

# Signature of ecological partitioning in the maintenance of damselfly diversity

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## Summary

1. Ecological differences among co-occurring taxa are often invoked as an explanation for the maintenance of biodiversity. Whether these differences facilitate coexistence, which allows unequal competitors to remain in systems and thus maintain biodiversity, is still unclear.

2. Here, we used observational and experimental studies to test for ecological partitioning in ways that would promote coexistence among three co-occurring damselfly genera. We evaluated two necessary conditions for coexistence: (i) that the damselfly genera differ in their abilities to engage in interactions with other damselfly genera and environmental conditions such that their relative abundances covary differently along environmental gradients and (ii) that an increase in intra-generic abundance is more detrimental to performance-related demographic features of each genus than increases in intergeneric abundances.

3. Observational studies across 40 lakes showed that relative abundances of each genus covaried differently along an environmental gradient of lake abiotic and biotic features consistent with ecological partitioning. Field experiments in which we manipulated both intra- and intergeneric densities demonstrated that per capita growth rates of each genus are negatively density-dependent and are only limited by increases in intra- not intergeneric densities.

4. Collectively, these results show a clear signature of ecological partitioning among each genus, which should prevent competitive exclusion and maintain each genus in this system. The results do not guarantee local coexistence among the three genera but are consistent with criteria that should promote their coexistence. Our results also suggest that a food web model coupling keystone predation and apparent competition is likely necessary to explain the ecological dynamics of persistence among these genera.

**Key-words:** coexistence, competition, food web, species diversity, stabilizing effect

## Introduction

Disentangling the factors that structure and maintain biodiversity in ecological communities is an enduring problem, as many ecosystems harbour spectacular levels of local diversity. Ecological differences among species within local communities are often invoked as an explanation for the maintenance of biodiversity (MacArthur 1972; Chesson 2000; Chase & Leibold 2003). However, species cannot merely be different from one another to promote diversity maintenance. Rather, the ecological differences among species must be translated into differences in the demographic performances of species in relation to how they interact with the environment (Chesson 2000; Leibold & McPeck 2006). Specifically, species must (i) differ in ways that make them differentially successful in engaging in interactions with other species and dealing with abiotic factors (e.g. acquiring

resources, avoiding predators, combating diseases and interacting with mutualists), such that (ii) each species is limited more by their own abundance than by the abundances of other similar species within the same trophic level (MacArthur 1972; Chesson 2000). These ecological differences need not operate at the species level, as it has become apparent that such differences are not always manifested with such phylogenetic affinities (e.g. Siepielski & McPeck 2010).

Indeed, one of the central conceptual tenants of community ecology is that ecological differences among species in local assemblages promote their long-term coexistence (Hutchinson 1959; MacArthur 1972; Chesson 2000, 2008; Chase & Leibold 2003). Yet despite its prominence and widespread acceptance as an important mechanism maintaining biodiversity, empirical evidence for coexistence is surprisingly scarce (Siepielski & McPeck 2010). One reason for the lack of empirical evidence is that demonstrating the necessary and sufficient conditions for local coexistence is logistically infeasible in many systems and will generally require a

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number of coordinated and complementary studies (Chesson 2000, 2008; Adler, HilleRisLambers & Levine 2007; Adler, Ellner & Levine 2010; Siepielski & McPeck 2010). The ultimate test that taxa are locally coexisting is a series of 'invasibility' experiments in which each species is systematically removed from the community and then reintroduced; if each species can increase when rare, the collection of species is coexisting (MacArthur 1972; Chesson 2000; Chase & Leibold 2003; Leibold & McPeck 2006; Adler, HilleRisLambers & Levine 2007; Levine & Hille Ris Lambers 2009; Adler, Ellner & Levine 2010; Siepielski & McPeck 2010).

In lieu of direct tests of invasibility, we can, however, focus on testing some of the necessary conditions for coexistence (Siepielski & McPeck 2010). As noted earlier, two necessary conditions are (i) species differ in their abilities to engage in interactions with other species and abiotic factors and (ii) an increase in intraspecific abundance is more detrimental to the demographic rates of each species (i.e. survival, growth and fecundity) than increases in interspecific abundances (MacArthur 1972; Chesson 2000). If these conditions are not met, then coexistence (i.e. reciprocal invasibility) is impossible. Thus, although indirect, evaluating these conditions is, nevertheless, important for understanding how ecological differences combine to maintain biodiversity.

The first of these conditions implies ecological partitioning by species such that each is regulated by different relative combinations of interactions with other species and abiotic factors. A test of this condition is to evaluate whether species' relative abundances and demographic rates covary along environmental gradients (Chesson 2000; Siepielski & McPeck 2010). For example, the relative abundances of resource competitors should change predictably along supply gradients of their limiting resources (e.g. Tilman 1982), or the relative abundances of 'apparent' competitors should covary along a gradient of their shared predator's abundance (e.g. Holt 1977). Identifying the ecological gradients along which relative abundances and demographic rates covary will provide essential insights to identify the possible mechanisms of coexistence.

The second condition implies that ecological partitioning, if present, maintains species diversity by causing the demographic performance of each species to be limited more by the ecological consequences of increasing intraspecific abundance than by the consequences of increasing the abundances of other species (so-called stabilizing effects; Chesson 2000). If fitness differences among species are not too great, this self-limitation prevents competitive exclusion. Thus, a demographic signature of ecological partitioning that would facilitate, but not guarantee coexistence, is that increases in intraspecific abundance should be more detrimental to the survival, growth and fecundity of each species than increases in interspecific abundances (Chesson 2000). This is an important test because such differences must be apparent, regardless of the specific coexistence mechanism operating in the system (Levine & Hille Ris Lambers 2009).

Here, we combine observational and experimental studies to evaluate these two conditions for damselflies that are

found living together in eastern North American ponds and lakes that support fish as top predators. Three genera in two families of damselflies (Odonata: Zygoptera) are numerically dominant in these lakes: *Enallagma* and *Ischnura* species in the Coenagrionidae and *Lestes* in the Lestidae. Typically, 5–12 of *Enallagma*, 2–3 of *Ischnura* and 1–2 of *Lestes* can be found together in the littoral zones of lakes across eastern North America, and all can be found together in very close proximity (Johnson & Crowley 1980; McPeck 1998; Stoks & McPeck 2003). For example, a single 1-m-long net sweep through the littoral zone will frequently result in a sample containing multiple species of all three of these genera. All these species are common mid-trophic-level consumers, where they spend 10–11 months as aquatic larvae, are fed upon by fish predators and feed on a variety of smaller invertebrate prey (McPeck 1998; Westfall & May 2004; Stoks & McPeck 2006). Our previous studies indicated that species within the genus *Enallagma* are in fact not coexisting with one another, but rather are ecologically equivalent to one another, so that the total abundance of all individuals of all *Enallagma* is the regulated group (a functional group), not each individual species (e.g. ecological drift; Siepielski *et al.* 2010). Given these results for species within one genus, in this paper, we present the results of a study to examine whether these genera are ecologically differentiated in ways necessary for their coexistence.

To do this, we conducted a large-scale observational study across 40 lakes to examine whether relative abundances of the genera vary differently across an environmental gradient of lake abiotic and biotic features. We also experimentally tested for stabilizing effects among the three genera by manipulating intra- and interspecific densities to determine whether the different genera are more strongly affected by their own abundance than the abundances of other species. In this experiment, we specifically focused on the potential role of differential resource use, which would be reflected in per capita growth rates, because resources are limiting to the growth of damselflies in fish lakes and our previous studies have already focused on a role for predation (e.g. McPeck 1998; Stoks & McPeck 2006). Growth rates are also a key vital rate for damselflies, because variation in growth rates will determine the length of time larvae are exposed to their predators (e.g. McPeck & Peckarsky 1998), and only individuals that have grown to a sufficient size can metamorphose into adults, when reproduction occurs.

## Materials and methods

### RELATIVE ABUNDANCE ALONG AN ENVIRONMENTAL GRADIENT

We conducted an observational study to explore how damselfly relative abundances varied across environmental gradients. Our approach here was to broadly estimate values for most of the trophic levels in the littoral zone food web of each lake, as variation in environmental features within and across trophic levels can either directly or indirectly affect species demographic performance and thus their relative abundances (e.g. Chesson & Huntly 1997; Chesson 2000).

### Study sites

We conducted this study at 40 ponds and lakes throughout New Hampshire and Vermont, USA, during 2008 and 2009 (Table S1, Supporting information). Water bodies were chosen based on the following criteria: (i) easy access, (ii) the presence of fish as the top predators in the system (i.e. no lakes lacking fish were included) and (iii) a well-developed littoral zone, because damselfly larvae are found clinging to the plants in the littoral zone.

### Water chemistry

Water chemistry could be an important factor influencing damselfly relative abundances because various attributes of water chemistry either directly or indirectly determine the flow of nutrients, oxygen and other potentially important abiotic factors into damselflies themselves or their predators or prey within the food web. During August, we measured several water chemistry attributes. We used hand-held probes to record dissolved O<sub>2</sub> (%), conductivity (µs) (model YSI 85 handheld probe; YSI incorporated, Yellow Springs, OH, USA) and pH (Milwaukee model SM102 handheld probe; Milwaukee Instruments incorporated, Rock Mount, NC, USA) of littoral water from three locations spaced 20 m apart within each lake (means were used in the analyses). Total nitrogen and total phosphorus of lake water were estimated using standard methods following persulfate digestion. Particulate carbon was estimated using a gravimetric approach, based on filtering 500–1000 mL of littoral lake water through glass filters (method described later as for quantifying productivity).

### Lake productivity

We quantified the net primary productivity of the littoral food web of each lake by estimating the growth rates (mg C day<sup>-1</sup>) of attached algae on clay tiles. We placed a small floating plexiglass rack (57 × 42 × 19 cm) with 12 unglazed clay tiles in the littoral zone of each lake in early September. The rack held the tiles 19 cm below the water's surface and maintained them unshaded. The rack was housed in an enclosure made of fine mesh netting (0.1 mm mesh) that excluded all but the smallest grazers but permitted water movement. Every 3 days for 12 days, two tiles were chosen at random and removed from the grid. The upper surface was scrubbed with a toothbrush to remove all attached algae and other microbes. Although some particulate organic matter from the water column likely settled on the tiles, we suspect most organic matter was from growth on the tiles, as this settling organic matter would have washed off as the tiles were removed. The removed material was then filtered through glass fibre filters (47 mm; Pall Corporation, Ann Arbor, MI, USA), and the ash-free dry mass (AFDM) of the removed sample was determined. AFDM was estimated as the difference between the combined weight of the filter and filtrate before and after combustion at 500 °C. We then regressed AFDM against sampling date; productivity was defined as the slope of this regression in the initial linear portion of the curve (typically the first three sampling dates plus zero on day 0). We also estimated chlorophyll-A in the water column by filtering 250–500 mL of littoral lake water across glass filters; the filters were then left immersed and undisturbed for 24 h at 4 °C in methanol to extract chlorophyll-A. We then used a fluorometer (model TD 700; Turner Designs, Sunnyvale, CA, USA) to record the fluorescence of the chlorophyll-A extractant; raw fluorescence values were then converted to estimates of µg/L of chlorophyll-A using the standard Porra equations.

### Fish and other predator densities

Fish and, to a lesser extent, newts (*Notophthalmus viridescens*) are the main vertebrate predators of damselflies in lakes with fish (Blois-Heulin *et al.* 1990; McPeck 1998). During July–September, we estimated the densities and diversity of these predators in each lake by seining. At each lake, we took four standardized seine hauls (using a 4.5 m × 1.5 m beach seine, with 5-mm mesh) through the major vegetation types constituting the littoral zone of each lake. Sampling locations were at least 10 m apart (means were used in the analyses). All seined fish were counted, identified to species and immediately returned alive to the lake.

### Macrophyte density

Macrophytes provide cover and are not only the main foraging surface for damselfly larvae (Crowley & Johnson 1992) but also the primary oviposition site for adult damselflies (Butler & deMaynadier 2008). During July–September, we quantified macrophyte density and species composition in 0.5 m × 0.5 m quadrats randomly placed along transects through the littoral zone of each lake. Within each quadrat, all species were counted and identified to genus or species. The length of transects and the number of quadrats depended on the extent of the littoral zone in each lake and consisted of between 10 and 20 replicates. Because individual macrophyte species differ in a number of features (shape and surface area), this measure provides only an index of overall structural habitat for damselflies.

### Damselfly prey abundance

During July–early September, damselfly prey abundances in each lake were quantified by taking 10 replicate samples with a 6-L box sampler (100-µm mesh) (Downing 1986) (means were used in the analyses). All sampling for a given lake was completed on a single day. Although it would have been ideal for all sampling to have been completed in a shorter window of time to avoid any potential seasonal effects, this was not logistically possible. However, we note that no obvious seasonal trends were present as we found no correlation between the sample date and the indexes of prey abundance we describe below (for prey PCI  $r_s = -0.231$ ,  $P = 0.152$ ; for prey PC2  $r_s = -0.156$ ,  $P = 0.336$ ). The sampler was placed over the macrophytes (e.g. where damselflies forage), and the invertebrates were trapped in the sampler. Prey sampling locations were stratified among the various macrophyte types present in each lake, based on the macrophyte sampling. Captured animals were washed into bottles and preserved in 70% ethanol. Samples were sorted in the laboratory, and all captured prey were identified to the lowest possible taxonomic unit (family in most cases).

### Damselfly abundances

During September, we sampled damselfly larvae to estimate their densities and relative abundances. We also recorded the abundances of other large odonates (e.g. dragonflies) captured in this sampling method. We sampled damselfly larvae using standardized 1-m-long sweeps with a D-frame dip-net (28-cm net opening, 1 × 1 mm mesh) at a depth between 0.25 and 0.50 m; this method gives highly repeatable estimates of odonate abundances (Crowley & Johnson 1992; Stoks & McPeck 2003). On each sampling date, we took 10 replicate sweeps from throughout the macrophytes within the littoral zone of each lake. All captured animals were preserved in 70% ethanol. Because relative abundances of damselflies are highly consistent across

years (Crowley & Johnson 1992), this should not confound year and space effects, and the aforementioned environmental parameters measured in 1 year should be representative of the ecological conditions potentially affecting damselfly abundances in that lake.

### Analysis

We performed two related analyses. First, we examined whether densities among any of the genera across lakes were positively correlated. Given that lakes exhibit environmental heterogeneity, if different environmental features regulate populations of the different genera, positive correlations should be absent. This is an important test, because it does not require that important environmental features be measured, but it does assume that we sampled along a sufficiently wide gradient of lake environmental features, and that differences in environmental features between lakes are greater than variation within lakes. Second, to evaluate how relative abundances covaried with environmental features, we examined simple pairwise correlations between the relative abundance of each genus and lake features and damselfly prey abundances. While this is an unsophisticated analysis, it is a simple and direct one. We also used linear regression to evaluate second-order terms and simple graphical analyses, because species could perform best at intermediate values and not simply positively or negatively covary with different environmental features. However, we found no consistent patterns and so do not report the results of those analyses here. To reduce the overall dimensionality of the environmental variable and prey abundance data sets, we also conducted principal component (PC) analyses on each of these data sets separately. We used the correlation matrix of the environmental data set for the first PCA because the data were of variable units of measure. In the second PCA, we combined all taxa that were potential prey of damselflies (based on body size and their natural histories) and conducted the analysis on the covariance matrix because the data were all counts on similar ranges. In all of the aforementioned correlational analyses, we used Spearman rank correlations, because some variables were non-normally distributed.

### EXPERIMENTAL TEST OF STABILIZING EFFECTS

We used a modified target-neighbour design experiment (e.g. Goldberg & Werner 1983) to test whether manipulations of intraspecific density were more detrimental to per capita growth rates than manipulations of interspecific density in representative species of the three damselfly genera. Importantly, previous experiments have shown that the growth rates of damselflies are resource limited in fish lakes (McPeck 1998). In this experiment, we used *E. vesperum*, *I. verticalis* and *L. vigilax* as representative of each genus. All of these species are common in permanent lakes with fish throughout the study region.

The experiment was performed September–October 2009 at McDaniel's Marsh (Enfield, New Hampshire, USA 43°31'40"N, 72°04'40"W), where we placed wire cages stocked with macrophytes (*Chara vulgaris*) and appropriate densities of *Enallagma*, *Ischnura* and *Lestes* larvae from the littoral zone of this lake. The cages were 1.2-m high × 0.3-m-diameter cylinders made of poultry netting covered with 1.2-mm mesh nylon netting; this allowed all naturally occurring prey to readily colonize the experimental cages and all cages to experience similar local conditions (e.g. water chemistry and temperature). Larvae of each of these three genera are typically found together in areas much smaller than these experimental cages. The cages were closed at the bottom with a plastic dish, extended out of the water, and were open above. Cages were placed in c. 0.75-m-deep water in the littoral zone of the lake.

In our experimental design, every cage had all three species present, two at low abundance and the third at a higher abundance. Specifically, we placed six individuals of each of two 'target' species in each cage, and we placed either 12, 20, 40 or 60 individuals of the third 'neighbour' species. We also added a treatment to this design, in which six individuals of each of the three species were present. This range of densities was chosen because it encompasses and exceeds the natural densities of damselflies commonly found in lakes in this region, which should facilitate detecting competitive interactions. The full design included gradients, so that each species was a neighbour species. Thus, one set of cages had *Enallagma* as the neighbour, a second set had *Ischnura* as the neighbour, and a third had *Lestes* as the neighbour. This design manipulates each species across an intraspecific abundance gradient, while holding the other two species at low abundances along each species' abundance gradient. The 12 treatments with the neighbour species at abundances of 12–60 individuals (i.e. 3 species × 4 neighbour abundances) were each replicated twice, and the one treatment with each species at an abundance of six larvae per cage was replicated four times. Two other cages had no larvae added at the start of the experiment and served as controls to determine whether non-experimental animals infiltrated cages. This gave a total of 30 cages in the experiment.

We used per capita growth rates as a measure of performance, which likely has demographic consequences, as growth rates generally reflect the abilities of organisms to effectively use environmental resources (e.g. prey) and are an important component of fitness. Per capita growth rates were calculated as:  $[(\text{mean } \ln L \text{ of recovered larvae}) - (\text{mean } \ln L \text{ of larvae in initial sample})] / (\text{duration of experiment in days})$  and is expressed in units of  $\text{day}^{-1}$ , where  $L$  is body length. Body length is highly correlated with body mass in damselflies (typically  $r^2 \approx 0.95$  for regressions of dry mass on body length; McPeck 2004). This metric assumes a growth model of  $L(t) = L(0) \exp(gt)$ , where  $g$  is the growth rate and is independent of the initial sizes of species.

To examine whether per capita growth rates differed generally among the three species, we used an ANOVA model with the per capita growth rate as the response and species as the explanatory terms; no terms for densities were included in this model. To examine how each species' per capita growth rate varied in response to intraspecific and interspecific densities, we constructed ANOVA models with a given species per capita growth rate as the response and the target density treatments of the three different species as explanatory variables. In cages (two *Enallagma* treatments) where a negative growth rate was estimated (i.e. the initial sample length was larger than the recovered larvae, presumably owing to contamination by very small larvae), these estimates were excluded. All analyses were performed in SAS (V9.1 SAS Institute, Cary, NC, USA).

We specifically excluded fish or other possible predators from this experiment design. Changes in abundance and growth rates caused by fish predation in cages would greatly obscure our ability to detect potential differences among the genera in their responses to intraspecific and interspecific competition (McPeck 1990, 1998). We already know from previous studies that *Ischnura* species experience significantly higher per capita mortality from fish predation than do *Enallagma* and *Lestes* species, but *Enallagma* and *Lestes* species experience similar levels of per capita mortality from fish predation (cf. mortality rates in McPeck 1990, 1998; Stoks & McPeck 2003). Also, the various fish species found in these lakes have similar preferences and biases in their abilities to capture and consume different damselfly species (McPeck 1990). In addition, among fish-lake damselflies, other potential predators (e.g. dragonflies, newts, hemipterans and

dytiscids) do not significantly affect damselfly mortality rates (e.g. McPeck 1998).

## Results

### RELATIVE ABUNDANCE ALONG AN ENVIRONMENTAL GRADIENT

The genera differed greatly in their densities among lakes (generalized linear model:  $\chi^2 = 91.68$ , d.f. = 2,  $P < 0.0001$ ). The rank order of densities (mean number of individuals per 1-m sweep) was *Ischnura* (mean =  $7.96 \pm 1.10$  [SE]) > *Enallagma* ( $3.45 \pm 0.54$ ) > *Lestes* ( $0.37 \pm 0.12$ ). In addition, the densities of the different genera among lakes were uncorrelated: *Ischnura* vs. *Enallagma* ( $r_s = 0.061$ ,  $P = 0.708$ ), *Lestes* vs. *Ischnura* ( $r_s = 0.241$ ,  $P = 0.133$ ) and *Enallagma* vs. *Lestes* ( $r_s = 0.292$ ,  $P = 0.068$ ). The slight but non-significant positive correlation for *Enallagma* and *Lestes* is largely driven by one site (Russell Pond, NH), which when removed weakens the positive trend (correlation with outlier removed,  $r_s = 0.234$ ,  $P = 0.15$ ).

The 40 lakes differed considerably in environmental features (Fig. 1a, Table S2, Supporting information). Overall, the environmental PCA captured variation mainly along lake water chemistry factors. The first PC explained 29% of the total variation, with increasing values of PC1 meaning higher water pH, conductivity, oxygen and greater productivity. The second PC explained about 16% of the total variation, with increasing values of PC2 meaning lakes with more particulate C, nitrogen, phosphorus and chlorophyll-A (Fig. 1a; Table S2, Supporting information).

The relative abundances of the damselfly genera showed different patterns of variation along these lake environmental features (Fig. 1). The relative abundance of *Lestes* decreased with increasing values of PC1, whereas the relative abundance of *Enallagma* marginally decreased and *Ischnura* increased (Fig. 1b, Table 1); no genera covaried significantly with PC2 (Table 1). Consideration of individual lake variables reveals more precisely how the relative abundances of each genera differed. For example, *Lestes* relative abundance tended to covary more with lake water chemistry features (e.g. dissolved oxygen, pH and conductivity, Fig. 1c,e,g, respectively; Table 1). Interestingly, *Lestes* relative abundance was also positively correlated with dragonfly abundance (Fig. 1f). In contrast, *Enallagma* and *Ischnura* tended to covary more consistently with measures reflecting productivity of lakes (e.g. lake productivity, chlorophyll-a and prey density; Fig. 1c, Table 1).

Lakes also varied considerably in the assemblages of potential damselfly prey (Fig. 2a, Table S3, Supporting information). The first and second PC's of the prey PCA accounted for 62% and 12%, respectively, of the total variation, with increasing values of PC1 meaning lakes that had higher abundances of Cladocerans, and increasing values of PC2 meaning lakes with higher abundances of Gammaridae, Ostracoda, and lower abundances of Annelida (Fig. 2a, Table S3, Supporting information).

The relative abundances of the genera covaried differently with prey assemblages among lakes. *Lestes* relative abundance declined with increasing values of prey PC1, whereas *Enallagma* and *Ischnura* did not, and the latter two genera relative abundances covaried in opposite ways with prey PC2 (Fig. 2, Table 1). Thus, *Lestes* segregates most strongly along prey PC1, whereas *Enallagma* and *Ischnura* segregate in opposing ways along prey PC2.

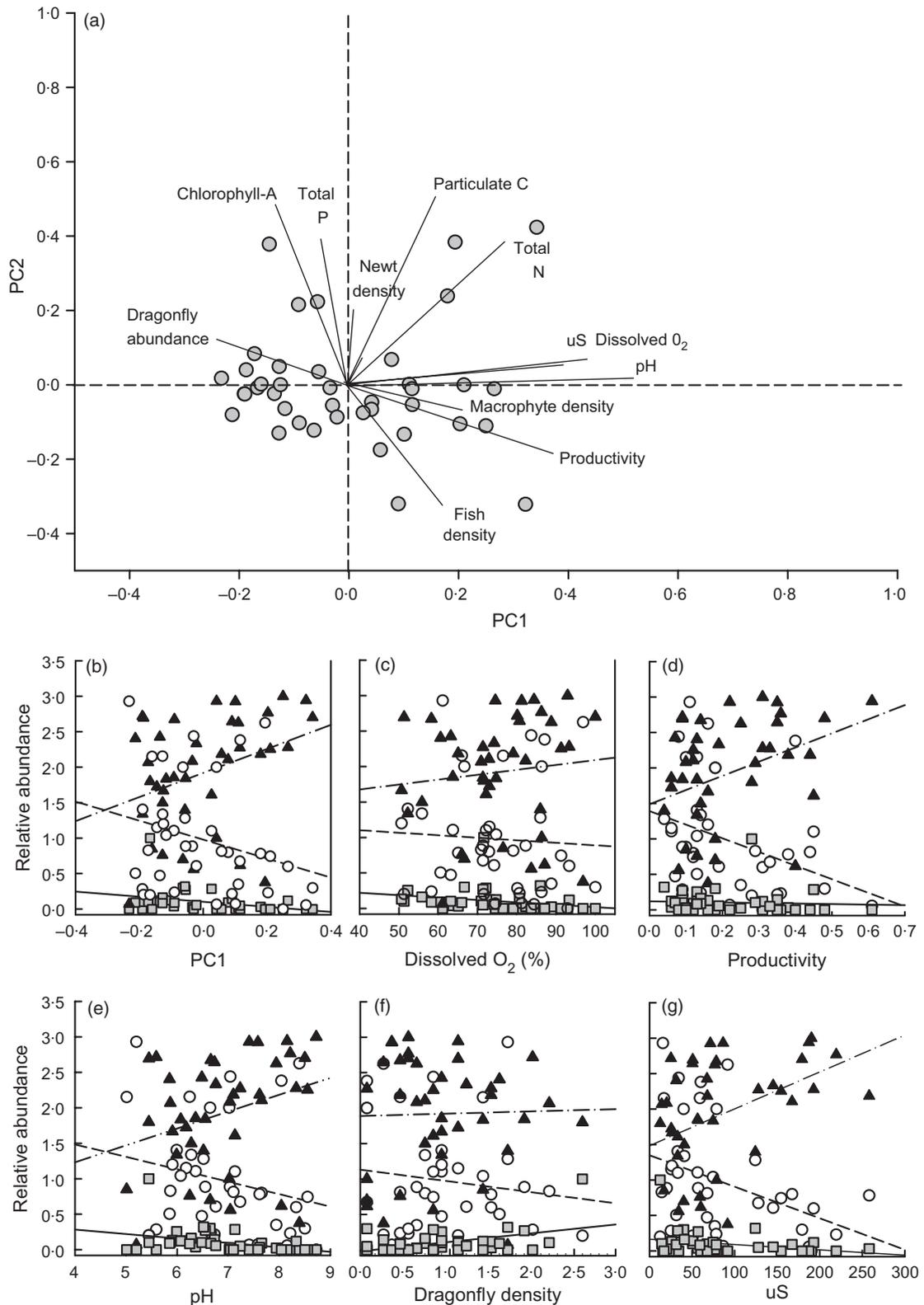
### EXPERIMENTAL TEST OF STABILIZING EFFECTS

Our experiment was designed to determine how per capita growth rates of each genus varied in response to manipulations of intra- and interspecific densities. Four cages (one 40 *Enallagma* treatment, one 20 *Ischnura* treatment and two cages with six individuals of each genus) were removed from this set of analyses because the cages were damaged (mainly from Trichoptera chewing holes in the mesh netting) and individuals presumably escaped. Despite these removals if these damaged cages were included, the results of all of the below analyses remain qualitatively the same and statistically significant. The three genera differed in overall per capita growth rates when averaged across all cages without respect to treatment ( $F_{2, 66} = 22.61$ ,  $P < 0.0001$ ), with *Ischnura* having a higher growth rate than both *Enallagma* or *Lestes* ( $P < 0.05$ , Tukey's HSD) and *Enallagma* and *Lestes* not being significantly different from each other ( $P > 0.05$ , Tukey's HSD; Fig. 3).

For *Enallagma*, per capita growth rates differed overall among the density manipulations ( $F_{3, 17} = 4.47$ ,  $P < 0.02$ ). *Enallagma* per capita growth rate declined only with increases in *Enallagma* density ( $F_{1, 17} = 8.98$ ,  $P < 0.01$ ) and was not affected by changes in the densities of either *Ischnura* ( $F_{1, 17} = 0.61$ ,  $P > 0.40$ ) or *Lestes* ( $F_{1, 17} = 0.03$ ,  $P > 0.85$ ) (Fig. 4a).

Because *Ischnura* were still quite small when the experiment was established, a number of small individuals contaminated the experimental cages: we recovered more *Ischnura* larvae than were introduced in most cages. *Enallagma* and *Lestes* were too large at the start of the experiment for any contamination. Thus, our results for *Ischnura* are suspect, because we cannot be sure of the initial *Ischnura* size distributions in cages. However, we report the results here for completeness. In contrast to the two other genera, the density manipulations had no statistically significant overall effect on *Ischnura* per capita growth rates ( $F_{3, 20} = 1.66$ ,  $P > 0.20$ ). Although *Ischnura* per capita growth rates declined with increasing *Ischnura* density, the relationship was not statistically significant ( $F_{1, 20} = 0.29$ ,  $P > 0.75$ ). Increases in the densities of the other two genera did not cause a reduction in *Ischnura* growth rates (Fig. 4b). *Ischnura* growth rates did, however, tend to increase with increases in *Enallagma* density ( $F_{1, 20} = 4.23$ ,  $P = 0.053$ ), but *Lestes* density had no effect ( $F_{1, 20} = 1.29$ ,  $P > 0.20$ ).

Similar to *Enallagma*, the per capita growth rate of *Lestes* was significantly affected by the density manipulations overall ( $F_{3, 20} = 4.00$ ,  $P < 0.05$ ). *Lestes* per capita growth rates declined with increases in the density of *Lestes* ( $F_{1, 20} =$



**Fig. 1.** Overall variation in lake environmental features based on a principal component analysis (a). Grey symbols are individual lakes ( $n = 40$  lakes), and the lengths of the solid lines emanating from the origin are proportional to their loadings on each PC (see Table S2, Supporting information). Also shown are comparisons of the relative abundance of each genus in relation to specific lake environmental features (b-g), which shows that the different genera generally respond differently to such features. Least squares regression lines are shown only to aid interpretation (*Enallagma*: open circles and dashed line; *Ischnura*: black triangles and dash-dot line; *Lestes*: grey squares and solid line).  $P$  values and correlation coefficients are given in Table 1.

**Table 1.** Correlation coefficients (Spearman rank) between damselfly relative abundances and lake environmental factors and prey abundances from 40 lakes throughout New Hampshire and Vermont, USA

Factor	Damselfly genus		
	<i>Enallagma</i>	<i>Ischnura</i>	<i>Lestes</i>
Productivity (mg C day <sup>-1</sup> )	<b>-0.363</b>	<b>0.323</b>	-0.209
Dissolved O <sub>2</sub> (%)	-0.136	0.176	<b>-0.521</b>
pH	-0.265	<b>0.342</b>	<b>-0.445</b>
µS	<b>-0.358</b>	<b>0.446</b>	<b>-0.321</b>
Particulate C (mg L <sup>-1</sup> )	0.183	-0.127	-0.131
Total N	-0.066	0.018	0.010
Total P	0.064	-0.054	0.250
Chlorophyll-A (µg L <sup>-1</sup> )	<b>0.346</b>	<b>-0.334</b>	<b>0.326</b>
Macrophyte density (stems m <sup>-2</sup> )	-0.188	0.239	-0.099
Fish density (no./haul)	-0.265 <sup>†</sup>	0.221	-0.123
Newt density (no./haul)	0.099	-0.166	-0.042
Dragonfly density (larvae/1-m sweep)	-0.037	0.033	<b>0.326</b>
Invertebrate prey density (no./6-L sample)	<b>-0.350</b>	<b>0.317</b>	0.109
PC1 (of prey abundances)	-0.213	0.242	<b>-0.401</b>
PC2 (of prey abundances)	<b>-0.330</b>	<b>0.303</b>	0.052
PC1 (of lake environmental variables)	-0.287 <sup>†</sup>	<b>0.345</b>	<b>-0.417</b>
PC2 (of lake environmental variables)	0.252 <sup>†</sup>	-0.254 <sup>†</sup>	0.184

Bold =  $P < 0.05$ , <sup>†</sup> $0.05 < P < 0.10$ .

11.22,  $P < 0.005$ ), but not *Enallagma* ( $F_{1, 20} = 0.43$ ,  $P > 0.50$ ) or *Ischnura* ( $F_{1, 20} = 0.19$ ,  $P > 0.65$ ) (Fig. 4c).

## Discussion

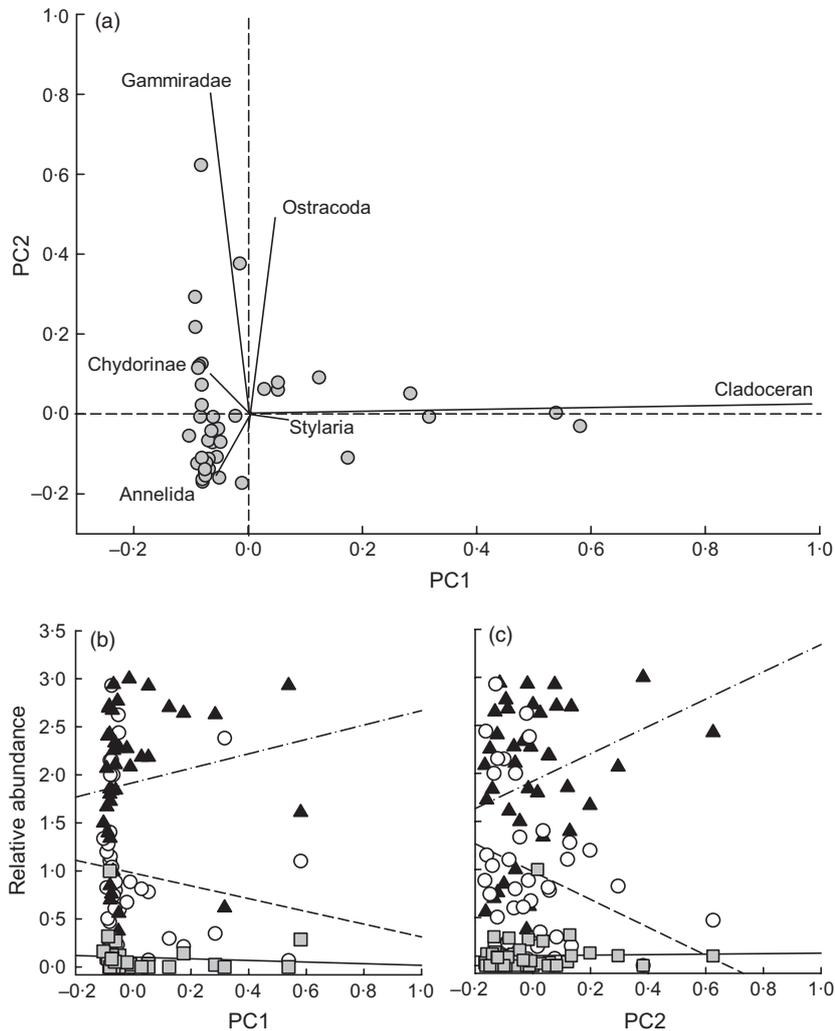
Our combined observational and experimental studies demonstrated two key signatures of ecological partitioning as an important mechanism that may act to maintain local damselfly diversity. First, our observational study showed that densities of the genera were not correlated with each other and that the relative abundances of each genus covaried along environmental gradients of lake abiotic and biotic features in different ways, suggesting that environmental features affected the demographic performance of each genus differently. Second, our experimental results demonstrated that per capita growth rates of two of the three genera were negatively density-dependent and decline only with increases in their own abundances and not with increases in the abundances of species in other genera. This implies that each genus interacts with the environment in different ways, so that per capita growth rates of species in each genus are regulated by these differences in the way they utilize the local environment. Given that previous experiments have shown these damselflies in fish lakes to be food limited, competition for limiting resources is likely the mechanism causing the growth responses we detected in the experiment (McPeck 1998). These results fulfil two key conditions that have been outlined to show that ecological differences among species can act to maintain biodiversity in local communities

(Chesson 2000; Chase & Leibold 2003; Leibold & McPeck 2006).

In most communities, species have different average fitnesses. If these fitness differences are too large, the competing species with the highest population growth rate should eventually exclude other species from the community (Chesson 2000). Our experimental study showed that *Ischnura* per capita individual growth rates (not to be confused with population growth rates) were about 50% greater than those of the other two genera (Fig. 3), consistent with previous field (McPeck 1998) and laboratory experiments (McPeck 2004). These elevated growth rates are a consequence of physiological mechanisms that make *Ischnura* very effective in converting food resources into their own biomass (McPeck 2004). Essentially, this means that *Ischnura* is able to convert the same amount of a shared resource into more of its own biomass. *Lestes* also has a higher assimilation efficiency than *Enallagma* (Stoks & McPeck 2006) but not as high as *Ischnura* [cf. McPeck (2004) and Stoks & McPeck (2006)]. Not surprisingly, *Ischnura* relative abundances were positively correlated with lake productivity and invertebrate prey abundance (Fig. 1, Table 1). Although we failed to detect negative density dependence in the per capita growth rates of *Ischnura* in our experiments because of *Ischnura* contamination, the trend was negative (Fig. 4b). Indeed, other field experiments have shown that *Ischnura* per capita growth rates decline with increasing density (McPeck 1998).

In the absence of any mechanism promoting coexistence, the higher per capita growth rates of *Ischnura* on shared resources could lead to the exclusion of *Enallagma* and *Lestes*, because these higher per capita growth rates through assimilation efficiencies mean that *Ischnura* populations should be able to increase in abundance on less food resources. However, our experimental results showed that *Ischnura* abundance did not negatively affect the per capita growth rates of either *Enallagma* or *Lestes* (Fig. 4). Instead, only intrageneric densities generated negative density dependence in per capita growth rates. The consistent lack of any negative effects of increases in intergeneric densities on per capita growth rates indicates that intergeneric competition is either absent or very weak. Although we found evidence that each genus is more strongly self-regulated, our experiment was only conducted in a single lake. Our observational study revealed that the relative abundances (and presumably the demographic rates) of each genus covaried differently along environmental gradient as well. Consequently, the magnitude of competitive interactions is also likely to covary along these environmental gradients if the competitive interactions are context-dependent (e.g. dependent on abiotic or biotic features of the lake). However, we suspect that while these possible context-dependent competitive interactions may shift the relative magnitudes of competitive effects, it is unlikely that this would necessarily cause intergeneric effects to become stronger than intraspecific effects.

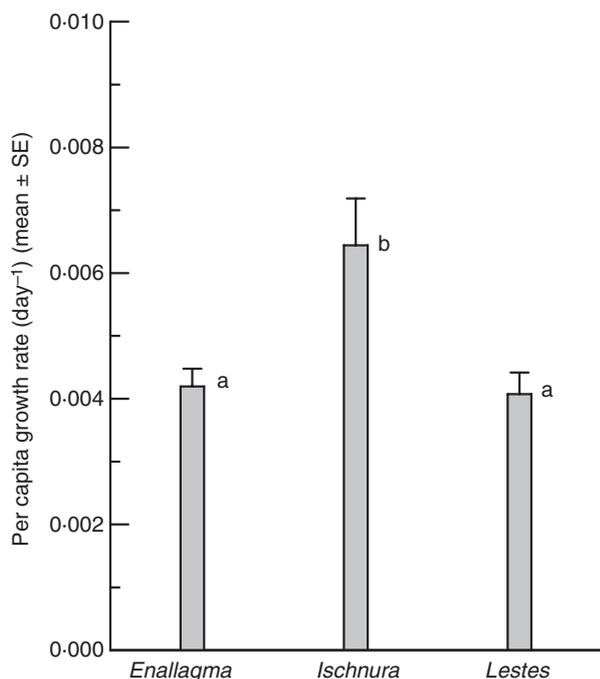
Hence, these genera appear to be differentiated into different components of the prey resources available to them. *Ischnura* and *Enallagma* are comparable in overall size (e.g.



**Fig. 2.** Overall variation in the abundance of different damselfly prey items based on a principal component analysis (a). Grey symbols are individual lakes ( $n = 40$  lakes), and the lengths of the solid lines emanating from the origin are proportional to their loadings on each pc (only those prey items with high loadings (eigenvectors  $> |0.06|$ ) are shown; see Table S3, Supporting information). Also shown are comparisons of the relative abundance of each genus in relation to the first (b) and second (c) of the PCA, which shows that the different genera generally respond differently to prey abundances among lakes. Least squares regression lines are shown only to aid interpretation (*Enallagma*: open circles and dashed line; *Ischnura*: black triangles and dash-dot line; *Lestes*: grey squares and solid line).  $P$  values and correlation coefficients are given in Table 1.

Westfall & May 2006) and thus can feed on many of the same prey. However, *Ischnura* and *Enallagma* relative abundances covary differently with prey abundances (Fig. 2). Even species that overlap considerably in food resources may coexist by partitioning how these resources are used for growth. For example, coexistence of grasshoppers that feed on the same plants has been attributed to each species extracting different amounts of macronutrients (proteins and carbohydrates) in different amounts that maximize each species growth rate (Behmer & Joern 2008). These species-specific, 'cryptic nutritional niches' may be important for allowing generalist consumers to coexist. If *Ischnura* per capita growth rates did indeed increase with increases in *Enallagma* density (Fig. 4b), this also suggests the possibility that *Ischnura* and *Enallagma* use different prey, but these different prey compete themselves. Increases in *Enallagma* density would decrease the abundance of one of the prey, thus freeing more of the shared resource for the prey that *Ischnura* use. However, in this scenario, we would have expected that *Enallagma* per capita growth rates would have increased with increases in *Ischnura* densities. Although we did find such a positive trend, it was not statistically significant (Fig. 4a).

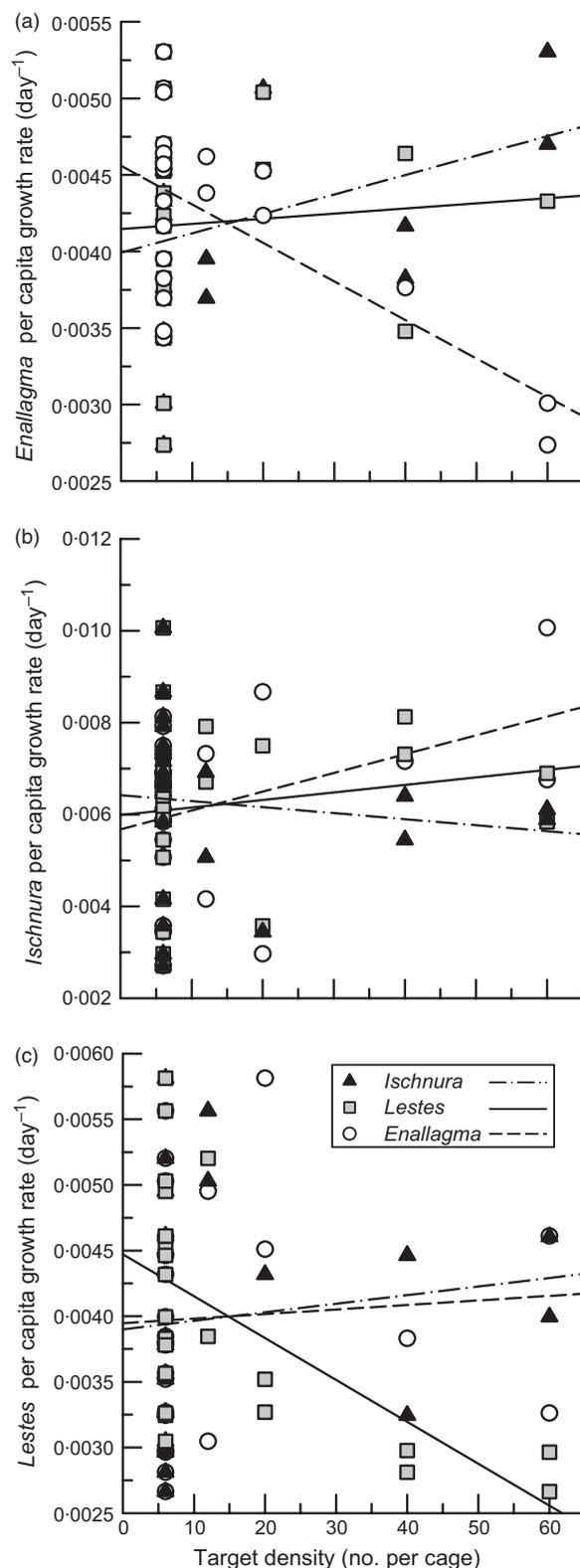
*Lestes* are much larger than either *Enallagma* or *Ischnura* (e.g. Westfall & May 2006) and may simply feed on larger prey items not accessible to *Enallagma* or *Ischnura*. We have found that only *Lestes* is capable of feeding on some of the larger prey items (e.g. *Hyaella*) in this system (A. Siepielski and M. McPeck, unpublished data). If *Lestes* are biased towards feeding on larger prey, this might also help account for the positive correlation between *Lestes* relative abundances and dragonfly abundances (Fig. 1, Table 1). In fish lakes, dragonflies do not impose significant mortality on damselflies (McPeck 1998). However, like *Lestes*, dragonflies are much larger than *Ischnura* and *Enallagma* and so presumably use larger prey. Consistent with this, the abundances of *Lestes* and dragonflies both decrease with increasing values of prey PC1 (Table 1; dragonflies:  $r_s = -0.525$ ,  $P = 0.0005$ ), whereas such correlations were absent for *Enallagma* and *Ischnura* (Table 1). Consequently, the ability of *Lestes* to use larger prey items seems to be critical in regulating its demographic performance, and the use of these items must come at the expense of being able to utilize prey used by *Enallagma* and *Ischnura*. However, the potential differences in prey use among these three genera, and the extent to which they cause each genus to be strongly



**Fig. 3.** Differences in per capita growth rates among genera of damselflies in the experimental communities. Different letters indicate post hoc comparisons (Tukey's HSD) and are significantly different from one another ( $P < 0.05$ ).

self-regulated, are likely to vary with ontogeny. Although *Lestes* larvae are much larger during later instars (i.e. those used in our experiments), during early instars their body size overlaps considerably with both *Enallagma* and *Ischnura*. Consequently, during these early life stages, it is likely that all three genera may compete for prey resources. This kind of age-structured component of resource competition and niche overlap is clearly important and may represent an important demographic bottleneck (e.g. Wilbur 1980; Polis 1984; Werner & Gilliam 1984).

Our experiments only considered interactions among the damselflies in the absence of predation and thus focus on differences in resource use. When combined with our previous results on differences in susceptibility to predation, we can develop a more comprehensive picture of the ecological differences that may promote coexistence among these genera. Differences in predator susceptibility along with the resource differences identified in this study appear to promote coexistence between *Enallagma* and *Ischnura* via keystone predation (Holt, Grover & Tilman 1994; Leibold 1996; McPeck 1996). *Enallagma* are better at avoiding predators, whereas *Ischnura* species are better at converting prey into their own biomass (McPeck 1998). This trade-off between these two vital rates (predation and growth) gives them countervailing demographic advantages and disadvantages that presumably prevent each from excluding the other from the system. The importance of possible prey segregation, or along other relevant ecological gradients we identified here, should only reinforce the keystone predation mechanism, although the ways in which different coexistence



**Fig. 4.** The effects of experimental manipulations of intra- and inter-specific density on damselfly per capita growth rates show that each genus most strongly regulates its own per capita growth rate – a signature of ecological partitioning. Each panel shows the per capita growth rates for different genera in relation to manipulations of the densities of all three genera. The different symbols and lines represent different genera (*Enallagma*: open circles and dashed line; *Ischnura*: black triangles and dash-dot line; *Lestes*: grey squares and solid line).

mechanisms interact has not been investigated. Indeed, we do not know whether multiple coexistence mechanisms actually increase the potential for coexistence (i.e. the effects of coexistence mechanisms are additive) or perhaps decrease the potential for coexistence (i.e. the effects of coexistence mechanisms are antagonistic somehow).

A distinct role for predation seems unlikely as an important niche axis for *Lestes*. *Lestes* is very similar to *Enallagma* in phenotypic traits that influence predator susceptibility (Stoks & McPeck 2006) and as a consequence, experiences very similar per capita mortality rates to *Enallagma* from fish predation (McPeck 1990, 1998; Stoks & McPeck 2003). Thus, the difference in resource use appears to be the primary way *Lestes* is ecologically differentiated from *Enallagma* and *Ischnura*.

Our results therefore suggest that a food web model including all three genera could involve keystone predation (Holt, Grover & Tilman 1994; Leibold 1996; McPeck 1996) coupled with apparent competition (Holt 1977). In this configuration, all three genera experience mortality imposed by fish, with the trade-off between mortality and competition for one set of limiting food resources (prey PC2) fostering coexistence between *Enallagma* and *Ischnura* (McPeck 1998) and *Lestes* use of an alternative set of prey (prey PC1) promoting its coexistence. Combining such trophic modules is an important step for understanding the complexities inherent in the assembly, structure and persistence of multispecies interaction webs (Holt, Grover & Tilman 1994; Holt 1997).

## Conclusions

For over a century, interest and expectations of the importance of ecological differentiation as an explanation for the maintenance of species diversity have waxed and waned (Hubbell 2001; Chase & Leibold 2003). Hundreds of studies have now identified ecological differences – behavioural, morphological and physiological – and trade-offs between traits affecting the demographic performances of species within communities and along ecological gradients of co-occurring taxa. Concomitantly, numerous theoretical models have now identified more exactly the ways in which species must be ecologically different to promote coexistence through ecological partitioning (Chesson 2000; Leibold & McPeck 2006). These studies are critical, but this focus on identifying ecological differences and possible mechanisms alone has simply left the field with ample evidence that species are quite different, but whether these differences actually matters for stabilizing species interactions is still largely untested (Silvertown 2004; Leibold & McPeck 2006; Adler, HilleRisLambers & Levine 2007; Siepielski & McPeck 2010). The approach taken here helps clarify the role of ecological partitioning as an important mechanism maintaining biodiversity. As with other recent studies adopting this general approach (e.g. Levine & Hille Ris Lambers 2009), we have not definitively tested whether these genera do in fact coexist. Nevertheless, by focusing more generally on testing for a demographic signature of

ecological partitioning, our study provides evidence that ecological differentiation can promote the maintenance of biodiversity.

## Acknowledgements

We thank K. Cottingham for help with some of the water chemistry analyses. L. Symes and several anonymous reviewers provided helpful comments on an earlier draft. Thanks to the New Hampshire Fish and Game Department and the Vermont Fish and Wildlife Department for research permits. This work was supported by National Science Foundation grant DEB-0714782.

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Received 7 February 2011; accepted 21 April 2011  
 Handling Editor: Frank Johansson

## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Table S1.** List of ponds and lakes sampled in New Hampshire and Vermont, USA, during 2008 and 2009.

**Table S2.** Eigenvectors of the first and second principal component analysis of a PCA (correlation matrix) of lake environmental variables from 40 lakes throughout New Hampshire and Vermont, USA. Per cent variance of each PC explained in parentheses.

**Table S3.** Eigenvectors of the first and second principal components of a PCA (covariance matrix) of prey abundances from 40 lakes throughout New Hampshire and Vermont, USA. Per cent variance explained in parentheses.

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