

# Dispersal strength influences zooplankton co-occurrence patterns in experimental mesocosms

BRANDON S. SCHAMP,<sup>1,3</sup> SHELLEY E. ARNOTT,<sup>2</sup> AND KATELYN L. JOSLIN<sup>2</sup>

<sup>1</sup>*Department of Biology, Algoma University, Sault Ste. Marie, Ontario P6A 2G4 Canada*

<sup>2</sup>*Department of Biology, Queen's University, Kingston, Ontario K7L 3N6 Canada*

**Abstract.** Negative co-occurrence patterns are intriguing because they may reflect the outcome of interspecific interactions and therefore signal how competition shapes communities. However, other factors also contribute to these patterns. For example, theoretical studies as well as two survey-based studies have all suggested that dispersal may also impact these patterns. While natural communities commonly have nonrandom patterns of negative co-occurrence, understanding how different processes drive these patterns requires further research. We tested the influence of dispersal on co-occurrence patterns using a zooplankton mesocosm experiment with four different dispersal treatments varying in the number of dispersers delivered into mesocosms on regular intervals. Our dispersal treatments were intended to adjust the relative importance of dispersal and competition experienced within mesocosms (i.e., high dispersal results in a relatively low influence of competition on species composition and vice versa). Higher dispersal translated into increased zooplankton species richness and inter-mesocosm compositional similarity, and also changed species occupancy patterns such that species occurrences were more even across mesocosms in higher-dispersal treatments. Dispersal treatments also differed markedly in species co-occurrence patterns. Negative co-occurrence patterns were significant for all but the lowest-dispersal treatment, peaked in the intermediate-dispersal treatments, and declined in the highest-dispersal treatment. Stability analyses illustrate that co-occurrence differences are robust to the exclusion of any single mesocosm in null model analyses. Dispersal treatments did not significantly differ with respect to abiotic variation, which has been recognized as a potential driver of negative co-occurrence patterns. These results suggest that not only can dispersal influence patterns of negative co-occurrence via changes to species richness and distribution (occupancy patterns among mesocosms), but the degree to which they do so varies nonlinearly with the strength of dispersal. Critically, because negative co-occurrence patterns were nonsignificant when the contribution of dispersal was lowest, it is possible that dispersal contributes strongly to many observed patterns of negative co-occurrence. Consequently, great care should be taken prior to interpreting significant co-occurrence tests as a product of species interactions.

**Key words:** community assembly; competition; dispersal; Killarney Provincial Park, Ontario, Canada; negative co-occurrence; null model; predation; species association; species segregation.

## INTRODUCTION

A continuing goal in ecology is to understand how processes like species interactions and dispersal contribute to observed patterns of species abundance and distribution in natural communities (e.g., Diamond 1975, Shurin and Allen 2001, Gotelli and McCabe 2002, Seabloom et al. 2005). Considerable effort has been put into trying to understand whether species interactions result in distinct patterns of negative species co-occurrence within natural systems (e.g., Gotelli and McCabe 2002).

Studies of species co-occurrence patterns in communities were brought to prominence by Diamond (1975), and are of interest because it has been proposed that they are principally driven by species interactions, specifically competition. When species are recorded in samples (e.g., sites, plots, islands), patterns of negative co-occurrence can be quantified for the community by assessing the degree to which a presence-absence matrix is characterized by checkerboard patterns of species distribution. For example, for a given pair of sites and species, a checkerboard pattern consists of the presence of each species in one site of the pair with neither occurring in the same site. Co-occurrence patterns can also be positive, with the same pair of species present in one site, but jointly absent from another. These patterns can be quantified for a focal community using a variety of co-occurrence metrics (Stone and Roberts 1990, Gotelli 2000). Some approaches have also been devel-

Manuscript received 12 June 2014; revised 22 September 2014; accepted 23 September 2014. Corresponding Editor: H. Hillebrand.

<sup>3</sup> E-mail: brandon.schamp@algomau.ca

oped that can be used to assess particular pairs of species (e.g., Sfenthourakis et al. 2004, Veech 2013); however, community-scale analyses are the focus here because of their potential for understanding the overall contribution of species interactions to community structure.

Considerable effort has gone into determining what methods are required to discern whether patterns of negative co-occurrence are sufficiently different from patterns that might be expected to result from chance (e.g., Connor and Simberloff 1979, 1983, Stone and Roberts 1990, Manly 1995, Sanderson et al. 1998, Gotelli 2000, Gotelli and Entsminger 2001, Fayle and Manica 2010, Gotelli and Ulrich 2012). This work has contributed to gains in our understanding of the merits of particular analyses (e.g., Gotelli 2000); however, there remain significant challenges (Gotelli and Ulrich 2012). It has long been recognized, for example, that processes other than competition may contribute to patterns of negative co-occurrence (Diamond 1975, Gilpin and Diamond 1982, Ulrich 2004, Bell 2005, Dullinger et al. 2007, Reitalu et al. 2008, Heino 2013). More work is needed to understand how these different processes can contribute to patterns of negative co-occurrence. Of particular interest here are recent suggestions, both from theoretical analyses (Ulrich 2004, Bell 2005) and two survey-based studies (Zalewski and Ulrich 2006, Heino 2013), that dispersal may contribute to these patterns.

Dispersal is recognized as an important process capable of shaping ecological communities (MacArthur and Wilson 1967, Belyea and Lancaster 1999, Leibold et al. 2004, Cadotte 2006, Flinn et al. 2010). The contribution of dispersal to community composition varies, in some cases playing a major role (e.g., Ozinga et al. 2005, McCauley 2006, Zalewski and Ulrich 2006, Beaudrot and Marshall 2011), while in others playing a minor one (e.g., Cottenie and De Meester 2004, Forrest and Arnott 2006, Shurin et al. 2009, Heino et al. 2012, Warfe et al. 2013). Increasing the local arrival of species through dispersal has the potential to alter species richness and abundance patterns in such a way that species co-occurrence patterns may also be impacted.

It is well understood that dispersal can impact community assembly by determining the pool of available species that can colonize a given site (e.g., Belyea and Lancaster 1999). Recent work has suggested that dispersal can indeed contribute to species co-occurrence patterns. For example, theoretical explorations have revealed that neutral dynamics may contribute to negative co-occurrence patterns (e.g., Ulrich 2004, Bell 2005). Similarly, a study of co-occurrence patterns on a series of islands that vary in relative isolation also suggests that dispersal may contribute to co-occurrence patterns, particularly for species that are found in few sites (i.e., satellite species; Zalewski and Ulrich 2006). Heino (2013) observed that dispersal mode, which can limit dispersal of some species to some sites, impacts co-occurrence patterns of benthic invertebrates in a stream metacommunity. Finally, other work suggests that mass

effects may reduce competitive exclusion and contribute to co-occurrence within macroinvertebrate communities (Heino and Grönroos 2013). Consequently, the influence of dispersal on co-occurrence patterns may make these patterns unsuitable for understanding the contribution of species interactions to species organization within communities/metacommunities.

While it is clear that dispersal can impact co-occurrence patterns, there remains much to understand about how it can do so. It is possible, for example, that dispersal could slow or stop the process of competitive exclusion, effectively keeping pairs of species together that competition would otherwise drive apart, or making co-occurrence more random. In either circumstance, dispersal may result in either neutral (Zalewski and Ulrich 2006) or more positive co-occurrence patterns within a community. However, as theoretical studies have indicated, dispersal can also contribute to negative co-occurrence patterns under neutral dynamics. Additionally, differences among species in dispersal mode (e.g., Heino 2013) can limit the potential of some species such that they cannot reach all sites, which can result in negative co-occurrence patterns (Pielou and Pielou 1968). Even if the study system is sufficiently small that dispersal limitations are unlikely and all species may clearly disperse to all sites, dispersal may still result in negative co-occurrence patterns. Limited dispersal, for example, may continually maintain species in a community at a low frequency (i.e., in a few samples). This can contribute to negative co-occurrence patterns, as these species will negatively co-occur with all species not found in that location. Stronger dispersal may increase the number of sites a species is found in, which may contribute to neutral co-occurrence patterns, or even positive co-occurrence patterns. While it is clear that dispersal can influence species co-occurrence patterns, it is not clear how co-occurrence patterns will be affected along a gradient of dispersal strength, and to our knowledge, no direct test has been conducted.

In this paper, we describe a direct experimental test of the influence of dispersal on zooplankton co-occurrence patterns using groups of mesocosms varying in the degree to which dispersal supplies new species and individuals. These treatments effectively represent a gradient in the relative influence of dispersal and species interactions on zooplankton community structure. Zooplankton systems are ideal for such a test because there is sufficient evidence that dispersal and species interactions are important in these systems (Shurin 2000, Shurin and Allen 2001, Cottenie and De Meester 2004).

## METHODS

### *Experimental design*

To evaluate the effects of dispersal on species co-occurrence patterns and zooplankton community structure, we conducted a 12-week field mesocosm experiment in Killarney Provincial Park, Ontario, Canada, where we set up four dispersal treatments, each

consisting of 15 mesocosms. All mesocosms were similar in temperature and water chemistry throughout the experiment, as well as in initial zooplankton community structure. Treatments only differed in the number of dispersers received throughout the experiment.

On 14 May 2010, 60 378-L Rubbermaid stock tanks were filled with 350 L of water from George Lake, Ontario that was filtered through a 50- $\mu$ m mesh net to remove zooplankton but allow most phytoplankton to pass through. Mesocosms were placed in a shaded area more than 500 m from the nearest body of water and covered with a 1-mm mesh to minimize exogenous colonization from large insects and other animals during the experiment. Nutrients (535.9 mg  $\text{NaNO}_3$  and 17.4 mg  $\text{NaH}_2\text{PO}_4$ ) were initially added to each mesocosm to achieve the average nutrient level of the five lakes used for the dispersal treatment. Every 7 d throughout the experiment, 18.76 mg  $\text{NaNO}_3$  and 0.59 mg  $\text{NaH}_2\text{PO}_4$  were added to each mesocosm to make up for loss of N and P to periphyton. On 16 May 2010, each mesocosm was inoculated with zooplankton of ambient density from George Lake.

Four dispersal treatments (0.1%, 1.3%, 2.5%, and 9.6% of the mesocosm volume, every 11 d) were distributed haphazardly across the tanks and were chosen to cover a range of natural dispersal levels (Michels et al. 2001). Dispersers were collected from five nearby lakes that varied in their physical and chemical attributes (Appendix: Table A1) to simulate dispersal from a diverse regional species pool to individual lakes. Zooplankton were collected from each lake by taking two 4-m vertical hauls with a 35 cm diameter, 50- $\mu$ m mesh net. Zooplankton were placed in two 2-L containers of lake water and transported to the mesocosm site in coolers. All dispersers were combined in 60 L of 50- $\mu$ m filtered George Lake water.

Aliquots for each mesocosm were taken from the pool of dispersers, and based on calculating the volume of water filtered from each lake, compared to the volume of the mesocosm. We used an aliquot that represented 70 mL from each lake (0.1% dispersal by volume in total) for low dispersal, 91 mL (1.3%) for medium dispersal, 1750 mL (2.5%) for high dispersal, and 6720 mL (9.6%) for very high dispersal.

Each week, starting on 17 May 2010, we measured temperature, dissolved oxygen (DO), pH, and conductivity (starting 16 June) in the center of the mesocosm using either a YSI 550A DO probe (YSI, Yellow Springs, Ohio, USA) and a Hanna pHep probe (Hanna Instruments, Woonsocket, Rhode Island, USA; 17 May–10 June) or a YSI 600R multiparameter probe (16 June–11 August).

The experiment ended after 88 d, 10 d following the final dispersal event. Crustacean zooplankton and Rotifera zooplankton in each mesocosm were sampled at that time. Mesocosms were sampled starting with the lowest-dispersal treatment and finishing with the highest-dispersal treatment. Each mesocosm was gently

mixed with a canoe paddle before taking two samples with a 3-L tube sampler. The water in the tube sampler was gently poured through a 50- $\mu$ m mesh. Zooplankton retained on the mesh were anesthetized with Alka Seltzer (Bayer Schering, Berlin-Wedding, Germany) and preserved with 70% ethanol.

Water samples for chlorophyll *a* analysis were collected  $\sim$ 10 cm below the surface in each mesocosm at the end of the experiment. Phytoplankton were collected by passing 300 mL of water through a GF/C Whatman (Sigma-Aldrich, St. Louis, Missouri, USA) glass fiber filter. Filters were stored in the freezer, then chlorophyll *a* was extracted in methanol for 24 h, and analyzed using a TD 700 Fluorometer (Turner Designs, Sunnyvale, California, USA; Welschmeyer 1994).

Zooplankton were identified and counted in five or six subsamples ( $\sim$ 60% of the total sample), and the remainder of the sample was examined for rare species. Samples were examined at 20–25 $\times$  magnification using a MZI25 dissecting microscope (Leica, Solms, Germany). Cladoceran species were identified using the keys of Edmondson (1959), Witty (2004), and Balcer et al. (1984), and DeMelo and Hebert (1994) was used to identify bosminids. Smith and Fernando (1978) and Hudson et al. (1998) were used for the identification of copepods.

Smaller subsamples ( $\sim$ 3% of the total sample) were examined to identify members of the Rotifera. Six successive subsamples (number determined based on the asymptote of species accumulation curves) were examined in full at 200 $\times$  magnification, using a Leitz Biomed compound microscope (Leica). Rotifers were identified to genus using Edmondson (1959) and Stemberger (1979).

From counts of all adults in samples, we determined zooplankton species richness and species evenness using species abundance data for each mesocosm. We also calculated community compositional distances based on Sørensen similarity, and further explored compositional similarity among mesocosms within dispersal treatments. We used  $E_{\text{var}}$  as a measure of evenness because it is uncorrelated with species richness (Smith and Wilson 1996), and the Sørensen coefficient to measure compositional similarity between all pairs of mesocosms in each treatment. The Sørensen coefficient ( $S_S$ ) was calculated as  $S_S = 2c/(a + b + 2c)$ , where  $c$  is the number of species that two mesocosms share,  $a$  is the number of species unique to the first mesocosm, and  $b$  is the number that are unique to the second mesocosm (Legendre and Legendre 2012). Compositional similarity was calculated using presence–absence data because these were the data that contributed to any observed differences in co-occurrence patterns. Total abundance in each mesocosm was the sum of all adult individuals in samples. We also examined the abundance of predatory species per mesocosm to better understand how dispersal may shape systems via changes in predators.

### Statistical analyses

We used repeated-measures analysis of variance (RM ANOVA) to ensure abiotic conditions (temperature, DO, pH, and conductivity) were similar among treatments, as it is recognized that environmental variation can contribute to zooplankton community structure (e.g., Cottenie et al. 2003). Chlorophyll *a*, an indication of food resources for herbivores, was compared among dispersal treatments on the last day of the experiment using a one-way ANOVA. Because previous work has indicated that dispersal can influence community structure (Shurin and Allen 2001, Cottenie and De Meester 2004), we used one-way ANOVAs to assess differences in species richness, total abundance, and species evenness among the dispersal treatments. Differences in species composition among dispersal treatments were assessed using the nonparametric MANOVA method described by Anderson (2001), using the *adonis* function from the *vegan* package. All ANOVAs were run using R version 3.0.1 (R Core Team 2013).

### Species co-occurrence

We used an established null model test to determine whether co-occurrence patterns among mesocosms within our dispersal treatments were significantly different than what would be expected by chance. These analyses were performed twice, first on all zooplankton, and then separately on only herbivorous zooplankton, to understand the contribution of predators to co-occurrence patterns. We used the *C* score metric as our measure of co-occurrence within treatments; this metric is recognized as having good statistical properties (Gotelli 2000). The *C* score quantifies the mean number of checkerboards observed for all pairs of species and is calculated as  $\sum(S_i - Q)(S_j - Q)/((R)(R - 1)/2)$ , where  $S_i$  is the sum of row *i*,  $S_j$  is the sum of row *j*,  $Q$  is the number of sites in which both species in a given pair are present, and  $R$  is the number of rows in the matrix (Stone and Roberts 1990). We compared the *C* score for each treatment to null distributions of 5000 *C* scores generated by randomizing the presence-absence matrix using the fixed-fixed independent swap algorithm with 30 000 swaps (Lehsten and Harmand 2006). A dispersal treatment was considered to have significant negative or positive co-occurrence patterns when the *C* score for that treatment was greater than (significant negative co-occurrence), or less than (significant positive co-occurrence) 97.5% of *C* scores for 5000 randomized matrices (a two-tailed *P* value). We compared co-occurrence patterns across dispersal treatments using the standardized effect size (SES; e.g., Sanders et al. 2003). This measure is calculated as the number of standard deviations that the observed *C* score ( $C_{\text{obs}}$ ) for the study community is away from the mean *C* score in the null distribution ( $C_{\text{sim}}$ ), defined here by 5000 randomized *C* scores using  $(C_{\text{obs}} - C_{\text{sim}})/S_{\text{sim}}$ , with  $S_{\text{sim}}$  representing the standard deviation of simulated *C* scores (Sanders et al. 2003).

Because co-occurrence tests produce a single value for each treatment, we were unable to test for statistical differences between co-occurrence patterns among our dispersal treatments. However, we tested the stability of the SES for each of our dispersal treatments using a jackknifing procedure in which we systematically re-ran our null model tests for each treatment in the absence of a single mesocosm (i.e., all combinations of 15 – 1 mesocosms). This method gives a measure of how stable our co-occurrence estimates are by determining how sensitive they are to the inclusion of any single mesocosm, and consequently allows an improved evaluation of whether differences among treatments are spurious or reliable. Thus, we ran 15 null model tests for each treatment. While this use of jackknifing is not common for co-occurrence patterns, it is a tool that is used to examine the stability of phylogenetic hypotheses (Lanyon 1985).

## RESULTS

### Environmental variables

Temperature, pH, dissolved oxygen, and conductivity varied significantly ( $P < 0.05$ ) over the course of the experiment (RM ANOVA, time,  $P < 0.001$  for all factors) but were not significantly different among dispersal treatments (RM ANOVA, time  $\times$  dispersal for temperature,  $P = 0.43$ ; pH,  $P = 0.75$ ; dissolved oxygen,  $P = 0.53$ ; conductivity,  $P = 0.43$ ; Appendix: Table A2). Environmental variables showed no significant difference between dispersal treatments at the end of the experiment (ANOVA,  $df = 3, 56$ ; temperature,  $P = 0.81$ ; pH,  $P = 0.79$ ; dissolved oxygen,  $P = 0.77$ ; conductivity,  $P = 0.39$ ; chlorophyll *a*,  $P = 0.15$ ).

### Community structure and dispersal

A total of 42 species of Crustacea and 20 genera of Rotifera were identified among the 60 mesocosms. At the conclusion of the experiment, species richness significantly differed ( $P < 0.05$ ) across dispersal treatments (ANOVA,  $df = 3, 56$ ,  $P = 0.004$ ; Fig. 1a), with richness higher in 2.5% compared to 0.1% treatments (Tukey HSD,  $P = 0.04$ ), and 9.6% higher than 0.1% treatments (Tukey HSD,  $P = 0.003$ ). There was no significant difference in mean species evenness per mesocosm across treatments (ANOVA,  $df = 3, 56$ ,  $P = 0.16$ ; Fig. 1b). Compositional similarity was generally higher in higher-dispersal treatments, although the lack of independence among mesocosm pairs makes statistical comparisons inappropriate (Fig. 1c). This similarity was driven in large part by changes in species richness, as mesocosms in the high-dispersal treatment contained a significantly larger proportion of the species pool. There was, however, a difference in species composition across dispersal treatments (nonparametric ANOVA,  $df = 3, 56$ ,  $P < 0.001$ ), with the highest-dispersal treatment (9.6%) having different species composition from the lowest-dispersal treatment (nonparametric pairwise comparison,  $P < 0.001$ ) and the second-highest-disper-

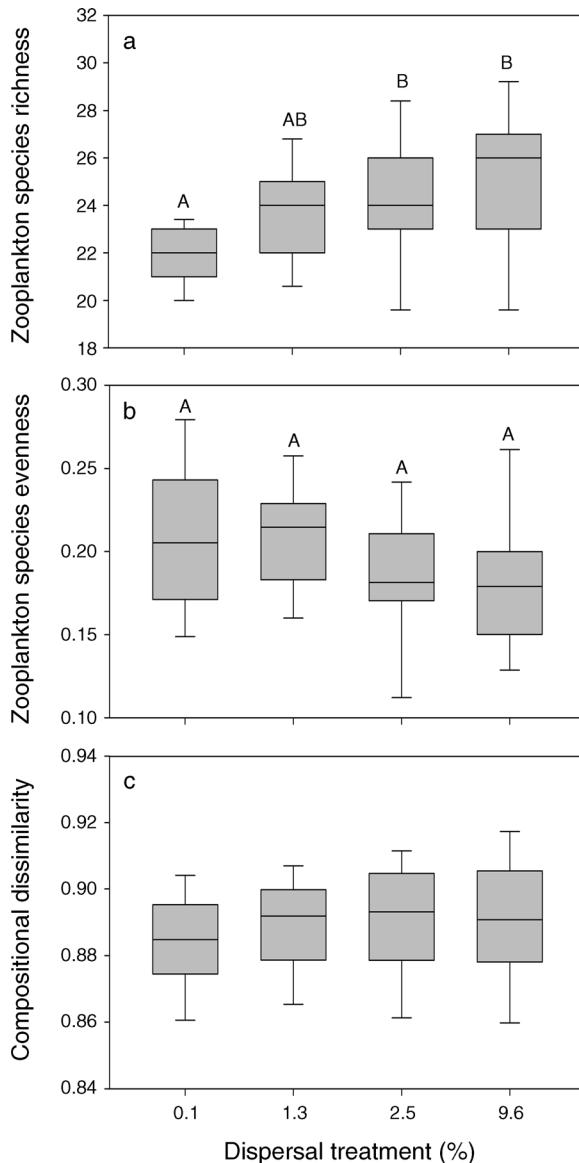


FIG. 1. Boxplots illustrating (a) the median species richness per mesocosm (number of species), (b) the median species evenness ( $E_{var}$ ) per mesocosm in each dispersal treatment, and (c) the median compositional similarity among pairs of mesocosms (Sørensen coefficient) for zooplankton under four different dispersal treatments. Zooplankton addition treatments are percentage of the mesocosm volume. Horizontal lines in the center of each box indicate the 50th percentile (median) of the data, the extent of the boxes indicates the 25th and 75th percentiles of the data, and the whiskers indicate the 5th and 95th percentiles. Boxes with different letters above them were significantly different ( $P < 0.05$ ) according to a Tukey HSD test. No statistical test was applied to (c) compositional similarity data because of the lack of independence among pairs of mesocosms.

sal treatment (2.5%) being different from the low- and medium-dispersal treatments (nonparametric pairwise comparisons,  $P < 0.001$  and  $P = 0.043$ , respectively; Appendix: Fig. A1).

Predatory zooplankton abundance was significantly greater in mesocosms in the three highest-dispersal treatments (Kruskal-Wallis ANOVA on ranks,  $df = 3$ ,  $P < 0.001$ ; Fig. 2b), although there was no significant difference between the richness of predatory zooplankton species across dispersal treatments (Kruskal-Wallis ANOVA on ranks,  $df = 3$ ,  $P = 0.08$ ). Total zooplankton abundance in mesocosms did not differ significantly across dispersal treatments (ANOVA,  $df = 3$ ,  $56$ ,  $P = 0.46$ ; Fig. 2a).

#### Co-occurrence patterns and dispersal

Co-occurrence patterns were evaluated at the end of the experiment using final census for all 15 mesocosms in each treatment. When all zooplankton were included, analyses of the full mesocosm data sets for each treatment highlighted significant ( $P < 0.05$ ) negative co-occurrence patterns for the 1.3% dispersal ( $P < 0.001$ ), 2.5% dispersal ( $P < 0.001$ ), and 9.6% dispersal ( $P = 0.005$ ) treatments (Fig. 3a). The weakest dispersal treatment (0.1%) was also characterized by negative co-occurrence patterns; however, patterns were not significantly different from what was expected by chance ( $P = 0.078$ ; Fig. 3a). This pattern was similar when only herbivorous zooplankton were included in the analysis; however, the highest-dispersal treatment also did not have significant negative co-occurrence ( $P = 0.13$ ; Fig.

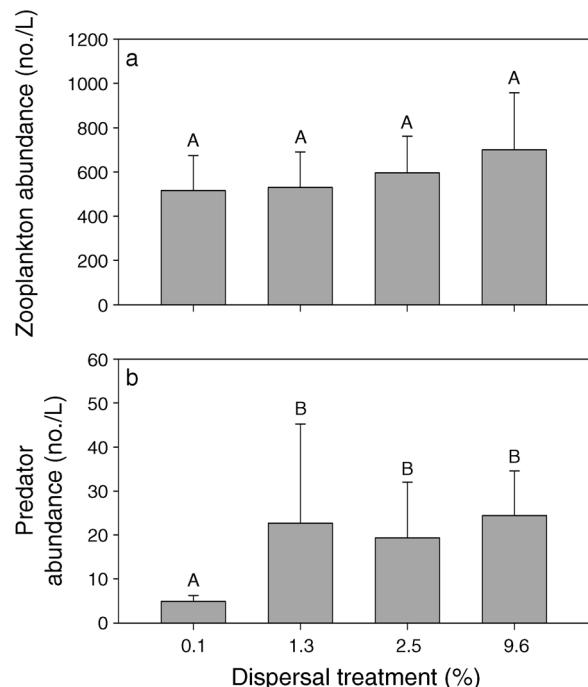


FIG. 2. (a) Mean abundance of zooplankton in mesocosms and (b) mean abundance of predatory zooplankton in mesocosms across the four dispersal treatments. Error bars identify the 95% confidence limits; bars with different letters above them were significantly different ( $P < 0.05$ ) according to a Tukey HSD test (on ranks for panel b).

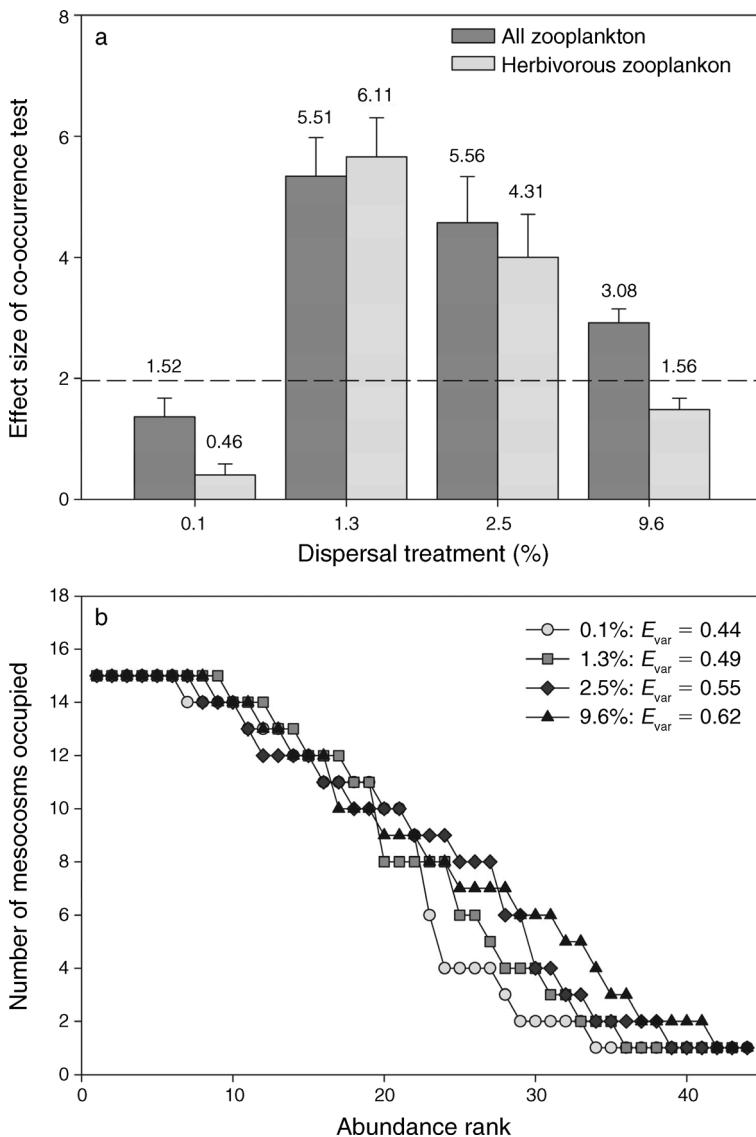


FIG. 3. (a) Standardized effect sizes for co-occurrence null model tests for each of the four dispersal treatments in the experiment. The first, in dark gray, represents scores for all zooplankton species; the second, in light gray, illustrates scores for only herbivorous zooplankton. Null model tests used the *C* score metric and the independent swap algorithm with 30 000 swaps. Error bars represent 95% confidence intervals for effect sizes of co-occurrence tests. These intervals result from a jackknifing procedure in which co-occurrence tests were performed for each dispersal treatment for all possible combinations of mesocosms with a single mesocosm removed. This was done to establish the stability of the co-occurrence score for each treatment; no test for significant differences among treatments was performed, as there was a single metacommunity replicate per dispersal treatment. Values presented above each bar represent the standardized effect size for co-occurrence tests of all 15 mesocosms per treatment. The dashed horizontal line is at 1.96, which is the 97.5 percentile point for a standard effect size (*z* score). (b) Rank abundance distribution for all zooplankton species (predators and herbivores) in each dispersal treatment. Abundance is here measured as the number of mesocosms each species occupies, an important component of variation in the presence-absence matrices that are responsible for co-occurrence patterns. We also report in the key how evenly species occurrences are distributed among mesocosms in each treatment ( $E_{var}$ ).

3a). The stability analysis of our co-occurrence effect sizes suggests that differences in co-occurrence patterns across experimental dispersal treatments are not sensitive to the inclusion of any single mesocosm in analysis; this was consistent, regardless of whether predatory species were included or excluded from the analysis (Fig. 3).

Rank abundance distributions, using the number of occurrences of each species across mesocosms in each treatment as a measure of abundance, differed across dispersal treatments (Fig. 3b). Higher-dispersal treatments were characterized by a more even distribution of species occurrences among mesocosms (Fig. 3b). Differences in rank abundance patterns across dispersal

treatments were greatest for species with lower abundance ranks. These species were found in more mesocosms in the higher-dispersal treatments (Fig. 3b).

## DISCUSSION

### *Community structure and dispersal*

Abiotic variables did not significantly differ across dispersal treatments, indicating that the observed differences in community structure (Fig. 1) and composition (Appendix: Fig. A1) were the result of dispersal treatments rather than physical or chemical differences that developed over the course of the experiment. This is important, as it has been observed that zooplankton composition is responsive to such differences (e.g., Cottenie et al. 2003), and abiotic differences among sites can translate into differences in species co-occurrence patterns (e.g., Dullinger et al. 2007, Reitalu et al. 2008, Heino 2013). Thus, we can accept that the observed differences in co-occurrence patterns are primarily the result of our treatments, not variation in the suitability of species to mesocosms in different treatments (Pielou and Pielou 1968).

Zooplankton species richness per mesocosm was significantly different among treatments, with higher dispersal generally associated with higher species richness (Fig. 1a). This supports the assertion that dispersal can maintain species within mesocosms that might otherwise be eliminated through antagonistic interactions among species. Evidence has accumulated in support of dispersal as a mechanism for increasing the number of species within a system (Cadotte 2006, Myers and Harms 2009). We also found that dispersal influenced species composition, resulting in species differences between treatments with high and low levels of dispersal. Zooplankton species evenness within mesocosms, measured using abundance data, did not significantly change across dispersal treatments, indicating that dispersal did not significantly impact the distribution of resources across species. In part, this may be explained by the fact that increased richness in mesocosms in higher-dispersal treatments appears to have increased compositional similarity (Fig. 1c). This suggests that dispersal had a homogenizing impact on our experimental zooplankton communities (e.g., Pedruski and Arnott 2011, Vogt and Beisner 2011). These results conform to the general finding that dispersal can make a significant contribution to community structure (e.g., Zalewski and Ulrich 2006, Beaudrot and Marshall 2011, Heino 2013). Regional-scale processes like dispersal can be expected to positively impact species richness in contrast to the likely negative impact of local-scale, antagonistic interactions (e.g., Ricklefs 1987, Cornell and Lawton 1992, Caley and Schluter 1997, Olsen and Klanderud 2014).

Total zooplankton abundance per mesocosm did not significantly differ across dispersal treatments, suggesting that mesocosms had reached carrying capacity (Fig. 2c). Because zooplankton species vary considerably in

size, we also tested whether total mesocosm estimates of biomass differed across treatments. This post hoc assessment compiled biomass values calculated from length–weight regressions for our species (J. A. Rusak, B. E. Beisner, M. J. Paterson, and S. E. Arnott, *unpublished data*), and indicated that mean total biomass per mesocosm did not significantly differ across dispersal treatments (ANOVA,  $df = 3, 56, P = 0.86$ ). Based on these results, it appears that our dispersal treatments all reached a carrying capacity at which interactions can be expected to have played a role.

Predation can promote local diversity among prey species in aquatic systems (e.g., Shurin and Allen 2001) through a number of mechanisms associated with reducing competitive interactions (Chase et al. 2002), although there is ample evidence that the introduction of predators can also reduce species richness (e.g., Yan et al. 2002, Cadotte et al. 2006). It is possible that the observed increase in species richness in higher-dispersal treatment mesocosms (Fig. 1a) resulted from a combination of increased predation and dispersal. In our experiment, mesocosms in the three highest-dispersal treatments contained significantly more predators (Fig. 2b), but did not have a lower total abundance of herbivores, suggesting that the effect of predators on herbivorous zooplankton diversity was limited.

### *Co-occurrence patterns and dispersal*

Dispersal treatments had a significant impact on co-occurrence patterns (Fig. 3). More pronounced and significant patterns of negative co-occurrence were observed for the two intermediate-dispersal treatments, as well as in the highest-dispersal treatment when predators were included in the analysis (Fig. 3). Interestingly, patterns of zooplankton co-occurrence were nonsignificant in the lowest-dispersal treatment, in which the most pronounced effects of interspecific interactions that could contribute to negative co-occurrence were anticipated (Fig. 3). It is also possible that interactions were less pronounced in the low-dispersal treatment because not all species were as likely to reach mesocosms where competitive interactions could occur. While it might be expected that increased dispersal would produce positive co-occurrence patterns, this was not the case; rather, higher-dispersal treatments were generally characterized by significant negative co-occurrence patterns (Fig. 3). Our results clearly indicate that dispersal can have clear and significant impacts on species co-occurrence patterns, and may be a prominent contributor to previously reported significant negative co-occurrence patterns (e.g., Gotelli and McCabe 2002). The impact of dispersal treatments on co-occurrence patterns does not appear to have been mediated by predatory zooplankton. The top three dispersal treatments did not significantly differ in predator abundance or species richness, but clearly differed in patterns of co-occurrence. Furthermore, patterns of co-occurrence followed a similar trend

whether predatory zooplankton were included or excluded from analysis (Fig. 3a).

Gotelli and Rohde (2002) noted that a lack of significant co-occurrence patterns may be normal for small-bodied taxa with low vagility as a consequence of reduced likelihood of niche saturation and low population densities at which interspecific interactions are weak. However, there is some evidence of significant negative co-occurrence patterns for small organisms (e.g., Heino 2013). Additionally, it is not likely that this applies to zooplankton systems, as they are not recognized as being poor dispersers; furthermore, given the evidence suggesting our mesocosms reached carrying capacity, it is not likely that a low potential for interactions can explain insignificant co-occurrence patterns in our low-dispersal treatment.

The impact of dispersal on co-occurrence patterns peaked in low-intermediate-dispersal treatments, and then declined (Fig. 3). This may be related to the fact that the three highest-dispersal treatments did not significantly differ in species richness, although there was a general trend upward (Fig. 1a). Thus, it appears clear that the impact of dispersal on co-occurrence patterns will not simply be a positive function of dispersal strength. The impact of dispersal may level off as it increases, with further increases having a limited or negligible effect. This may result from the expected homogenization at very high levels of dispersal. Crucially, all significant negative co-occurrence patterns were in the three highest-dispersal treatments. Given the observed influence of dispersal on co-occurrence patterns and the recognition that dispersal varies in influence among communities (e.g., Ozinga et al. 2005, Heino et al. 2012), it may not be possible to attribute significant negative co-occurrence patterns observed in nature to antagonistic interactions among species, even if other processes recognized as contributing to negative co-occurrence patterns could be controlled for.

Our results are consistent with theoretical explorations that indicate that stochastic processes such as dispersal can drive significant negative co-occurrence patterns (e.g., Ulrich 2004, Bell 2005), as well with field tests for the potential influence of dispersal on co-occurrence patterns (Zalewski and Ulrich 2006, Heino 2013). Our study is an experimental extension of the work by Zalewski and Ulrich (2006), who explored co-occurrence patterns on islands that varied in their relative isolation and consequently in the relative importance of dispersal and local interactions. In that case, co-occurrence patterns were a function of the relative isolation of particular communities. One difference, however, is that Zalewski and Ulrich's results (2006) may have been impacted by differences among species in how suitable they were for each habitat (e.g., Pielou and Pielou 1968, Gotelli and Rohde 2002), while abiotic similarity among dispersal treatments in this study circumvents this potential influence on co-occurrence patterns. Additionally, we were able to control for

rates of dispersal in our study, something not possible using the field approach taken by Zalewski and Ulrich (2006). Our results are the first experimental assessment of the impact of dispersal on co-occurrence patterns, and confirm the potential for dispersal to interfere with the ability of researchers to attribute significant negative co-occurrence patterns to interspecific interactions.

There are two central elements of the presence-absence matrix that contribute to negative co-occurrence patterns: species richness per mesocosm (Fig. 1a), and the distribution of species occupancies among mesocosms (Fig. 3b). While it is unclear at this time how subtle changes in these characteristics translate into changes in the significance of co-occurrence patterns, it is clear that dispersal can impact these two characteristics of natural systems in ways that are of importance in this regard. The solution to this problem lies in a general need for understanding how particular changes in the richness and abundance distributions (i.e., occupancy patterns) among sites (mesocosms/habitats/plots) in the presence-absence matrix contribute to changes in the significance of relevant co-occurrence null model tests.

Out of practical necessity, our experiment was not replicated beyond a single metacommunity per dispersal treatment. Consequently, we are restricted to demonstrating that dispersal can strongly impact co-occurrence patterns. However, higher dispersal in our experiment resulted in increased species richness and modest increases in inter-mesocosm similarity. Evidence suggests that dispersal regularly influences systems in this way (Cadotte 2006, Myers and Harms 2009, Pedruski and Arnett 2011, Vogt and Beisner 2011); as such, we feel our findings are sound and broadly applicable. Furthermore, the stability of our observed patterns in the face of our jackknifing procedure suggests that differences in co-occurrence across our treatments are not spurious.

### *Conclusions*

Variation in the number of dispersers significantly impacted the species richness and composition of zooplankton in our experimental mesocosms. Changes in community structure elicited by our dispersal treatments also had a powerful impact on zooplankton species co-occurrence patterns. The lowest-dispersal treatment, under which species interactions should theoretically have been most important, was the only treatment for which there was consistently no evidence of significant negative co-occurrence patterns; higher dispersal generally resulted in more significant and pronounced negative co-occurrence patterns, although this declined at the highest dispersal level. Our results suggest that even in the absence of significant environmental heterogeneity, which is known to impact co-occurrence patterns (e.g., Dullinger et al. 2007, Reitalu et al. 2008, Heino 2013, McCreadie and Bedwell 2013), dispersal appears capable of driving patterns of signif-

icant negative co-occurrence. Previously, dispersal limitation has been identified as a process that can influence co-occurrence patterns; however, it is clear from our results that dispersal can contribute to these patterns even when all dispersers are equally capable of reaching all sites. Consequently, we recommend that extreme caution be taken when interpreting significant negative co-occurrence patterns, even in relatively homogeneous, small-scale communities, as evidence of the importance of antagonistic interactions among species.

#### ACKNOWLEDGMENTS

We thank D. Gray, C. Symons, A. Jokela, K. Lemmon, A. Bresenhan, M. Yuille, and P. Turko for help setting up the experiment as well as T. Nash for assistance with data collection and processing. J. Sinclair helped with some statistical analysis. Comments by two anonymous reviewers improved the manuscript. This research was supported by the Natural Sciences and Engineering Research Council of Canada grants to B. Schamp and S. Arnott.

#### LITERATURE CITED

- Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32–46.
- Balcer, M. D., N. L. Korda, and S. I. Dodson. 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. University of Wisconsin Press, Madison, Wisconsin, USA.
- Beaudrot, L., and A. Marshall. 2011. Primate communities are structured more by dispersal limitation than by niches. *Journal of Animal Ecology* 80:332–341.
- Bell, G. 2005. The co-distribution of species in relation to the neutral theory of community ecology. *Ecology* 86:1757–1770.
- Belyea, L. R., and J. Lancaster. 1999. Assembly rules within a contingent ecology. *Oikos* 86:402–416.
- Cadotte, M. W. 2006. Dispersal and species diversity: a meta-analysis. *American Naturalist* 167:913–924.
- Cadotte, M., A. Fortner, and T. Fukami. 2006. The effects of resource enrichment, dispersal, and predation on local and metacommunity structure. *Oecologia* 149:150–157.
- Caley, M. J., and D. Schluter. 1997. The relationship between local and regional diversity. *Ecology* 78:70–80.
- Chase, J. M., P. A. Abrams, J. P. Grover, S. Diehl, P. Chesson, R. D. Holt, S. A. Richards, R. M. Nesbit, and T. J. Case. 2002. The interaction between predation and competition: a review and synthesis. *Ecology Letters* 5:302–315.
- Connor, E. F., and D. Simberloff. 1979. The assembly of species communities: chance or competition? *Ecology* 60:1132–1140.
- Connor, E. F., and D. Simberloff. 1983. Interspecific competition and species co-occurrence patterns on islands: null models and the evaluation of evidence. *Oikos* 41:455–465.
- Cornell, H. V., and J. H. Lawton. 1992. Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. *Journal of Animal Ecology* 61:1–12.
- Cottenie, K., and L. De Meester. 2004. Metacommunity structure: synergy of biotic interactions as selective agents and dispersal as fuel. *Ecology* 85:114–119.
- Cottenie, K., N. Nuytten, E. Michels, and L. De Meester. 2003. Zooplankton metacommunity structure: regional vs. local processes in highly interconnected ponds. *Ecology* 84:991–1000.
- DeMelo, R., and P. D. N. Hebert. 1994. A taxonomic reevaluation of North American Bosminidae. *Canadian Journal of Zoology* 72:1808–1825.
- Diamond, J. M. 1975. Assembly of species communities. Pages 342–444 in M. L. Cody and J. M. Diamond, editors. *Ecology and evolution of communities*. Harvard University Press, Cambridge, Massachusetts, USA.
- Dullinger, S., et al. 2007. Weak and variable relationships between environmental severity and small-scale co-occurrence in alpine plant communities. *Journal of Ecology* 95:1284–1295.
- Edmondson, W. T. 1959. *Ward and Whipple's freshwater biology*. John Wiley and Sons, New York, New York, USA.
- Fayle, T. M., and A. Manica. 2010. Reducing over-reporting of deterministic co-occurrence patterns in biotic communities. *Ecological Modelling* 221:2237–2242.
- Flinn, K. M., T. C. Gouhier, M. J. Lechowicz, and M. J. Waterway. 2010. The role of dispersal in shaping plant community composition of wetlands within an old-growth forest. *Journal of Ecology* 98:1292–1299.
- Forrest, J., and S. E. Arnott. 2006. Immigration and zooplankton community responses to nutrient enrichment: a mesocosm experiment. *Oecologia* 150:119–131.
- Gilpin, M. E., and J. M. Diamond. 1982. Factors contributing to non-randomness in species co-occurrences on islands. *Oecologia* 52:75–84.
- Gotelli, N. J. 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81:2606–2621.
- Gotelli, N. J., and G. L. Entsminger. 2001. Swap and fill algorithms in null model analysis: rethinking the knight's tour. *Oecologia* 129:281–291.
- Gotelli, N. J., and D. J. McCabe. 2002. Species co-occurrence: a meta-analysis of J. M. Diamond's assembly rules model. *Ecology* 83:2091–2096.
- Gotelli, N. J., and K. Rohde. 2002. Co-occurrence of ectoparasites of marine fishes: a null model analysis. *Ecology Letters* 5:86–94.
- Gotelli, N. J., and W. Ulrich. 2012. Statistical challenges in null model analysis. *Oikos* 121:171–180.
- Heino, J. 2013. Environmental heterogeneity, dispersal mode, and co-occurrence in stream macroinvertebrates. *Ecology and Evolution* 3:344–355.
- Heino, J., and M. Grönroos. 2013. Does environmental heterogeneity affect species co-occurrence in ecological guilds across stream macroinvertebrate metacommunities? *Ecography* 36:926–936.
- Heino, J., M. Grönroos, J. Soinen, R. Virtanen, and T. Muotka. 2012. Context dependency and metacommunity structuring in boreal headwater streams. *Oikos* 121:537–544.
- Hudson, P., J. Reid, L. Lesko, and J. Selgeby. 1998. *Cyclopoid and harpacticoid copepods of the Laurentian Great Lakes*. Ohio Biological Survey, Ohio State University, Columbus, Ohio, USA.
- Lanyon, S. M. 1985. Detecting internal inconsistencies in distance data. *Systematic Zoology* 34:397–403.
- Legendre, P., and L. Legendre. 2012. *Numerical ecology*. Elsevier, Amsterdam, Netherlands.
- Lehsten, V., and P. Harmand. 2006. Null models for species co-occurrence patterns: assessing bias and minimum iteration number for the sequential swap. *Ecography* 29:786–792.
- Leibold, M. A., et al. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7:601–613.
- MacArthur, J., and E. Wilson. 1967. *The theory of island biogeography*. Princeton University Press, Princeton, New Jersey, USA.
- Manly, B. F. J. 1995. A note on the analysis of species co-occurrences. *Ecology* 76:1109–1115.
- McCauley, S. 2006. The effects of dispersal and recruitment limitation on community structure of odonates in artificial ponds. *Ecography* 29:585–595.
- McCreadie, J. W., and C. R. Bedwell. 2013. Patterns of co-occurrence of stream insects and an examination of a causal mechanism: ecological checkerboard or habitat checkerboard? *Insect Conservation and Diversity* 6:105–113.

- Michels, E., K. Cottenie, L. Neys, and L. De Meester. 2001. Zooplankton on the move: first results on the quantification of dispersal of zooplankton in a set of interconnected ponds. *Hydrobiologia* 442:117–126.
- Myers, J. A., and K. E. Harms. 2009. Seed arrival, ecological filters, and plant species richness: a meta-analysis. *Ecology Letters* 12:1250–1260.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens and H. Wagner. 2013. *vegan*: community ecology package. R package version 2.0-10. <http://CRAN.R-project.org/package=vegan>
- Olsen, S. L., and K. Klanderud. 2014. Biotic interactions limit species richness in an alpine plant community, especially under experimental warming. *Oikos* 123:71–78.
- Ozinga, W., J. Schaminée, R. Bekker, S. Bonn, P. Poschold, O. Tackenberg, J. Bakker, and J. van Groenendael. 2005. Predictability of plant species composition from environmental conditions is constrained by dispersal limitation. *Oikos* 108:555–561.
- Pedruski, M. T., and S. E. Arnott. 2011. The effects of habitat connectivity and regional heterogeneity on artificial pond metacommunities. *Oecologia* 166:221–228.
- Pielou, D. P., and E. C. Pielou. 1968. Association among species of infrequent occurrence: the insect and spider fauna of *Polyporus betulinus* (Bulliard) Fries. *Journal of Theoretical Biology* 21:202–216.
- R Core Team. 2013. *R*: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Reitalu, T., H. C. Prentice, M. T. Sykes, M. Lonn, L. J. Johansson, and K. Hall. 2008. Plant species segregation on different spatial scales in semi-natural grasslands. *Journal of Vegetation Science* 19:407–416.
- Ricklefs, R. E. 1987. Community diversity: relative roles of local and regional processes. *Science* 235:167–171.
- Sanders, N. J., N. J. Gotelli, N. E. Heller, and D. M. Gordon. 2003. Community disassembly by an invasive species. *Proceedings of the National Academy of Sciences USA* 100:2474–2477.
- Sanderson, J. G., M. P. Moulton, and R. G. Selfridge. 1998. Null matrices and the analysis of species co-occurrences. *Oecologia* 116:275–283.
- Seabloom, E. W., O. N. Bjornstad, B. M. Bolker, and O. J. Reichman. 2005. Spatial signature of environmental heterogeneity, dispersal, and competition in successional grasslands. *Ecological Monographs* 75:199–214.
- Sfenthourakis, S., S. Giokas, and E. Tzanatos. 2004. From sampling stations to archipelagos: investigating aspects of the assemblage of insular biota. *Global Ecology and Biogeography* 13:23–35.
- Shurin, J. 2000. Dispersal limitation, invasion resistance, and the structure of pond zooplankton communities. *Ecology* 81:3074–3086.
- Shurin, J. B., and E. Allen. 2001. Effects of competition, predation, and dispersal on species richness at local and regional scales. *American Naturalist* 158:2624–2637.
- Shurin, J. B., K. Cottenie, and H. Hillebrand. 2009. Spatial autocorrelation and dispersal limitation in freshwater organisms. *Oecologia* 159:151–159.
- Smith, B., and J. B. Wilson. 1996. A consumer's guide to evenness indices. *Oikos* 76:70–82.
- Smith, K., and C. Fernando. 1978. A guide to the freshwater calanoid and cyclopoid copepod crustacean of Ontario. Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.
- Stemberger, R. S. 1979. A guide to rotifers of the Laurentian Great Lakes. EPA-600/4-79-021. U.S. Environmental Protection Agency, Cincinnati, Ohio, USA.
- Stone, L., and A. Roberts. 1990. The checkerboard score and species distributions. *Oecologia* 85:74–79.
- Ulrich, W. 2004. Species co-occurrences and neutral models: reassessing J. M. Diamond's assembly rules. *Oikos* 107:603–609.
- Veech, J. A. 2013. A probabilistic model for analysing species co-occurrence. *Global Ecology and Biogeography* 22:252–260.
- Vogt, R. J., and B. E. Beisner. 2011. Assessing the impact of dispersal on zooplankton community structure. *Journal of Plankton Research* 33:1757–1761.
- Warfe, D. M., N. E. Pettit, R. H. Magierowski, B. J. Pusey, P. M. Davies, M. M. Douglas, and S. E. Bunn. 2013. Hydrological connectivity structures concordant plant and animal assemblages according to niche rather than dispersal processes. *Freshwater Biology* 58:292–305.
- Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnology and Oceanography* 39:1985–1992.
- Witty, L. M. 2004. Practical guide to identifying freshwater crustacean zooplankton. Cooperative Freshwater Ecology Unit, Sudbury, Ontario, Canada.
- Yan, N. D., R. Girard, and S. Boudreau. 2002. An introduced invertebrate predator (*Bythotrephes*) reduces zooplankton species richness. *Ecology Letters* 5:481–485.
- Zalewski, M., and M. Ulrich. 2006. Dispersal as a key element of community structure: the case of ground beetles on lake islands. *Diversity and Distributions* 12:767–775.

## SUPPLEMENTAL MATERIAL

## Ecological Archives

The Appendix is available online: <http://dx.doi.org/10.1890/14-1128.1.sm>