased salamander abundance.

We expected top predator densities to negatively affect salamander densities via direct consumption. We also had direct links in the SEM between top predators and body size, though in this context it could be an indirect effect if they exploitatively competed with salamanders for prey (e.g., zooplankton) or a direct effect of interference, which our model cannot distinguish. We also expected indirect effects of top predators on body size, with the direction of the relationship depending in part on whether predators also affected density: The relationship with body size would be positive if predators also had a negative relationship with density (a density IC), or negative if predators had no effect on density, suggesting reduced foraging behavior (an interaction modification). For *A. maculatum*, which had two higher trophic levels (top predators, *A. annulatum* and *A. opacum* larvae), we evaluated interaction chains on both density and body size. However, because there was only one trophic level above *A. annulatum*, we could assess indirect predator pathways only on body size.

We considered Zygoptera, non-Aeshnidae dragonflies, and Ranidae to function as competitors and thus included a direct casual path between density and body size in the SEMs (Fig. 1a). Again, in an ecological sense, competition could be an indirect effect if it is exploitative in nature (Wootton 2002); the type of competition is unknown for many of the species pairs studied here. Previous work has shown that these organisms cannot or do not prey upon larval salamanders, and thus, no link was included to salamander density (Stretz et al. 2019). Graphical versions of the full models are shown in Appendix 1 (Fig. A1).

Analysis: We used three SEMs (two for body size, one for abundance) to evaluate our meta-model and determine the relative importance of direct and indirect effects from predators, competitors, and habitat features on larval body size and density. We did not construct latent variables because none of the variables were highly correlated (all $r < 0.4$). We also tried to use composite variables to represent our meta-model, but it resulted in a poor model convergence, so we used the individual variables. We fit all models using the ‘lavaan’ package in R (R Core Team 2020; Rosseel 2012).

We constructed two separate SEMs for body size of each of our focal species (Fig. 1b; Fig. A1). For the model of *A. annulatum* body size, we used density data from the spring sampling period, as that was the only relevant time point (i.e., abundance from the summer sampling could not affect larvae two months prior). For *A. maculatum*, we used their abundance and body size data from the summer, but predictor data from the spring sampling period, corresponding to when *A. maculatum* eggs were laid or hatching. Because *A. maculatum* body size increased over the course of each sampling season, we included sampling date as an additional exogenous variable in their size model; date effects were not present for *A. annulatum*. While species densities were included in the size models, we focused on the direct and indirect effects on salamander body size only because salamander absences were necessarily eliminated (i.e., when salamander density was zero, no size data was available). We evaluated effects on densities in these models only if they were linked to changes in body size. To assess direct and indirect effects on density, we fit one model for all species, where zeros were informative, using the spring sampling data for all taxa except *A. maculatum*. To equalize variances, we divided structure, substrate, and sampling date by 100, and *A. annulatum* body size, *A. maculatum* body size, *N. viridescens* abundance, Ranidae abundance, and *A. maculatum* abundance by 10.

We evaluated model fit using Chi-square statistic, with the test significance adjusted using the Bollen-Stine bootstrap. We also examined the comparative fit index and root-mean-square error of approximation. After determining our global models achieved satisfactory convergence, we then removed non-significant paths and evaluated the remaining

paths. We used a conservative threshold of $P < 0.10$ for remaining terms in the model. The qualitative conclusions do not change if we evaluate the relationships in the global models that retain all $P > 0.10$ paths, but we did so here to improve clarity of the models given the number of variables we were testing. We report the parameter estimates for the global models in Appendix 1 (Tables A3–A6).

We compared direct and indirect effects following previous studies (Cariveau et al. 2004; Matteson et al. 2013). Individual direct effects were the significant standardized regression coefficients. We also summed the significant pathways to obtain the total direct density and total direct habitat effects on each focal species' density and size, as well as the overall total direct effects on body size and density. To get indirect effects, we multiplied the regression coefficients of two direct pathways (see Fig. A2 for an example). We repeated this process for several indirect pathways for each species: each habitat effect mediated by densities of all taxa on body size (e.g., Fig. 1a, middle right boxes), each predator's effect mediated by densities of salamanders, Zygoptera, non-Aeshnidae dragonflies, and Ranidae on body size (Fig. 1a, middle left boxes), habitat mediated by top predators on salamander densities (Fig. 1a, far right boxes), and top predator effects mediated by *A. annulatum*/*A. opacum* densities on *A. maculatum* density (Fig. 1a, far left boxes). We also evaluated the indirect effects of *A. annulatum* and *A. opacum* densities on *A. maculatum* size, mediated by *A. maculatum* density (Fig. 1a, middle left boxes). We then summed the indirect effects corresponding to species' densities or habitat variables to create total effects of density ICs, habitat ICs, and overall indirect effect (habitat IC + density IC) pathways. Thus, for body size models, we examined six comparisons to determine the relative strength of direct vs indirect effects: (1) direct and indirect effects of individual variables; (2) direct density vs indirect density (density IC); (3) direct habitat vs indirect habitat (habitat IC); (4) direct density vs habitat; (5) density ICs vs habitat ICs; and (6) total direct vs total indirect effects. For the density model, comparisons 1, 2, and 6 were not made for *A. annulatum*

because density ICs were only possible for *A. maculatum*. We still examined comparisons 3–5 for *A. annulatum*.

We accounted for non-normality in the data using bootstrap standard errors ($n = 5000$) (Rosseel 2012), which also more appropriately test for significance in mediation analyses (Hayes and Scharkow 2013). Bootstrap standard errors also helped account for the non-independence of data from repeated sampling of some ponds across years by providing more conservative estimates of statistical significance, especially for the indirect effects (Hayes and Scharkow 2013). Because count data often have strong mean–variance relationships (Warton et al. 2012), we also examined whether we needed to stabilize the within-pond variance of each taxa prior to use in SEMs. Because some species exhibited strong within-pond patterns (i.e., a wedge shape), we examined whether log transformation of all species densities influenced the qualitative results of the three models; in all cases, the inferences remained the same, so we used the raw, untransformed data.

We also conducted a supplementary SEM analysis that accounted for the nested structure of data (multiple individuals from the same ponds and repeated surveys across years) using the 'survey' and 'lavaan.survey' packages (Lumley 2004; Oberski 2014). We used individual salamander measurements rather than pond averages to account for differences in sample sizes across ponds. These other packages do not allow bootstrap standard errors, so we instead used robust SEM, which also controls for non-normality in the data and model fit using the Satorra–Bentler scaled test statistic. These results were qualitatively similar to the models using bootstrap standard errors, so we do only report these model summaries in Appendix 1 (Tables A7–A10).

Results

Model Fit: After including several additional covariance links (Fig. A1), the global models did not significantly deviate from the data (Table 1). After removing the

Table 1 Fit statistics for both the global and reduced (non-significant paths removed) structural equation models of body size of each salamander species, and the density model

Model	Response	DF	Chi-square	Bollen-Stine P value	CFI	RMSEA
Global	<i>A. annulatum</i> size	15	29.391	0.441	0.957	0.060
	<i>A. maculatum</i> size	28	35.132	0.608	0.987	0.028
	Density	15	21.878	0.482	0.993	0.025
Reduced	<i>A. annulatum</i> size	11	22.533	0.279	0.916	0.072
	<i>A. maculatum</i> size	14	33.403	0.416	0.912	0.079
	Density	14	9.074	0.984	1.000	0.000

Each model was fit with bootstrapped standard errors and test statistics. Under this procedure, the Bollen-Stine P value indicates whether the model deviates from the data. Bootstrapping accounts for non-normality of data and helps with non-independence of data points (see *Methods*). A CFI > 0.95 and RMSEA < 0.05 indicate good model fit

non-significant paths, model fit remained good (Table 1). The body size models explained more than twice the amount of variability for *A. maculatum* ($R^2=0.430$) than *A. annulatum* ($R^2=0.148$). The density model also explained differing amounts of variability in each focal taxa's density: *A. maculatum* ($R^2=0.171$) and *A. annulatum* ($R^2=0.104$).

Overall, we observed our focal species to vary in terms of which individual variables and aggregated variables were important in explaining body size and density (Table 2; Fig. 2; Table A2). Below, we detail the six specific comparisons for direct and indirect effects outlined in our hypotheses. Note that some results are discussed multiple times as the same variables are used in different comparisons.

Direct and indirect effects of individual variables: The individual variables that had the strongest effect on each species' body size were both indicative of competition. The variable that had the strongest effect on *A. annulatum* size was *A. opacum* density and was negative in direction (Fig. 2a, Table A2). Only species' densities were directly linked to *A. annulatum* body size, with no strong direct habitat effects. Several habitat ICs were significant, though relatively weak in magnitude compared to the direct density effects (Fig. 2a,

Table A2). The only variable for which individual direct and indirect effects could be compared for *A. annulatum* size was from *N. viridescens*, with the indirect effect passing through *A. opacum* density. The magnitude of the negative direct effect on body size was five times greater than the magnitude of the positive indirect effect.

Intraspecific density had the strongest effect on *A. maculatum* size (Fig. 2b, Table A2) and was negative in direction. The magnitude of intraspecific density was four times greater than any other predictor (Fig. 2b, Table A2). As with *A. annulatum*, most effects on body size were from species' densities, with substrate being the only habitat variable to have a direct effect (Fig. 2b, Table A2). Aeshnidae and substrate also were the only variables to have both direct and indirect effects on body size. For Aeshnidae, the indirect effect passed through *A. maculatum* densities and was approximately equal in magnitude to the direct effect, but opposite in direction (Fig. 2b, Table A2). Substrate had a negative indirect effect on *A. maculatum* body size via its positive relationship with non-Aeshnidae dragonfly density that was 15% the magnitude of the direct effect of substrate (Fig. 2b, Table A2).

Table 2 Comparison of direct and net indirect effects of species densities and habitat variables, and the totals for each pathway on salamander body size and density

Species	Response	Effect source	Est	Lower	Upper	Std Est
<i>A. annulatum</i>	Body size	Habitat	0.000	0.000	0.000	0.000
		Density	-0.014	-0.070	0.032	-0.261
		Habitat IC	-0.051	-0.073	-0.033	-0.092
		Density IC	0.009	-0.003	0.019	0.019
		Total direct	-0.014	-0.070	0.032	-0.261
		Total indirect	-0.042	-0.064	-0.025	-0.074
<i>A. maculatum</i>	Body size	Habitat	-0.044	-0.070	-0.017	-0.153
		Density	-0.295	-0.430	-0.151	-0.516
		Habitat IC	-0.052	-0.072	-0.035	-0.124
		Density IC	0.102	0.053	0.161	0.064
		Total direct	-0.339	-0.474	-0.195	-0.669
		Total indirect	0.050	-0.0003	0.104	-0.060
<i>A. annulatum</i>	Density	Habitat	0.028	0.005	0.056	0.027
		Density	2.849	1.332	4.715	0.278
		Habitat IC	0.082	0.044	0.127	0.079
		Density IC	NA	NA	NA	NA
		Total direct	2.878	1.340	4.767	0.305
		Total indirect*	0.082	0.044	0.127	0.079
<i>A. maculatum</i>	Density	Habitat	0.252	0.197	0.311	0.334
		Density	-0.111	-0.331	0.090	0.111
		Habitat IC	0.024	0.004	0.046	0.044
		Density IC	0.000	0.000	0.000	0.000
		Total direct	0.141	-0.062	0.331	0.445
		Total indirect	0.024	0.004	0.046	0.044

Lower and upper confidence interval (CI) estimates are based on 5000 bootstrap samples. Std. Est is the standardized estimate, an approximation of effect size. Rows with all zeros indicate that the effects are not present, while NAs indicate where values could not be estimated (see Methods). Bold values indicate $P < 0.10$. *Total indirect effects for this species equal habitat ICs due to no density ICs

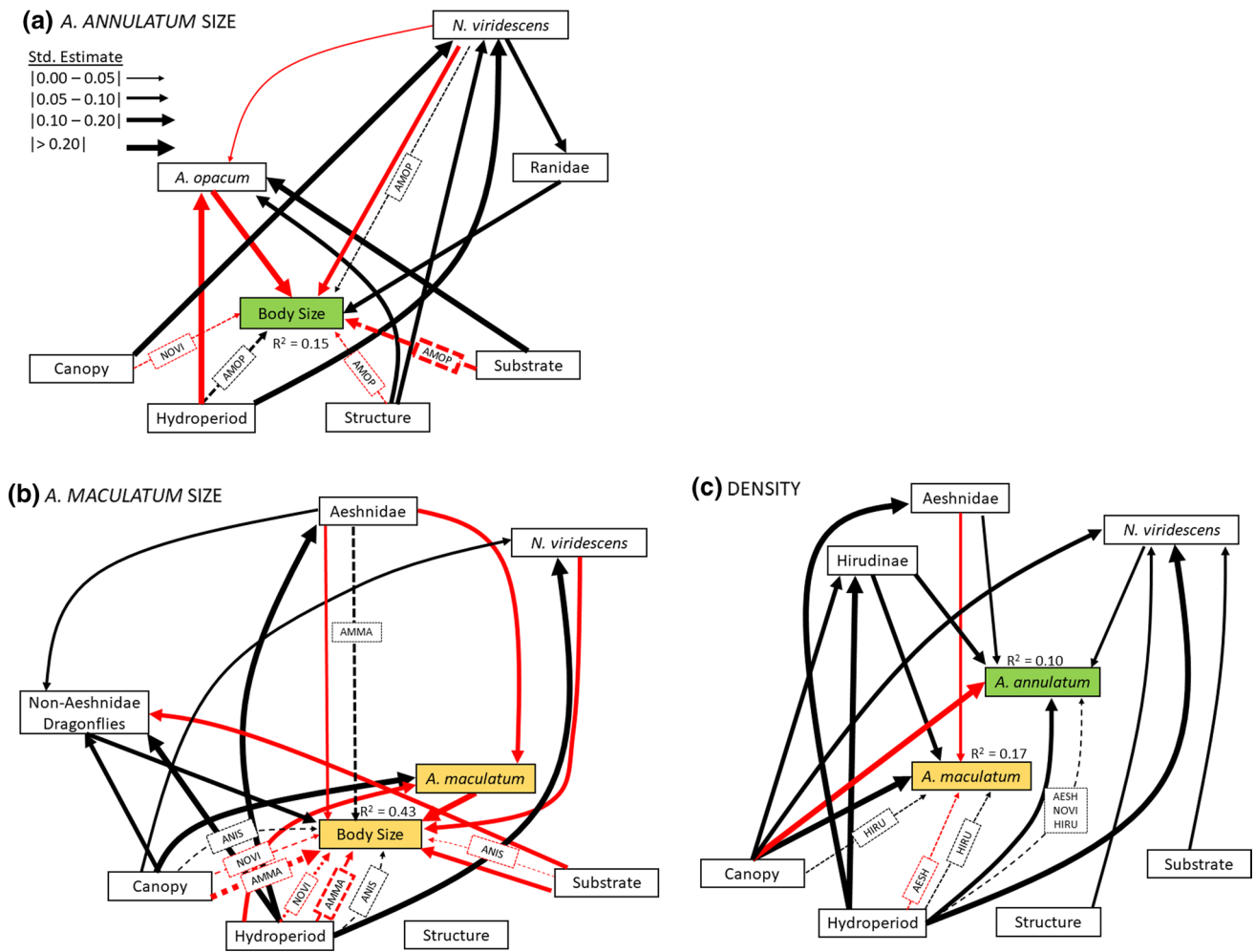


Fig. 2 Structural equation models for body size of **a** larval *A. annulatum* (N=202) and **b** *A. maculatum* (N=221), and **c** density of both taxa (N=494). Only paths that directly or indirectly affected the response variable of interest (e.g., body size or density) are shown ($P < 0.10$). Black lines indicate positive effects, and red lines indicate negative effects. Solid lines indicate direct relationships, and dashed lines indicate indirect relationships. Text boxes with dashed borders set on dashed lines indicate the mediating variable. Line width for all

variables scales with the magnitude of the standardized effect size, as shown in the legend. Unstandardized and standardized parameter estimates are shown in Table A2. Abbreviations include: AESH = Aeshnidae, AMAN = *A. annulatum*, AMMA = *A. maculatum*, AMOP = *A. opacum*, ANIS = Non-Aeshnidae dragonflies, HIRU = Hirudinae, NOTO = Notonectidae, NOVI = *Notophthalmus viridescens*. The variables with the largest standardized effects were **(a)** *A. opacum* density, **(b)** *A. maculatum* density, and **(c)** canopy for both species

Canopy cover had the largest standardized effect size on densities of both focal species (Fig. 2C, Table A2). However, the relationships were opposite in direction (positive for *A. maculatum* and negative for *A. annulatum*). Canopy cover also had indirect effects on *A. maculatum* density through its effects on Hirudinae, but the direct effect was substantially larger (standardized estimates = 0.340 vs 0.028, respectively). Hydroperiod had large direct and indirect effects (through Aeshnidae, *N. viridescens*, and Hirudinae) on *A. annulatum* density, with the direct effect being an order of magnitude larger than each indirect effect (Fig. 2c, Table A4). Hydroperiod also indirectly affected *A. maculatum* through its effects on Aeshnidae and Hirudinae (Fig. 2c), though direct effects of hydroperiod were absent.

Direct density vs indirect density (density IC): For body size of *A. annulatum*, both direct effects of density and density ICs were not different from zero (Table 2). For *A. maculatum* body size, the magnitude of direct density effects was substantially larger than density ICs and was opposite in direction (Table 2). The magnitude of the direct effects was an order of magnitude larger than the indirect effects (Table 2). Neither direct density nor density ICs were significant for explaining the density of *A. maculatum* (Table 2).

Direct habitat vs indirect habitat (habitat IC): Habitat ICs negatively affected *A. annulatum* body size, while direct habitat effects were not present (Table 2). The direct effect of habitat was slightly larger than habitat ICs for *A. maculatum* body size (Table 2). The effect of habitat ICs was three

times as large as the direct effect of habitat on *A. annulatum* density (Table 2). In contrast, direct habitat effects were substantially larger than habitat ICs for *A. maculatum* density (Table 2).

Direct density vs habitat: Neither direct density nor habitat were significant for *A. annulatum* body size (Table 2). For *A. maculatum* body size, direct density effects were five times larger than habitat effects (Table 2). The direct effect of density on *A. annulatum* density was an order of magnitude larger than the habitat effect, which was not significant (Table 2). In contrast, direct habitat effects were three times stronger for *A. maculatum* density than direct density effects (Table 2).

Density ICs vs habitat ICs: Habitat ICs were significant and negative for *A. annulatum* body size, and nearly an order of magnitude greater than the positive effects of density ICs. Negative effects of habitat ICs were twice as strong as the positive effects of density ICs on *A. maculatum* body size. No density ICs were present for *A. maculatum* density and were not possible for *A. annulatum*, so we could not quantitatively compare them to habitat ICs.

Total direct vs total indirect effects: For body size of *A. annulatum*, only total indirect effects were statistically different from zero (Table 2). For *A. maculatum* body size, total direct effects were an order of magnitude larger than total indirect effects (Table 2). For the density of *A. maculatum*, total direct effects were not different from zero, while indirect effects imparted a relatively weak effect, as compared to other individual variables (Table 2).

Discussion

Indirect effects are increasingly recognized as important processes that impact population and community dynamics, especially through species interactions (Werner and Peacor 2003; Wootton 1994a). Yet, quantifying their importance relative to direct effects from observational data is lacking for many systems, in part because such data may not be available for all systems. Tests of these pathways are needed to help understand when and where direct and indirect processes are most important, and whether they stem from abiotic or biotic sources. Here, we evaluated direct and indirect pathways derived from habitat variables and species densities on larval salamander body size and density in natural pond communities. While we cannot definitively assign causality based on observational data, several overall patterns emerged from our analysis: (1) Body size and density were explained through a combination of direct and indirect effects, with their relative importance changing based on the response variable and species; (2) individual competitor densities best explained body size, while habitat variables best explained species' densities, (3) when an individual

variable imparted both direct and indirect effects, the direct effect was stronger in six of seven cases; and (4) aggregated habitat and density effects in some cases negated one another to result in non-significant total effects. Overall, of the 10 comparisons we could make for the impacts of aggregated direct versus indirect effects of habitat, density, and total effects on salamander body size and density (points 2, 3 and 4 above), four showed direct effects were stronger, four showed indirect effects were stronger, and the remaining two were weak for both pathways. Thus, our results are one of few examples to show the relative magnitudes of direct and indirect effects in an observational setting that encapsulate natural heterogeneity of ecosystems (Figs. 2, 3). We discuss these overall patterns below within the context of our six research questions.

Individual variables: Our SEMs showed that different individual variables contributed to explaining body size and abundance of our focal species. Discussion of all variables is beyond the scope of this paper, so we instead highlight several notable results that relate to previous experimental results. First, intra- and interspecific density-dependent competition is often manipulated in experimental settings and typically has strong effects on body size at metamorphosis or individual growth rates (Scott 1990; Wilbur 1997). Consistent with these results, we observed competitor densities to have the largest standardized effect on body size: *A. opacum* density negatively affected *A. annulatum*, and intraspecific

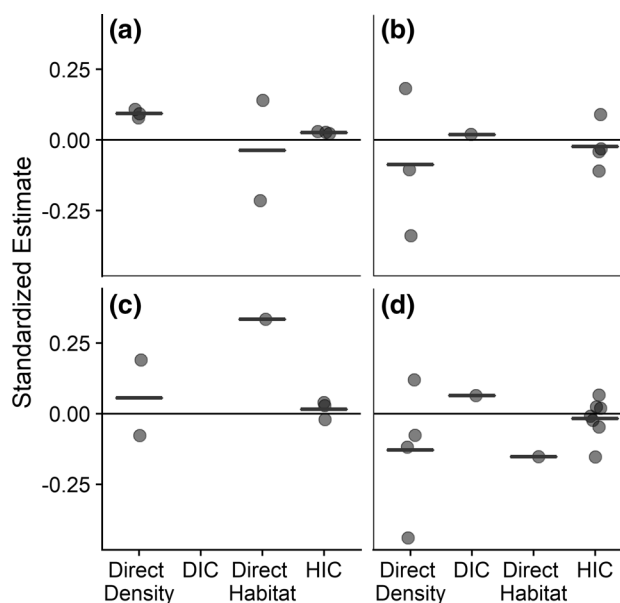


Fig. 3 Standardized estimates of direct and indirect effects of density and habitat variables on (a) *A. annulatum* density, (b) *A. annulatum* body size, (c) *A. maculatum* density, and (d) *A. maculatum* size. Points represent standardized parameter estimates from individual variables in Table A2, and the horizontal lines the mean value of the effect type (direct and indirect density and habitat)

density negatively affected *A. maculatum*. For *A. maculatum*, this is likely evidence of an indirect interaction, due to intraspecific competition being primarily exploitative in this species (Walls 1996). It is not known whether interference or exploitative competition would drive *A. opacum* and *A. annulatum* interactions. Aggression against conspecifics is well documented for *A. opacum* (Mott and Sparling 2010), however, suggesting they may be strong interference competitors, making their interactions with *A. annulatum* based more on interference. Second, larval Aeshnidae typically have strong consumptive and non-consumptive effects on amphibian prey (Peacor and Werner 1997; Relyea and Yurewicz 2002). Here, we observed patterns for Aeshnidae on *A. maculatum* body size consistent with a density IC: Aeshnidae negatively affected *A. maculatum* density, and conspecific density negatively affected body size of *A. maculatum*. Thus, Aeshnidae induced a positive indirect effect on body size, suggestive of thinning effects (Van Buskirk and Yurewicz 1998). This effect was approximately equal in magnitude to the direct effect of Aeshnidae on *A. maculatum* body size, which could result from either competition or *A. maculatum* reducing foraging in the presence of Aeshnidae, leading to smaller body sizes.

Canopy cover amount had the largest standardized effect size for the density of each species but affected them in opposite ways—the relationship was positive for *A. maculatum* and negative for *A. annulatum*. This is consistent with previous results showing *A. maculatum* is a more forest-dependent species (Anderson et al. 2017). The strong direct effect canopy cover had on *A. maculatum* density resulted in a relatively strong indirect effect on body size in this species. Of the five significant predictors of body size, only *A. maculatum* density had a larger standardized effect size than the indirect effect of canopy cover.

While some individual indirect effects were relatively strong, most imparted weak effects on our response variables. This was especially the case for when individual variables had both significant direct and indirect effects on body size or density of salamanders, allowing for direct comparisons. In nearly all cases, the direct effect had a larger standardized parameter estimate, and for several comparisons, by an order of magnitude. This occurred in contrast to comparisons of the aggregated impacts of direct and indirect effects, described in the next few sections. Overall, these individual results highlight that only certain variables strongly impact system dynamics, findings which should receive further scrutiny by other systems where data exist from which comparisons of direct and indirect effects from multiple variables could be made.

Direct density/habitat vs density IC/habitat ICs: We had expected direct habitat and direct density to be the best predictors of salamander density and body size, respectively, based on previous field studies of amphibians (Anderson

et al. 2017; Ousterhout et al. 2015; Van Buskirk 2005, 2009). While these expectations were borne out in the individual results discussed above, aggregating density and habitat effects from multiple variables yielded different patterns (Fig. 3). Habitat ICs had the largest effect on *A. annulatum* body size and density, while direct density and habitat best explained *A. maculatum* size and density, respectively. Habitat ICs were often relatively weak compared to the individual impacts of the strongest predictors. For example, *A. opacum* density effects alone were much stronger than habitat ICs for *A. annulatum* size and canopy cover effects much stronger than habitat ICs for *A. maculatum* density. In several cases, the direct effects of these aggregate variables were also non-significant, despite individual variables being strong predictors of a given response. For instance, *A. opacum* density had the largest effect on *A. annulatum* size, but total direct density was not significant. This likely occurred because positive and negative relationships of different species densities with *A. annulatum* size counteracted one another, resulting in non-significant overall density impacts. Overall, these diverse outcomes, both between species and when comparing aggregate vs individual impacts, are important because characterization of the factors structuring communities often includes both density and habitat as covariates. However, examinations typically do not include cumulative impacts and instead focus on the importance of individual variables. Further use of SEMs, where such aggregate impacts can be identified, would help highlight the conditions that would result in such patterns.

Density ICs vs Habitat ICs: For the responses where comparing habitat ICs and density ICs was possible, habitat ICs were either the only one that was significant (*A. annulatum* body size, *A. maculatum* density) or stronger in magnitude (*A. maculatum* body size). Understanding how habitat and/or climatic variables initiate interaction chains is important, especially for variables that may change with climate shifts or other anthropogenic changes (Ogilvie et al. 2017). However, creating and testing for indirect effects of habitat are less appreciated, at least compared to indirect effects in species interactions. For amphibians, as climate change is likely to impact hydrological regimes (Brooks 2009), subsequent deviations in interaction chains stemming from hydroperiod change will likely occur, especially for wetlands that have predators that require permanent water (Wellborn et al. 1996). Similarly, if canopy cover or forested areas surrounding wetlands change in structure or amount, such that larval or adult abundances are impacted, we hypothesize changes to interaction chains would likely ensue in addition to the direct impacts observed on species densities. Considering the importance of indirect effects of habitat and climatic factors will be especially important for predicting how climate change will restructure populations and communities (Blaustein et al. 2010).

Total direct vs indirect: Total direct and indirect effects were both significant only in explaining the body size of *A. maculatum*. In all other cases, only indirect effects were significant, likely due to direct habitat and density variables having opposing effects, as described above. However, total indirect effects often had low standardized effect sizes, again indicating they were of relatively minor importance compared to individual direct effects. Overall, our aggregate findings show that while individual relationships may have strong direct and/or indirect impacts, their aggregate impacts on a response may be negated by numerous opposing forces.

Conclusions: Indirect effects of species interactions may not occur if a community is composed of weakly interacting species or there is substantial environmental stochasticity (Wootton 1994a, b). We suspect that both conditions could occur in natural pond systems and partially explain the lack of strong indirect effects. Regarding interaction strengths, experimental evidence indicates that only Aeshnidae and Notonectidae act as strong predators of larval salamanders across ontogeny in these systems (Stretz et al. 2019) and were two of the numerically dominant taxa we observed. Thus, only certain taxa would likely initiate density ICs. However, only Aeshnidae affected *A. maculatum* density and was relatively weak compared to habitat variables. Therefore, these communities may be primarily composed of weakly interacting species, at least in terms of limiting species' densities. Hydroperiod length is one type of environmental variability that may strongly modulate both species' abundances and species interactions (Wellborn et al. 1996). For instance, hydroperiod affects amphibians directly by limiting recruitment, and indirectly by affecting whether fish can persist. We observed hydroperiod to be a significant predictor of densities of nearly all taxa (Table A4), and previous research in this system has found that when fish are present in long hydroperiod ponds, densities and occupancy rates of amphibians are lower (Anderson et al. 2015; Ousterhout et al. 2015; Peterman et al. 2014), indicative of the indirect effects that long hydroperiod ponds impart on amphibians.

While weak interactions among species and environmental heterogeneity may explain the lack of strong indirect effects, there are several caveats to interpreting our results. First, we lacked some data that would have allowed further tests of direct and indirect effects observed in other studies. For example, we were unable to evaluate trophic cascades onto plankton from amphibians (e.g., Blaustein et al. 1996; Holomuzki et al. 1994). It is also possible that we were missing important variables in our models that would explain some of the relationships we observed. In particular, we observed many positive relationships between species (Fig. 2), which are unlikely to be examples of facilitation or mutualisms and rather reflect both species responding positively to some unmeasured

environmental signal (Dormann et al. 2018). This may be especially relevant in our aggregation of direct and indirect effects—missing an important mediating variable may result in a biased portrayal of their true impacts. Our study did, however, include the majority of the important species and relevant habitat variables based on experiments and other observational studies. We also could not fully evaluate behavioral or morphological changes that are typically found in experimental studies (Peacor and Werner 1997; Relyea 2001; Van Buskirk and Schmidt 2000). Finally, we used snapshots of density without data on breeding effort—signals of indirect effects of predators and habitat were perhaps important earlier in ontogeny and/or would have been detectable if we were able to evaluate actual survival (i.e., larvae per egg numbers). Given the noise of observational systems, attempts to disentangle direct and indirect effects may be difficult, especially in data limited systems. Other approaches such as models that account for observation and measurement error (e.g., Marino et al. 2019) may advance our understanding of the importance of direct and indirect in effects in natural systems.

Evaluating the relative importance of how direct and indirect effects structure populations and communities is an important goal in ecology. Our results suggest that individual factors may have significant indirect effects that are of equal or lesser strength than their direct effect counterparts. The overall strength of each pathway may be muted in natural systems, however, due to multiple and sometimes opposing forces acting on demographic variables. Importantly, our study demonstrates these effects using observational data, which increase the realism under which such processes operate. Other studies in natural systems that use a similar in situ approach will further solidify how different biotic and abiotic contexts influence population and community dynamics due to this increased realism, though with greater care being needed to assign causality (Dormann et al. 2018). Given species-specific importance of direct and indirect effects has been observed in some studies (Menéndez et al. 2007) but not others (e.g., indirect effects always more important; Ogilvie et al. 2017), attempts to generalize how direct and indirect pathways impact a given system's dynamics may also require investigation of multiple species to examine this variation. Further linking whether factors identified in experimental settings remain important under the conditions organisms experience in natural settings is also critical (Kimbrow et al. 2017). Had we only focused on the indirect effects predicted to be important by experimental studies in our system (indirect effects of predators), we would have found statistically significant patterns in some cases (e.g., *A. maculatum* size), but would have missed that their relative importance was much less than direct effects, giving a false sense of their importance. Thus, increasing the degree of realism in experimental studies, or using observational

data as we have demonstrated here, will be an effective way to further tease apart the relative importance of direct and indirect effects.

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Author contribution statement TLA and BHO conceived the project; TLA, BHO, FER, DLD, and JJB collected the data; TLA analyzed the data and wrote the manuscript; and all authors contributed substantially to editing the manuscript.

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