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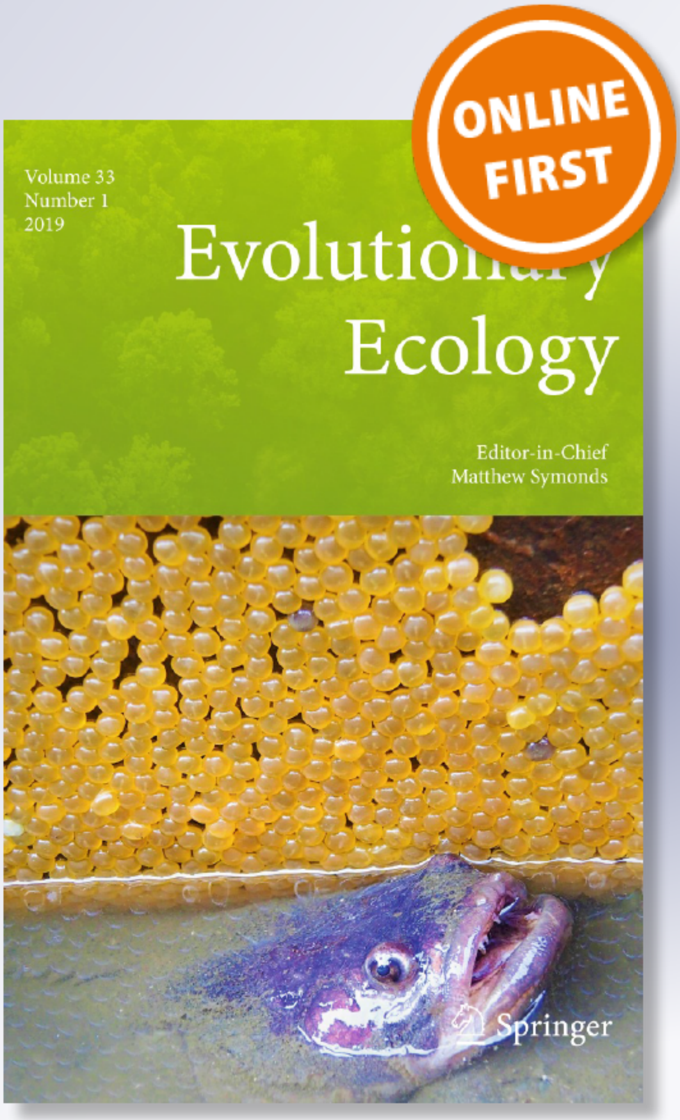
Evolutionary Ecology

ISSN 0269-7653

Evol Ecol
DOI 10.1007/s10682-019-09975-2



Springer

Evolutionary Ecology
https://doi.org/10.1007/s10682-019-09975-2

ORIGINAL PAPER

Predator driven niches vary spatially among co-occurring damselfly species

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Received: 12 November 2018 / Accepted: 9 February 2019

Abstract

Determining how niche differences contribute to local species coexistence is a vexing problem. Previous work has shown that the ecological and evolutionary processes shaping niche differentiation can vary among populations, suggesting that the strength of niche differences among species should likewise vary geographically. Most tests of this idea compare different species in different locations, not the same species in different locations. Thus, it is unclear whether niche differences vary spatially because of variation in community composition or because populations of the same species experience differences in the strength of niche effects. To test this latter hypothesis, we used field experiments to manipulate the relative abundances of the same pair of *Enallagma* damselfly species at two lakes. Manipulating relative abundances allowed us to quantify the demographic signature of niche differences that could stabilize coexistence, because if species are niche differentiated, they should experience lower mortality in response to their shared fish predator, and higher growth in the face of resource competition, when rare. We found that both species experienced lower mortality when rare in one location but not the other. No differences in growth were detected, indicating that competition for prey resources may not be a key factor affecting coexistence. These results suggest the species are ecologically differentiated among populations in ways shaping survivorship in response to a shared predator, which should promote their coexistence. We discuss several factors that could contribute to the differences we observed, focusing on the ideas that either (1) niche differentiation between species evolves locally, or that (2) spatial variation in environmental factors affects the manifestation of species niche differences. We therefore argue that the problem of 'species coexistence' is not a problem of species, but rather is one of understanding if species' populations coexist. Such results imply a role for microevolutionary processes in structuring communities.

Keywords Coexistence · Competition · Damselfly · Niche · Predation · Stabilizing effect

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Introduction

Considerable effort has been exerted to understand how species' ecological differences shape the potential for local coexistence to structure communities. Numerous studies have shown that co-occurring species differ in myriad ways that influence their abilities to use resources, their susceptibility to shared predators or pathogens, or their responses to abiotic factors (MacArthur 1972; Mittelbach 2012). However, simply showing that species are ecologically different does not mean that those differences promote coexistence (Siepielski and McPeek 2010; Cothran et al. 2015). Rather, the differences that promote coexistence are those that cause each species to limit their own population growth rates more than their heterospecific competitors (Chesson 2000b; Adler et al. 2007). The demographic signature of these differences is that per capita population growth rates (or key vital rates such as fecundity, individual growth, or survivorship) decline as species become common, but increase as they become rare (e.g., negative frequency dependent population growth, Chesson 2000b; Adler et al. 2007). For instance, when population regulation is strongly dependent on predator driven mortality (McPeek and Peckarsky 1998) and species exhibit different anti-predator behaviors or camouflage, per capita mortality should be lower when rare. Similarly, if resource competition drives coexistence and species segregate in resource use (e.g., consume different prey items, or are limited by different nutrients), per capita growth rates should be higher when rare (Siepielski et al. 2011).

By preventing any one species from dominating in a community, such frequency dependent demographic responses can stabilize community dynamics. In the absence of these 'stabilizing' effects (Chesson 2000b), species can be slowly lost from communities via ecological drift (Bell 2001; Hubbell 2001) or competitive exclusion (Chesson 2000b). The strength of stabilizing effects can be measured as the demographic advantage (e.g., lower mortality, or higher growth rates) when a species is rare and its competitors are common (Adler et al. 2007; Levine et al. 2008). While mechanisms reducing competitive fitness differences (inequalities in per capita population growth rates in the absence of any stabilizing effects) between species influence the strength of stabilization necessary for coexistence, such 'equalizing effects' cannot by themselves yield coexistence (Chesson 2000b; Adler et al. 2007). Thus, testing for the presence of stabilizing effects is a strong test of whether species exhibit ecological differences that would promote, but do not guarantee, coexistence.

Because stabilizing effects lead species' population growth rates to differ so that each is regulated by different environmental factors (Chesson 2000b; Siepielski and McPeek 2010), such effects have the potential to differ spatially. Indeed, several recent studies have found that stabilizing effects promoting competitor coexistence vary geographically (Germain et al. 2016; Bracewell et al. 2017; LaManna et al. 2017; Usinowicz et al. 2017; Svensson et al. 2018). Such spatial variation may be driven by local differences among communities in factors such as nutrients, prey abundance, or refugia from predators (Chesson 2000b; Cothran et al. 2015; Hart et al. 2017). Broader scale factors such as climate history, the evolutionary duration of species interactions, or the evolutionary history of the species pool could also influence the strength or occurrence of stabilizing effects (HilleRisLambers et al. 2012; Lessard et al. 2012; Germain et al. 2016; Bracewell et al. 2017). Species might also evolve niche differences geographically among local populations (Lankau 2011; Bassar et al. 2017; Germain et al. 2017). Although insightful, past studies investigating spatial variation in stabilizing effects have been conducted by comparing different species in different locations, confounding community composition and geography (but see Svensson et al. 2018). Thus, it is unclear whether stabilizing effects differ spatially because of variation in community composition, or because populations of the same species experience differences in the strength of stabilizing effects. Addressing this issue provides insight into both the spatial and evolutionary time scales at which niche differences contribute to coexistence and the structure of communities.

Here, we test the hypothesis that stabilizing effects between a pair of potentially coexisting species differs among geographically distinct populations. By 'differ', we explicitly mean that the potential demographic advantages when a species is rare (e.g., lower mortality and higher growth) could either be stronger, weaker, or absent among populations of the same pair of species. If stabilizing effects do differ among populations, this would imply that ecological niche differences are not a fixed property of species. Thus, the macroevolutionary processes generating species would not necessarily constrain the abilities for species to coexist (Peterson et al. 1999), and might instead depend more on local and microevolutionary dynamics (Wiens 2004; Siepielski et al. 2018). Alternatively, it may be that niche differences are fixed at the species level, as implicitly assumed in most coexistence models. Evaluating this hypothesis requires that tests for stabilizing effects are replicated in two or more populations for the same pair of species. Therefore, we conducted field experiments designed to detect the demographic signature of stabilizing effects with the same pair of *Enallagma* damselfly species at two locations.

Materials and methods

Study system

Enallagma damselflies are a diverse group of voracious predatory insects found in many lakes and ponds. Several species of *Enallagma* can typically be found co-occurring with one another in small areas (<1 m^2) of littoral habitats with fish predators throughout North America (McPeek 1990, 1998; McPeek and Brown 2000; Siepielski et al. 2010). Larvae inhabit plants, feed on small invertebrates, and are themselves fed upon by larger predators (principally fish) and other odonates. These species experience density-dependent predation and resource competition (damselflies are food limited), and ecological differences that could affect coexistence, such as activity levels, behavioral responses to predators, and prey foraging rates among species are detectable (McPeek 1990, 1998; McPeek et al. 2001; Stoks and McPeek 2006; Siepielski et al. 2011; Siepielski and McPeek 2013). Thus, this is a particularly suitable group of taxa to examine how stabilizing effects might differ between populations.

Experimental design

Because simultaneously manipulating many species in one experimental design would be prohibitive, we chose to include only two: *E. signatum* and *E. vesperum* (Fig. 1). We chose these species for two key reasons: (1) their ranges overlap in much of North America (Westfall and May 2006) (Fig. 2a), and (2) they commonly locally co-occur (McPeek 1990, 1998). Thus, a key strength of our experimental design is that we are able to determine how the strength of niche-based stabilizing effects differ among populations for the same pair of species, removing the possibility that differences observed among locations may be attributed to different species being investigated in different locations.

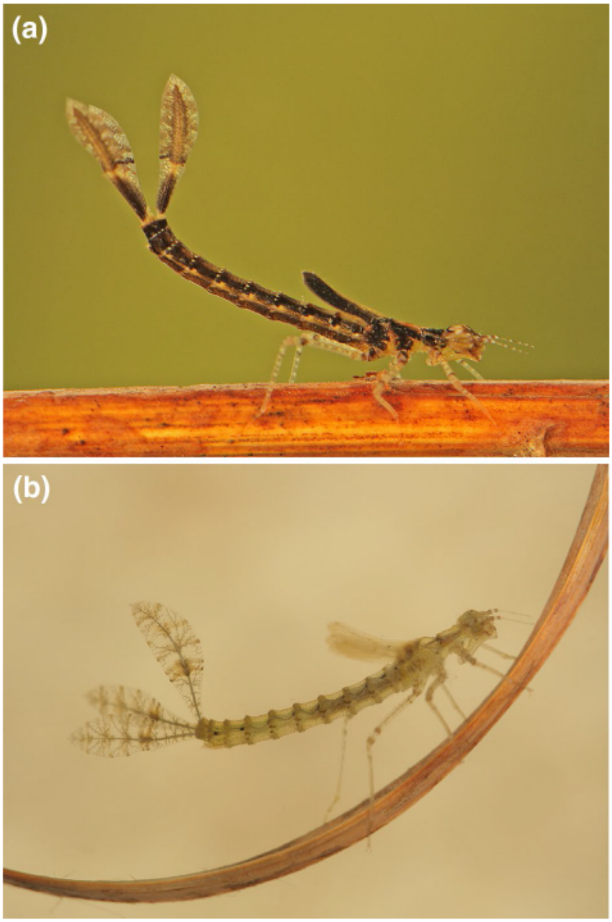

Fig. 1 Photographs of damselflies used in the experimental studies of spatial variation in niche differentiation: **a** *Enallagma signatum* and **b** *E. vesperum*. The length from the head to the end of the abdomen of each species is approximately 25 mm. Photographs by Steve Krotzer

The experiment was performed September–November 2016 at Palmatier Lake (Michigan, USA, 42.572, − 85.434; abbreviated MI), and 6.5° latitude to the south at Lake Wilson (Arkansas, USA, 35.998, − 94.1350; abbreviated AR) (Fig. 2a). These study locations were chosen because from previous sampling and studies (McPeek 1990, 1998; Siepielski unpublished data), we knew the focal taxa were present and sufficiently abundant to conduct the experiments.

As in previous experiments designed to capture the littoral zone environment where larval damselflies locally compete for food resources and attempt to avoid shared predators (McPeek 1990, 1998; Siepielski et al. 2010), we used cylindrical cages (54 cm diameter × 120 cm height) made of chicken-wire frames covered with mosquito netting (0.6 × 1.2 mm mesh). The cages were sealed on the bottom with plastic dishes filled with ~ 2 cm of lake sediment and placed linearly in each lake at a depth of ~ 90 cm with their open tops extending ~ 30 cm out of the water. To provide structure for damselflies, cages were stocked with macrophytes (*Chara* and *Najas*) at natural densities (e.g., at an amount equal to that found in the same area as the bottom of the cages) after odonates and other large invertebrates had been removed from the plants. The cages then stood for 3 days to allow prey to naturally colonize through the netting before treatments were

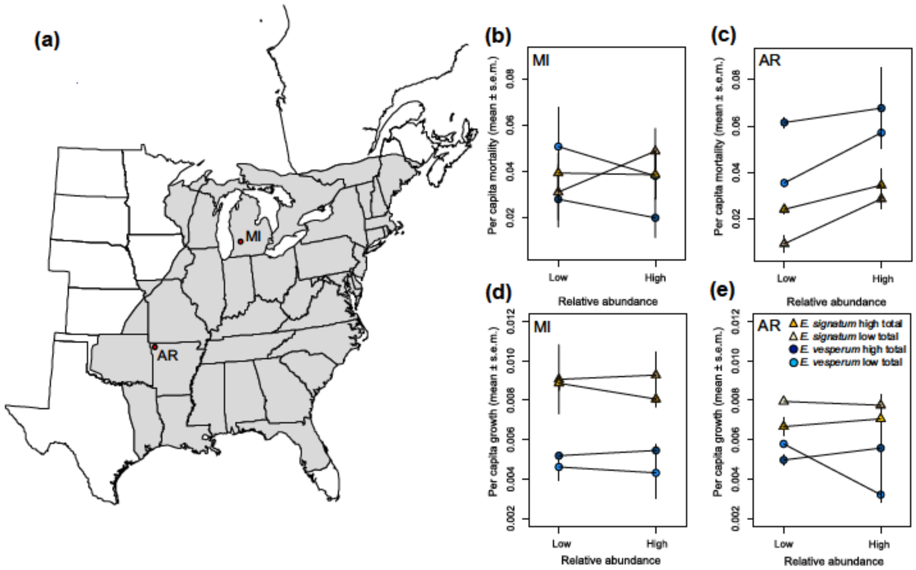

Fig. 2 Experimental evidence that the stabilizing effects of niche differences vary between populations. **a** The overlapping distributions of the two focal damselfly species (in light grey) and locations of experimental lakes (red dots) in Michigan (MI) and Arkansas (AR). No differences in per capita mortality **b** or growth rate **d** at low relative abundance or across total abundance treatments were detected in MI. **c** Per capita mortality rates were significantly lower at low relative abundance in AR in the low total abundance treatment, but not the high total abundance treatment. **e** Per capita growth rates did not differ at low relative abundance or across total abundance treatments in AR. Shown are mean ± standard error (s.e.m., $n=3$ replicates per treatment per location, except as noted in the main text where two cages were damaged); s.e.m.'s are smaller than the size of the symbol for some treatments. (Color figure online)

applied. At the end of the experiment, all cages contained abundant prey (i.e., annelids, copepods, cladocerans, chironomids, and ostracods). Importantly, *Enallagma* mortality and growth rates in these cages are very similar to those in natural populations (McPeek 1990, 2004) implying that they adequately capture the natural environment.

At each location, we manipulated total and relative abundances of *Enallagma* larvae in the same 2 × 2 factorial design, with total abundances of 45 or 135 *Enallagma*/cage, and relative abundance ratios of 23% *E. signatum* to 77% *E. vesperum* and vice versa. Treatments were randomly assigned to cages. Two cages served as controls (to determine if contamination from non-experimental individuals infiltrated cages), and each treatment was replicated three times in each location. Thus, there were a total of 14 cages established per location. Two cages (from different treatment levels) in AR were damaged due to vandalism and excluded from the analyses. Based on previous studies, the relative abundance levels were chosen to ensure adequate numbers of larvae survived to the end of the experiment to estimate per capita growth and mortality rates (Siepielski et al. 2010). The total abundance levels used are within the typical ranges of *Enallagma* in lakes in N. America (McPeek 1990, 1998; Siepielski et al. 2010). Although manipulating relative abundance is key to evaluating stabilizing effects (Chesson 2000b; Adler et al. 2007; Levine et al. 2008), we included the total abundance manipulation because equilibrium abundances are unknown, and because carrying capacities could differ between locations. Thus, by varying total abundance we guarded against the possibility that the relative abundances within these treatment levels consistently resulted in species being above their carrying capacities.

Damselflies were collected from each lake and added in appropriate numbers to the MI lake cages on 13 September 2016 and to the AR lake cages on 7 October 2016, after they were too big to pass through the netting. The experiment began 1 day after those dates when we added one bluegill fish (*Lepomis macrochirus*, ~65 mm in length) to each cage to assess mortality responses under the abundance manipulations. This fish density is within the natural range for lakes found both in MI (Mittelbach 1988) and AR (Siepielski unpublished data). Centrarchid fishes, especially *Lepomis*, are the primary predator of *Enallagma* larvae in the littoral zone throughout N. America (McPeek 1990, 1998; Siepielski et al. 2010). All damselflies, macrophytes, and fish were from their local lake (e.g., no transplants between locations).

The experiment ended 27 October 2016 in MI and 17 November 2016 in AR by removing cages from the lake and carefully sorting the contents to recover surviving damselflies. The duration of this experiment covered much of the larval phase of these species' life cycle, from approximately the fourth to the penultimate instar (*Enallagma* has 11 instars). Head widths of a subset of larvae (29 individuals in AR, 30 individuals in MI) at the start of the experiment, and all surviving larvae, were measured using a dissecting microscope fitted with a micrometer. As in our previous studies (McPeek 1990, 1998; Siepielski et al. 2010), mortality and growth rates for each species were calculated for each cage. Per capita mortality rate was estimated as mortality rate = –(ln(number recovered) – ln(initial number))/duration. Per capita growth rate was estimated as growth rate = (ln(mean head width of recovered individuals) – ln(mean head width of the sub-sample taken at the beginning of the experiment))/duration. This growth rate metric assumes a model of Head width(t) = Head width$(0)e^{(gt)}$, where g is the growth rate and is independent of the initial size of the species (McPeek 1998).

Although we cannot estimate per capita population growth rates directly, both per capita growth and especially per capita mortality, strongly contribute to damselfly population regulation (McPeek and Peckarsky 1998). Previous field experiments have shown that competition and predation impart strong, negative density-dependence on damselfly per capita growth and survival rates in fish lakes (McPeek 1990, 1998). Fish predation accounts for up to 80% of *Enallagma* mortality in fish lakes, and per capita mortality rates due to fish predation generally increase as damselfly abundance increases (McPeek 1990, 1998). Fish-lake *Enallagma* are also food limited and increasing *Enallagma* abundance in the presence or absence of fish decreases their per capita growth rates (McPeek 1990, 1998). Per capita growth rates are also a key vital rate for damselflies, because variation in growth rates determines the duration that larvae are exposed to their main predators (e.g., McPeek and Peckarsky 1998), and only individuals that have grown to a sufficient size can metamorphose into adults, when reproduction occurs. Thus, because predation and competition are the predominant factors affecting demographic rates of damselflies (McPeek 1990, 1998), and directly account for much of their population regulation (McPeek and Peckarsky 1998), we use these two demographic rates for each species as the response variables.

Frequency and density-dependent effects of both resource competition and fish predation can contribute to any growth and mortality responses expressed in this experimental design (Siepielski et al. 2010). For example, predation by fish could reduce damselfly densities and as a result influence the strength of competitive interactions (Chase et al. 2002). Our goal, however, was not to distinguish the relative contributions of growth and mortality rates, but rather to determine whether the species respond differently as their total and relative abundances changed, and if these changes varied among geographic locations.

Statistical methods

If these species experience spatial differences in stabilizing effects, they should exhibit significantly lower per capita mortality and/or higher per capita growth at low relative abundance, and these responses should differ between the two populations. Because both species were present in all cages, the mortality and growth responses are inherently multivariate. Thus, we first used MANOVA models to determine the effects of location, total and relative abundances, as well as interactions between these factors on log transformed per capita growth and mortality rates of both species. When MANOVA models were significant, individual ANOVA models for each species were subsequently used to determine whether any of the experimental treatments (as above) were driven by single species responses. We evaluated model assumptions of normality and heterogeneity of the residual variances, and found no strong patterns in the residuals or clear violations of parametric assumptions in any of the models. To determine if the strength of species interactions varied between species and locations, we used t-tests (Welch's t test where variances were slightly unequal, and paired t tests comparing species within locations because both species were present in each cage) to compare differences in mortality and growth rates between species and geographic locations.

Results

Mortality rates

The MANOVA showed that for both species' per capita mortality rates, there was an effect of location indicating differences in per capita mortality between locations (Table 1); however, the interaction term between relative abundance and location was not significant (Table 1). We suspect that because the effects of relative abundance were subtle (Fig. 2), detecting a significant interaction term suffered from low power, especially given the unfortunate loss of two cages (in separate treatments) because of vandalism. Indeed, a retrospective power analysis of the interaction term between location and relative abundance showed that power to detect this effect was quite low (retrospective power on the observed MANOVA relative abundance x location term, $1-\beta=0.08$). Thus, given our a priori interest in comparing the strength of stabilizing effects between locations, and the graphical analysis suggesting that the effects of relative abundance differed between locations (Fig. 2), we conducted separate MANOVAs for the AR and MI sites. This analysis showed a significant interaction term between total and relative abundances in AR (Table 1, Fig. 2c), but not MI (Table 1, Fig. 2b). That is, the effect of relative abundance on per capita mortality rates depended on total abundance in AR, but not MI.

In AR, for both species, per capita mortality rates were consistently lower at low relative abundance in the low total abundance treatment (MANOVA results in Table 1, Fig. 2c). This effect was not driven by a single species, because individual species ANOVAs showed that per capita mortality rates were 38.6% lower for *E. vesperum* (ANOVA $F_{1,3}=212.92$, $P=0.007$) and 55% lower for *E. signatum* (ANOVA $F_{1,3}=11.38$, $P=0.040$) at low relative abundance in AR (Fig. 2c). Although the per capita mortality rates were also lower at low relative abundance in the high total abundance treatment, this difference was not significant examining both species collectively (MANOVA results in Table 1, Fig. 2c), or individually

Table 1 Results from the MANOVA models comparing *Enallagma signatum* and *E. vesperum* per capita mortality and growth rates

Term	Per capita mortality				Per capita growth			
	Wilk's λ	F	df	P	Wilks λ	F	df	P
Overall model								
Total abundance	0.968	0.214	2, 13	0.81	0.893	0.59	2, 13	0.57
Relative abundance	0.955	0.306	2, 13	0.741	0.984	0.08	2, 13	0.924
Location	0.489	6.782	2, 13	0.009	0.937	0.33	2, 13	0.723
Total abundance × relative abundance	0.965	0.231	2, 13	0.796	0.851	0.87	2, 13	0.447
Total abundance × location	0.881	0.872	2, 13	0.441	0.897	0.57	2, 13	0.581
Relative abundance × location	0.961	0.259	2, 13	0.775	0.94	0.31	2, 13	0.737
Total abundance × relative abundance × location	0.917	0.281	2, 13	0.572	0.829	1.03	2, 13	0.393
Arkansas model								
Total abundance × relative abundance	0.274	6.61	2, 5	0.039				
Relative abundance at low total abundance	0.005	213.2	2, 2	0.005				
Relative abundance at high total abundance	0.204	3.89	2, 2	0.204				
Michigan model								
Total abundance	0.786	0.948	2, 7	0.432				
Relative abundance	0.927	0.276	2, 7	0.767				
Total abundance × relative abundance	0.865	0.542	2, 7	0.604				

The overall model had a significant location effect, thus separate MANOVA's were performed for AR and MI. Because the interaction term between total abundance × relative abundance effect was significant in AR, MANOVA's were performed separately for each total abundance

(*E. vesperum* ANOVA $F_{1,3}=0.100$, $P=0.772$; *E. signatum* ANOVA $F_{1,3}=3.75$, $P=0.148$). Neither total nor relative abundance affected per capita mortality rates in MI (Table 1). These results suggest stabilizing effects through survivorship are present for both species in AR, but not MI.

These species also differed in their per capita mortality rates between locations. Across relative and total abundance treatments, *E. vesperum* per capita mortality rates were about 50% greater than those of *E. signatum* (paired $t_{1,9}=7.67$, $P<0.0001$; Fig. 2c) in AR, with no differences in per capita mortality rates between the species in MI (paired $t_{1,11}=-0.89$, $P=0.389$; Fig. 2b). Per capita mortality rates were also higher for *E. vesperum* in AR relative to MI (Welch's $t_{1,19.14}=2.86$, $P=0.010$), but the opposite was true for *E. signatum* (Welch's $t_{1,18.72}=-2.57$, $P=0.010$; Fig. 2). The magnitude of per capita mortality thus differs among species, but these differences vary spatially.

Growth rates

The MANOVA showed that for both species' per capita growth rates, the interaction term between relative abundance and geographic location was not significant (Table 1). Similarly, there were no statistically significant effects of total or relative abundance (Table 1; Fig. 2d, e). Unlike the above analysis with mortality rates, because there was no effect of location we did not further pursue the breakdown of the MANOVA by location. Thus, no evidence of stabilizing effects through possible resource competition was evident for either species in both locations.

Across relative and total abundance treatments, per capita growth rates of *E. vesperum* were about 28% lower than those of *E. signatum* in AR (paired $t_{1,7} = -3.77$, $P = 0.007$; Fig. 2e) and about 44% lower in MI (paired $t_{1,10} = -7.13$, $P < 0.0001$; Fig. 2d). Per capita growth rates for *E. vesperum* did not differ between AR and MI (Welch's $t_{1,10.05} = 0.25$, $P = 0.805$), but for *E. signatum* they were lower in AR relative to MI (Welch's $t_{1,16.99} = -2.67$, $P = 0.016$; Fig. 2). As with mortality, per capita growth rates differ among species as well as spatially in one species.

Discussion

Both species experienced lower per capita mortality rates when rare in one location (AR) but not the other (MI), supporting the hypothesis that the stabilizing effects of ecological niche differences promoting coexistence differ among populations. This is a key finding because it suggests that population-level, not fixed species-level, comparisons are necessary to understand if local species diversity is being maintained. Had we only conducted this study in MI, we would have concluded that niche-based mechanisms are not maintaining damselfly diversity. Indeed, we previously reached this conclusion in an earlier study comparing two other *Enallagma* species (Siepielski et al. 2010). Likewise, had we only conducted this study in AR, we would have concluded that niche-based mechanisms are always important. These results therefore demonstrate that conclusions reached on the mechanisms promoting coexistence can benefit from considering population-level processes in a geographic context (Chesson 2000a; Hart et al. 2017). Below, we discuss several factors that could contribute to the patterns we observed. We focus on the ideas that either (1) niche differentiation between species evolves locally, or that (2) spatial variation in environmental factors affects the manifestation of species' niche differences.

Our finding of population-level differences in stabilizing niche effects is consistent with recent studies that have investigated spatial variation in local species coexistence. For example, LaManna et al. (2017) found that the strength of conspecific negative density-dependence relative to heterospecific negative density-dependence in forest plants increased with decreasing latitude. Similarly, Usinowicz et al. (2017) found that geographic variation in the storage effect caused the ratio of interspecific relative to intraspecific competition in forest plants to vary among locations. Bracewell et al. (2017) showed that the competition-colonization tradeoff, a mechanism promoting species coexistence, varied spatially in sessile marine invertebrates. All of these studies, however, compared different groups of species in different locations. Our study avoided this complicating issue, strengthening our argument that the propensity of species to exhibit stabilizing effects is likely not a fixed property of species.

Our results suggest that spatially varying ecological differences affect whether stabilizing effects operate to facilitate local coexistence, and we offer two explanations. First, it may be that local adaptive niche evolution in *E. signatum* and *E. vesperum* has occurred, allowing them to differ ecologically among populations in ways that promote their coexistence (Lankau 2011; Bassar et al. 2017; Germain et al. 2017; Siepielski et al. 2018). Although most studies of niche-based mechanisms of coexistence have emphasized a role for resource competition, predation can also play an important role (Caswell 1978; Vance 1978; Chase et al. 2002; Chesson and Kuang 2008). Finding significant mortality and no detectable growth responses suggests *E. signatum* and *E. vesperum* largely differ in ways promoting survival with fish predators, rather than differentiation to moderate resource competition. It may be that in AR *E. signatum* and *E. vesperum* have evolved different behavioral responses (e.g., hiding tactics or reducing activity levels) to the threat of predation such that fish develop different search images depending on the abundances of each species (Martin 1988). Such a mechanism would readily work in a frequency-dependent manner to reduce damselfly mortality (Vance 1978; Allen 1988), and negative frequency dependence is often important in maintaining diversity in insects subject to predation (Nosil et al. 2018). Although we lack a common-garden approach, which is ultimately necessary to determine if the difference in stabilizing effects between locations is driven by local predator driven niche evolution, traits mediating predator defenses in damselflies (e.g., behavioral responses such as reducing activity) are heritable and frequently under selection (Shama et al. 2011; Strobbe et al. 2011; Siepielski et al. 2014; Swaegers et al. 2017; Ousterhout et al. 2018), often leading to local adaptation (Siepielski et al. 2016).

Local divergence of *E. signatum* and *E.vesperum* in phenotypes to reduce competition, such as utilization of alternative prey resources, seems unlikely as we detected no increased per capita growth rate at low relative abundance. However, our experiment was not designed to parse out the relative contributions of mortality and growth as we lacked a no predator treatment. Previous studies have shown that damselflies are generalist predators and typically consume prey in proportion to their local abundance (Thompson 1978; Corbet 1999). However, these latter inferences are from studies in a single location, which precluded the potential to investigate spatial variation in prey specialization. The number of possible prey species available to *Enallagma* is probably in the hundreds, if not thousands, in the littoral zone where *Enallagma* dwell (Thorp and Covich 2009). Thus, despite an astounding diversity of prey available for consumption by these species, there seem to be few realized opportunities for resource specialization (McPeek 2017). Such a scenario matches that proposed by Meyer and Kassen (2007), who suggested that predation can drive niche divergence when resource specialization is unlikely, although future studies further investigating this possibility are necessary.

An alternative, although not mutually exclusive, explanation for spatial variation in the occurrence of stabilizing effects is that the phenotypes shaping the ecological differences that promote this niche differentiation are fixed at the species level. That is, the species have not diverged between these two locations in the phenotypes shaping their niches. Rather, the ecological opportunity for these fixed niche differences to be expressed simply varies among locations. For example, in MI, the fish predator may not recognize or respond to differences in *Enallagma* traits (e.g., coloration or activity levels) that differentially affect their susceptibility to predation. Differences in prey availability could also affect activity levels of damselflies between these two locations, and thus influence their susceptibility to predation. The importance of ecological opportunity for promoting niche differentiation is exemplified by comparative studies that have shown repeated instances of evolution of parallel niche differences when similar ecological opportunities exist (Schluter 2000; Stroud and Losos 2016; McPeek 2017). Disentangling these various possibilities will require understanding when and where niche differences have evolved. This would involve mapping the evolution of the traits that generate niche differences to the location and timing of population differentiation, a goal we are pursuing.

Despite finding that stabilizing niche effects (as revealed through differences in survivorship in response to relative abundance manipulations) varied spatially, we found no evidence that the strength of species interactions (e.g., per capita mortality and growth) covaried with the strength of niche differences. Instead, we found substantial differences in these demographic rates between the two species among these locations. Per capita growth rates were lower for *E. signatum* in AR than MI, but did not differ for *E. vesperum*. By contrast, per capita mortality rates were higher for *E. vesperum* in AR relative to MI, but were lower for *E. signatum* in MI relative to AR. Overall, though, *E. signatum* experienced both lower mortality and higher growth than *E. vesperum* at the AR location. In the absence of any stabilizing effects, such exaggerated asymmetries in these vital rates would likely lead to the local loss of *E. vesperum*. Consequently, the stronger stabilizing effects we found in the AR site are likely critical in maintaining these species. Whether or not the observed strength of stabilizing effects in AR is sufficient to overcome the differences in mortality rates between these two species to yield stable coexistence cannot be ascertained. Determining this would require estimating the extent of fitness differences (inequalities in per capita population growth rates when the effects of stabilizing effects are absent) and then evaluating if the observed stabilizing effects are strong enough to overcome the estimated fitness differences between these species (Chesson 2000a, b).

The absence of stabilizing effects in the MI location suggests that *E. signatum* and *E. vesperum* there are prone to ecological drift (Hubbell 2001; Siepielski et al. 2010). Should one species become extremely rare, it would gain no demographic advantages (e.g., reduced mortality or increased growth) that would rescue it from local extirpation, and when combined with demographic stochasticity the species could eventually be lost (Hubbell 2001; Siepielski et al. 2010, 2018; Haney et al. 2015; McPeek 2017; Svensson et al. 2018). Dispersal during the adult life stage from nearby lakes could potentially allow these species to persist for some time, but then any co-occurrence is not because of niche differentiation and would instead reflect mass effects within a metacommunity (Shmida and Ellner 1984; Leibold and Chase 2017). Even low levels of dispersal, which is typical of *Enallagma* and of damselflies in general (McPeek 1989), could greatly slow the loss of any species. Overall, given these observed patterns, the observed strength of species interactions per se does not seem to be a critical factor promoting niche differentiation between these locations. Thus, it remains unclear the extent to which stronger species interactions necessarily lead to stronger niche differentiation.

Our study focused on only a single pair of species within a single genus, yet within these lakes are other *Enallagma* species and damselfly genera (especially *Ischnura* and *Lestes*), as well as other mid-level consumers that play similar functional roles (Wellborn et al. 1996; McPeek 1998; Stoks and McPeek 2006). However, we can develop some insights about the mechanisms regulating potential coexistence among this diverse group of consumers. Our previous studies have shown that stabilizing niche effects promote coexistence among *Enallagma*, *Ischnura*, and *Lestes*, with each genus acting as a distinct functional group regulated by different ecological factors (Siepielski et al. 2011). Large-bodied *Lestes* consume larger prey items than small-bodied *Enallagma* and *Ischnura*, which are of similar size and feed on similar prey items (Siepielski et al. 2011). Coexistence between *Enallagma* and *Ischnura* arises instead via a tradeoff between growth and predation from fish, as predicted by the keystone predation model (Holt and Lawton 1994; Leibold 1996; McPeek 1996, 2004; Siepielski et al. 2010). Other potential intraguild predators (e.g., dragonflies, newts, hemipterans, dytiscids), although absent from our experiments, do not appear to strongly affect damselfly mortality rates in lakes with fish (McPeek 1998), although the importance of any such mortality may depend on the size structure of these interacting species, especially among odonates (Wissinger 1992). However, these other guild members likely compete for prey resources and may generate feeding interference (e.g., McPeek 1998). This full suite of species undoubtedly shapes the competitive landscape, and it may very well be that complex interactions among these diverse community members via interaction chains and higher order interactions are critical (Rudolf 2007; Levine et al. 2017).

The maintenance of species diversity in biological communities is a vexing problem. Our results suggest that this problem is even more nuanced by finding that a key factor maintaining species diversity—stabilizing niche effects—differs spatially among populations. We therefore argue that the problem of 'species coexistence' is perhaps not really a problem of species, but rather is one of understanding if populations of species coexist. Ecological differences are rarely fixed at the species level, and evolutionary change principally occurs at the population level (Thompson 2005). As a result, this potential evolutionary malleability of populations may be critical in shaping species diversity across the landscape. We simply should not expect that species will uniformly differ ecologically across their ranges in ways that promote or constrain their abilities to coexist.

Acknowledgements We thank R. Bassar, J. Beaulieu, R. Germain, J. P. Lessard, B. Ousterhout, J. Usinowicz, and several anonymous reviewers from previous submissions for thoughtful comments on earlier versions. Conversations with Mark McPeek were especially insightful on the ideas presented here. We also thank Gary Mittelbach and Kellogg Biological Station for allowing access to their facilities. Earl Werner kindly granted us access to Lake Palmatier. M. Chrietzberg, S. Graham, A. Hasik, A. Heffington, B. Ousterhout, A. E. Siepielski, L. Siepielski, J. Senn, and M. Serrano helped with picking bugs and building experimental cages. This work was supported by NSF (DEB 1620046, 1748945) awarded to AMS.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Data accessibility All data will be deposited at Datadryad.

Ethical statement We adhered to all animal care guidelines pertaining to the use of fish in our experiments as set forth under approval of the University of Arkansas IACUC (#17059).

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