THE MAINTENANCE AND GENERATION OF FRESHWATER DIVERSITY FROM THE LOCAL TO THE GLOBAL SCALE

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ABSTRACT

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The distribution of biological diversity is markedly uneven across the world. Despite the seemingly endless variety of forms and adaptations that have evolved and continue to evolve, large differences in the numbers and characteristics of species remain among locales. These differences are often not random; e.g., the Earth's tropics are disproportionately rich in the diversity of life and large areas harbor more species than small areas. Observations of these general biogeographic patterns are some of the oldest contributions by early naturalists and ecologists, yet explanations for these patterns are a recurring topic of debate. Generality of pattern (with room for exceptions to the rule) has been reached for large-scale gradients in diversity but not generality of the theories that underlie these patterns.

Unlike diversity at the biogeographic scale, even the generality of pattern at the local scale of species interactions remains elusive. Although numerous investigations into how potential drivers of local diversity such as productivity, isolation, and disturbance influence diversity have been made, a unifying consensus to describe general patterns of local diversity has not emerged. Today, increasing emphasis is being placed on the importance of the interaction between local and regional scales in influencing local diversity.

The thesis first introduces and summarizes attempts to describe and explain biodiversity patterns at both the local and regional scales. Chapter 1 describes a study of the role of dispersal and assembly history in influencing species diversity in natural and experimental pond communities of plankton under the metacommunity framework. A further test of the role of

dispersal and assembly history is presented in Chapter 2 using experimental pond mesocosms. This study evaluates the role of ecosystem size on the assembly and structure of a multitrophic community of both plankton and macroinvertebrates while concurrently varying nutrient input rate and initial assembly. Chapter 3 empirically illustrates variation in the dormancy-dispersal strategies used by freshwater zooplankton that can lead to interspecific differences in the degree and type of dispersal limitation. In Chapter 4, I use the distribution of single lake endemic fish in the largest lakes in the world to estimate the relative contributions of lake surface area, age, and latitude on diversification.

I dedicate this dissertation to my parents, Joseph and Ernestine.

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I am grateful for the years of support, guidance, and discussion from my advisor, Gary Mittelbach. May I be as giving to others, as warm-hearted, and as dedicated to the scientific pursuit in my own career. I also acknowledge comments and critique from my other committee members, Elena Litchman, Christopher Klausmeier, and Orlando Sarnelle. Finally, I thank my wife, Melanie, for her selfless support of me in all things.

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INTRODUCTION

Despite centuries-old knowledge of general biodiversity patterns, the search for cohesive, general frameworks to describe both the maintenance and generation of these patterns continues (e.g., Hubbell 2001, Ricklefs 2004, Scheiner and Willig 2008, McGill 2010, Vellend 2010). Many local and regional processes that can lead to differential speciation, extinction, and species coexistence have been long-known (MacArthur 1972), but a single, unified framework for understanding Earth's diversity seems to always remain just beyond the horizon. Indeed, even some of the strongest drivers of local diversity such as productivity vary in the shape of their effect (Mittelbach et al. 2001). While local determinism in the abundance and diversity of species must always be the case to some degree, context-dependence is ubiquitous and is necessarily tied to regional processes (Ricklefs 1987).

Chapters 1 & 3

The metacommunity framework (Leibold et al. 2004) unites a number of pre-existing of multiple community theories that influence species coexistence and diversity patterns, initially using the four paradigms of patch dynamics, mass effects, species sorting, and neutral theory. The metacommunity approach provides a common framework for ecologists to study how species abundances and distributions are formed through the interdependence of processes at different spatial scales. In its simplest form, a metacommunity is a number of communities connected by dispersal (Gilpin and Hanski 1991) and may be better seen as a philosophical approach rather than an actual suite of theories. Indeed, recent effort has been made to generalize

the framework and to prevent the pigeonholing of metacommunity studies into tests of which of the four original paradigms is most important (Leibold and Chase 2017).

Dispersal is the single ecological constant in the metacommunity framework, however, its magnitude and role in shaping metacommunity diversity patterns are not well-established. The majority of experimental studies on metacommunities do not utilize natural dispersal rates (Grainger and Gilbert 2016), which are challenging to measure in multispecies systems (Heino et al. 2017). In Chapter 1, I measure dispersal rates in the field for 79 plankton taxa while measuring the influence of these dispersal rates on natural ponds in a metacommunity as well as experimental pond communities that either contain a fully assembled community or are unassembled. Chapter 3 combines dispersal rate data with evidence on the production and dormancy of resting stages in zooplankton taxa to assess for interspecific differences in dormancy-dispersal tradeoffs in pond metacommunities.

Chapter 2

The oldest known empirical biodiversity pattern is the species-area relationship (SAR) for British plants that was described by H. C. Watson in 1859 (Rosenzweig 1995). The SAR describes the common observation that biotic diversity scales with the spatial extent area sampled. The most commonly applied relationship between diversity and area to describe the SAR is the power function of Preston (1960), which states that the number of species (N) is related to area (A) according to the constants k and z:

$$N=kA^z$$

Preston considered the possibility that a "canonical" relationship between the power scaling (z slope) of species number and area may exist (1962) and that this value may be approximately

0.26 (Preston 1962) or range from 0.20 to 0.35 (Wilson and MacArthur 1967). Although neither the power function or a particular *z* slope should be considered a "best" model to describe diversity (Connor and McCoy 1979), the diversity of many biological systems fits such a canonical relationship. Species-area relationships can be a useful way to compare the scaling of diversity across systems or under different ecological conditions (e.g., McGuinness 1984, Powell et al. 2013).

Despite the utility of the species-area relationship, it may not be suitable to describe the full scaling of diversity from local to global scales. The "small island effect" describes the observation that there is often an absence or a marked difference in the slope of the species-area relationship over small ecosystem sizes (Lomolino 2000, Triantis et al. 2006). Further, the process by which species are sampled can lead to considerable variation in the perceived species-area relationship (Palmer and White 1994) and scale-dependency of the slope of the species-area relationship can be common (Dengler 2009). Finally, which some consideration has been given to the interplay of the species-area relationship with evolution (e.g., Losos and Schluter 2000, Triantis et al. 2008), characterization of differences in slope among trophic levels (Ryberg and Chase 2007), as well as observations that steeper species-area curves are found at lower latitudes (Drakare et al. 2006), a full integration of the major drivers of diversity into a species-area relationship framework from the very local to the global scale does not exist.

Few experimental manipulations of ecosystem size in a multitrophic context in a semi-natural setting exist and a major knowledge gap is an explanation for the "small island effect", which has previously been attributed to stochasticity (Lomolino 2000) rather than ecological process. In Chapter 2, I present results from an experimental pond mesocosm study where ecosystem size, productivity, and assembly status are manipulated in a multitrophic system of

plankton and macroinvertebrates. I evaluate the species-area relationships observed over this small-scale ecosystem size gradient alongside the potential effects of productivity, dispersal limitation, and changes to trophic structure.

Chapter 4

Like the species-area relationship, the observation that there are more species in the tropics (the latitudinal diversity gradient) dates to the time of early naturalists and is a general pattern that has remained persistent in the face of over 100 years of scrutiny. While a handful of taxa have weak or negative latitudinal diversity gradients, it is remarkably consistent across the many forms of life on Earth (Hillebrand 2004). Potential drivers of the latitudinal diversity gradient include the non-mutually exclusive theories that diversification rates are higher in the tropics, that the tropics are able to support more individuals and species, and that the tropics have historically been larger and more widespread over Earth's evolutionary history (Mittelbach et al. 2007). These drivers that potentially influence latitudinal diversity may interact with regional differences such as temperature and rainfall (MacArthur 1972) as well as with local differences such as the strength of predation and competition (Pianka 1966) and the prevalence of certain biotic interactions (Schemske et al. 2009).

Tests of theories to explain the latitudinal diversity gradient have focused primarily on the potential for a difference in diversification rate with latitude – either through higher rates of speciation in the tropics or higher rates of extinction outside the tropics (or both). Mammals, for example, have both higher speciation rates and lower extinction rates in the tropics (Rolland et al. 2014). While evolutionary explanations for the latitudinal diversity gradient have a strong basis, ecological processes must still be considered as any differences in species number due to

diversification must still be maintained through coexistence (Schemske and Mittelbach 2017). Moreover, while large-scale processes such as climate stability are important in structuring the ranges of species through abiotic filtering, the influence of stability can be just as important in determining species interactions at local scales.

Notably difficult in latitudinal diversity gradient studies is the ability to test the effects of age and area alongside differences in diversification rates or species coexistence. While evidence for an area and time-integrated effect on the latitudinal diversity gradient exists (Fine and Ree 2006), there are no global-scale studies that simultaneously evaluate the effects of age, area, and latitude while also incorporating local scale data on abiotic factors and the biotic community context. In Chapter 4, I construct and analyze a data set of the endemic fish species in the largest lakes in the world (> 50 km^2) for which I also collected ages of continuous water occupancy as well as a number of biological, physical, and chemical variables. I estimate the joint effects of age, area, and latitude on endemism (a proxy for *in situ* speciation) while testing for differences among tropical versus extratropical fish families and the number of fish lineages across latitude.

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CHAPTER ONE

THE INFLUENCE OF DISPERSAL ON THE REALIZED TRAJECTORY OF A POND METACOMMUNITY

Abstract

Dispersal rates play a critical role in metacommunity dynamics, yet few studies have attempted to characterize dispersal rates for the majority of species in any natural community. Here we evaluate the relationship between the abundances of 179 plankton taxa in a pond metacommunity and their dispersal rates. We find the expected positive relationship between the regional abundances of phytoplankton, protozoa, and metazoan zooplankton, which is suggestive of dispersal being a density-independent per capita rate for these groups. When we tested to see if the rates of dispersing taxa predicted changes in community composition, we found that dispersers had no measurable impact on the short-term trajectory of local pond communities or mesocosm communities established experimentally (assembled communities), but became increasingly represented in the overall pond metacommunity during the course of the full growing season. In comparison, the composition of experimental mesocosms that lacked any initial zooplankton community (unassembled communities) were found to be driven by dispersal measured at the local pond community but not by dispersal observed across the overall metacommunity. These results suggest that the role of dispersal may shift from a contributor to local, ecological dynamics to that of metacommunity-wide, colonization-extinction dynamics as communities assemble.

Introduction

Dispersal, the movement of individuals and species, binds together the fates of communities, allowing local interactions and dynamics to scale up to the entire landscape. Although dispersal rate is arguably the most important parameter affecting metacommunity dynamics (Leibold et al. 2004), it is a notoriously difficult parameter to quantify in nature (Jacobson and Peres-Noto 2010). Recent advances in quantifying dispersal in nature have been made using indirect methods such as molecular approaches (Werth et al. 2006) and by partitioning diversity (Vandvik and Goldberg 2006), but there remain few measurements of species' dispersal rates at the metacommunity level in relation to species abundances and alongside data on the temporal dynamics of local community composition. This paucity in the measurement of dispersal rates in ecological communities persists despite long-standing knowledge of its interspecific variability and importance in colonization, including in freshwater plankton communities (Maguire 1963). Instead, most studies examining the effects of dispersal in a metacommunity are necessarily theoretical or experimentally impose dispersal rates on experimental systems that may or may not approximate those found in nature. Further, both theoretical models and empirical studies typically assume that all species within a community have the same per capita ability to disperse, ignoring that dispersal itself may be a highly variable trait (Lowe and McPeek 2014). This simplifying assumption ignores the potential of organisms to exhibit tradeoffs between colonization and competitive ability (Hanski and Ranta 1983, Cadotte et al. 2006b), or for dispersal to vary with local community conditions (Benard and McCauley 2008, Fronhofer et al. 2015).

Plankton communities in small ponds are ideal systems to study dispersal at the metacommunity level; plankton disperse readily, share a similar suite of passive dispersal

mechanisms such as by wind and animal vectors (Kristiansen 1996), and most species have rapid generation times that allow ecological interactions to play out over short timespans. Freshwater plankton also exhibit a wide range of life histories strategies for maximizing dispersal and comprise multiple trophic levels. This diversity in the functional traits of plankton presents multiple opportunities to investigate how different ecological factors may interact with dispersal to affect community dynamics.

Empirical evidence and the natural history of many organisms suggest that dispersal ability varies widely among freshwater plankton (Cáceres and Soluk 2002), as taxa differ in their ability to survive gut passage in animals, resist desiccation, and to be carried by wind (Havel and Shurin 2004). For example, the viability of eggs of different copepod species following fish consumption ranges from zero to greater than 90% (Conway et al. 1994). In cladoceran zooplankton, dispersal probabilities can be affected by differences in ephippia (diapausing egg) morphology (Hanski and Ranta 1983) as well as behaviour; *Daphnia* typically deposit ephippia on the water surface (Ślusarczyk and Pietrzak 2008), whereas chydorid ephippia are attached to the substrate (Fryer 1972). Such differences potentially increase the per capita dispersal rate by wind or animal vectors of the former relative to the latter. This evidence suggests that even in passively dispersing plankton, life history and behavioural differences among species can affect the propensity of a species to disperse.

Experimental manipulations of dispersal rates (e.g., Kneitel and Miller 2003, Cadotte et al. 2006a, Howeth and Leibold 2010) have generally found a strong role of dispersal in structuring local communities, demonstrating the importance of species dispersal rates to understanding community dynamics. However, significant challenges confront our ability to directly measure dispersal rates in nature. Dispersal limitation at the metacommunity level is

often inferred from the realized spatial patterns of species, where species that exhibit spatial autocorrelation or whose distributions are structured spatially (but not environmentally), are considered dispersal limited (Shurin et al. 2009, Frisch et al. 2012). However, classifying dispersal ability in this manner does not measure dispersal rate in the absolute sense of the tendency of an organism to move, which can cause these measures to be confounded with similar, yet conceptually distinct, processes such as the probability of establishment.

The collection of dispersal data in aerially transported plankton through windsocks, stick traps, water-filled containers, and newly constructed ponds (e.g., Jenkins 1995, Jenkins and Buikema 1998, Louette and De Meester 2005, Vanschoenwinkel et al. 2009, Lopes et al. 2016) is a widespread, but variable practice that has contributed to a renewed appreciation of how dispersal varies interspecifically among plankton, by dispersal vector, and with distance from source populations. Nevertheless, these practices of measuring natural dispersal are rarely incorporated into experiments on how dispersal shapes metacommunity diversity, which almost exclusively use the direct transfer of water or the manipulation of connectance through tubes (Grainger and Gilbert 2016). Here, we concurrently sample the dispersal rates of freshwater plankton (algae, protists, and zooplankton) and their abundances in a natural metacommunity at fine temporal resolution over a full growing season. We then evaluate how the measured dispersal frequencies of different plankton taxa in the metacommunity are related to the abundances of each taxa and test if these dispersal frequencies are predictive of the trajectories of established communities as well as during the assembly of experimental mesocosms that lack an initial community. We look to see whether certain major taxonomic groupings tend to be overrepresented in local communities or amongst the dispersers, and if patterns of dispersal in

the constituent taxa of different taxonomic groups reflect tradeoffs between local competitive ability and dispersal tendency.

Methods

Experimental design

Ten freshwater communities (ponds and near-shore sites of lakes) at the Kellogg Biological Station's Lux Arbor Reserve (Barry County, Michigan, USA) were selected for study. The Lux Arbor Reserve (LAR) is ideal for testing questions about freshwater metacommunities, as it contains approximately 30 distinct natural water bodies within 445 hectares. Logistically, we were unable to sample the entire metacommunity, however, the sites chosen roughly approximate the range and quantity of different near-shore habitats in the area. We sampled each of the ten local communities eight times (approximately every two weeks) during 24 May 2011 to 17 September 2011. Sampling was staggered throughout this time period, with a maximum of two sites sampled on any given day owing to the need to live count non-metazoan taxa, which a pilot study showed was necessary to accurately identify soft-bodied, non-loricate protists.

Phytoplankton and protozoa were sampled at each site by taking a 25 mL sample of the whole water column at four haphazardly chosen points with a depth of 0.4 – 0.5 m. The four samples were then pooled and filtered through a 16-μm mesh screen. The taxa in a tenth of the resulting volume (10 mL total) were then identified and enumerated using a Palmer counting cell at up to 400X magnification using light microscopy. Additional clarifying identification was made using confocal microscopy at the Michigan State University Center for Advanced Microscopy. Metazoan zooplankton were sampled at each site by taking a 250 mL sample of the

whole water column at the same four points using a PVC tube integrated sampler. The resulting sample was filtered through 80-μm mesh screen, preserved in 2% acid Lugols solution, and fully enumerated under a dissecting microscope.

Taxa with a body or resting stage length <16 μm were excluded from our analyses as individuals from these taxa may pass through filtering and be mistakenly identified as dispersing. Numerically important taxa excluded by this criterion include the green alga *Chlorella* and the flagellate protozoan *Bodo*. Larger members of the plankton, such as larvae of *Chaoborus* (phantom midge), *Ochlerotatus triseriatus* (mosquito), as well as Hydracarina (water mites) were also excluded since they are not generally dispersed by wind and can exhibit non-passive dispersal involving habitat choice (Resetarits et al. 2005, Vonesh et al. 2009). Immature forms of taxa that could not be identified to the same level as mature forms (e.g., copepod nauplii) were also excluded from our analyses.

To measure dispersal rate at a sampling site, a plastic container with a height of 0.4 m and a volume of 50 liters was placed within a few meters of each local pond community. About 50 L of water from the adjacent community was filtered through 16-μm plankton mesh and added to the container in late-May or early-June. Water from ponds was used to provide initial resources comparable to local communities and to prevent artificial abiotic environmental filters from influencing our estimates of dispersal rates. Dispersal containers were sampled at the same time as the adjacent local pond community. Any large debris that had fallen into the containers (e.g., leaves) was removed prior to sampling. Containers were first fully filtered with 80-μm plankton mesh and then fully filtered with 16-μm plankton mesh to produce two samples analogous to those used for phytoplankton/protists and metazoan zooplankton from the natural surveys. This filtration process (sometimes taking up to an hour per sample) as well as the live

sampling of phytoplankton and protists prevented the sampling of all ponds and containers simultaneously. Following the sampling process, the containers were replaced with new, empty containers that were again filled with pond water filtered through 16-μm mesh from the adjacent pond. These two samples were enumerated in the same manner as in the natural surveys. Taxa were enumerated up to a total of 1,000 individuals in a container, whereupon the volume sampled was noted to extrapolate to a total abundance in the full sample volume. A follow-up study found qualitatively similar results using a 1-μm filter, indicating that a 16-μm filter was not influencing our dispersal estimates for the target organisms.

Although measuring plankton dispersal in this manner is not free from error (e.g., loss of taxa between sampling points and potential growth following arrival) we believe that it is preferable to other methods such as using windsocks or measuring new species encountered in an established community. Using this technique, plankton disperse into a depauperate community where biotic interactions may have less influence on dispersal estimates and where abiotic conditions are comparable to local community conditions. Therefore, our measurement of dispersal is expected to most closely approximate the dispersal experienced by local communities prior to biological filtering. These methods are comparable to those used to measure dispersal in newly constructed pools and ponds (e.g., Jenkins and Buikema 1998, Louette and De Meester 2005) but have the added benefit of measuring dispersal into a new, vacant community at each time step at the same location. A potential concern is that the numbers of individuals observed dispersing into containers may be influenced by *in situ* growth or extinction in those containers, which may lead to our dispersal measurements incidentally incorporating some degree of establishment success, particularly for phytoplankton and protozoa taxa with short generation times. For this reason, we pair our statistical analyses on the

abundances of dispersing taxa with those that only account for the presence of absence of dispersing taxa, which are only susceptible to error in cases where taxa go completely extinct following dispersal. Moreover, the surface area of our dispersal containers is substantially smaller than the ponds, which may cause our measure of dispersal to be more stochastic than that realized naturally in the pond communities.

To evaluate how plankton dispersal influences the assembly of pond communities over a growing season, three experimental mesocosms were established within 1 m of each dispersal sampling container. These treatments were: (1) a mesocosm containing water from the adjacent pond that was coarsely filtered through 1.5-mm mesh to remove macroinvertebrates and debris but retain most of the plankton community (an "assembled community"), (2) a mesocosm containing water from the adjacent pond that was filtered through 16-μm mesh to exclude the focal plankton (body size and resting stage size > 16 μm) of this study (an "unassembled community"), and (3) a mesocosm containing well water to act as a standardized habitat across locales (an "unassembled community"). All mesocosms were identical in surface area, height (0.4 m), and volume (50 L) to the dispersal sampling containers. Mesocosms at a site were established and sampled concurrently, but only for zooplankton (in contrast to the natural ponds and dispersal samples), in a manner identical to the adjacent pond community using the methods described previously.

Statistical analyses

Permutational multivariate ANOVAs (PERMANOVA) using the Jaccard's distance metric (presence/absence of phytoplankton, protozoa, and metazoan zooplankton taxa) were used

to test whether the composition of the metacommunity differed from the composition of dispersers, and whether local communities received distinct suites of incoming dispersers depending on location or time period. Because the composition of natural pond communities is not independent across sampling periods, we do not evaluate for compositional differences within the pond communities over time and we look at the influence of spatial and temporal factors on the dispersers in isolation. To test for a difference in the composition of natural pond communities and dispersers, we compare the centroids for each natural pond community's composition over all eight sampling periods is compared to the compositions of disperser samples. The relative compositions of local communities and dispersers were visualized using a non-metric multidimensional scaling (NMDS) plot using the Jaccard's distance metric, a form of ordination which aims to best represent the difference in similarity between any two points as the Euclidean distance between the two points.

To better understand what species are contributing to the difference between local communities and dispersers, the Similarity Percentage (SIMPER) was calculated between the two groups using the Jaccard's distance metric. The individual contribution of each taxon (phytoplankton, protozoa, and metazoan zooplankton) in the metacommunity was determined, allowing us to assess whether taxa that tended to be overrepresented or underrepresented in the dispersers (presence/absence in dispersal samples pooled across the growing season) were more likely to be from particular taxonomic groupings of freshwater plankton. We tested whether taxa that were overrepresented in the dispersers in the SIMPER analysis were more likely to have an increase in community occupancy from the beginning to the end of the growing season using Pearson's product-moment correlation.

To test whether dispersal as a whole influenced the composition of communities, we evaluated whether communities tended to become more similar to either the taxa dispersing locally into an area or the overall composition of taxa dispersing regionally across the season. We did this by using what we are calling the *attraction coefficient* (AC), which estimates whether communities tend to become compositionally more similar to incoming dispersers over a given time step. The AC is the standardized change in the compositional distance of a community to the composition of dispersers over some time period:

$$AC = \frac{Dist(t, disp) - Dist(t+1, disp)}{Dist(t, t+1)}$$

where Dist(*t*, *disp*) is the distance between the community at time *t* and the composition of dispersers, Dist(*t* + 1, *disp*) is the distance between the community at time *t* + 1 and the composition of dispersers, and Dist(*t*, *t* + 1) is the distance between the community at time *t* and *t* + 1. The AC ranges from -1 to +1, where complete attraction of a community toward dispersers is given by +1 and complete repulsion of a community away from dispersers is given by -1 (Figure 6). For example, if the Jaccard's dissimilarity index for a pond at the first time period and the second time period was 0.4 (the denominator of the AC) and the dissimilarity index for the pond at the first time period and the intervening dispersers was 0.4 while it was 0.3 for the pond at the second time period, then the pond has become more similar to the incoming dispersers over the time period (AC = (0.4 – 0.3)/0.4 = 0.25). Community trajectories that are neutral with respect to the incoming dispersers (i.e. dissimilarity remains constant) have an AC value of 0. Since communities contain a finite number of species with finite possible

dissimilarities from dispersers, not all values of AC will be possible for a given trajectory. However, the maximum value of +1 is always achievable in the trivial case where a community is completely replaced by incoming dispersers and the minimum value of -1 is always achievable when community composition is equivalent to disperser composition and then deviates from that state.

We calculated the AC using the Bray-Curtis dissimilarity metric for each local community at each of the seven measured time steps based on the incoming dispersers to only that local community to see if compositional changes attributable to dispersers were visible at time scales of approximately two weeks. We visualize the time series of each community's trajectory relative to local dispersers over the growing season and use a student's t-test to evaluate whether the pooled AC is significantly different from zero, which would indicate an overall tendency of communities to become more similar to incoming dispersers over short timescales. To test whether there is a longer timescale effect of dispersers, we rerun this analysis using the initial and final local communities and the sum total of all dispersers over the growing season. Because there was a potential for population growth to occur in our dispersal sampling containers we also ran this analysis using the Jaccard's dissimilarity metric, which is only based on the presence and absence of taxa.

To test whether dispersal was density-independent or density-dependent in either phytoplankton/protozoa or metazoan zooplankton, we evaluated generalized linear models with a Gaussian link function on the log-transformed abundances of taxa in the metacommunity versus disperser samples pooled across all locations and sampling periods. Three statistical models were constructed: one where disperser abundance was constant and unrelated to metacommunity abundance, one where disperser abundance increased linearly with metacommunity abundance

(density-independent per capita rate), and one where disperser abundance could vary nonlinearly with metacommunity abundance by introducing a quadratic term (density-dependent per capita rate). Akaike's Information Criterion (AIC) was used for model selection separately for each taxonomic group. Eight taxonomic groupings of plankton (amoeboids, cladocerans, chlorophytes, ciliates, copepods, cryptophytes, euglenoids, and rotifers) had sufficient constituent taxa to attempt to evaluate whether the per-capita dispersal rate of taxa within a group exhibited density dependence, however, the amount of variation and outliers within groups made them sensitive to changes in the statistical model and data transformations being employed, with no model achieving singular support using AIC. Therefore, our main conclusions regarding density-dependent dispersal are based on two broad taxonomic groups (phytoplankton/protozoa or metazoan zooplankton).

To test whether the assembly and trajectory of communities was influenced by dispersers, we calculated the AC of each experimental mesocosm ("assembled" or "unassembled") at each time step relative to both the local and metacommunity-wide composition of dispersers observed during that time step. The AC values of the two types of mesocosm communities lacking initial zooplankton were indistinguishable and so we grouped them together into a single category ("unassembled"). Similarly, the AC values of the mesocosms that contained an initial zooplankton community were indistinguishable from those of the local ponds and so we grouped them into a second, combined category ("assembled"). A generalized linear model was used to detect whether unassembled and assembled communities experienced observable attraction toward the composition of dispersers and whether this tendency varied throughout the growing season.

Results

We encountered a total of 179 algal, protozoan, and metazoan plankton taxa meeting our criteria for inclusion in the study (Data to be made available from the Dryad Digital Repository). Of these 179 taxa, 138 (77.1%) dispersed into our experimental containers from late-May to early-September based on a sample total estimated to be in excess of three million individuals. With the exception of desmids, algae and protozoa were typically identified to the generic level with some taxa only identified to phylum. Metazoan zooplankton were mostly identified to the species level with the exception of some rotifer taxa.

Natural pond community trajectory

The composition of local pond communities was significantly different from that of the dispersing taxa (PERMANOVA: F ratio = 11.35, R^2 = 0.0806, P = 0.001; Table 1, Figure 1). The composition of dispersers also varied by location (F ratio = 1.26, R^2 = 0.1582, P = 0.001), indicating consistent spatial heterogeneity in dispersal across the metacommunity. Temporal differences in disperser composition across sampling periods were not significant (F ratio = 1.02, R^2 = 0.0860, P = 0.395).

Contributions to the difference among community and disperser composition were widely distributed across taxa. 102 taxa are required to explain 80% of the difference between the composition of communities and dispersers with no one species explaining more than 1.62% of the difference (Table 2). Overall, metazoan zooplankton were underrepresented in the dispersers relative to phytoplankton and protozoa (Figure 2). Within phytoplankton groups, only cryptophytes were broadly overrepresented in the dispersers. However, the bulk of the

compositional difference between natural communities and dispersers (>25%) was from the chlorophytes, whose constituent taxa were equally likely to be over- and under-represented in the dispersers. On a per taxon basis, higher contribution via SIMPER to the dispersers was not correlated with an increase in community occupancy from the beginning to the end of the growing season (Pearson's r = 0.081, t = 1.079, df = 177, P = 0.28). In other words, the taxa that had the greatest contribution to the difference between initial and final metacommunity composition over the course of the growing season were not, on the whole, the taxa that had disproportionately high dispersal.

The trajectory of individual local communities was overwhelmingly neutral over each sampling period with respect to the composition of the dispersers (Figure 3a). Individual communities tended to alternate between becoming more compositionally similar to dispersers and becoming more compositionally dissimilar with no clear pattern (student's t-test: t-value = 0.39, P = 0.7) based on the attraction coefficient using the Bray-Curtis distance metric. However, the trajectory of the entire metacommunity (all 10 ponds sampled) at the time scale of the growing season was significantly directed toward the composition of the incoming dispersers (student's t-test: t-value = 3.44, P < 0.01; Figure 3b). Results using the Jaccard's distance metric that reflects only the presence and absence of taxa were qualitatively similar.

Density dependence of plankton dispersal

A simple linear model without density dependence was best supported according to AIC for the relationship between the metacommunity abundance of plankton and their observed dispersal (positive slope, P < 0.001 for both sets of plankton, Figure 4). The per capita dispersal

tendencies of the eight dominant taxonomic groups were split equally between being density independent (as evidenced by a positive linear relationship between proportional abundance in the metacommunity and proportional abundance in the disperser sample; Figure 7) and showing evidence of negative density dependence at high densities (evidenced by a significant negative quadratic relationship; Figure 7). Taxonomic groups varied in the exact shape of their model fits and the relationships showed considerable scatter, but all non-intercept parameters were significant ($P < 0.05$ for all comparisons, Table 3). Each best-fit model had an intercept not significantly different from zero, indicating no strong signal of species dispersing from outside the sampled metacommunity ($P > 0.05$ for all comparisons).

Experimental mesocosm assembly

Data from experimental mesocosms using only metazoan zooplankton (not phytoplankton or protozoa, for which data were not collected) showed that mesocosms that were initiated without zooplankton ("unassembled communities"; i.e., those filled either with well water or 16-µm mesh filtered water) consistently became more similar to local dispersers over each time step (GLM on attraction coefficient using Jaccard's index: *t*-value = 2.77, $P < 0.006$; GLM on attraction coefficient using Bray-Curtis index: *t*-value = 2.73, $P < 0.007$l; Figure 5a), whereas "assembled communities" (i.e., experimental mesocosms containing an initial zooplankton community and the ten natural pond communities) did not ($P > 0.5$). However, when comparing community trajectories to the metacommunity-wide composition of dispersers for zooplankton there was no significant compositional attraction found for zooplankton communities that were initially unassembled (GLM on AC using Jaccard's: *t*-value = 1.07, $P = 0.285$; GLM on AC using Bray-Curtis: *t*-value = 1.15, $P = 0.251$; Figure 5b) or were assembled

with an initial community ($P > 0.5$). Therefore, dispersal over ecological timescales in this metacommunity appears to be substantially more important to the assembly of new communities than the trajectory of established communities.

Discussion

This study estimates the overland dispersal of plankton taxa relative to their abundances in a natural pond metacommunity, while simultaneously tracking the trajectory of local communities for phytoplankton, protozoan, and zooplankton taxa (179 taxa in all). Such broad taxonomic comparisons are underrepresented in the literature relative to those for zooplankton alone and are important for making multitrophic comparisons. Our sampling program also allowed us to assess the contributions of rare as well as abundant taxa, suggesting the level of sampling effort needed to capture the dispersal of rare taxa. For example, repeated, random resampling (N = 100) of our dispersal data (N = 70) shows that a subsample of 5 L (10% of the total volume) of our dispersal containers would, on average, capture only about a quarter of phytoplankton/protozoa disperser richness and about a third of metazoan plankton disperser richness in each container, respectively (Figure 8). A sample of about 37.5 L (75% of the total volume) is required to capture an average of 90% of the dispersing taxa for either phytoplankton/protozoa or metazoan zooplankton.

More than 70 percent of the taxa in the metacommunity were found to disperse during a single growing season. Thus, plankton as a whole appear to experience relatively little dispersal limitation within this pond metacommunity, although there may still exist general limitation if dispersal rates are too low to overcome stochasticity during establishment. Dispersal may also be

limiting for some of these taxa if there are windows of establishment opportunity that taxa may miss if their dispersal rate is sufficiently low. Moreover, dispersal limitation at larger spatial scales may of course be important when considering species that are outside the range of this study's metacommunity that could potentially establish if they were in the species pool.

Our study sampled a broad segment of the pond plankton community and attempted to capture the passive dispersal rates of freshwater plankton without bias toward a particular dispersal mode. However, if plankton exist in our system that disperse primarily via the movement of macroinvertebrates or waterfowl, they may be underrepresented in our dispersal estimates. We observed visitation to the disperser sampling units by macroinvertebrates such as Odonata and even colonization by small *Laccophilus* diving beetles. However, there was no observed visitation by some common macroinvertebrate taxa including *Notonecta*, which are known dispersers of cladoceran ephippia (Van de Meutter et al. 2008), or by waterfowl. Since macroinvertebrate and waterfowl visitation is mediated by the conditions of a given habitat (Kaminski and Prince 1981; Haas et al. 2007), effectively sampling plankton dispersed by these vectors would need to control for (and potentially manipulate) potential drivers of macroinvertebrate and waterfowl habitat choice. Taxa with known active habitat choice also were excluded from our comparisons. For example, some dipterans (e.g., phantom midges and mosquitoes) are planktonic as larvae but disperse as adults, and are selective in their oviposition habitat choice (Berendonk 1999, Resetarits and Silberbush 2016).

Although numerous models and some empirical freshwater plankton studies (Schamp et al. 2015) show the potential importance of dispersal to local community structure, our study found no signal of dispersal in the compositional changes of local, established communities over time. Instead, we observed that the trajectories of assembled local plankton communities were

random with respect to dispersal (Figure 3). One potential explanation is that community structure in this metacommunity could be predominantly driven by local factors beyond species dispersal rates (i.e., species sorting). Previous research in freshwater plankton communities has found a similar importance of local factors even at high dispersal rates (Cottenie et al. 2003, Howeth and Leibold 2008, Vanormelingen et al. 2008). Local factors also have been found to be of prime importance in structuring freshwater taxa that actively disperse and can make habitat choices such as midges (Garcia and Mittelbach 2008), aquatic beetles (Binkley and Resetarits 2005), and damselflies (Stoks and McPeek 2003). Other empirical work in coastal dune plants (Brunbjerg et al. 2012) and island woody plants (Lu et al. 2011) have also found species sorting to dominate over dispersal as the source of metacommunity structure. Collectively, these studies point to species sorting being the major driver of community composition in established freshwater and perhaps other systems. Of note is the potential for many of our study taxa to "disperse" through time by forming resting stages, with the potential to link community dynamics over temporal scales of hundreds of years (Hairston 1996, Gyllström and Hansson 2004). Therefore, these pond communities experience an analogous input of new individuals and taxa that may alter the relative importance of spatial versus temporal dynamics but is not measured by our study.

Interestingly, despite local community dynamics in natural ponds that did not reflect the composition of dispersers, a clear signal of dispersal was found at the scale of the whole metacommunity across the full growing season (Figure 3b). Taxa with greater dispersal ability appear to gain some net advantage over the general species pool, as the composition of the metacommunity moved towards that of the disperser taxa by the end of the summer. However, the precise mechanism is unknown and taxa that were overrepresented in dispersal samples were

not similarly overrepresented in local community samples at the end of the growing season. One possibility is that species that disperse more readily are more likely to recolonize a local community in which they became extinct, thereby increasing success over long timescales despite the effect being invisible at short timescales. In addition, inter-annual coexistence tradeoffs may exist, wherein some taxa expand their prevalence throughout a metacommunity during the benign conditions of the growing season but are more susceptible to overwintering mortality.

In contrast to the trajectory of established pond communities, the assembly and temporal trajectory of our experimental zooplankton communities that initially lacked zooplankton were consistently influenced by the local dispersers arriving during each time step (Figure 5). This result suggests disparate roles of dispersal during the assembly process when compared to the process by which taxa turnover in an established metacommunity, which may potentially be explained by priority effects and monopolization (Loeuille and Leibold 2008, Urban and De Meester 2009). Interestingly, only local and not metacommunity-wide measures of dispersal were predictive of assembly dynamics within the relatively limited scale of this pond metacommunity (maximum distance between ponds = 2.75 km). This spatial scale is well within the scale of <10 km considered by Havel and Shurin (2004) to be where the supply of colonizing zooplankton should not be limiting. Moreover, each of our ponds would be considered within the species pool (within 3 km radius) of each other in the framework employed by Louette and De Meester (2005) to characterize the dispersal and colonization of cladoceran zooplankton into new communities. Thus, the scale of dispersal that is relevant to assembly in plankton communities in ponds appears to be even more local than often considered – perhaps due to fine-scale dispersal barriers such as foliage or surface topography. This result also emphasizes that the use of single,

global measures of dispersal at the metacommunity scale may be inappropriate to capture the local heterogeneity of dispersal and its influence on freshwater plankton communities, which can markedly alter the role of dispersal in metacommunity dynamics. For example, a manipulation of dispersal in protists and rotifers has previously found differences in the diversity patterns generated by homogenous global dispersal versus dispersal that is influenced locally through directional biases (Altermatt et al. 2011).

Overall, the dispersal rates of plankton taxa were highly related to their metacommunity abundances in a density independent manner (Figure 4) despite many individual taxa varying substantially from this general fit in what may potentially represent biological variation in dispersal tendencies. Although the amount of variation in our data prevents any confident interpretations of the density dependence of dispersal within taxonomic groups, one set of statistical models does show that the apparent per capita dispersal rates (including bias from possible *in situ* growth and extinction) of eight plankton taxonomic groups were found to be either density independent (four groups; Figure 7a) or to show some negative density dependent (four groups; Figure 7b). Theory suggests that density dependence may evolve in metapopulations where species evolve to decrease dispersal rate when populations are below their local carrying capacity and evolve to increase dispersal rates when local carrying capacities are exceeded (Travis et al. 1999). Experiment evidence also exists for positive density-dependence in the ephippia (resting eggs) of some cladoceran species (Carvalho and Hughes 1983, Smith et al. 2009). We did not estimate the carrying capacities of taxa in our study, however, none of our plankton groups showed evidence of increased per capita dispersal rates at high abundance (and thus most likely to be at or above carrying capacity).

At the metacommunity level, negative density-dependent per capita dispersal may represent a competition-colonization tradeoff where ecologically similar taxa are able to coexist when species that are locally the best competitors are the worst dispersers, and vice versa. However, this cannot be the only diversity-maintaining force in our metacommunity since it is unlikely to occur in the taxa with density-independent dispersal rates. Previous research on similar taxa also found mixed results: ciliates lacked a general competition-colonization tradeoff (Limberger and Wickham 2011) whereas Hanski and Ranta (1983) found a tradeoff between local competitive ability and dispersal tendency within three species of *Daphnia* in a rock pool metacommunity. A myriad of coexistence mechanisms, including those involving dispersal, may be expected when considering diverse metacommunities where it is highly improbable that all individual species pairs or multispecies interaction modules are being maintained through similar mechanisms. In this context, the absence of positive density dependent per capita dispersal in plankton groups to potentially lower intraspecific competition is interesting and may imply a lack of selective pressure toward this strategy due to an outsized fitness cost for passively dispersing through a completely non-viable (terrestrial) matrix.

Metacommunity models in which the constituent species have similar dispersal abilities make clear, general predictions for the effect of dispersal rate on species richness at local and regional scales. For example, Mouquet and Loreau (2003) showed that local community richness is a hump-shaped function of overall dispersal rate, and that regional species richness is maximized at intermediate rates of dispersal (but see Haegeman and Loreau 2014 for conditions under which this prediction is altered). However, general results such as these may be less likely when dispersal rates stem from a mixture of metacommunity abundance, differences in the presence of density dependence as abundance changes, and differences in the general tendency

of broad taxonomic groups to disperse, as we found here. We found dispersal rates to vary significantly among taxa in at least some groups, suggesting that patterns of local and regional species richness are unlikely to scale simply with a univariate measure of dispersal (e.g., connectance) within a plankton metacommunity.

Previous work (Bie et al. 2012) in aquatic metacommunities suggests that the body size of passively dispersing species such as plankton may be positively correlated with dispersal limitation; i.e., large-bodied species disperse at lower rates. This body size/dispersal rate relationship may stem either from the lower population sizes of larger-bodied taxa compared to smaller-bodied taxa, or the tendency of smaller-bodied taxa to disperse through passive means at a higher per capita rate. Our results confirm that body size may generally decrease dispersal tendency as evidenced by the tendency of poorly dispersing taxa to be overrepresented by metazoan zooplankton and highly dispersing taxa to be dominated by phytoplankton (Table 2; Figure 2). Although the influence of these differences of body size on dispersal limitation is apparent in the coarse scale difference between phytoplankton and metazoan zooplankton, finer scale size differences among individual taxa within these two groupings were less apparent or nonexistent. No effect of body size on dispersal tendency was seen within the highly dispersal-variable cladocerans (P.J.H., analysis not shown) and many qualitatively similar taxa, including some pairs in the same genera, had remarkably dissimilar dispersal tendencies.

A key implication of our study is that species that are found to rapidly colonize new habitats are not necessarily more capable dispersers and that there is more to establishing in a community than simply arriving there. For example, both Shurin (2000) and Cáceres and Soluk (2002) found the rotifer *Brachionus angularis* to be an apt colonizer of new habitats, but we found it to have one of the greatest discrepancies between its occupancy in the metacommunity

(52.5%) and its commonness in disperser samples (2.86%). Thus, in metacommunities such as ours where there is strong connectivity among locales, differences in the propensity of certain taxa to successfully establish in a community may be more important than dispersal tendency in determining the overall colonization ability of freshwater plankton (e.g., Shurin 2000) or other taxa (e.g., Case 1975, Gross 1982, Gill and Marks 1991). Importantly, this phenomenon may vary depending on the scale at which a metacommunity is defined. For example, a study in a rock pool zooplankton metacommunity found significantly more dispersal limitation across larger spatial extents of rock pools than within smaller spatial scales (Ng et al. 2009). Establishment limitation following dispersal could be mediated through differences in competitive ability, initial growth rate, differences in maintaining adequate body condition during dispersal, or an Allee effect in sexually reproducing species when the availability of mates is limiting (Sarnelle and Knapp 2004). A study quantifying dispersal in lichens reached similar conclusions regarding the potential for establishment limitation at the local scale to be at least as important as dispersal (Werth et al. 2006).

It is clear from our study that dispersal rates alone are unlikely to explain the trajectory of local, established plankton communities. Rather, in this and other study systems, a combination of forces is likely to shape community structure and these forces may be context-dependent. For example, empirical work in both freshwater bacterial communities (Lindström and Östman 2011) and aquatic plants (Akasaka and Takamura 2011) found that the effect of dispersal was dependent on both dispersal rate and local environment, supporting a combination of factors contributing to metacommunity structure. Our study evaluates the dispersal rates of each taxon relative to its own abundance and occupancy within the metacommunity, but recent studies have shown that dispersal rates in freshwater protists (Fronhofer et al. 2015) and nematodes (De

Meester et al. 2015) can be altered by interspecific competition. Yet, despite these caveats, our study found clear patterns in the dispersal patterns of taxonomic groups relative to their metacommunity abundances and an effect of dispersal on metacommunity composition that cannot be explained by short-term dynamics. Future empirical and theoretical work on metacommunities cannot discount the possibility of dispersal rates that are not simply density-independent and, for freshwater plankton metacommunities, should increase exploration of the relative importance of long-term compositional changes to established metacommunities through colonization-extinction dynamics as well as quantifying and understanding the inflection point where the short-term impact of dispersal during the assembly process gives way to these longer-term dynamics.

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APPENDIX

Table 1. Permutational multivariate analysis of variance (PERMANOVA) output table evaluating whether plankton composition differed between local communities and dispersers, as well whether the composition of dispersers was significantly different across sampling periods and location in the metacommunity. P-values < 0.05 are in bold.

Source of variation	df	SS	MS	Psuedo-F	R^2	P(perm)
Community vs. Dispersers	1	2.3756	2.3758	6.8372	0.0806	**0.001**
Residuals	78	27.1008	0.3475		0.9194	
Total	79	29.4763			1	

Source of variation	df	SS	MS	Psuedo-F	R^2	P(perm)
Time (Dispersers only)	6	2.2325	0.3721	1.0242	0.0860	0.395
Location (Dispersers only)	9	4.1048	0.4561	1.2554	0.1582	**0.001**
Residuals	54	19.6179	0.3633		0.7558	
Total	69	25.9552			1	

Table 2. Similarity Percentage (SIMPER) results showing the species responsible for the greatest difference between the composition of local communities and dispersers, along with their percent contribution to this difference. The top ten species underrepresented in the dispersers relative to their metacommunity occupancies are shown above and the top ten overrepresented species are shown below.

Low Dispersing Taxon	Classification	Contribution	SD	SD Ratio	Avg. Community Occurrence	Avg. Disperser Occurrence
Ceriodaphnia reticulata	Cladoceran	1.62%	0.0101	1.6017	78.75%	4.29%
Ostracod	Ostracod	1.52%	0.0108	1.4066	77.50%	12.86%
Chydorus sphaericus	Cladoceran	1.49%	0.0109	1.3671	75.00%	12.86%
Acanthocyclops vernalis	Copepod	1.41%	0.0112	1.2617	77.50%	22.86%
Diacyclops thomasi	Copepod	1.35%	0.0111	1.2215	65.00%	4.29%
Frustulia	Diatom	1.23%	0.0113	1.0836	67.50%	30.00%
Spirogyra	Chlorophyte	1.12%	0.0114	0.9847	60.00%	38.57%
Tropocyclops prasinus	Copepod	1.12%	0.0111	1.0040	53.75%	5.71%
Brachionus angularis	Rotifer	1.11%	0.0114	0.9763	52.50%	2.86%
Cosmarium sp. B	Chlorophyte	1.08%	0.0115	0.9331	61.25%	52.86%

High Dispersing Taxon	Classification	Contribution	SD	SD Ratio	Avg. Community Occurrence	Avg. Disperser Occurrence
Unidentified Ciliate A	Ciliate	1.09%	0.0113	0.9635	45.00%	55.71%
Hyalotheca	Chlorophyte	1.08%	0.0114	0.9458	48.75%	52.86%
Coelastrum cambricum	Chlorophyte	1.03%	0.0112	0.9186	37.50%	44.29%
Fragilaria sp. A	Diatom	0.91%	0.0109	0.8407	12.50%	41.43%
Halteria	Ciliate	0.86%	0.0107	0.8071	25.00%	32.86%
Cryptomonas sp. B	Cryptophyte	0.76%	0.0108	0.7033	21.25%	24.29%
Scenedesmus arcuatus	Chlorophyte	0.73%	0.0102	0.7195	21.25%	25.71%
Radiofilum	Chlorophyte	0.72%	0.0106	0.6777	13.75%	27.14%
Chlorobotrys	Eustigmatophyte	0.65%	0.0097	0.6636	18.75%	21.43%
Bambusina	Chlorophyte	0.63%	0.0097	0.6509	13.75%	24.29%

Table 3. Fitted models describing the relationship between the metacommunity abundance of taxa within eight plankton taxonomic groups and their measured dispersal rates throughout the growing season. P-values < 0.05 are in bold.

Dispersal model	Taxonomic group	Parameter	Estimate	Std. Error	t value	P-value
$Dispersal = \alpha + \beta_1(Abundance) + \beta_2(Abundance)^2$	Amoeboids	α	0.0037	0.0041	0.9130	0.3661
		β_1	0.8107	0.1988	4.0780	**0.0002**
		β_2	-3.5933	0.9720	-3.6970	**0.0006**
	Chlorophytes	α	0.0007	0.0035	0.2040	0.8390
		β_1	1.9418	0.2434	7.9780	**< 0.001**
		β_2	-7.0829	1.7775	-3.9850	**0.0001**
	Ciliates	α	0.0000	0.0000	-0.2540	0.8003
		β_1	0.0338	0.0145	2.3370	**0.0228**
		β_2	-11.2800	5.4980	-2.0510	**0.0447**
	Copepods	α	0.0015	0.0016	0.9180	0.3600
		β_1	1.1551	0.2375	4.8640	**0.0000**
		β_2	-11.3467	2.7803	-4.0810	**0.0001**
$Dispersal = \beta_1(Abundance)$	Cladocerans	β_1	0.0108	0.0049	2.2210	**0.0287**
	Cryptophytes	β_1	1.8329	0.5072	3.6140	**0.0007**
	Euglenoids	β_1	0.3448	0.0892	3.8650	**0.0003**
	Rotifers	β_1	0.0184	0.0092	2.0060	**0.0459**

Figure 1. Non-metric multidimensional scaling (NMDS) ordination plot showing the difference in composition between local communities (green circles) and dispersers (blue crosses) across all sampling periods. The composition of the ten local communities on the final sampling date (i.e., the final metacommunity) is represented by the filled circles (black), which show a visible attraction toward the composition of dispersers that is confirmed by the subsequent analysis using the attraction coefficient (AC).

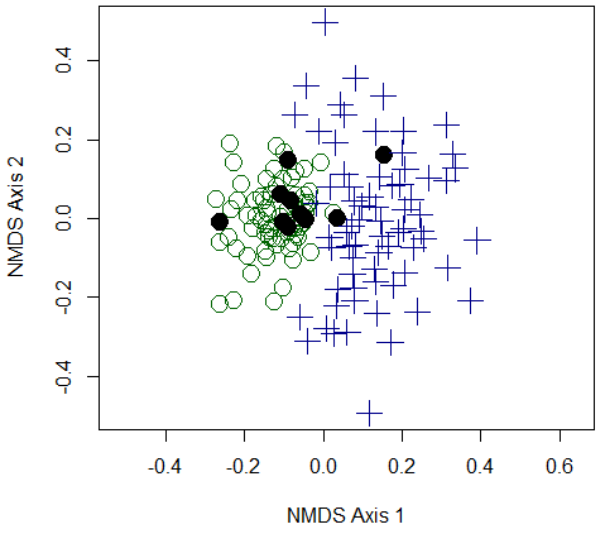


Figure 2. Boxplots showing how broad taxonomic groups varied in their percent contribution to the difference between the metacommunity and the dispersers and the degree to which each group was overrepresented or underrepresented in the dispersers. The zero line indicates taxa that were equally represented in the metacommunity and in the dispersers.

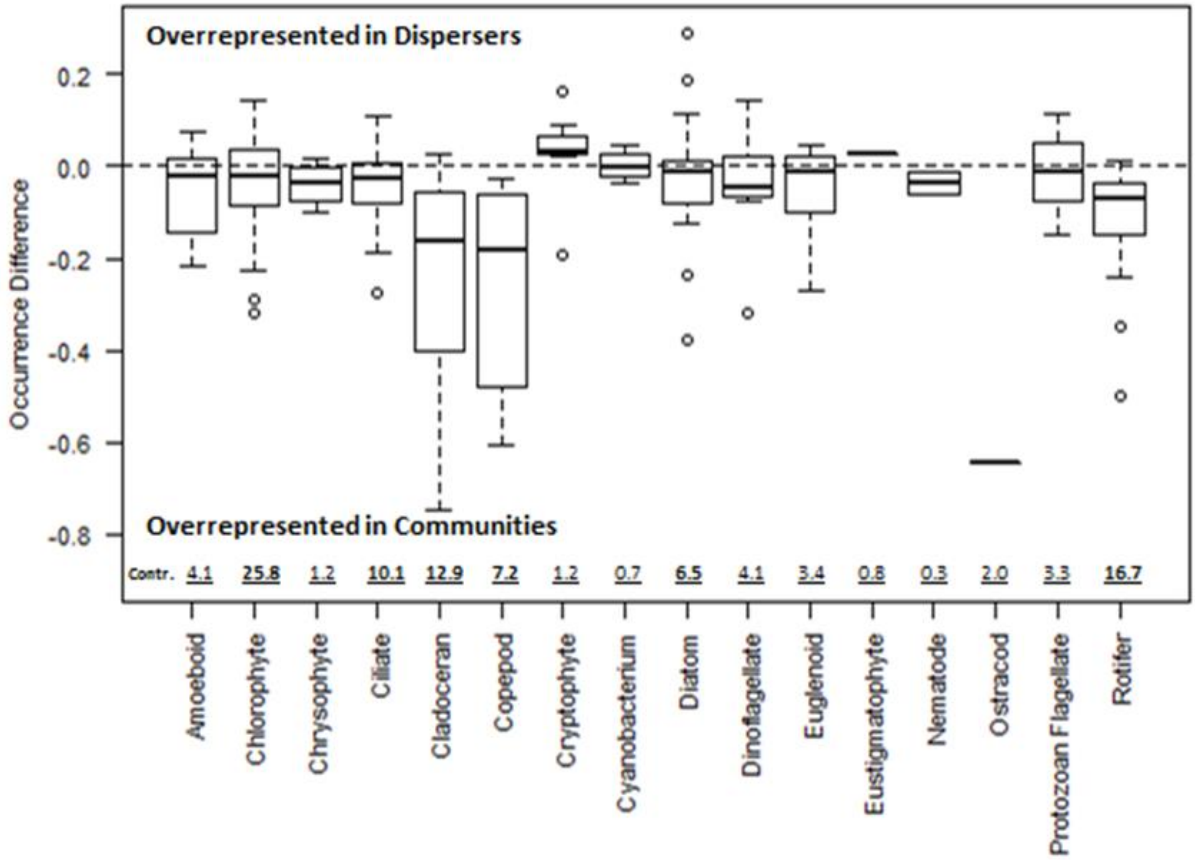


Figure 3. Plot showing how each local community varied in the degree to which it became more similar to its local incoming dispersers at each of the seven time steps according to the attraction coefficient (AC) based on the Bray-Curtis dissimilarity index (a). Boxplots showing the attraction of local communities toward incoming local dispersers at each individual time step and the attraction of the metacommunity toward all dispersers over the course of the entire growing season according to the AC (b).

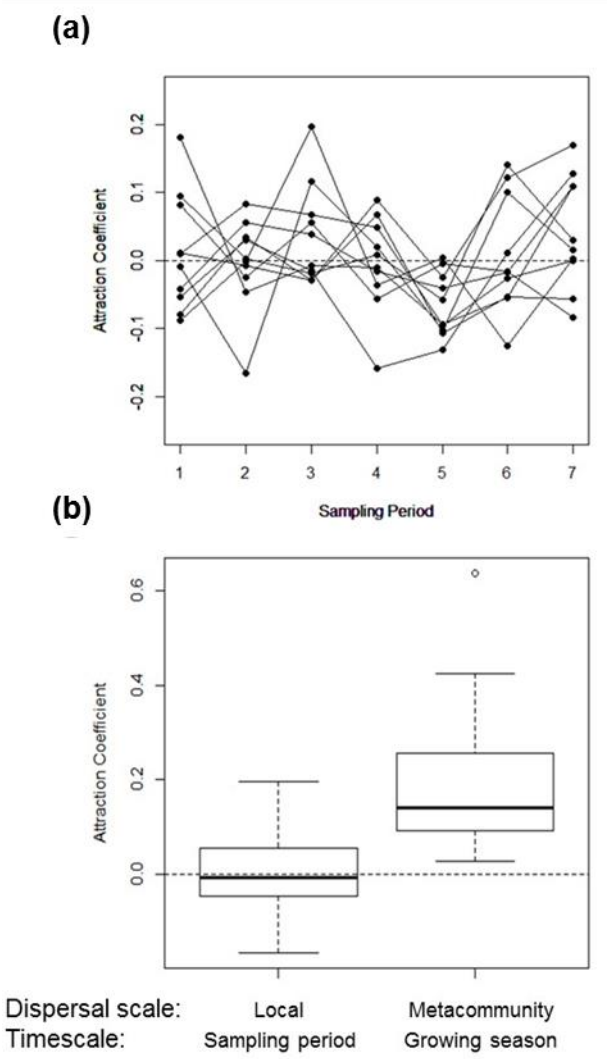


Figure 4. Relationship between the abundance of taxa in the pond metacommunity and the number of observed dispersers for phytoplankton and protozoa (a, Estimate = 1.01, t = 8.11, $P < 0.001$) as well as metazoan zooplankton (b, Estimate = 0.81, t = 5.54, $P < 0.001$). Each point represents an individual taxon. Solid lines indicate the best fit linear relationships between metacommunity and disperser abundances with corresponding 95% confidence bands (dashed lines).

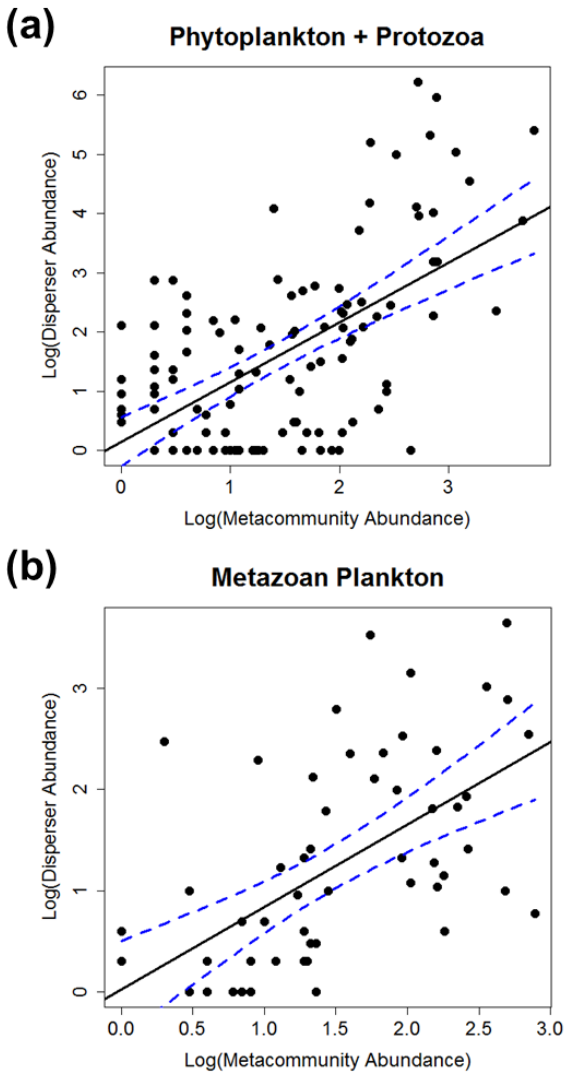


Figure 5. The compositional attraction of assembled (red) and unassembled (blue) zooplankton communities toward the suite of dispersing zooplankton observed locally (a) and meta-community wide (b) over seven time steps based on the Bray-Curtis dissimilarity index. Assembled communities – both in natural ponds and experimental mesocosms – showed no net attraction toward either local ($P = 0.52$) or metacommunity-wide dispersers ($P = 0.68$). Unassembled communities exhibited a consistent net attraction toward local dispersers ($P < 0.007$) but no net attraction toward metacommunity-wide dispersers ($P = 0.25$).

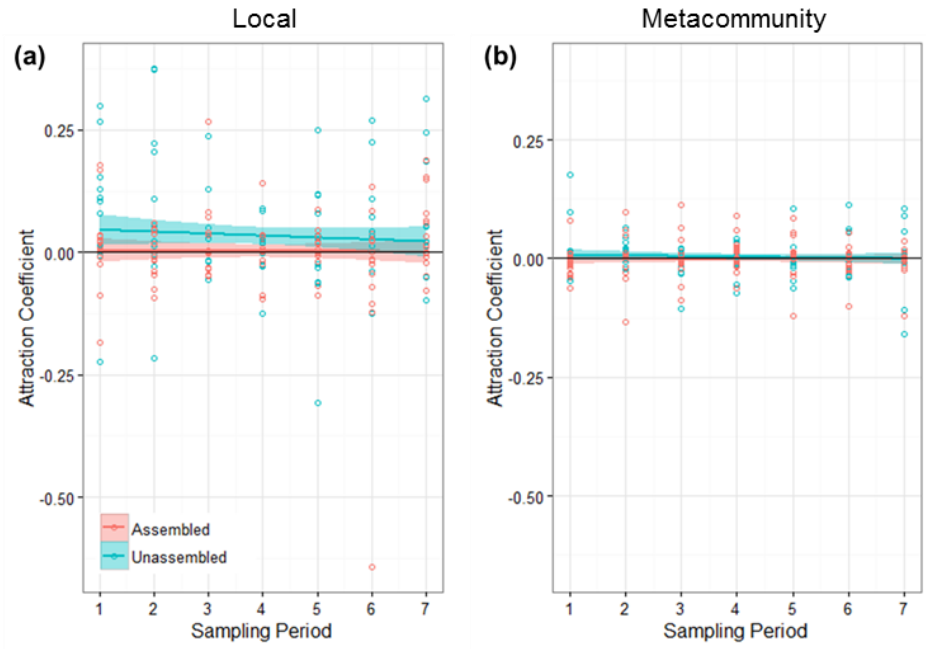


Figure 6. Conceptual figure depicting scenarios in idealized ordination space that would lead to the maximum, zero, and minimum values of the attraction coefficient, respectively. T represents the community composition at a given time point, T+1 represents the composition of the same community after some time period, and D represents the composition of the dispersers during that time period. Numeric values represent the distances (e.g., the Jaccard's or Bray-Curtis dissimilarity indices) among these three compositions.

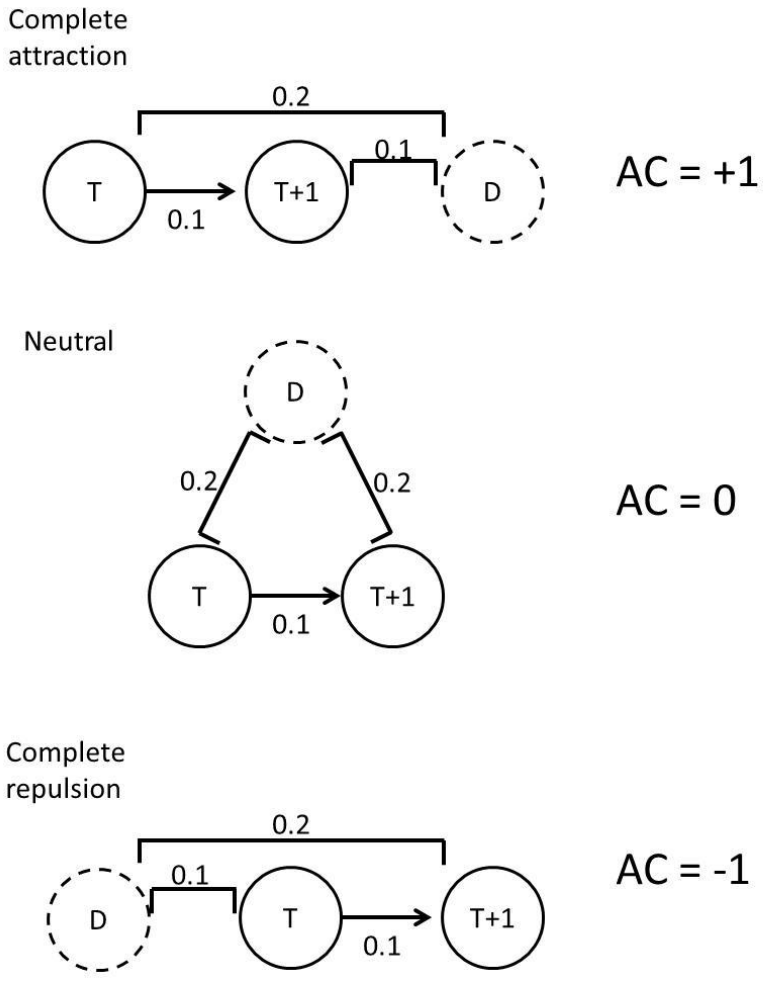


Figure 7. Best AIC models of the relationship between metacommunity abundance and dispersal rate for the eight dominant taxonomic groups in the study. Taxonomic groups that exhibited density-independent per capita dispersal rates (a). Taxonomic groups that exhibited negative density-dependent per capita dispersal rates (b). Intercepts for all groups were not significantly different from zero.

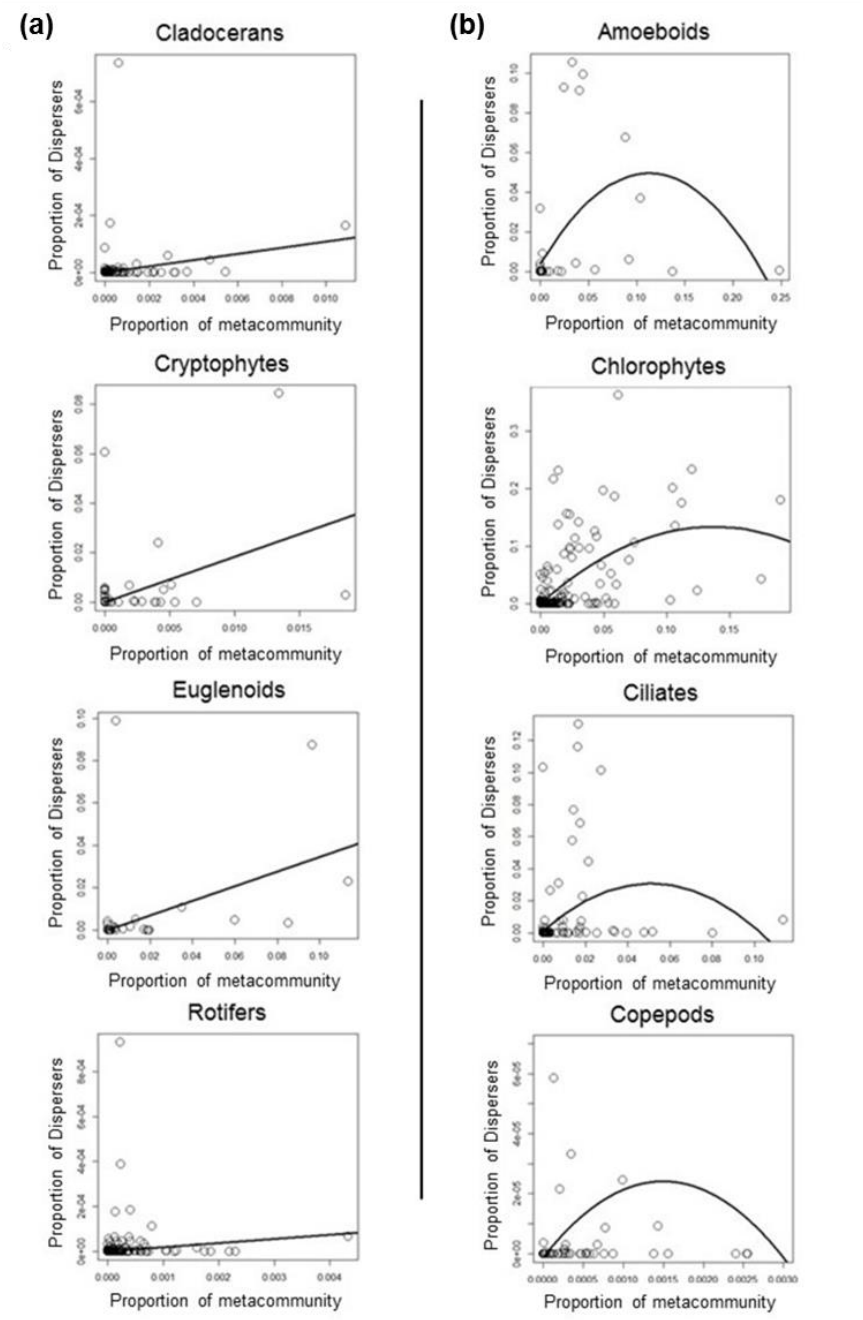
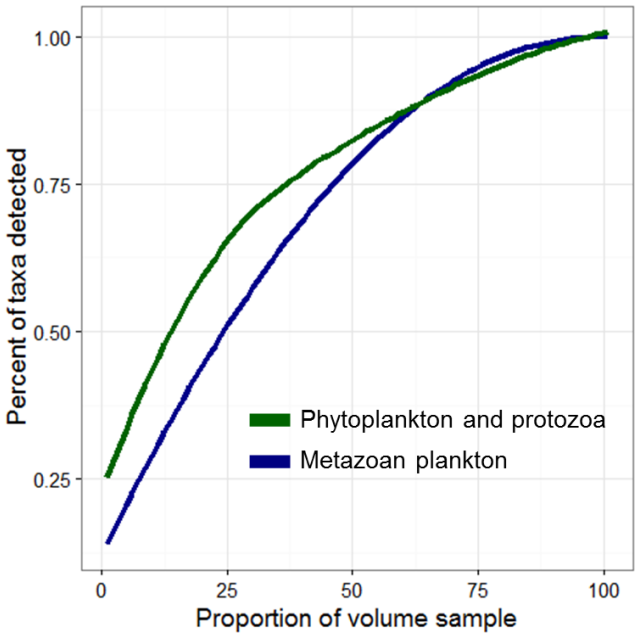


Figure 8. Species accumulation curves for dispersal samples generated by randomly resampling each of the 70 samples 100 times from 1% to 100% of the total volume sampled (50 liters). On average, enumeration of approximately 75% of the sample volume is required to capture 90% of unique dispersing taxa for phytoplankton and protozoa (green line) as well as metazoan plankton (blue line).



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CHAPTER 2

THE INFLUENCE OF ECOSYSTEM SIZE ON TROPHIC STRUCTURE AND DIVERSITY DURING AQUATIC COMMUNITY ASSEMBLY

Abstract

The role of ecosystem size in shaping species diversity and trophic structure has rarely been tested experimentally under field conditions. Here, we examine how ecosystem size affects community assembly, endpoint diversity and trophic structure over ~3.5 orders of magnitude in volumetric size in experimental freshwater pond mesocosms. Five mesocosms sizes (3 L, 16 L, 80 L, 333 L, 1,000 L) were factorially crossed with two nutrient input levels and initiated with either no starting community or a starting community approximating the natural density and trophic diversity found locally in freshwater ponds. Over the 12-week experiment, ecosystem size directly determined the maximal trophic level of communities. Overall species richness of macroinvertebrates increased with ecosystem size without mediation by the level of nutrient input. Zooplankton species richness, however, was not strongly affected by ecosystem size. Thus, the overall species richness increase with ecosystem size occurred through the addition of trophic complexity since the diversity of individual, lower trophic groups did not increase with size. Further, the structure of the community food webs in terms of densities of major taxonomic groups could be attributed to differences in their success across the ecosystem size gradient, but not to differences in assembly state or nutrient input level. Communities that began with a starting community had elevated endpoint zooplankton species richness compared to those without an initial starting community due to the influence of a handful of taxa with low dispersal

rates, while macroinvertebrate species richness was indistinguishable based on assembly state by the midpoint of the experiment. Overall, ecosystem size was the dominant factor structuring the diversity and trophic structure of communities, with convergence over time likely through a combination of differential mortality, establishment probability, and active dispersal into and out of mesocosms. These results emphasize the heterogenous influence of ecosystem size across trophic levels and the taxonomic groups within trophic levels and suggest that non-stochastic processes can drive the "small island" effect in species-area relationships.

Introduction

The species-area relationship, the scaling of species richness with ecosystem size, is one of the most fundamental and general patterns in all of ecology (Schoener 1976, Rosenzweig 1995). Species richness often scales linearly with area on a log-log plot, but the slope of this relationship may vary depending on taxa, location (e.g., tropics vs temperate), and the range of ecosystem areas sampled (Rosenzweig 1995). Likewise, ecosystem size can play a major role is determining community trophic structure, with the number of trophic levels (or average food-chain length) often scaling positively with ecosystem size (Post et al. 2000, McHugh et al. 2010, Sabo et al. 2010). Yet, despite decades of work on the effects of ecosystem size on community structure (richness, trophic links, etc.), important questions remain that highlight our limited understanding of the mechanisms driving these patterns. For example, species-area relationships tend to break down at small ecosystem sizes, the "small island effect" of Wilson and MacArthur (1967). The absence of a significant species-area effect at small ecosystem sizes was found by Hassall et al. (2011) for plants and macroinvertebrates in 425 ponds less than 2 hectares and by Wang et al. (2016) in 104 of 211 islands in their dataset. Our own review of the literature of species-area relationships in freshwater ecosystems found that significant species-area relationships were more likely as maximal surface area increased and as the total range of surface areas sampled increased (Appendix). Yet, despite its prevalence, the small island effect is often overlooked (Lomolino 2000), is mechanistically poorly understood (Triantis and Sfenthourakis 2012), and there is no consensus on its causes or if it is predictable (Triantis et al. 2006).

Debate remains whether the small island effect stems from stochasticity associated with sampling small areas or if there is a mechanistic explanation for why species-area relationships

occur irregularly across smaller spatial extents. Possible explanations for the small island effect include the accumulation of habitat types with area (an idea dating back to Wilson and MacArthur 1967) or by minimum area requirements for particular species (Turner and Tjørve 2005). These predictions have empirical basis in systems where habitat heterogeneity has been found to be more important than area in explaining species richness (Báldi 2008) and the observation that habitat heterogeneity scales with area for many ecological systems (Boecklen 1986, Kerr and Packer 1997). Habitat heterogeneity may be particularly important driver of species richness if habitat specialists are unable to persist in smaller communities (Schuler et al. 2017).

Higher trophic levels appear to be especially sensitive to ecosystem size (Roslin et al. 2014). This may partially explain the observation that ecosystem size can be a strong predictor of the length of trophic structure in both aquatic (Post et al. 2000, Fukami 2004, Vander Zanden and Fetzer 2007, McHugh et al. 2010) and terrestrial (Takimoto et al. 2008) systems. These minimum area requirements may be influenced by the productivity of an ecosystem independent of size as a greater number of individuals may be possible in a more productive ecosystem, lowering extinction risk from demographic stochasticity. Dispersal limitation can also act as a driver of the species-area relationship (Shen et al. 2009) through the "target effect" (Gilpin and Diamond 1976) where immigration rates are higher into larger ecosystem. Small habitats may have limited ability to experience a "target effect" as dispersal may be more stochastic and lead to higher probabilities of priority effects (Fukami 2004) where assembly history becomes an important predictor of diversity.

These multiple potential drivers of both the small island effect and the relationship between ecosystem size and trophic structure, argue for the importance of manipulative

experiments to tease apart the potential interactions. Here, we establish a mesocosm size gradient from 3 L to 1000 L to investigate how species richness and trophic structure change across ecosystem size in communities at low and high nutrient input levels, and that vary in whether they have been initiated with a fully assembled community or are assembling from an empty state. In our experiment, pond mesocosms increase in size without the accumulation of additional new habitats and we control for a "target effect" by manipulating the presence of an initial, fully assembled community. Having two nutrient levels (high and low) also allows us to examine whether productivity interacts with ecosystem size to affect species richness and trophic structure. We followed the trajectory of the mesocosm communities for 12 weeks and report results on species richness and densities of groups from multiple trophic levels to evaluate how ecosystem size influences the diversity and trophic structure of freshwater pond communities and whether the effect of size is influenced by the interactive effects of dispersal limitation and productivity.

Methods

Mesocosm experiment

A mesocosm experiment mimicking freshwater habitats of different sizes (i.e., tree holes to small ponds) was established at Michigan State University's Kellogg Biological Station Experimental Pond Facility (Hickory Corners, MI, USA; 42.41° N, 85.39° W). Mesocosms were of five sizes (3 L, 16 L, 80 L, 333 L, and 1,000 L) fully crossed by two nutrient input levels and the presence/absence of initial metazoan zooplankton and macroinvertebrate colonizers ("assembled" and "unassembled"). Each mesocosm treatment was replicated four times for a total of 80 mesocosms. Mesocosms were set up with no substrate and were filled with well water

and inoculated with bacteria and phytoplankton on 6 May 2014. “Assembled” mesocosms were inoculated with a mixture of pond water from eight natural ponds at Lux Arbor Reserve (Barry County, MI, USA; 42.29° N, 85.45° W). Nutrients initially added to “high” nutrient mesocosms were 10.4 mg/L $NaNO_3$ and 0.33 mg/L NaH_2PO_4 followed by weekly supplementation at a rate of 2.46 mg/L $NaNO_3$ and 0.12 mg/L NaH_2PO_4 (after Shurin 2001). “Low” nutrient mesocosms were fertilized and supplemented at one-quarter of this “high” nutrient rate. The addition of zooplankton and macroinvertebrates into “assembled” mesocosms took place on 13 May 2014. Target volumes were maintained by weekly additions of well water.

Mesocosms that were “assembled” were seeded with an initial zooplankton community containing two common cladoceran species (*C. reticulata* and the hybrid *Daphnia pulex* × *D. pulicaria*), one copepod species (*Acanthocyclops vernalis*), and five rotifer species (*Brachionus angularis, Brachionus quadridentatus, Lecane luna*, *Monostyla bulla*, *Platyias patalus*) in proportion to the total mesocosm volume. Zooplankton were collected from eight natural ponds at Lux Arbor Reserve. Approximately three-quarters of inoculated zooplankton were D. *pulex* × *D. pulicaria*, which were added to mesocosms at a rate of approximately seven individuals per liter of volume. Larvae of the dipteran *Chaoborus* (a zooplankton predator) were also added to each “assembled” mesocosm at a rate of approximately one individual per liter of mesocosm volume. Macroinvertebrates added to “assembled” mesocosms were from five groups: water boatmen (one species; *Hesperocorixa* sp.), backswimmers (two species; *Notonecta irrorata* and *N. undulata*), damselflies (one species; *Ischnura verticalis*), dragonflies (one species; *Leucorrhinia frigida*), and aquatic beetles (five species; *Dytiscus* sp., *Gyrinus* sp., *Laccophilus* sp., *Tropisternus* sp., and Hydrophilidae sp.). Individual macroinvertebrates were added to mesocosms at a rate of approximately one individual per 3 L water volume. 3 L mesocosms were

inoculated with one random individual from one of the five groups (total individuals = 1), 16 L mesocosms with one individual from each of the five groups (total individuals = 5), 80 L mesocosms with five individuals from each of the five groups (total individuals = 25), 333 L mesocosms with 21 individuals from each of the five groups (total individuals = 105), and 1000 L mesocosms with 63 individuals from each of the five groups (total individuals = 315).

The mesocosm experiment lasted for 12 weeks following the addition of zooplankton and macroinvertebrates and was terminated on 5 August 2014. Macroinvertebrates were fully sampled without permanent removal in each mesocosm on the Monday, Wednesday, and Friday of each week through a combination of visual surveys (to minimize handling when possible) and temporary removal using dip nets (when needed to enumerate high densities or to confirm identities). Zooplankton were sampled on the Tuesday and Thursday of each week by taking three equally spaced, vertically integrated 1 L water samples. Each resulting sample was filtered through 40 µm mesh to create a 50 mL volume sample, which was fully enumerated following preservation in 2% acid Lugol's solution in the 80 L, 333 L, and 1000 L mesocosms and counted live under a dissecting microscope in 5-10 mL subsamples and returned to the source mesocosm along with the 2.95 L of filtered water in the case of 3 L and 16 L mesocosms. A 3 L sample represents the full volume of the smallest mesocosms and nearly 20% of the volume of the second smallest mesocosms. Therefore, care was taken to minimize the disturbance of sampling on the zooplankton in these smallest mesocosms; zooplankton samples were taken singly and enumerated immediately in a building adjacent to the mesocosm array.

Data formatting and manipulation prior to analysis

In all, a total of 35 zooplankton taxa (Appendix) were observed in the experimental mesocosms: 14 Rotifera, 1 Calanoida, 16 Cladocera, and 3 Cyclopoida as well as Ostracoda (not identified to species and considered a single taxon in this paper). Of the inoculated species, only the rotifer *Brachionus quadridentatus* was not immediately observed during mesocosm sampling (although it was observed in multiple mesocosms starting on 3 June 2014) and may not have had sufficient opportunity to establish at the start of the experiment. Only one individual was estimated to be inoculated per 3 L of mesocosm volume and *B. quadridentatus* may have simply been a rare species in the sampled inoculatum. A total of 21 macroinvertebrate taxa (Appendix) were found in the experiment: 4 Odonata, 5 Hemiptera, 5 Coleoptera, 5 Diptera as well as Baetidae and Hydrachnidia.

For characterization of the ecosystem food web, species were categorized into four macroinvertebrate groups (the insect orders Coleoptera, Diptera, Hemiptera, and Odonata) and four zooplankton groups (the phylum Rotifera and the crustacean orders Calanoida, Cladocera, and Cyclopoida). Due to the trophic diversity within these groups, feeding relationships were only considered between distinct trophic levels (e.g., between macroinvertebrates and microcrustacean zooplankton) and not within trophic levels where feeding relationships are not unidirectional (e.g., odonates and hemipterans).

Because macroinvertebrates were sampled three times per week and zooplankton only two times per week, temporal sampling of zooplankton and macroinvertebrates did not map one-to-one with each other. Therefore, to minimize creating non-independence among sampling points, the third macroinvertebrate sampling point (Friday) each week was dropped from analyses. Monday macroinvertebrate and Tuesday zooplankton samples were considered as

single time points and Wednesday macroinvertebrate and Thursday zooplankton were considered as single time points for the purpose of analysis.

Prior to analysis, all abundance data were converted into density data for each mesocosm for both zooplankton and macroinvertebrates in terms of individuals per L. These density data were then square-root transformed prior to analysis. Ecosystem sizes in terms of the volumetric sizes in L were log-transformed prior to analysis.

Structural equation modeling

Structural equation models were used to assess how ecosystem size, nutrient input level, and assembly status treatments influenced both community diversity and the food web structure of communities in terms of the densities of different taxonomic groups during the time course of the experiment. Two sets of models were specified using the three treatments as exogenous variables: (1) a diversity model where treatments can predict the separate species richness of zooplankton and macroinvertebrates with the potential for a top-down effect of macroinvertebrate diversity on zooplankton diversity, and (2) a food web model where treatments can predict the densities of any of the eight major taxonomic groups in the experiment and where top-down effects of predator densities are possible on prey densities.

Endpoint comparison of composition

Permutational multivariate analysis of variance (PERMANOVA, Anderson 2005) was used to test whether the final composition of zooplankton and macroinvertebrate communities differed across ecosystem size, nutrient input level, and initial assembly status. PERMANOVAs were run separately for zooplankton and macroinvertebrates using the Bray-Curtis index of

dissimilarity and the differences among endpoint communities were visualized using non-metric multidimensional scaling plots.

Because many of the smallest size mesocosms contained no macroinvertebrates by the end of the experiment, only the composition of endpoint macroinvertebrate communities for the two largest size mesocosms (333 L and 1000 L) were evaluated using PERMANOVA. Further, two of the 3 L mesocosms were compositionally identical at the endpoint (contained the same density of one species) and so the abundance value for one mesocosm was increased by 0.001 to make the NMDS computation possible.

Results

Effect of ecosystem size, nutrient input, and assembly status on diversity

Macroinvertebrate communities had significantly higher endpoint species richness (Table 4; estimate = 4.08, $P < 0.0001$) and density (Table 5; estimate = 7.15, $P = 0.0005$) in larger mesocosms, whereas there were no significant effects of the other treatments (nutrient addition, assembly) in either model ($P > 0.09$ for all coefficients). The endpoint richness of the zooplankton community showed no response to any treatment or interaction among treatments (Table 6; $P > 0.16$ for all coefficients), while the endpoint density of zooplankton decreased with increasing mesocosm size (Table 7; estimate = -4.92, $P = 0.001$) and with higher nutrient input rate (estimate = -9.43, $P = 0.03$). The SEM combining species richness of both main trophic groups and the main (non-interactive) effects of treatments corroborate the strong effect of mesocosms size on macroinvertebrate richness (Figure 9, estimate = 3.91, $P < 0.001$) and the model explains a substantial proportion of the variation in macroinvertebrate richness (R-squared = 0.78). In contrast to the results from macroinvertebrates, for zooplankton the endpoint species

richness SEM showed no mesocosm size effect (P = 0.14) and instead indicates a positive effect of the assembly treatment on final zooplankton richness (estimate = 1.41, P = 0.002) with the overall model explaining a modest amount of explained variation (R-squared = 0.24). Removing the interaction terms from the linear model on zooplankton species richness also reveals a positive effect of the assembly treatment on final zooplankton richness (estimate = 1.38, P = 0.0006) and this simplified linear model should be given identical consideration as the fully interactive linear model according to Akaike information criterion model selection (delta AIC = 0.22).

Influences on food web structure

The endpoint food web SEM model (using densities of the major trophic groups in the mesocosm experiment (Figure 10) showed that ecosystem size positively influenced the densities of the three highest trophic level macroinvertebrate groups (Coleoptera: estimate = 0.19, P < 0.001; Odonata: estimate = 0.06, P < 0.001, Hemiptera: estimate = 0.08, P < 0.001) as well as the predatory calanoid copepods (estimate = 0.37, P < 0.001). Smaller mesocosms contained higher densities of rotifers than larger mesocosms (estimate = -2.00, P = 0.004). The assembly treatment at the start of the experiment had a positive effect only on the final density of the calanoid copepods (estimate = 0.85, P < 0.001). The nutrient input rate into mesocosms was not found to influence the final densities of any of the taxonomic groups (P > 0.30 for all regressions). Three feeding interactions were found to be significant: the negative effect of dipteran density (estimate = -1.82, P = 0.03) and calanoid copepod density (estimate = -1.31, P = 0.002) on cladoceran density and the negative effect of hemipteran density on cyclopoid copepod density (estimate = -1.81, P = 0.04). Additionally, two groups were found to significantly covary in density within a

trophic level: cladoceran and rotifer densities covaried negatively (estimate = -2.74, P = 0.04) while odonate and hemipteran densities positively covaried (estimate = 0.002, P = 0.04). The SEM model explained an average of 27.8% of the variation within taxonomic groups (R-squared values: Coleoptera 0.21, Hemiptera 0.36, Odonata 0.37, Diptera 0.10, Calanoida 0.38, Cladocera 0.15, Cyclopoida 0.18, Rotifera 0.41). Expanding the richness SEM model to add either interactive effects of treatments or an interaction of treatments on the potential limitation of zooplankton richness by macroinvertebrates were not informative (delta AIC > 200 for both models).

At the endpoint of the experiment, zooplankton communities varied significantly in their composition (Table 8, Figure 11) across both ecosystem size (PERMANOVA: F = 5.08, P = 0.001) and assembly status (F = 2.06, P = 0.03), as well as marginally significantly with the interaction between ecosystem size and assembly status (F = 1.85, P = 0.06). A main effect of nutrient input level was not important in determining zooplankton community composition (P = 0.31) but nutrient input may have had an interactive effect with size on composition (F = 1.67, P = 0.08). Endpoint macroinvertebrate composition was significantly different between the two largest mesocosm sizes (Table 9; PERMANOVA: F = 2.32, P = 0.03) with no influence from other predictors or their interactions (P > 0.12). This PERMANOVA on macroinvertebrate species richness excludes the three smallest ecosystem sizes as too few contained macroinvertebrates to compare composition.

Temporal trends of effects on diversity and trophic structure

The initial species richness of both plankton and macroinvertebrates were significantly elevated by the assembly treatment at the first sampling point following inoculation (Figure 12;

zooplankton SEM regression estimate: 3.25; macroinvertebrate SEM regression estimate: 6.60; $P < 0.05$ for both regressions). A size effect was also initially observable for macroinvertebrates but not zooplankton. The positive influence of starting with an assembled community on zooplankton species richness was maintained throughout the experimental duration, only losing significance during a single sampling point. No other treatments were found to significantly influence zooplankton species richness throughout the experiment. For macroinvertebrates, the effect of initial assembly dissipated over time and became nonsignificant by the midpoint of the experiment. Size positively influenced macroinvertebrate species richness throughout the experiment, whereas there was no influence of nutrient input level.

Zooplankton densities were idiosyncratically predicted by treatments during the time course of the experiment (Figure 13). Calanoid copepod densities rapidly became positively influenced by both size and assembly status while cyclopoid densities were positively influenced by assembly status at some time points but never by size. Both cladocerans and rotifers lost the positive effect of assembly status on their densities rapidly. In general, cladoceran densities were not influenced by ecosystem size, although a transient but significant and positive effect of size did occur around the midpoint of the experiment. Rotifer densities became negatively influenced by size (beyond any top-down feeding effects) about one-quarter into the experimental duration and this effect was mostly consistent in its significance for the remainder of the experiment. Nutrient input rate was only found to have a significant, positive effect on cladoceran densities at a single time point.

Macroinvertebrate densities were all significantly elevated in mesocosms that started with an initial community (Figure 14), but this effect dissipated over time for all four major macroinvertebrate groups. Dipteran densities became rapidly unrelated to assembly status while

it took to about the experimental midpoint for assembly status to no longer be a significant predictor of density for odonates and coleopterans. Hemipterans took until nearly the endpoint of the experiment for the influence of assembly status to disappear. Odonates, hemipterans, and coleopterans all had significantly higher densities in larger size mesocosms by about one-third through the experiment and this effect became persistent. Dipteran densities were never found to be significantly influenced by size and dipteran density actually became negatively influenced by assembly status at some time points. The level of nutrient input only had a significant, positive effect on hemipteran densities at a single time point.

The observed species-area relationship

A species-area relationship was present for both macroinvertebrates and zooplankton at the end of the experiment (Figure 15; $P < 0.05$ for both groups). The observed species-area relationship had a much higher slope (0.60) for macroinvertebrates than for zooplankton (0.09). Although significant, the species-area relationship for zooplankton explained a relatively low amount of variation in species richness across the ecosystem size gradient (R-squared = 0.09).

Discussion

Effect of ecosystem size on species richness

Ecosystem size had a strong, positive effect on the species richness and densities of macroinvertebrates, which constituted the top trophic levels in the experiment. Thus, there was a clear effect of ecosystem size on trophic complexity and food chain length. Moreover, macroinvertebrates that were initially added to the two smallest mesocosm sizes (3 L and 16 L) rarely persisted for more than a few weeks, disappearing through a combination of mortality and

active dispersal. Only a limited suite of macroinvertebrate species was found to actively disperse or oviposit into these smallest mesocosms. In general, midges and small-bodied beetles were the only macroinvertebrates that actively established into 3 L and 16 L mesocosms. Conversely, macroinvertebrates from all taxonomic groups actively dispersed into larger mesocosms and all 333 L and 1000 L mesocosms contained macroinvertebrates by the end of the experiment. Thus, larger ecosystems clearly favored that establishment of higher trophic level species.

Although our experimental design caused an initial effect of size on species richness (because more species were added to 80 L, 333 L, and 1000 L mesocosms than 3 L and 16 L mesocosms due to the limited number of individuals that could be added to the smallest containers), there was a considerable amount of macroinvertebrate turnover during the experiment in these largest mesocosms. Further, these large, pre-assembled communities ended with similar species richness to those communities that initially contained no macroinvertebrates, confirming that the positive effect of size on macroinvertebrate species was not an artifact of our experimental design.

Zooplankton species richness was not significantly influenced by ecosystem size at any point in the experiment. Of note is the potential increasing trend toward zooplankton richness being higher in larger mesocosms (Figure 12), but this effect never reached significance. Unlike with macroinvertebrates, communities that began assembled had persistently elevated zooplankton species richness that remained significant throughout the experiment, except for a single sampling point. This effect was largely driven by elevated presence in assembled mesocosms of the two dominant copepod species, *Acanthocyclops vernalis* and *Epischura lacustris*, as well as the cladoceran *Ceriodaphnia reticulata*. While *E. lacustris* was not found in the sampled zooplankton inoculatum used in the assembly treatment, it was immediately found

in subsequent samples of the experimental mesocosms that started assembled, implying that there is a high probability that the species may have been abundant in the inoculatum as eggs or early instar nauplii.

Macroinvertebrates may have limited any potential increase in zooplankton diversity with ecosystem size, but this effect is difficult to distinguish in our experiment since all of the two largest mesocosm sizes contained macroinvertebrates. Although some top-down predation effects were observable at the endpoint of the experiment (Figure 10), these effects were not shared across all zooplankton and calanoid copepods were more successful in larger ecosystems despite higher densities of macroinvertebrates. In natural lakes and ponds, the effect of predators on prey diversity are idiosyncratic: smaller lakes have been found to contain higher invertebrate diversity in the absence of fish (Scheffer et al. 2006)**,** but higher predation or herbivory does not necessarily lead to a reduction in biodiversity of lower trophic levels in aquatic ecosystems. For example, grazing pressure from zooplankton can promote phytoplankton diversity by allowing more inedible phytoplankton species to persist (McCauley and Briand 1979) and there appears to be no general relationship between phytoplankton and zooplankton diversity in marine systems (Irigoien et al. 2004). Previous work using similar communities to our experiment has found that the presence versus absence of notonectid predators had no net effect on zooplankton diversity since notonectids facilitated the invasion of as many species as they extirpated (Shurin 2001). However, not all predation leads to facilitation and elevated zooplankton species richness. The introduction of the zooplankton predator *Bythotrephes longimanus* has led to permanent reductions in zooplankton species in some lakes (Yan et al. 2002). Other examples on islands include the loss of spider diversity from lizard introductions (Schoener and Spiller 1996) and the loss of bird diversity from snake introductions (Savidge 1987).

Ecosystem size has been found to have no effect on zooplankton species richness across multiple studies in small ponds and lakes (Hessen et al. 2006, Longmuir et al. 2007, Mazaris et al. 2010, although a positive effect of ecosystem size on zooplankton richness has also been observed in small lakes (Reche et al. 2005). Across a larger spectrum of lake sizes up to the size of Lake Superior (~82,000 km^2), zooplankton species do exhibit a classic species-area relationship where more species are found in larger lakes (Dodson 1992).

The lack of clear scaling of zooplankton richness with size in our experiment may represent a small island effect, but the cause of this effect is not simply a "target effect", a result of minimum area requirements, or dispersal limitation. Zooplankton composition turned over across mesocosm sizes (Figure 11) and the diversity of smaller ecosystems was not simply a nested subset of larger ecosystems. Larger communities were much more likely to contain calanoid copepods, which may represent a minimum area threshold for that group across the size range of our experiment. Further, the smallest size ecosystems contained a very limited suite of cladocerans, suggesting a minimum area requirement for a significant proportion of taxa. While rotifers themselves were not influenced by minimum area requirements in our experiment, they did appear to be negatively influenced by the minimum area requirements of cladocerans, as they had dramatically lower and negatively covarying density with cladocerans in all but the smallest size mesocosms. The exclusion of rotifers through interference competition with larger cladocerans (e.g., *Daphnia*) is well-documented (Gilbert 1985).

An interesting contrast to our results are those of Blakely and Didham (2010) who manipulated size and resource concentration in aquatic microcosms (up to ~3 L volume) and found a negative effect of ecosystem size and a positive effect of increasing basal resources on insect species richness. While there may have been no proximate mechanism for an effect of size

per se in their tree hole analogue study system, which contained almost exclusively dipteran species, many species in our study system are known to have oviposition and dispersal preferences for larger size habitats. For example, notonectids may preferentially colonize larger habitats (Wilcox 2001)

Effect of ecosystem size on trophic structure

Like our study, others have found that the number of predatory macroinvertebrate species increases with lake and pond area (Heino 2000, Kadoya et al. 2004) and with increasing size of microcosms and mesocosms (Harlan and Paradise 2006), although the strength of the species-area relationship can be quite weak for some groups (Oertli et al. 2002). In our experiment, macroinvertebrate species preferentially dispersed out of small mesocosms and preferentially dispersed into large mesocosms. While this result lends some credence to a "target effect" occurring in this system, the influence of minimum area requirements also exists. Odonates, which can only actively disperse following emergence as adults, performed poorly in small mesocosms with no odonates emerging from 3 L mesocosms and only a single *Leucorrhinia frigida* successfully emerging from a 16 L mesocosm. Conversely, odonates regularly oviposited in larger mesocosms and successfully emerged. *Libellula* dragonflies, which were not a component of our assembly treatment, oviposited in 20 mesocosms over the summer: 0/16 3L, 0/16 16 L, 1/16 80 L, 6/16 333 L, and 13/16 1000 L mesocosms. Successful emergence of the initial stocking of *Leucorrhinia frigida* in 333 and 1000 L mesocosms reached >50% of individuals.

Matching the results from lakes (Post et al. 2000), larger ecosystems in our experiment contained longer food chain length. The smallest (3 and 16 L) mesocosms typically contained

high densities of rotifers (in 3 L) or cladocerans (in 16 L) with occasional occupancy by a limited suite of macroinvertebrates such as chironomid and chaoborid midge larvae, mosquito larvae of *Aedes triseriatus* and small-bodied aquatic beetles such as those of the genus *Laccophilus*. Large, secondary predators such as *Notonecta* and larger predaceous diving beetles such as *Dytiscus* were only found residually in smaller mesocosms from the assembly treatment and all individuals eventually actively dispersed away or were extirpated. Larger mesocosms (333 and 1000 L) not only contained secondary predators but often also contained predatory calanoid copepods as an intermediate trophic level between macroinvertebrates and non-predatory zooplankton. As ecosystem size in our experiment increased, both more trophic levels and the potential complexity of the food web (as implied from the increased macroinvertebrate diversity and addition of calanoid copepods) increased.

Lack of nutrient input level effect

Nutrient input rate had no measurable effects on either the diversity or densities of macroinvertebrates or zooplankton with exception of two significant but transient elevations of cladoceran and hemipteran densities due the first quarter of the experimental duration. This result contrasts with the classic pattern of an increase in zooplankton biomass in aquatic systems as nutrient concentrations increase (e.g., Pace 1986). However, some nutrient additions to aquatic systems have found that biomass may not always respond to enrichment when in a multitrophic context (e.g., Lynch and Shapiro 1981). While some research has found an increase in zooplankton species richness with increasing nutrients or ecosystem productivity (Hessen et al. 2006), species richness of zooplankton has more commonly been found to have a unimodal

relationship with productivity (Leibold 1999, Dodson et al. 2000, Longmuir et al. 2007) and in some cases even negative (Jeppesen et al. 2000).

It is not clear in our study if the nutrient subsidy failed to increase productivity generally or if we were unable to measure changes in productivity using species abundances of zooplankton and macroinvertebrates. Lower zooplankton density was observed at larger ecosystem sizes but this difference coincided with fewer small-bodied rotifers and more large-bodied cladocerans and copepods. Further, some of the potential increase in productivity may not have contributed to the measured community. Zooplankton (*Scapholeberis mucronata* in particular) produced resting stages during the experiment and some productivity may have been exported through the emergence of odonates and dipterans as well as the dispersal of other flight capable aquatic macroinvertebrates.

Conclusion

Small island effects and the lack of a clear scaling of species richness with area over size ranges of a few orders of magnitude are commonly observed in freshwater systems (Appendix). While stochasticity invariably plays some role in causing this effect, there are consistent changes in freshwater pond communities as ecosystem size increases that lead to diversity scaling that is trophically structured. Larger fishless pond ecosystems contain higher densities and species richness of predatory macroinvertebrates that limit regular scaling of zooplankton diversity with ecosystem size. Zooplankton densities are generally lower in the presence of macroinvertebrate predators and many taxa may be at higher extinction risk in larger rather than smaller ecosystems. This predation effect, as well as the accumulation of novel, competing zooplankton species as ecosystem size increases, may limit increases in species richness with size in fishless

freshwater pond communities. Our results highlight the differential scaling of diversity that can occur across trophic levels and the potential for higher trophic levels to limit the scaling of diversity of lower trophic levels.

APPENDIX

Table 4. Linear model of the influence of treatments on the final macroinvertebrate species richness of communities

	Estimate	Std. Error	t	*P*
(Intercept)	-2.83563	0.95781	-2.961	0.00416
Size	4.08247	0.47117	8.665	8.81E-13
Nutrient input	0.97006	1.35454	0.716	0.47621
Assembly	0.04305	1.35454	0.032	0.97473
Size x Nutrient input	-0.91693	0.66633	-1.376	0.17306
Size x Assembly	-0.24325	0.66633	-0.365	0.71614
Nutrient input x Assembly	-1.74795	1.91561	-0.912	0.36456
Size x Nutrient input x Assembly	1.61855	0.94234	1.718	0.09017

Table 5. Linear model of the influence of treatments on the final macroinvertebrate density of communities

	Estimate	Std. Error	t	*P*
(Intercept)	-4.7846	3.9901	-1.199	0.23442
Size	7.1495	1.9628	3.642	0.000506
Nutrient input	-3.6695	5.6429	-0.65	0.51757
Assembly	2.6791	5.6429	0.475	0.636389
Size x Nutrient input	2.6811	2.7759	0.966	0.337342
Size x Assembly	-2.7529	2.7759	-0.992	0.324656
Nutrient input x Assembly	0.4133	7.9802	0.052	0.958841
Size x Nutrient input x Assembly	-0.6854	3.9257	-0.175	0.861884

Table 6. Linear model of the influence of treatments on the final zooplankton species richness of communities.

	Estimate	Std. Error	t	*P*
(Intercept)	2.82443	0.84672	3.336	0.00135
Size	0.26111	0.41652	0.627	0.53272
Nutrient input	-0.49001	1.19744	-0.409	0.68359
Assembly	-0.79923	1.19744	-0.667	0.50662
Size x Nutrient input	-0.1702	0.58905	-0.289	0.77346
Size x Assembly	0.82314	0.58905	1.397	0.16658
Nutrient input x Assembly	1.31996	1.69343	0.779	0.43826
Size x Nutrient input x Assembly	0.01649	0.83304	0.02	0.98426

Table 7. Linear model of the influence of treatments on the final zooplankton density of communities.

	Estimate	Std. Error	t	*P*
(Intercept)	20.676	2.97	6.962	1.30E-09
Size	-4.918	1.461	-3.367	0.00122
Nutrient input	-9.427	4.2	-2.245	0.02786
Assembly	-3.489	4.2	-0.831	0.40886
Size x Nutrient input	3.282	2.066	1.589	0.1165
Size x Assembly	1.096	2.066	0.53	0.59754
Nutrient input x Assembly	8.735	5.939	1.471	0.14574
Size x Nutrient input x Assembly	-3.363	2.922	-1.151	0.25356

Table 8. PERMANOVA of final zooplankton communities.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	*P*
Size	1	1.756	1.756	5.0836	0.05973	0.001
Nutrient input	1	0.3826	0.3826	1.1076	0.01301	0.314
Assembly	1	0.7108	0.71084	2.0579	0.02418	0.026
Size x Nutrient Input	1	0.5766	0.57655	1.6691	0.01961	0.076
Size x Assembly	1	0.6387	0.63869	1.849	0.02173	0.055
Nutrient input x Assembly	1	0.2345	0.23454	0.679	0.00798	0.722
Size x Nutrient x Assembly	1	0.2275	0.22747	0.6585	0.00774	0.769
Residuals	72	24.8705	0.34542		0.84602	
Total	79	29.3972			1	

Table 9. PERMANOVA of final macroinvertebrate communities in the two largest mesocosms sizes.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	*P*
Size	1	0.5301	0.5301	2.32486	0.07215	0.035
Nutrient input	1	0.1868	0.18678	0.81917	0.02542	0.508
Assembly	1	0.4016	0.40159	1.76128	0.05466	0.122
Size x Nutrient Input	1	0.2291	0.22914	1.00494	0.03119	0.36
Size x Assembly	1	0.2828	0.28285	1.24049	0.0385	0.258
Nutrient input x Assembly	1	0.1368	0.13675	0.59977	0.01861	0.711
Size x Nutrient x Assembly	1	0.1074	0.10736	0.47086	0.01461	0.846
Residuals	24	5.4723	0.22801		0.74485	
Total	31	7.3469			1	

Figure 9. SEM model of the effects of treatments on endpoint species richness as well as the potential for interaction among zooplankton and macroinvertebrate richness. SEM regressions that are significant at $P < 0.05$ level are shown in bold. Ecosystem size had a strong, positive effect on macroinvertebrate richness but no significant effect on zooplankton richness. For zooplankton communities, richness was higher in mesocosms that began assembled. Nutrient input rate had no effect on the richness of either trophic group and no effect on macroinvertebrate richness on zooplankton richness was found.

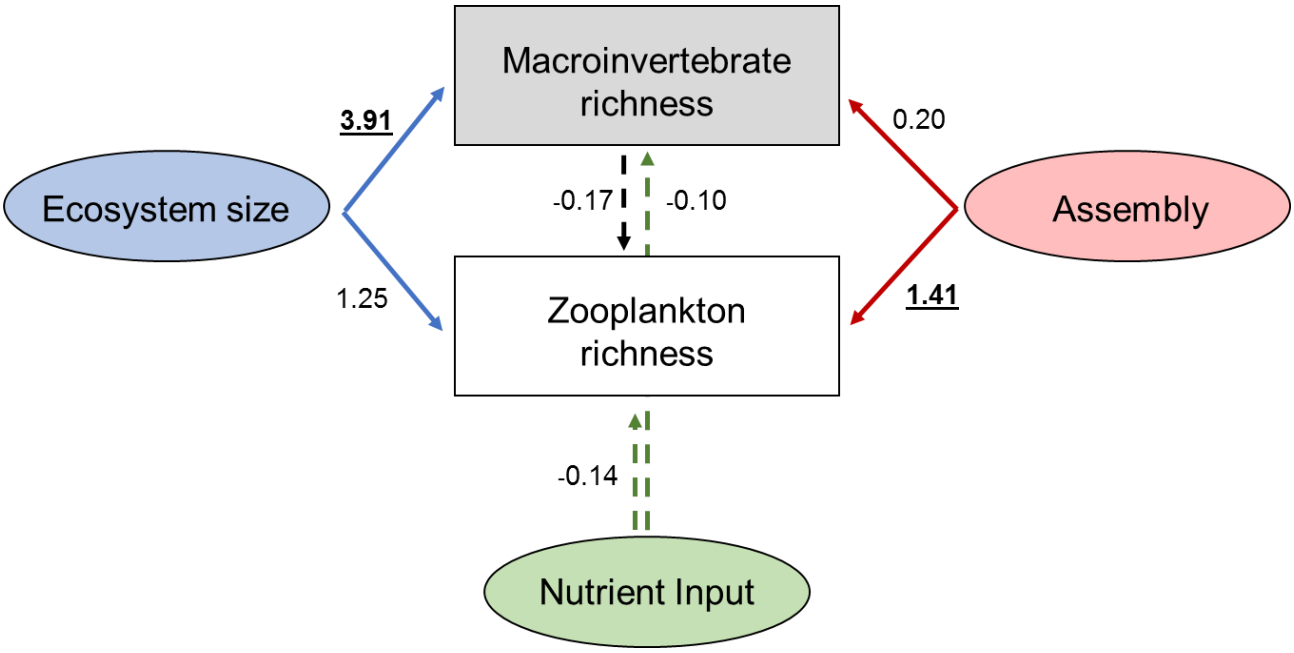


Figure 10. SEM food web model on densities of the major trophic groups incorporating feeding linkages and the effect of treatments. Only SEM regressions that are significant at the $P < 0.05$ level are shown. There were positive effects of ecosystem size on the densities of the macroinvertebrate groups Odonata, Hemiptera, and Coleoptera as well as the calanoid copepods. A negative effect of ecosystem size on rotifers was also observed. The assembly status of communities generally had no effect on the densities of taxonomic groups with the one exception of denser calanoid copepod populations in communities that began assembled.

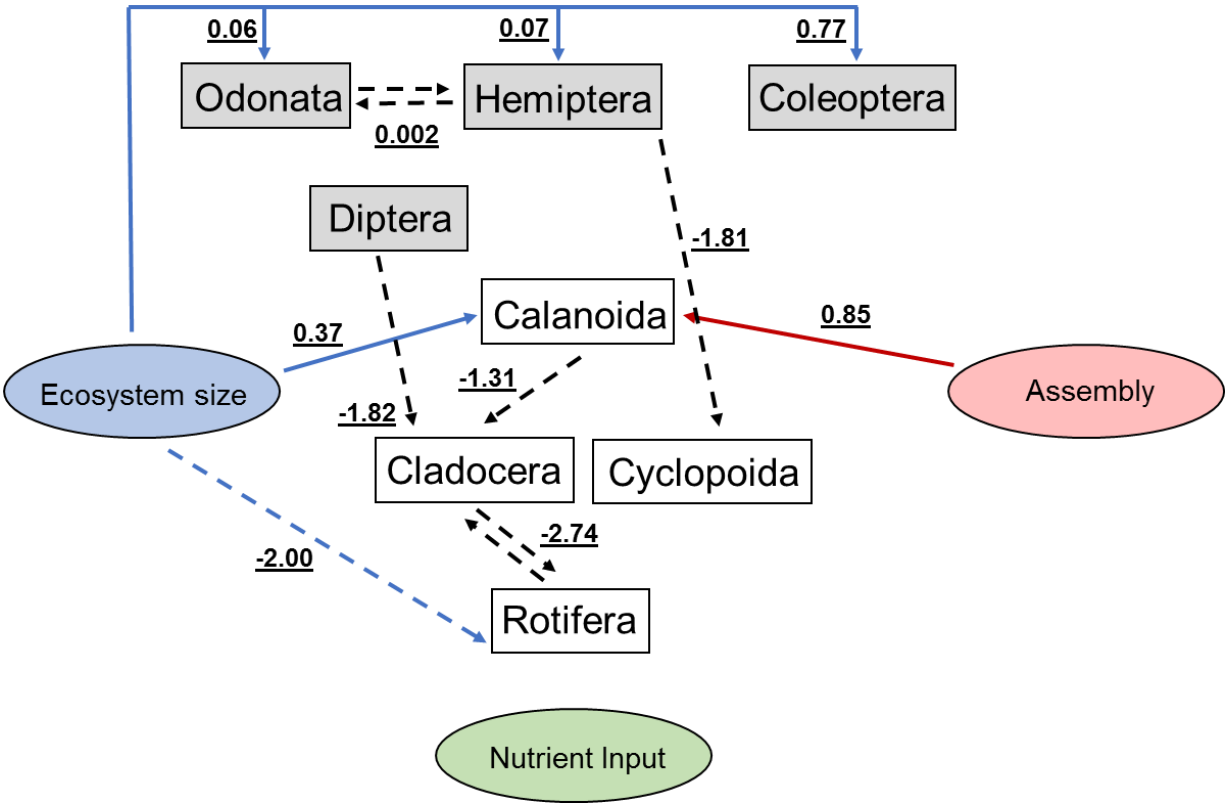


Figure 10 (cont'd). The nutrient input rate did not significantly affect the densities of any taxonomic group. Three significant top-down feeding effects were observable in the endpoint food web: negative effects of both dipteran and calanoid copepod density on cladoceran density, as well as a negative effect of hemipteran density on cyclopoid copepod density. Two within trophic level groups were also found to negatively covary with each other: odonates with hemipterans and cladocerans with rotifers.

Figure 11. NMDS plot of the endpoint composition of zooplankton (left panel) and macroinvertebrate (right panel) communities across treatment groups.

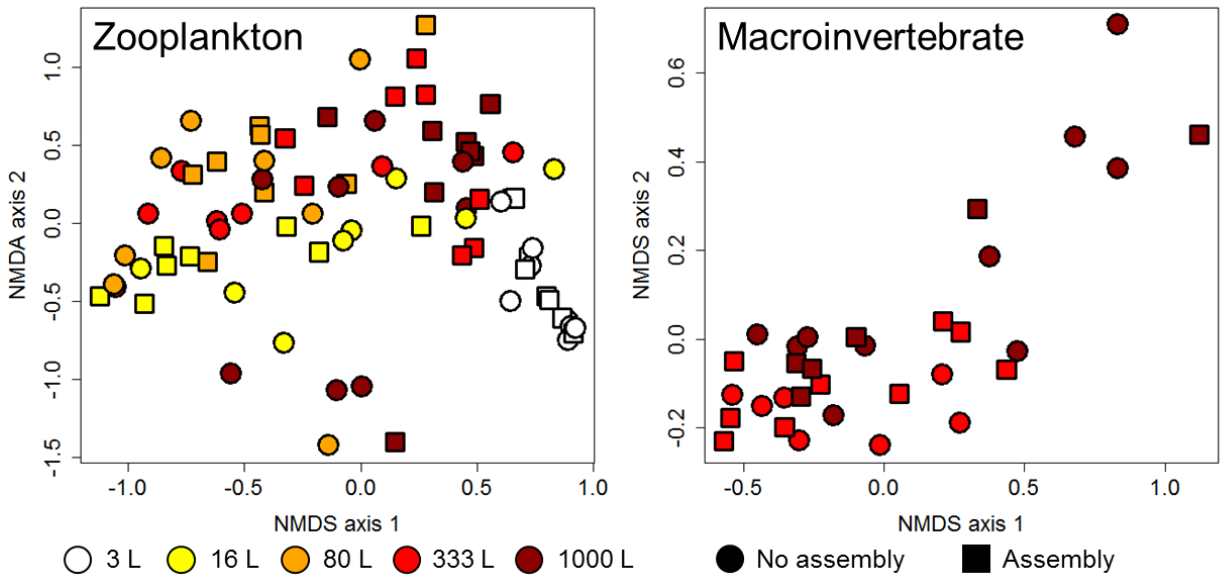


Figure 12. SEM regression values of the effects of treatments on zooplankton and macroinvertebrate species richness over the time course of the mesocosms experiment. Zooplankton richness remained significantly elevated in communities that began assembled over the duration of the experiment while ecosystem size and the nutrient input rate never had significant effects. Macroinvertebrate richness was initially higher in communities that began assembled but this effect disappeared by the midpoint of the experiment. The positive effect of ecosystem size on macroinvertebrate richness was consistent throughout the experiment. Thick lines indicate SEM regression values that are significant at $P < 0.05$.

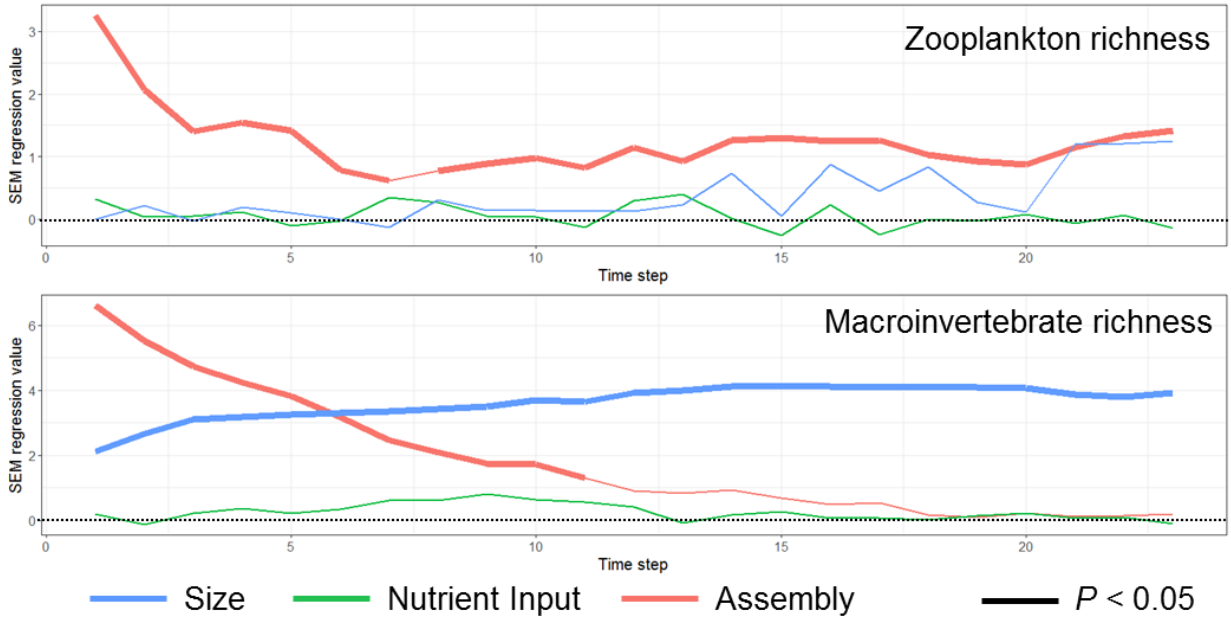


Figure 13. SEM regression values of the effects of treatments on the densities of the four major taxonomic groups of zooplankton in communities over the duration of the mesocosm experiment. Calanoid copepods rapidly became more abundant in larger size communities and communities that began assembled and this effect persisted until the experimental endpoint. Cyclopoid copepods were generally denser in communities that began assembled and this effect was intermittently significant throughout the experiment.

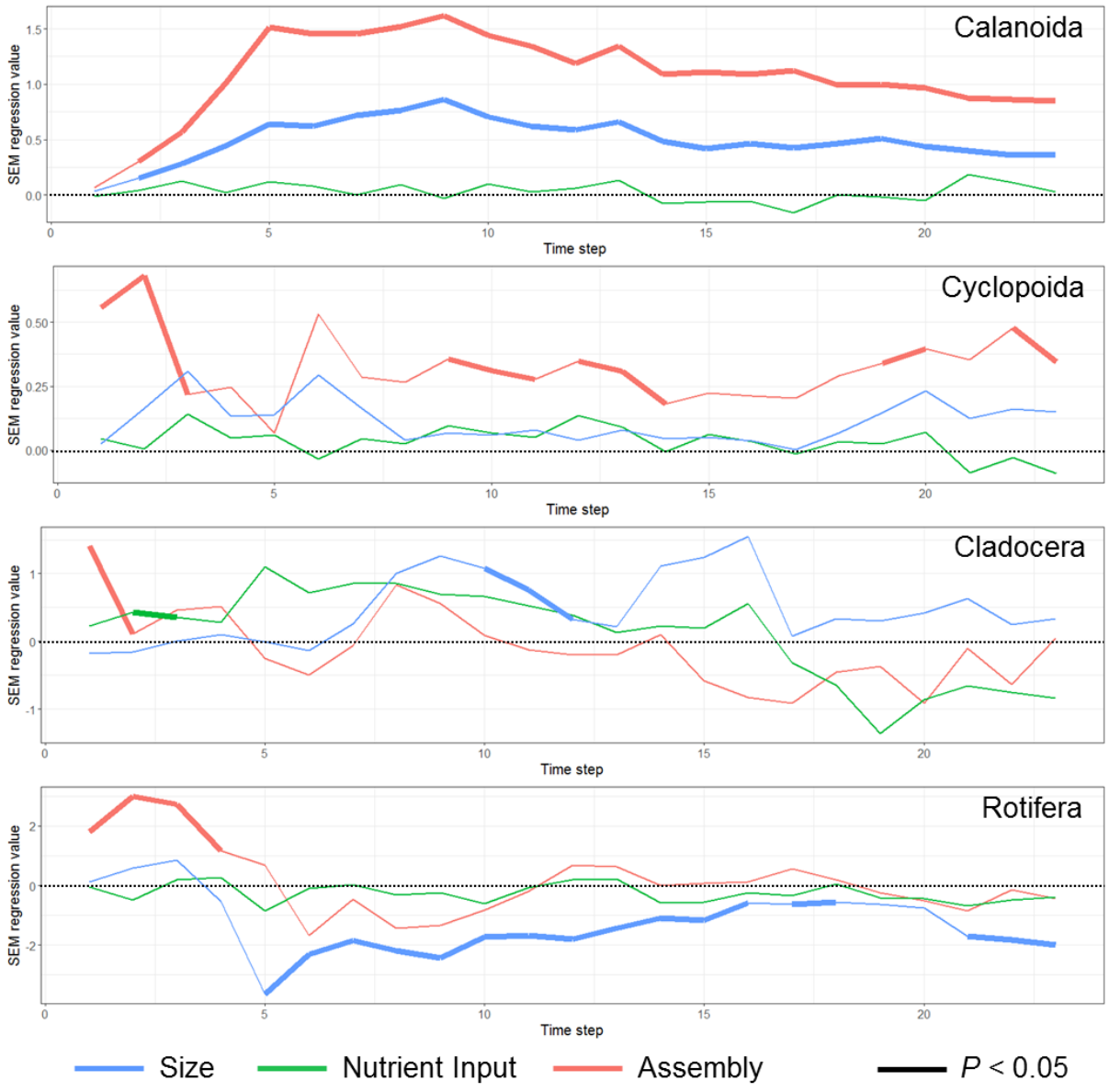


Figure 13 (cont'd). Cladocerans were occasionally found to be significantly affected by treatment groups but no general patterns were observed. Rotifers began to experience a consistent, significant, and negative effect of ecosystem size on their density starting at about one-quarter through the experiment. Thick lines indicate SEM regression values that are significant at $P < 0.05$.

Figure 14. SEM regression values of the effects of treatments on the densities of the four major taxonomic groups of macroinvertebrates in communities over the duration of the mesocosm experiment. All four groups were at significantly higher densities in communities that began assembled but this effect diminished and became unimportant throughout the duration of the experiment at varying speeds. Odonates, hemipterans, and coleopterans densities all rapidly became positively affected by ecosystem size and this effect became stable by the midpoint of the experiment.

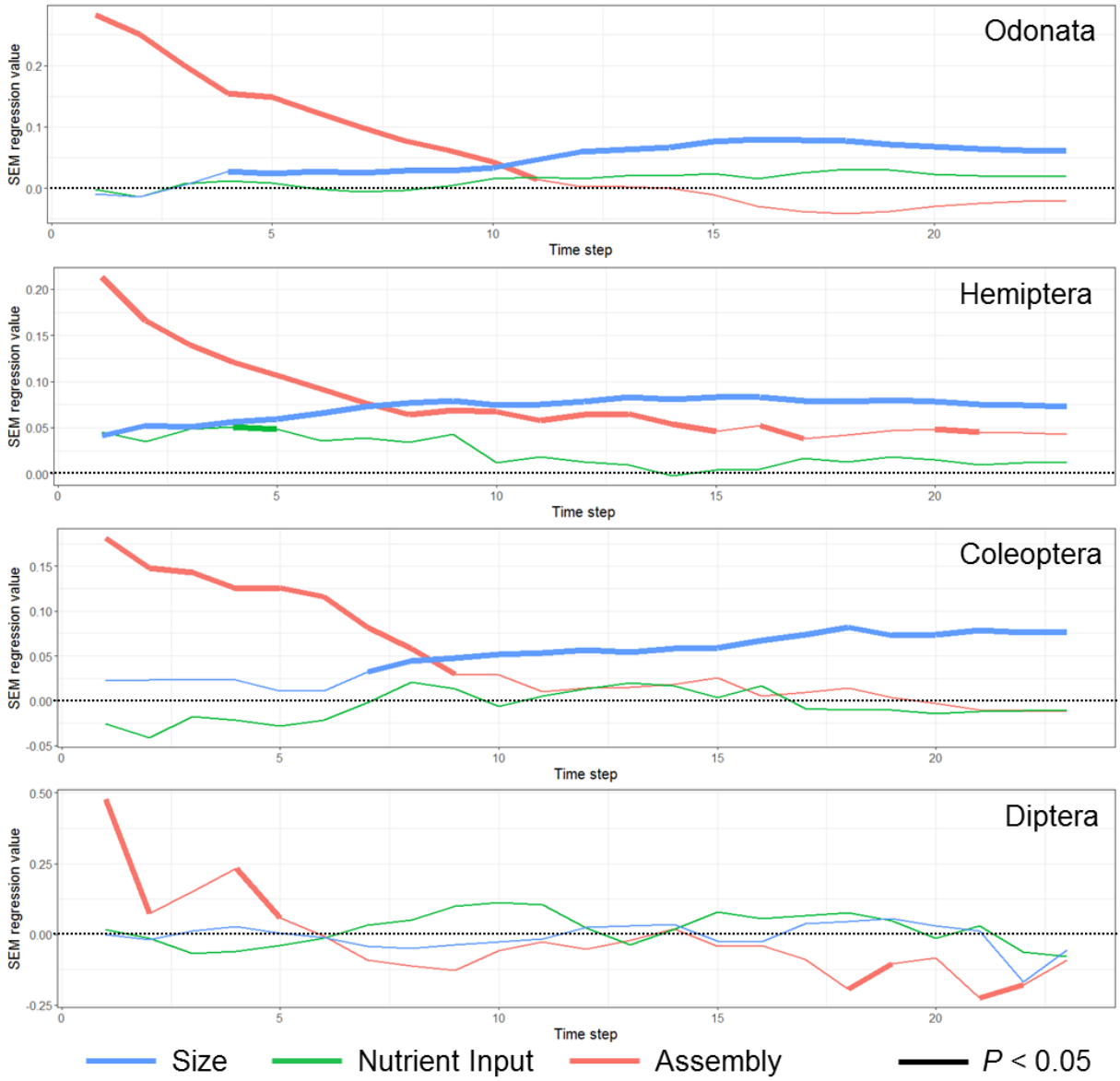
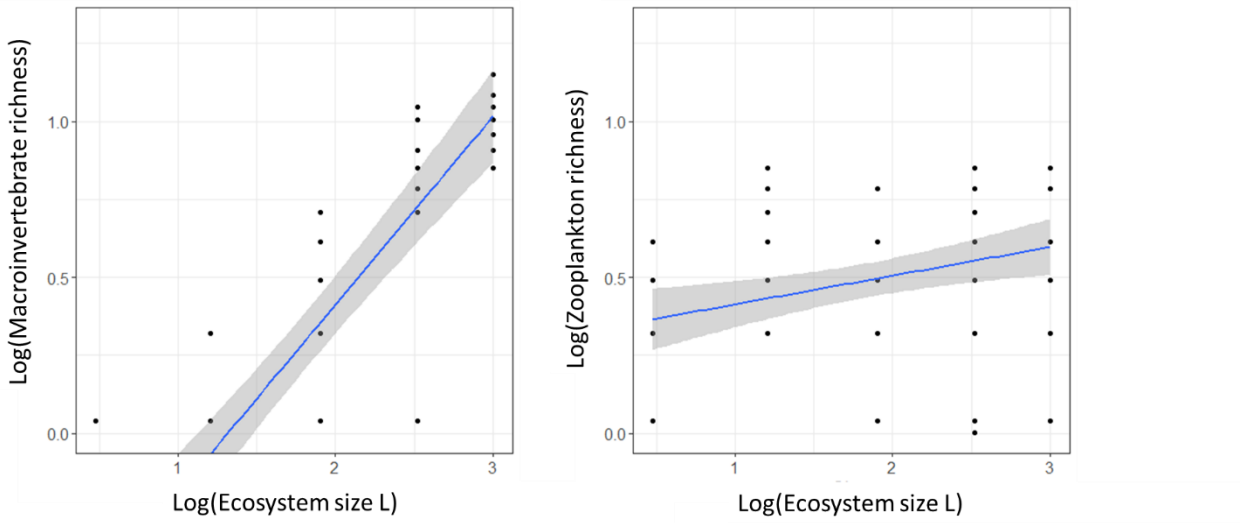


Figure 14 (cont'd). Except for a single time point near the start of the experiment, the nutrient input rate was not observed to have significant effects on macroinvertebrate densities. Thick lines indicate SEM regression values that are significant at $P < 0.05$.

Figure 15. Species-area relationships for macroinvertebrates (left; slope = 0.60) and zooplankton (right, slope = 0.09) taxa at the endpoint of the mesocosm experiment. Shaded area represent the 95% confidence bands of the regression lines.



Freshwater species-area relationship review methods

A Google Scholar search was performed on 6 June 2017 for ["species area" slope aquatic] yielding a total of 5,085 literature results. These results were partitioned into multiple date ranges for assessment as Google Scholar only displays the first 1,000 results from a given query. For assessment, we added to these results the combined 3,085 papers referencing Barbour and Brown (1974), Connor and McCoy (1979), Dodson (1992), and Lomolino (2000). Titles and abstracts were evaluated for potential primary data analyses used to determine whether a species-area relationship was observed in freshwater aquatic habitats. Papers passing this initial screen were then assessed for where the slope (or lack thereof) of the log-transformed surface area versus log-transformed species richness could be determined and was tested for significance. Papers were also required to have available data on the surface area range of freshwater habitat areas that were evaluated.

A total of 49 studies fit these screening criteria and for which the slope of the species-area relationship, a test of its significance, and the surface area range it was calculated were all available. Two binomial general linearized models were performed to detect whether studies in smaller-sized ecosystems (in log-transformed km^2) and over smaller total size ranges (in log units) were less likely to find a classic species-area relationship.

Review of species-area relationships found in freshwater ecosystems results

A total of 49 tests of a species-area relationship for freshwater ecosystems reporting the range of surface areas used were identified. 33 out of 49 (67.3%) had species-area relationships that were significant and positive while 16 out of 49 were either nonsignificant or negative. Separate binomial GLMs found that the observation of a species-area relationship was more likely as the maximal surface area sampled increased (Figure 1; estimate = 0.31, $P = 0.03$) and as

the total range of surface areas sampled in log units increased (estimate = 0.53, $P = 0.04$). Significant species-area relationships were always observed when the upper bound of surface areas investigated exceeded 2,000 km^2 or when the range of surface areas investigated exceeded 3.8 log units.

Figure 16. Literature data on the significance and slope of the species-area relationship (SAR) for freshwater taxa. The majority of data (33 out of 49) shows a significant, positive species-area relationship but the probability of finding a significant relationship is greater as the maximal (estimate = 0.31, $P = 0.03$) and range (estimate = 0.53, $P = 0.04$) of surface areas increases.

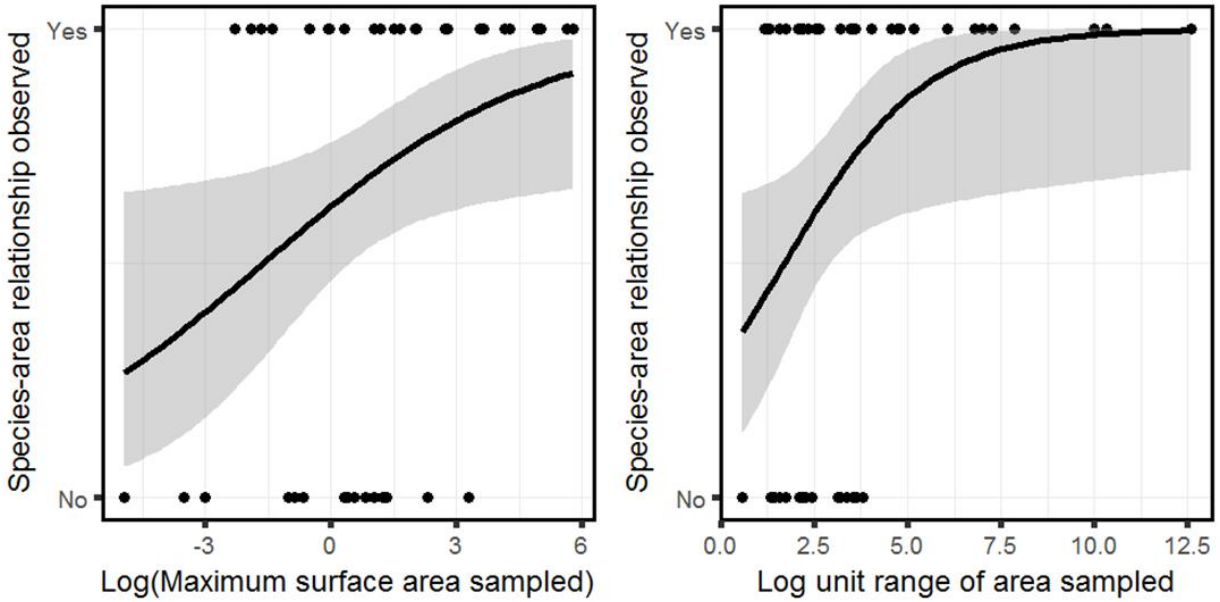


Table 10. Slopes of the species-area relationship derived from the literature for freshwater groups with the range of surface areas used in each study. NA indicates a lack of a species-area relationship.

Taxa	Low size (km^2)	High size (km^2)	SAR slope	Reference
Bacteria	0.0001	0.021	0.161	Reche, I., Pulido-Villena, E., Morales-Baquero, R., & Casamayor, E. O. (2005). Does ecosystem size determine aquatic bacterial richness?. *Ecology*, *86*(7), 1715-1722.
Bacteria	0.04	10.91	-0.151	Logue, J. B., Langenheder, S., Andersson, A. F., Bertilsson, S., Drakare, S., Lanzén, A., & Lindström, E. S. (2012). Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species–area relationships. *The ISME journal*, *6*(6), 1127-1136.
Bacteria	0.04	10.91	-0.151	Logue, J. B., Langenheder, S., Andersson, A. F., Bertilsson, S., Drakare, S., Lanzén, A., & Lindström, E. S. (2012). Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species–area relationships. *The ISME journal*, *6*(6), 1127-1136.
Fish	0.01	100000	0.25	Wagner, C. E., Harmon, L. J., & Seehausen, O. (2014). Cichlid species-area relationships are shaped by adaptive radiations that scale with area. *Ecology letters*, *17*(5), 583-592.
Fish	0.01	616	0.338	Minns, C. K. (1990). Patterns of distribution and association of freshwater fish in New Zealand. *New Zealand journal of marine and freshwater research*, *24*(1), 31-44.
Fish	10	105560	0.322	Watters, G. T. (1992). Unionids, fishes, and the species-area curve. *Journal of Biogeography*, 481-490.
Fish	0.000044	0.000961	NA	Uchida, Y., & Inoue, M. (2010). Fish species richness in spring-fed ponds: effects of habitat size versus isolation in temporally variable environments. *Freshwater Biology*, *55*(5), 983-994.
Fish	0.02	0.3	0.18	Maltchik, L., Lanés, L. E. K., Stenert, C., & Medeiros, E. S. (2010). Species-area relationship and environmental predictors of fish communities in coastal freshwater wetlands of southern Brazil. *Environmental Biology of Fishes*, *88*(1), 25-35.
Fish	8	436000	0.15	Barbour, C. D., & Brown, J. H. (1974). Fish species diversity in lakes. *The American Naturalist*, *108*(962), 473-489.

Table 10 (cont'd).

Fish	0.009	31.57	0.2	Matuszek, J. E., & Beggs, G. L. (1988). Fish species richness in relation to lake area, pH, and other abiotic factors in Ontario lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*(11), 1931-1941.
Fish	0.024	0.898	0.455	Tonn, W. M., & Magnuson, J. J. (1982). Patterns in the species composition and richness of fish assemblages in northern Wisconsin lakes. *Ecology*, *63*(4), 1149-1166.
Fish	0.001	18000	0.209	Eadie, J. M., Hurly, T. A., Montgomerie, R. D., & Teather, K. L. (1986). Lakes and rivers as islands: species-area relationships in the fish faunas of Ontario. *Environmental Biology of Fishes*, *15*(2), 81-89.
Fish	0.0283	44.73	0.39	Eadie, J. M., & Keast, A. (1984). Resource heterogeneity and fish species diversity in lakes. *Canadian Journal of Zoology*, *62*(9), 1689-1695.
Fish	0.002	0.869	0.36	Magnuson, J. J., Tonn, W. M., Banerjee, A., Toivonen, J., Sanchez, O., & Rask, M. (1998). Isolation vs. extinction in the assembly of fishes in small northern lakes. *Ecology*, *79*(8), 2941-2956.
Fish	0.03	10.55	0.141	Eckmann, R. (1995). Fish species richness in lakes of the northeastern lowlands in Germany. *Ecology of Freshwater Fish*, *4*(2), 62-69.
Macroinvertebrate	0.00006	0.0943	NA	Oertli, B., Joye, D. A., Castella, E., Juge, R., Cambin, D., & Lachavanne, J. B. (2002). Does size matter? The relationship between pond area and biodiversity. *Biological conservation*, *104*(1), 59-70.
Macroinvertebrate	0.01	2.138	0.114	Brönmark, C. (1985). Freshwater snail diversity: effects of pond area, habitat heterogeneity and isolation. *Oecologia*, *67*(1), 127-131.
Macroinvertebrate	10	105560	0.343	Watters, G. T. (1992). Unionids, fishes, and the species-area curve. *Journal of Biogeography*, 481-490.
Macroinvertebrate	0.0011	0.0216	0.445	Driver, E. A. (1977). Chironomid communities in small prairie ponds: some characteristics and controls. *Freshwater Biology*, *7*(2), 121-133.
Macroinvertebrate	0.0000921	0.00496	0.36	Ruggiero, A., Céréghino, R., Figuerola, J., Marty, P., & Angélibert, S. (2008). Farm ponds make a contribution to the biodiversity of aquatic insects in a French agricultural landscape. *Comptes Rendus Biologies*, *331*(4), 298-308.

Table 10 (cont'd).

Macroinvertebrate	0.0001	0.021	0.3	Reche, I., Pulido-Villena, E., Morales-Baquero, R., & Casamayor, E. O. (2005). Does ecosystem size determine aquatic bacterial richness?. *Ecology*, *86*(7), 1715-1722.
Macroinvertebrate	0.19	549	0.175	Sabetta, L., Barbone, E., Giardino, A., Galuppo, N., & Basset, A. (2007). Species-area patterns of benthic macro-invertebrates in Italian lagoons. *Lagoons and Coastal Wetlands in the Global Change Context: Impacts and Management Issues*, 127-139.
Macroinvertebrate	0.00001	0.04	0.175	King, J. L., Simovich, M. A., & Brusca, R. C. (1996). Species richness, endemism and ecology of crustacean assemblages in northern California vernal pools. *Hydrobiologia*, *328*(2), 85-116.
Macroinvertebrate	3640	589806	0.26	Ribera, I., Foster, G. N., & Vogler, A. P. (2003). Does habitat use explain large scale species richness patterns of aquatic beetles in Europe?. *Ecography*, *26*(2), 145-152.
Macroinvertebrate	0.000008	0.0003	NA	Towers, N. M. (2004). *Invertebrate community structure along a habitat-patch size gradient within a bog pool complex* (Doctoral dissertation, University of Edinburgh).
Macrophyte	0.01	2.138	0.129	Brönmark, C. (1985). Freshwater snail diversity: effects of pond area, habitat heterogeneity and isolation. *Oecologia*, *67*(1), 127-131.
Macrophyte	0.0001	0.012	0.286	Bosiacka, B., & Pieńkowski, P. (2012). Do biogeographic parameters matter? Plant species richness and distribution of macrophytes in relation to area and isolation of ponds in NW Polish agricultural landscape. *Hydrobiologia*, *689*(1), 79-90.
Macrophyte	0.004	17.3	NA	Vestergaard, O., & Sand-Jensen, K. (2000). Aquatic macrophyte richness in Danish lakes in relation to alkalinity, transparency, and lake area. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(10), 2022-2031.
Macrophyte	0.016	0.3	0.364	Rolon, A. S., Lacerda, T., Maltchik, L., & Guadagnin, D. L. (2008). Influence of area, habitat and water chemistry on richness and composition of macrophyte assemblages in southern Brazilian wetlands. *Journal of Vegetation Science*, *19*(2), 221-228.
Macrophyte	0.0007	4380	0.123	Rørslett, B. (1991). Principal determinants of aquatic macrophyte richness in northern European lakes. *Aquatic Botany*, *39*(1-2), 173-193.

Table 10 (cont'd).

Macrophyte	0.0001	14.77	0.018	Jones, J. I., Li, W., & Maberly, S. C. (2003). Area, altitude and aquatic plant diversity. *Ecography*, *26*(4), 411-420.
Macrophyte	0.02	3.7	NA	James, C., Fisher, J., Russell, V., Collings, S., & Moss, B. (2005). Nitrate availability and hydrophyte species richness in shallow lakes. *Freshwater biology*, *50*(6), 1049-1063.
Macrophyte	0.0001	0.226	NA	Jackson, S. T., & Charles, D. F. (1988). Aquatic macrophytes in Adirondack (New York) lakes: patterns of species composition in relation to environment. *Canadian journal of botany*, *66*(7), 1449-1460.
Macrophyte	0.0001	114	0.403	Weiher, E., & Boylen, C. W. (1994). Patterns and prediction of α and β diversity of aquatic plants in Adirondack (New York) lakes. *Canadian Journal of Botany*, *72*(12), 1797-1804.
Phytoplankton	3.8E-09	14100	0.134	Smith, V. H., Foster, B. L., Grover, J. P., Holt, R. D., & Leibold, M. A. (2005). Phytoplankton species richness scales consistently from laboratory microcosms to the world's oceans. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(12), 4393-4396.
Phytoplankton	0.0000079	591.56	0.043	Bolgovics, Á., Ács, É., Várbíró, G., Görgényi, J., & Borics, G. (2016). Species area relationship (SAR) for benthic diatoms: a study on aquatic islands. *Hydrobiologia*, *764*(1), 91-102.
Phytoplankton	0.000000075	0.0000117	NA	Soininen, J., & Meier, S. (2014). Phytoplankton richness is related to nutrient availability, not to pool size, in a subarctic rock pool system. *Hydrobiologia*, *740*(1), 137.
Phytoplankton	0.00000001	100	0.043	Bolgovics, A., Acs, E., Varbiro, G., Goergenyi, J., & Borics, G. (2016). Species area relationship (SAR) for benthic diatoms: a study on aquatic islands. *Hydrobiologia*, *764*(1), 91.
Phytoplankton	0.1	3582	0.043	Jankowski, T., & A Weyhenmeyer, G. (2006). The role of spatial scale and area in determining richness-altitude gradients in Swedish lake phytoplankton communities. *Oikos*, *115*(3), 433-442.
Zooplankton	0.0001	0.021	0.094	Reche, I., Pulido-Villena, E., Morales-Baquero, R., & Casamayor, E. O. (2005). Does ecosystem size determine aquatic bacterial richness?. *Ecology*, *86*(7), 1715-1722.

Table 10 (cont'd).

Zooplankton	0.33	44.5	0.09	Lepère, C., Domaizon, I., Taïb, N., Mangot, J. F., Bronner, G., Boucher, D., & Debroas, D. (2013). Geographic distance and ecosystem size determine the distribution of smallest protists in lacustrine ecosystems. *FEMS microbiology ecology*, *85*(1), 85-94.
Zooplankton	0.000004	82000	0.094	Dodson, S. (1992). Predicting crustacean zooplankton species richness. *Limnology and Oceanography*, *37*(4), 848-856.
Zooplankton	0.06	210	NA	Hessen, D. O., Faafeng, B. A., Smith, V. H., Bakkestuen, V., & Walseng, B. (2006). Extrinsic and intrinsic controls of zooplankton diversity in lakes. *Ecology*, *87*(2), 433-443.
Zooplankton	15	1892	NA	De los Ríos, P., & Soto, D. (2007). Crustacean (Copepoda and Cladocera) zooplankton richness in Chilean Patagonian lakes. *Crustaceana*, *80*(3), 285-296.
Zooplankton	0.000405	2.54	NA	Dodson, S. I., & Silva-Briano, D. (1996). Crustacean zooplankton species richness and associations in reservoirs and ponds of Aguascalientes State, Mexico. *Hydrobiologia*, *325*(2), 163-172.
Zooplankton	0.0001	0.1365	NA	Drenner, S. M. (2008). Crustacean zooplankton community structure in temporary and permanent ponds in a Texas grassland [electronic resource]. *UMI thesis.*
Zooplankton	0.239	6.544	NA	Dodson, S. I., Newman, A. L., Will-Wolf, S., Alexander, M. L., Woodford, M. P., & Van Egeren, S. (2008). The relationship between zooplankton community structure and lake characteristics in temperate lakes (Northern Wisconsin, USA). *Journal of Plankton Research*, *31*(1), 93-100.
Zooplankton	0.4	21.6	NA	Van Egeren, S. J., Dodson, S. I., Torke, B., & Maxted, J. T. (2011). The relative significance of environmental and anthropogenic factors affecting zooplankton community structure in Southeast Wisconsin Till Plain lakes. *Hydrobiologia*, *668*(1), 137-146.
Zooplankton	0.568	2.159	NA	Echaniz, S. A., Vignatti, A. M., José de Paggi, S., Paggi, J. C., & Pilati, A. (2006). Zooplankton Seasonal Abundance of South American Saline Shallow Lakes. *International Review of Hydrobiology*, *91*, 86-100.

Table 11. List of taxa observed in the mesocosm experiment

Rotifera
Asplanchna sp.
*Brachionus angularis**
*Brachionus quadridentatus**
Euchlanis sp.
Hexarthra mira
Keratella cochlearis
Keratella quadrata
*Lecane luna**
*Monostyla bulla**
Philodina sp.
*Platyias patalus**
Polyarthra sp.
Testunidella sp.
Trichocerca sp.

Cladocera
Acroperus harpae
Alona affinis
Alonella nana
Bosmina longirostris
*Ceriodaphnia reticulata**
Chydorus sphaericus
Daphnia ambigua
Daphnia dubia
*Daphnia pulex x D. pulicaria**
Diaphanosoma brachyurum
Macrothrix sp.
Moina micrura
Pleuroxus denticulatus
Scapholeberis mucronata
Sida crystallina
Simocephalus vetulus

Cyclopoida
*Acanthocyclops vernalis**
Mesocyclops edax
Tropocyclops prasinus

Calanoida
Epischura lacustris

Other zooplankton
Ostracod

Odonata
*Ischnura verticalis**
Lestes dryas
*Leucorrhinia frigida**
Libellula sp.

Hemiptera
Gerridae
Hesperocorixa sp.*
*Notonecta irrorate**
*Notonecta undulata**
Sigara sp.

Diptera
Aedes triseriatus
Anopheles quadrimaculatus
Chaoborus sp.*
Chironomidae
Culiseta inornata

Coleoptera
Dystiscus sp.*
Gyrinus sp.*
Hydrophilidae*
Laccophilus sp.*
Tropisternus sp.*

Other macroinvertebrates
Baetidae mayfly
Hydrachnidia

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CHAPTER 3

A NEW LOOK AT THE ROLE OF EPHIPPIA AS A DORMANCY-DISPERSAL STRATEGY IN THE CLADOCERAN (*SCAPHOLEBERIS MUCRONATA*)

Abstract

Ephippia, the diapausing stages of freshwater cladoceran zooplankton, provide a mechanism for dispersal in both space and time. Although much is known about ephippia production in the genus *Daphnia*, where it serves primarily as a means to escape unfavorable environmental conditions, almost nothing is known about how ephippia function in the life histories of most other cladoerans. This study investigates ephippia production as a life-history strategy in the cladoceran *Scapholeberis mucronata* using a combination of field observations and mesocosms experiments. Unlike *Daphnia*, the production of ephippia by *Scapholeberis* appears to be primarily a colonization mechanism, enhancing dispersal under favorable as well as unfavorable environmental conditions.

The ephippia of *S. mucronata* are relatively short-lived, persisting for a maximum of about two years. *S. mucronata* populations exhibit a peak of ephippia production during the summer growing season and prior to autumn overwintering in temperate ponds and lakes, which implies a function beyond the temporal hedging of adverse seasonal conditions. In an observational study, I found that *S. mucronata* exhibited the highest absolute and relative dispersal rates of eight common cladoceran species coexisting in a natural freshwater pond metacommunity. In a mesocosm experiment, this high dispersal rate led to rapid, non-selective

colonization that was not influenced by habitat size, community assembly history, or nutrient level.

The per capita production of ephippia by *S. mucronata* populations in mesocosms during their summer peak was only marginally affected by local conditions. There was a barely detectable decrease of ephippia production in *S. mucronata* populations from larger habitats and higher nutrient levels and this effect was not related to the success of these populations. This contrasts sharply with what is known about ephippia production in *Daphnia*, where the cues that trigger the onset of ephippia production are closely linked to local environmental conditions.

S. mucronata produced ephippia that hatched immediately following drying and re-immersion in water (i.e., no dormancy). On average, 4.8% of the ephippia produced hatched immediately and this hatching rate varied markedly among source populations (range 0% to 78% hatching). Ephippia from populations experiencing negative population growth rates hatched at a lower rate than those from increasing populations. Further, no immediately-hatching ephippia were produced by the nine *S. mucronata* populations that went extinct in the mesocosms, suggesting that *S. mucronata* may alter its hedging of temporal variability to match the favorability of local conditions by varying the percentage of ephippia that hatch immediately.

These results characterize a dormancy-dispersal strategy that may promote the success of *S. mucronata* at the metacommunity scale when integrated across a growing season. When conditions are favorable locally, *S. mucronata* ephippia hatch immediately after drying and reaching a new locale, increasing their hedging of spatial variability. But, when local conditions are not favorable, ephippia tend to hedge against temporal variability by not hatching. These observations suggest a much broader range of dispersal strategies among ephippia-producing zooplankton species than has previously been recognized.

Introduction

The production of dormant life stages in freshwater zooplankton provides a means to escape adverse seasonal and interannual conditions and enhances the capacity for dispersal (Brendonck and DeMeester 2003; Panov and Cáceres 2007). Dramatic examples of long-lived diapausing eggs have been recorded for copepods (e.g., viable eggs of *Diaptomus sanguineus* found in 300+ year old lake sediements; Hairston et al. 1995) and *Daphnia* (e.g., viable ephippia collected from 125 year old lake sediments; Cáceres 1998). The deposition of zooplankton resting eggs into the sediments of lakes can generate a massive egg bank (densities of up to a million eggs per meter squared; Hairston 1996), providing a large reservoir of genetically diverse propagules from which future populations may be regenerated. As noted by Brendonck and DeMeester (2003: 73), most cladocerans ….are cyclically parthenogenetic and sexual resting eggs (ephippia) are generally only produced when environmental conditions deteriorate … the stimulus that triggers the production of diapausing eggs … usually acts prior to the onset of deteriorating environmental conditions". Thus, the production of resting eggs is generally viewed as a means of escaping an adverse local environmental, either in time or in space.

Comparative data suggests that there is a tradeoff between the tendency of zooplankton species to buffer against the adverse effects of deteriorating local conditions either through long-lived diapausing stages or longer adult lifespans. A review of diapause in crustaceans, including many zooplankton orders, found an inverse relationship between life span and the duration of diapause (Hairston and Cáceres 1996). Some copepods exhibit extremely long-lived diapausing eggs while others only express “active diapause”, where free-living life stages reduce their metabolic rate (Brendonck and De Meester 2003). In rotifers, there exists a mutually exclusive tradeoff between dormancy and lifespan where relatively short-lived monogonont rotifers

produce long-lived diapausing eggs while long-lived bdelloid rotifers can enter short-lived quiescence (Ricci 2001). Interestingly, no rotifer species is known to both produce diapausing eggs and have the capacity for quiescence.

The capacity for cladocerans to produce diapausing eggs, ephippia, is an ancient innovation that exists in the fossil record from at least 145 Mya and there is even fossil evidence of induction by the presence of Chaoboridae predators (Kotov and Taylor 2011). Ephippia is presumably the dominant life stage involved in long-distance dispersal in cladocerans, as evidenced by the likely introduction of many invasive cladoceran species as ephippia (Panov et al. 2004). Thus, ephippia allow populations to avoid locally adverse conditions not only through diapause in place, but by enabling dispersal to new water bodies. Cladoceran ephippia can be transported following the survival of gut passage through fish, birds, amphibians, and mammals (Mellors 1975, Frisch et al. 2007) and may also be dispersed via attachment to aquatic invertebrates (Van de Meutter et al. 2008), mammals (Waterkeyn et al. 2010), and waterfowl (Figuerola and Green 2002).

In natural systems, both the emergence and production of cladoceran ephippia can vary substantially from year to year (Cáceres 1998). Ephippia production in cladocerans has been linked to population density (e.g., Carvalho and Hughes 1983, Larsson 1991, Zadereev and Lopatina 2006, Smith et al. 2009), food availability (e.g., Carvalho and Hughes 1983, Smith et al. 2009) and short photoperiods (e.g., Carvalho and Hughes 1983). In *Daphnia*, ephippia production can also be induced by predator cues alone (Slusarczyk 1995, Pijanowska and Stolpe 1996, Dzialowski et al. 2003). Although the causes and consequences of ephippia production in *Daphnia* are well-described, comparable experimental evidence is lacking for other cladoceran species. This lack of knowledge limits our understanding of the various roles that ephippia may

play in the life histories of zooplankton species, particularly their relatively effects on dispersal in time and in space, and leads to the potential for overgeneralizing the observations from *Daphnia* to all cladocerans.

Scapholeberis mucronata (O. F. Müller) is a surface-film specialist present in both small ponds and large lakes (Fryer 1985) that is widely distributed across North America and Eurasia (Dumont and Pensaert 1983). *S. mucronata* has been previously found to passively disperse at least 60 meters from source ponds (Cohen and Shurin 2003) and can be a major contributor to the colonization of new ponds (Cáceres and Soluk 2002, Louette and De Meester 2005). *Scapholeberis* produce ephippia with one egg (Dumont and Pensaert 1983) and their ephippia tend to float at the surface where they may be readily dispersed by wind and animals (REF). *S. mucronata* populations have been observed to undergo two seasonal peaks in ephippia production: a smaller peak during May-June, followed by a larger ephippia production event during September-December (Green 1963). *Scapholeberis* ephippia appear short-lived, typically undergoing diapause for only about two years (Hairston and Cáceres 1996) and in one study S. *mucronata* eggs did not appear to be viable after 12 years (Meijering 2003), although this finding could result from the absence of appropriate hatching stimuli. While viable *S. mucronata* ephippia have been observed in the egg banks of lakes (Vandekerkhove et al. 2005a), their short viability matches theoretical predictions of a short dormancy period in an organism that is a successful disperser (Venable and Lawlor 1980).

While the importance of ephippia to cladoceran population dynamics through dormancy and dispersal are well-established, interspecific differences in the relative tradeoff between dormancy and dispersal among cladoceran species outside the genus Daphnia are less understood. Here I investigate the colonization ability of *S. mucronata* into freshwater pond

mesocosms of varying productivity, assembly state, and size as well as the subsequent population success and extent of the spring/summer ephippia production event following establishment.

Methods

Survey of natural ponds and cladoceran dispersal assessment

In a previous study, I measured the abundances of zooplankton in a metacommunity of ten freshwater ponds in southwestern Michigan and quantified species-specific dispersal over a four month period during the summer growing season (see Hanly and Mittelbach 2017 for complete experimental design and analysis). *S. mucronata* was the third most numerically abundant cladoceran in this pond metacommunity. Here, I compare the dispersal ability of *S. mucronata* (measured as the number of dispersing individuals per individual *S. mucronata* in the metacommunity) to the eight most abundant cladoceran species: *Alona affinis*, *Alonella nana*, *Bosmina longirostris*, *Ceriodaphnia reticulata*, *Chydorus sphaericus*, *Diaphanosoma brachyurum*, and hybrid *Daphnia pulex* × *D. pulicaria*. For these same species, I also compare the proportion of disperser samples in which at least one individual of a species was observed relative to the number of local pond community samples in which at least one individual of that species was observed.

Outdoor mesocosm experiment

A mesocosm experiment mimicking freshwater habitats of different sizes (i.e., tree holes to small ponds) was established at Michigan State University's Kellogg Biological Station Experimental Pond Facility (Hickory Corners, MI, USA; 42.41° N, 85.39° W). Mesocosms were

of five sizes (3 L, 16 L, 80 L, 333 L, and 1,000 L) fully crossed by two nutrient input levels and the presence/absence of initial metazoan zooplankton and macroinvertebrate colonizers ("assembled" and "unassembled"). Each mesocosm treatment was replicated four times for a total of 80 mesocosms. Mesocosms were set up with no substrate and were filled with well water on 6 May 2014 and "assembled" mesocosms were inoculated with a mixture of pond water from eight natural ponds at Lux Arbor Reserve (Barry County, MI, USA; 42.29° N, 85.45° W). Nutrients initially added to "high" nutrient mesocosms were 10.4 mg/L $NaNO_3$ and 0.33 mg/L NaH_2PO_4 followed by weekly supplementation at a rate of 2.46 mg/L $NaNO_3$ and 0.12 mg/L NaH_2PO_4 (after Shurin 2001). "Low" nutrient mesocosms were fertilized and supplemented at one-quarter of this "high" nutrient rate. The addition of zooplankton and macroinvertebrates into "assembled" mesocosms took place on 13 May 2014. Target volumes were maintained by weekly additions of well water.

Mesocosms that were "assembled" were seeded with an initial zooplankton community containing the two cladoceran species (*C. reticulata* and the hybrid *Daphnia pulex* × *D. pulicaria*), one copepod species (*Acanthocyclops vernalis*), and five rotifer species (*Brachionus angularis*, *Brachionus quadridentata*, *Lecane luna*, *Monostyla bulla*, *Platyias patalus*) in proportion to the total mesocosm volume that were collected from eight natural ponds at Lux Arbor Reserve. Approximately three-quarters of inoculated zooplankton were D. *pulex* × *D. pulicaria*, which were added to mesocosms at a rate of approximately seven individuals per liter of volume. Predatory larvae of the dipteran *Chaoborus* were also added to each "assembled" mesocosm at a rate of approximately one individual per liter of mesocosm volume. Macroinvertebrates added to "assembled" mesocosms were from five groups: water boatmen (one species; *Hesperocorixa* sp.), backswimmers (two species; *Notonecta irrorata* and *N.*

undulata), damselflies (one species; *Ischnura verticalis*), dragonflies (one species; *Leucorrhinia frigida*), and aquatic beetles (five species; *Dytiscus* sp., *Gyrinus* sp., *Laccophilus* sp., *Tropisternus* sp., and Hydrophilidae sp.). Individual macroinvertebrates were added to mesocosms at a rate of approximately one individual per 3 L water volume. 3 L mesocosms were inoculated with one random individual from one of the five groups (total individuals = 1), 16 L mesocosms with one individual from each of the five groups (total individuals = 5), 80 L mesocosms with five individuals from each of the five groups (total individuals = 25), 333 L mesocosms with 21 individuals from each of the five groups (total individuals = 105), and 1000 L mesocosms with 63 individuals from each of the five groups (total individuals = 315).

The mesososm experiment lasted 12 weeks from the addition of zooplankton and macroinvertebrates until terminated on 5 August 2014. Macroinvertebrates were fully sampled without permanent removal in each mesocosm on the Monday, Wednesday, and Friday of each week through a combination of visual surveys (to minimize handling when possible) and temporary removal using dip nets (when needed to enumerate high densities or to confirm identities). Zooplankton were sampled on the Tuesday and Thursday of each week by taking three equally spaced, vertically integrated 1 L water samples. Each resulting sample was filtered through 40 µm mesh to create a 50 mL volume sample, which was fully enumerated following preservation in 2% acid Lugol's solution in the 80 L, 333 L, and 1000 L mesocosms and live under a dissecting microscope in 5-10 mL subsamples and returned to the source mesocosm along with the 2.95 L of filtered water in the case of 3 L and 16 L mesocosms. A 3 L sample represents the full volume of the smallest mesocosms and nearly 20% of the volume of the second smallest mesocosms. Therefore, care was taken to minimize the disturbance of sampling on the zooplankton in these smallest mesocosms; zooplankton samples were taken singly and

enumerated immediately in a building adjacent to the mesocosm array. Chlorophyll *a* measurements were taken *in vivo* using a self-contained underwater fluorescence apparatus (SCUFA, Turner Designs, Sunnyvale, CA) on 14 July 2014 at the midpoint of ephippia collection.

Ephippia collection and hatching

S. mucronata quickly colonized the experiment and after 30 days half of the mesocosms contained *S. mucronata*. Daily observations revealed a burst in *S. mucronata* ephippia production in mid-July. Ephippia were collected daily from 11 July 2014 through 17 July 2014 during the observed peak in ephippia production across the experiment. Limited ephippia production (< 15 ephippia per mesocosm) was observed during the preceding week but these ephippia were not removed until the collection period. Collected ephippia were segregated by mesocosm and stored in 250 mL containers at 25 °C without water until 21 July 2014. The immediate hatching ability of ephippia was tested by filling each of the 250 mL containers with well water on 21 July 2014 and observing hatching rates over the following three days. Each day containers were observed under a dissecting microscope and newly hatched *S. mucronata* were removed.

Statistical analyses

The probability of *S. mucronata* colonizing a mesocosm was examined using a binomial generalized linear model (GLM) with habitat size, nutrient input rate, and initial assembly status as predictor variables and a logit link function. For the probability of ephippia production by *S. mucronata* populations, a second binomial GLM using the same predictor variables as well as their interactions was utilized, excluding mesocosms where no *S. mucronata* colonized.

The mean density and average daily population growth rate of *S. mucronata* was calculated from the six zooplankton sampling times that encompassed peak ephippia production: 8, 10, 15, 17, 22, and 24 July 2014. The mean density of macroinvertebrate predators was determined from the nine macroinvertebrate sampling times that occurred a day prior or after each of those six dates. Another GLM incorporating habitat size, nutrient level, and initial assembly status and their interactions was used to test whether any of these variables were predictive of the per capita ephippia production rate within *S. mucronata* populations. This GLM was repeated on the residuals of the linear regression between *S. mucronata* density and ephippia density as well as with the additional predictor variables of the percent change in *S. mucronata* density, the mean density of cladocerans other than *S. mucronata*, the mean density of macroinvertebrate predators, and chlorophyll *a*.

Results

Pond survey

S. mucronata was observed in nine out of ten natural ponds at Lux Arbor Reserve at least once during repeated surveys conducted from June-September 2011. *S. mucronata* was observed dispersing into 17.1% of dispersal traps compared to an average of 7.3% (SE 1.32) of traps for the eight other most locally abundant cladocerans in natural ponds. Moreover, 4,421 *S. mucronata* individuals were collected from dispersal traps, which is over ten times the 347 individuals observed dispersing for *C. sphaericus*, the next most numerically abundant cladoceran disperser. Thus, *S. mucronata* had by far the highest dispersal rate of any of the cladoceran species in this pond metacommunity and its dispersal rate could not be explained by

either: 1) its total abundance in the pond metacommunity or, 2) by its occupancy of local communities (Figure 17).

Mesocosm experiment

S. mucronata dispersed into 58 out of 80 experimental mesocosms (54 of 64 mesocosms larger than 3 L). *S. mucronata* was observed in the smallest-size mescososms (volume = 3 L) and no successful establishment of persistent populations or the production of ephippia were observed in the 3 L mesocosms. Thus, this habitat size appears to be below the minimum required to maintain a population of *S. mucronata* and so these mesocosms were excluded from the GLM analysis of colonization. Excluding the smallest mesocosms, I found that *S. mucronata* colonization was not significantly influenced by mesocosm size, nutrient level, initial assembly status, or by any interaction of these factors (GLM: $P > 0.6$ for all comparisons).

S. mucronata produced a total of 8,922 ephippia in 48 mesocosms. The majority of ephippia collected were freely floating on the water surface or were loosely attached to the sides of the tank, including above the waterline. Less than 10 ephippia (< 0.1% of the total) were negatively buoyant and found at the bottom of mesocosms. In mesocosms where *S. mucronata* colonized, the number of ephippia produced was not significantly influenced by any of the mesocosm treatments ($P > 0.3$ for all comparisons).

The densities of *S. mucronata* in mesocosms varied widely from 0.333 individuals/L to 262.8 individuals/L with a mean density of 50.35 individuals/L (SE = 8.83). *S. mucronata* produced a mean of 0.095 ephippia (SE 0.025) per capita in mesocosms where they colonized. *S. mucronata* density and ephippia density were highly correlated (Pearson's $r = 0.69$, $P < 0.0001$; Figure 18), but per capita production was not significantly affected by mesocosm size, nutrient

level, initial community assembly, or by their interactions (GLM: $P > 0.2$ for all comparisons). However, when using the residuals of the relationship between *S. mucronata* adult density and ephippial density, there was a significant, negative influence of habitat size on the residuals of per capita ephippia production (GLM: Estimate -1.03, $t = -2.19$, $P = 0.03$) as well as a marginally significant, negative effect of the level of nutrient input on per capita ephippia production (GLM: Estimate -2.49, $t = -1.76$, $P = 0.08$). No effects of the initial community assembly status, the density of other cladocerans, or the density of macroinvertebrate predators were detected on per-capita ephippial production ($P > 0.1$ for all comparison). Using measured chlorophyll *a* instead of the nutrient input level as a proxy for productivity leads to a nonsignificant relationship between productivity and the residuals of the relationship between adult and ephippia density ($P = 0.36$). The residuals of the regression of *S. mucronata* density and ephippia density also could not be explained by the average daily population growth rate during the 16 days spanning the peak of ephippia production ($P = 0.88$; Figure 19).

A total of 503 ephippia (5.6% of the 8,922 collected) hatched over a 72 hr observation period under uniform conditions. Populations of *S. mucronata* from different mesocosms produced ephippia with hatching that varied from 0% to 78% (mean hatching rate = 4.8%). The probability that a non-zero number of ephippia hatched from a source population was unaffected by either mesocosm size or nutrient level experienced by source populations (binomial GLM: $P > 0.5$). However, the average daily population growth rate of the *S. mucronata* source population strongly influenced the probability of ephippia hatching (binomial GLM: Estimate = 3.52, $z = 2.73$, $P = 0.006$; Figure 19). Ephippia hatched from just 15% of source populations that were declining in abundance (n = 26), but over 63% of source populations that were growing (n = 19). In addition, ephippia from source mesocosms that contained an assembled community of

organisms at the start of the experiment were less likely to exhibit non-zero hatching rates over (binomial GLM: Estimate = -1.83, z = -2.06, P = 0.04; Figure 20).

Discussion

In the combined field survey and dispersal assessment, *S. mucronata* had the greatest capacity for dispersal both relative to its occupancy and abundance and in the absolute abundance of dispersers when compared to the other seven most common cladocerans found in these natural ponds. This high inferred colonization capacity is confirmed by the mesocosm experiment where *S. mucronata* populations successfully established from surrounding water bodies without aid into 54 out of 64 mesocosms with volumes between 16 L and 1,000 L and successfully completed gamogenetic reproduction in 48 out of the 64. Wind was the presumed dispersal vector for *S. mucronata* in this experiment as *S. mucronata* was often observed colonizing in mesocosms without concurrent colonization by aquatic macroinvertebrates and no observed visitation by waterfowl. Colonization by *S. mucronata* was unaffected by the experimental treatments imposed on the mesocosms (i.e., habitat size, nutrient level, or community assembly state). The fact that the probability of *S. mucronata* colonization was unaffected by local habitat conditions stands in contrast to the general results of zooplankton colonization experiments summarized by Havel and Shurin (2004). However, despite the lack of variation in colonization success with local conditions, the success of *S. mucronata* as measured by its abundance in mesocosms was significantly influenced by local mesocosm conditions (P.J.H., unpublished data).

The midsummer ephippia production peak observed in this study was much greater than that observed by Green (1963), where only a small fraction of *S. mucronata* exhibited midseason

gamogenic reproduction. The relatively large fraction of *S. mucronata* ephippia that hatched immediately in my study (average of 4.8% over 72 hr; range 0-78% among populations) suggests that diapause lasting months or years is not the fate of all or even most ephippia. Rather, some ephippia have the potential to contribute immediately to the dynamics of the source population and perhaps more importantly, colonization and population growth in surrounding habitats in the pond metacommuntiy. This ephippia emergence rate result contrasts sharply with those found in *Daphnia galeata* and *D. pulicaria*, where less than 1% were found to emerge each year (Cáceres 1998). Further, it appears that the duration of ephippial diapause in *S. mucronata* is strongly influenced by the local success of source populations.

S. mucronata populations all produced ephippia during the middle of the growing season in a putatively phenological manner and per-capita ephippia production was unrelated to the growth rate of the source population, suggesting that *S. mucronata* are not employing ephippia strategically to track local environmental conditions. Instead of varying ephippia production – which may be fixed – source populations of *S. mucronata* produced ephippia that varied widely in their immediate 72 hr hatching percentage, from as low as 0% to as high as 78%. In contrast to results from ephippia production, ephippia emergence increased dramatically at higher growth rates of source populations. If this result represents a fitness maximizing strategy for *S. mucronata*, it may suggest that a significant proportion of dispersing ephippia make it only a short distance from source populations where the likelihood of success post-colonization may be spatially autocorrelated.

The exceptional low fraction of non-buoyant ephippia observed suggests that *S. mucronata* does not make a significant allocation to the sediment egg bank, at least during the summer production peak under the conditions of the experiment. This result contrasts with those

found in *Daphnia pulicaria* in south-western Michigan lakes where 60-100% of ephippia were non-bouyant (Cáceres et al. 2007). A laboratory experiment using four species of *Daphnia* found rates of negative buoyancy from 100% in *D. magna* to only as low as 80% in *D. longispina* (Ślusarczyk and Pietrzak 2008). In *Daphnia* species there appears to be genetic and environmental variation in the buoyancy of ephippia (Cáceres et al. 2007) where females can deposit ephippia at the water surface to induce positive buoyancy (Ślusarczyk and Pietrzak 2008). In the marine calanoid copepod *Centropages tenuiremis*, eggs that are able to undergo immediate development had a slower sinking rate than those in diapause (Wang et al. 2005). The results presented here of overwhelmingly buoyant ephippia in *S. mucronata* are strongly suggestive of a tendency toward ephippia dispersal rather than dormancy in the egg bank.

Previous trap data suggests that while most aerial dispersing cladocerans are in an active life stage, the relative dispersal ability of ephippia is greater than that of adults (Allen 2007). The density of *S. mucronata* ephippia in the current study occasionally exceeded that of adults in individual mesocosms, suggesting that *S. mucronata* dispersal capacity via ephippia has the potential to be substantial relative to their local population dynamics. There is no indication that this observation represents a competition-colonization tradeoff with other metazoan zooplankton, as *S. mucronata* were frequently at the highest relative abundance in mesocosms, particularly in those without significant numbers of macroinvertebrate predators.

The production of ephippia comes at a cost to local population growth rate as cladoceran ephippia are costlier than parthenogenic eggs and may not hatch immediately. Typically, ephippia are viewed in terms of their contribution to the local egg bank as a form of hedging against seasonal and interannual variation in environmental conditions (Hairston 1996, Brendonck and De Meester 2003): “dispersal in time”. Here, *S. mucronata* populations –

regardless of the favorability of local conditions – are shown to produce substantial quantities of ephippia that are unlikely to contribute to local egg banks and are much more likely to be dispersed than those of better-studied *Daphnia* species. Further, ephippia vary predictably in their immediate hatching rates depending on the success of the *S. mucronata* populations from which they are sourced while remaining similarly buoyant and able to be dispersed. These combined results suggest an integrated strategy of dispersing in both time and space that is informed by source habitat conditions.

APPENDIX

Figure 17. The relative dispersal tendencies of the eight most common cladoceran species in a pond metacommunity. **Top panel:** *Scapholeberis mucronata* individuals were found 8.99 times more often in dispersal traps than in local ponds over a growing season (May-September). The y-axis represents the total number of each cladoceran found dispersing divided by the total number of each cladoceran found in local pond samples.

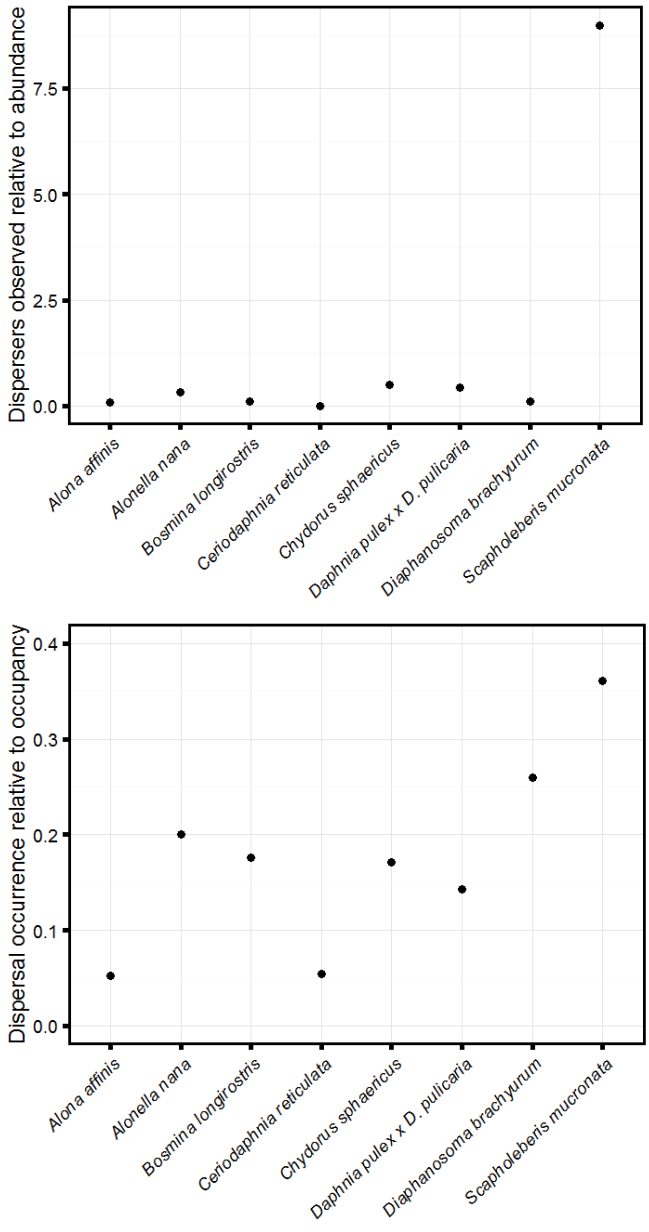


Figure 17 (cont'd). **Lower panel:** The proportion of dispersal traps containing at least one *S. mucronata* individual relative to *S. mucronata* occupancy in the metacommunity was 0.36, greater than any other common cladoceran. The y-axis represents the number of dispersal samples each cladoceran was found in divided by the number of local pond samples where each cladoceran was found.

Figure 18. The production of *S. mucronata* ephippia (ephippia per L) in experimental mesocosms was strongly related to the density of adult *S. mucronata* (individuals per L).

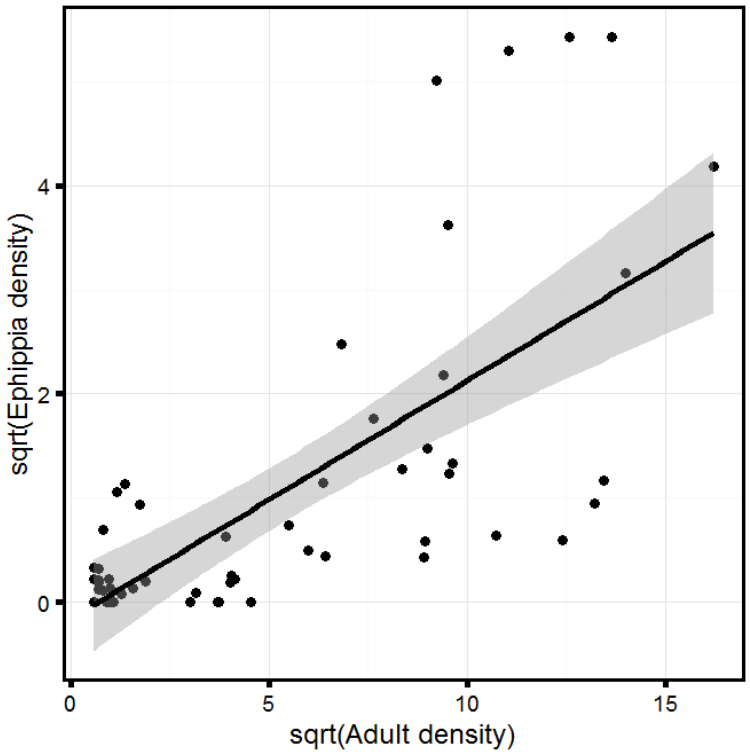


Figure 19. The average population growth rate during the spring-summer ephippial production peak in *Scapholeberis mucronata* was not found to mediate the relationship between adult density and the production of ephippia per capita (**left panel**; GLM: t = -0.150, P = 0.88). However, the 72 hr hatching rate of *S. mucronata* ephippia under common conditions was positively linked to the average daily population growth rate in the mesocosms in which they were produced (**right panel**; binomial GLM: t = 2.727, P = 0.006).

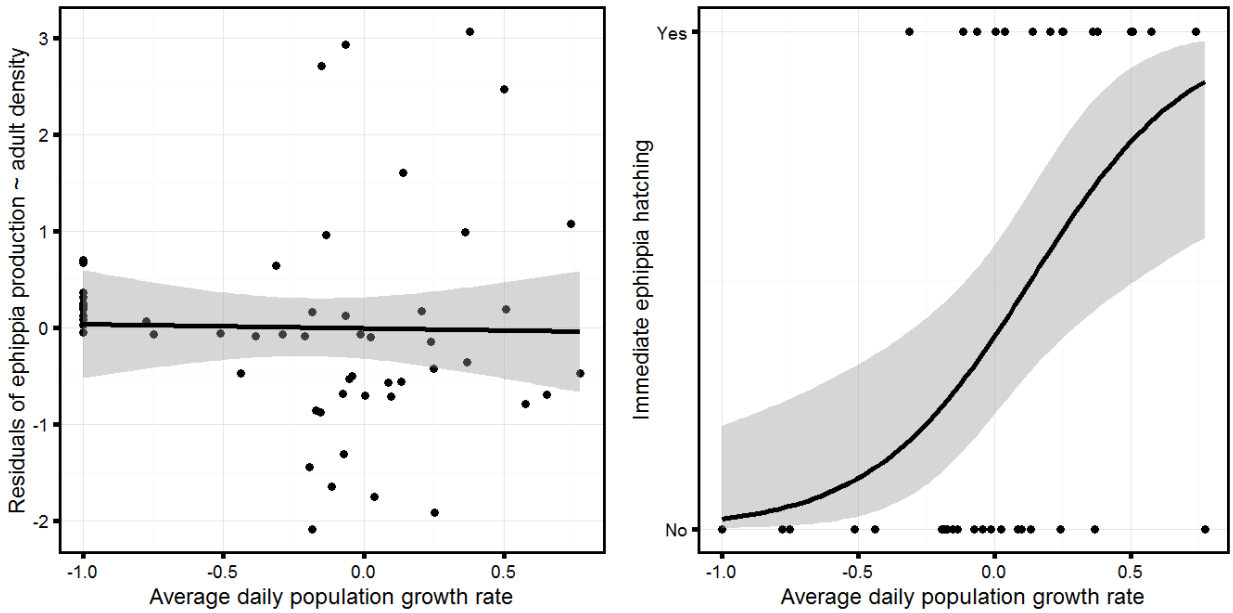
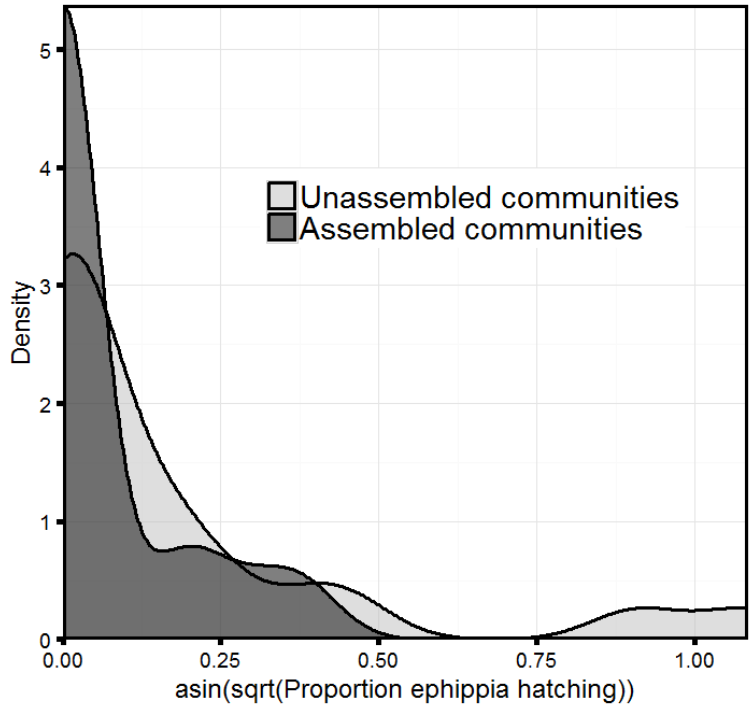


Figure 20. *Scapholeberis mucronata* in experimental mesocosms that were initiated with a starting, assembled community were less likely to produce ephippia that hatched within 72 hr under common conditions (binomial GLM: t = -2.056, P = 0.04).



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CHAPTER 4

SPECIATION AND THE LATITUDINAL DIVERSITY GRADIENT: INSIGHTS FROM THE GLOBAL DISTRIBUTION OF ENDEMIC FISH

Abstract

The nearly universal pattern that species richness increases from the poles to the equator (the latitudinal diversity gradient, LDG), has been of intense interest since its discovery by early natural-history explorers. Among the many hypotheses proposed to explain the LDG, latitudinal variation in 1) productivity, 2) time and area available for diversification, and 3) speciation and/or extinction rates have recently received the most attention. Because tropical regions are older and were formerly more widespread, these factors are often intertwined, hampering efforts to distinguish their relative contributions to the LDG. Here we examine the global distribution of endemic lake fishes to determine how lake age, area, and latitude each affect the probability of speciation and the extent of diversification occurring within a lake. We analyzed the distribution of endemic fishes worldwide (1,933 species and subspecies from 47 families in 2,746 lakes) and find that the probability of a lake containing an endemic species and the total number of endemics per lake increase with lake age and area, and decrease with latitude. Moreover, the geographic location of endemics in 34 of 41 families are found at lower latitudes than that of non-endemics. We propose that the greater diversification of fish at low latitudes may be driven in part by ecological opportunities promoted by tropical climates and by the coevolution of species interactions.

Introduction

The rich diversity of life at tropical latitudes is remarkably consistent across habitats and taxonomic groups (Hillebrand 2004), establishing the latitudinal diversity gradient (LDG) as Earth's dominant biogeographic pattern. Although explanations for the LDG date back to the time of Wallace and Darwin, no consensus on the drivers of elevated tropical diversity has yet emerged. Current hypotheses for the LDG focus primarily on temperate/tropical differences in productivity (energy), historical time and area, and rates of speciation or extinction (Gaston 2000; Mittelbach et al. 2007; Brown 2014; Fine 2015; Pigot et al. 2016), but disentangling these and other potential hypotheses for the LDG is challenging. The Earth has a single, shared history that can limit the ability to make inferences when potential drivers are inseparable (e.g., the greater age and area of the tropics relative to extratropical regions; Mittelbach et al. 2007).

Latitudinal variation in rates of speciation and extinction figure prominently in many hypotheses for the latitudinal diversity gradient (Mittelbach et al. 2007; Brown 2014) and phylogenetic inference is increasingly employed to estimate these rates for different taxa and apply them to studies of the latitudinal diversity gradient (Ricklefs 2007; Morlon 2014). It is perhaps surprising then that current phylogenetic analyses have yielded little consensus on either the magnitude or direction of latitudinal differences in rates of speciation or extinction. For example, analyses of bird phylogenies (probably the best-studied of all taxonomic groups) have differentially found higher speciation rates at low latitudes (Ricklefs 2006), higher recent speciation at high latitudes (Weir and Schluter 2007), and little difference in speciation rates across latitude (Rabosky et al. 2015; Pulido-Santacruz and Weir 2016). There are many challenges to estimating geographical variation in speciation and extinction rates from phylogenetic data (Morlon 2014; Rabosky and Goldberg 2015) and evolutionary biologists

continue to develop new methods to address these challenges (e.g., Rabosky and Huang 2016). In this study, we take a different approach to the question of whether latitude affects speciation by examining the global distribution of endemism in freshwater fish.

Fish represent the bulk of the planet's vertebrate diversity, with nearly 29,000 described species (Froese and Pauly 2016), 41% of which are found in freshwater. Like other vertebrates, freshwater fish diversity is greatest in the tropics (Tisseuil et al. 2013) and the diversity of fish increases with area in lakes (Barbour and Brown 1974) and river basins (Oberdorff et al. 1995). Owing to the restricted dispersal of lake fish, a measure of the generation of new species *in situ* can be obtained by identifying single-lake endemics (defined as species and subspecies confined to a single lake; hereafter simplified to "endemic") which can persist in their natal lakes sometimes for millions of years (as in the case of deep-water sculpins in Lake Baikal (Sherbakov 1999)). The global distribution of endemic fish thus provides a unique record of speciation events, with lakes containing endemic fish found from the equator to latitudes as high as 67.5 °N (Lake El'gygytgyn, Siberia). Moreover, lakes with endemic fish range in age from a few thousand to millions of years old, and provide natural replicates of lake ages and sizes across latitude (Figure 27). Thus, it is possible to estimate the relative importance of time, biogeography, and environment in the evolution of endemic freshwater fish, providing an opportunity to examine longstanding questions about the relationship between latitude and speciation. Our analysis of *in situ* speciation at the scale of individual lakes (and river basins) complements studies of endemism and speciation on islands (reviewed in Warren et al. 2015), as well as recent studies on global gradients in vertebrate diversity conducted at the scale of bioregions (Jetz and Fine 2012; Belmaker and Jetz 2015).

As Tedesco et al. (2012; pages 977-978) note, "…endemic species have always been fascinating because they should reflect the roles of speciation, extinction and dispersal ultimately responsible for their restricted distribution …". The restricted distribution of endemics within particular geographic regions that are often of known history makes it possible to relate both the presence of an endemic species (i.e., evidence of a speciation event) and the number of endemic species (a measure of diversification) to potential drivers of diversity. Recent studies of the distribution and abundance of endemic species in light of factors thought to influence evolutionary rates, including area, age, isolation, and environment have demonstrated the positive effects of area and isolation on speciation leading to endemicity in multiple taxa (e.g., *Anolis* Lizards on Caribbean islands, Losos and Schluter 2000; *Tetragnatha* spiders in the Hawaiian archipelago, Gillespie and Baldwin 2010; multiple taxa on islands worldwide, Kisel and Barraclough 2010; reviewed in Warren et al. 2015; flora on islands and mountains worldwide, Steinbauer et al. 2016; angiosperms on islands worldwide, Weigelt et al. 2016).

In fishes, Tedesco et al. (2012) found the global richness of riverine endemic species was positively related to drainage basin area and climatic stability, Wagner et al. (2014) demonstrated strong effects of lake area and depth on the number of cichlid species arising via *in situ* speciation in African lakes, and Doi et al. (2012) hypothesized that lake age and endemism affect food chain length based on isotopic analysis of fish from young and ancient lakes. Although some of the above studies suggest a greater preponderance of endemic species in the tropics compared to the temperate zone (e.g., see figure 2 in Tedesco et al. 2012), no studies to our knowledge have directly quantified how endemicity varies with latitude, age, and area. Here we develop a data set on the distribution of endemic fish in the world's largest lakes to examine this question.

We compiled data on native fish diversity (endemic and non-endemics), lake age (continuous occupancy), area (and perimeter), latitude, elevation, maximum depth, pH, and productivity (chlorophyll *a*, total phosphorus, Secchi depth) from 1,949 published sources for 2,746 natural lakes with a surface area ≥50 km^2 that are listed in the Global Lakes and Wetlands Database (Lehner and Döll 2004). These data were analyzed to determine how age, area, and latitude together with potential physical, chemical, and biological factors contribute to 1) the probability of at least one endemic occurring in a lake, and 2) the total number of endemic fish in a lake (a measure of net diversification). Although we control for the effect of age in our analysis of endemism in lake fishes, we cannot assess the effects of latitude or area on speciation rate *per se* because the ages of fish species are unknown. Because we cannot estimate how extinctions may have influenced contemporary patterns of endemism, endemic species richness in a lake is best viewed as an estimate of the extent of diversification (speciation minus extinction) and the presence of an endemic species in a lake is evidence of a least one speciation event. To determine whether there is a geographic bias in the occurrence of new species relative to the background distribution of species, we performed a family-level analysis comparing the latitudinal distribution of endemic fish to that of non-endemic fish.

Methods

Database construction

A database of endemic fish distributions was assembled for the 2,746 largest natural lakes in the world. These lakes were selected from the Global Lakes and Wetland Database Level 1 (GLWD-1) (Lehner and Döll 2004), a compilation of water bodies larger than 50 km^2 in area that represents an unbiased sampling of lakes worldwide. After removing duplicates and manmade

reservoirs that were misattributed as natural lakes, our database has 321 fewer lakes than the GLWD-1. Latitude, longitude, surface area, perimeter, and elevation data for these lakes were obtained from GLWD-1 and we added data on lake age, depth, productivity, pH, temperature, as well as native and endemic fish distributions from a review of nearly 2,000 literature sources (data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.70sr1).

We performed a Google Scholar query for "[Lake Name]" (including name variations, when applicable) to identify the peer-reviewed literature for each lake. Articles were read until either all target data were collected or no more information could be obtained. In cases where complete data could not be obtained through peer-reviewed literature, an additional Google Search query for "[Lake Name] filetype:pdf" was used to locate grey literature such as technical reports and government documents. Due to the scarcity of substantial literature published on most lakes, no quality screens were performed. As an additional verification measure to ensure the endemic status of each fish, a Google Scholar query for "[Fish Scientific Name]" was carried out to either corroborate or invalidate single lake endemicity.

We include as endemics those species and subspecies of fish found only in a single lake and its tributaries. We define lake age as the duration that a lake basin has been continuously occupied by water, as estimated from lake sediment cores and from the timing of tectonic activity, glaciation, volcanism, natural damming, and impact events. Non-endemic native fish species names were standardized across lakes using FishBase (Froese and Pauly 2016) to consolidate synonyms since original data sources spanned eight decades and encompassed many taxonomic revisions. Names of endemic fish were taken from the literature except where misapplication of a species name or taxonomic revision could be determined.

Analyses on lakes with age data

Presence/absence and the numbers of endemic fish taxa were evaluated for the 252 lakes with estimates of age and with complete data for all predictors. The distributions of age, area, and latitude for these lakes are given in Figure 28. A generalized linear model using the presence or absence of endemic fish as a binomially distributed response and a logit link function was constructed using the absolute value of lake latitude, hemisphere, log-transformed lake surface area, elevation, and age. A second model was constructed for the log-transformed number of endemic fish species or subspecies in lakes containing at least one endemic fish with the same predictor variables. Lake perimeter was not used in these models as it was strongly correlated with lake area when both were log-transformed and standardized for analysis of the probability that a lake contains an endemic ($r = 0.90$, $P < 0.0001$) and for the restricted data set of lakes that had age data and contained endemics ($r = 0.96$, $P < 0.0001$). To facilitate comparison of effect sizes, standardized z-scores were calculated for each variable except hemisphere prior to analysis by centering and scaling each variable based on its mean and standard deviation.

Multiple missing data imputation on the full lake dataset

To test for the potential effect of predictors for which data were limited (e.g., productivity, native species richness, maximum lake depth) a multiple missing data imputation was performed using chained equations (Buuren and Groothuis-Oudshoorn 2011). Multiple imputation repeatedly generates imputed data sets using all non-missing data (e.g., the presence and number of endemics for all 2,746 lakes in our case) but draws different, plausible values for missing data that reflect the range of uncertainty in those missing data values. Pooled estimates

are calculated on the set of multiple imputation results such that significant effects are only found when they are consistent across imputations.

We pooled results from generalized linear models on 100 imputed datasets, allowing us to obtain parameter estimates using all actual predictor values from the full database of 2,746 lakes. This imputation included lake age, maximum depth, mean pH, maximum surface water temperature, the richness of non-endemic native fish, chlorophyll *a*, total phosphorus, and maximum Secchi depth. All parameters except pH were log-transformed prior to imputation. Native fish species richness was only used for the 288 lakes where sources attempted to catalog all fish species to prevent including artificially low values for lakes where the full fish community (e.g., non-sportfish) is not well-documented. Generalized linear models predicting the probability that a lake contains an endemic and the number of endemics in a lake were evaluated using these additional predictors. We report the fraction of missing information (FMI), the ratio of the difference of information in the complete versus the incomplete data sets to the information contained in the complete data set, as well as the total variance due to missing data (λ) (Buuren and Groothuis-Oudshoorn 2011). Although λ was nontrivial and reached values up to 0.5, our use of 100 imputations (an atypically large number of imputations in practice) is approximately double the number needed to produce 95% confidence in confidence interval half-widths as well as the estimate of λ (Bodner 2008). Although no similar theoretical generalization on the number of imputations needed to achieve confidence in the estimate of *P* values is available, variability in the estimate is unlikely to alter the rejection of a null hypothesis at the 0.05 significance level when $P \leq 0.01$. This condition is satisfied for all our significant findings except for the effect of lake age ($0.01 \leq P \leq 0.05$) for which we have run a separate, explicit

analysis as previously described and, nevertheless, the variability in these estimates is minimized by the large number of imputations used.

Comparison to fish endemism patterns in rivers

The distribution of endemics at the regional, river-basin scale was evaluated using data from the Fish-SPRICH database (Brosse et al. 2013), which contains 4,193 endemic fish that are restricted to single river basins. Generalized linear models predicting the presence and number of endemic fish were performed using latitude, area, range of elevations within a basin, hemisphere, and native fish richness for 928 of 1,054 river basins without missing data. Age information for river basins is not available. Area, elevational range, and native fish richness were log-transformed.

Phylogenetic considerations and potential sampling bias

A species-level phylogeny for freshwater fish at a global scale does not exist. Therefore, we were unable to employ standard phylogenetic controls in our analyses. Instead, we used other means to examine the potential effects of sampling bias and evolutionary non-independence on our results. Speciation rates are often estimated per lineage in phylogenetic analyses to control for the number of lineages contributing to the overall speciation rate (Morlon 2014). We examined whether the effect of latitude on the probability that a lake contained an endemic species was the result of an increase in the number of fish lineages per lake at low latitudes relative to high latitudes. We estimated the number of lineages per lake by taking separate sums of the unique number of families and genera in lakes where complete fish species lists were

available, and tested whether the probability that a lake contains an endemic was influenced by the number of lineages in that lake using generalized linear models for families and genera.

To determine if our model output from the dataset of 252 lakes with age data was driven by the African Great Lakes, which display extreme fish endemism relative to other lakes, we reevaluated these models for both the presence and number of endemics after excluding: 1) the three largest and most speciose African Great Lakes (Victoria, Tanganyika, and Malawi), and 2) up to eight of the largest African Great Lakes.

To determine whether our results were robust to excluding the remarkable endemism exhibited by the family Cichlidae worldwide, we also reevaluated our original models after removing all endemic cichlids from the lake analysis. To further examine whether our results depend on the distribution of endemics within particular families, the latitudinal centers of distribution of both endemics and non-endemic natives within our lakes database were calculated for each of the 47 fish families containing at least one endemic. The mean latitude of endemic fish occurrence was compared to that of non-endemic native fish for the 41 families with data on both endemic and native distributions. The latitudinal center for each species or subspecies was determined by averaging the latitudes of each of the lakes in which it occurs. Families excluded from this analysis were entirely or predominantly comprised of endemics for which no native distribution could be calculated (e.g., the Abyssocottidae of Lake Baikal; Sherbakov 1999).

Finally, as in any geographic comparison of biogeography, our methods require that the division of taxonomic units into species and subspecies is comparable across latitudes such that lineages represent similar subsets of a phylogeny. Moreover, geographic differences in sampling intensity may introduce bias. To evaluate the possible effect of sampling bias, we compiled

records of the date of description of the endemic fish in our database and determined whether new endemic fish were being described at different rates in tropical versus extratropical regions.

Results

A total of 1,895 endemic species and 38 subspecies of fish were found, inhabiting 107 lakes worldwide (examples: Figure 21.; distribution: Figure 22). For the set of 252 lakes with associated age data, the probability that a lake contains an endemic fish and the total number of endemic fish per lake are significantly associated with increased lake age (GLM, $z = 5.225$, $P < 0.0001$), increased area (GLM, $z = 2.808$, $P = 0.005$), and lower latitude (GLM, $z = -4.679$, $P < 0.0001$; Figure 23), and the standardized effect sizes of these variables are similar in magnitude and statistically indistinguishable. Together, these effects are highly predictive of the probability that a lake contains an endemic fish (53.4% of total variance explained; Figure 24). Similarly, lake age (GLM, $z = 4.042$, $P < 0.001$), area (GLM, $z = 6.394$, $P < 0.0001$), and latitude (GLM, $z = -3.880$, $P < 0.001$) are jointly predictive of the number of endemic fish per lake (34.7% of total variance explained, Figure 25), with the probability that a lake contains an endemic increasing with lake age and area and decreasing with latitude. Lakes in the Western Hemisphere had a lower probability of containing an endemic fish (GLM, $z = -3.839$, $P < 0.001$), but for lakes with endemic fish there was no difference in the number of endemic fish between hemispheres (GLM, $z = -1.132$, $P = 0.26$; Figure 23).

Our analysis of total endemic species richness includes endemics that may have evolved via cladogenesis (i.e., one species evolves into two or more new species) or by anagenesis (i.e., one species evolves into a single new species). Cladogenesis is most often linked to diversification, as it increases species richness locally (within a lake in our case), whereas

anagenesis does not (although anagenetic speciation may increase regional species richness through increases in beta diversity). Coyne and Price (2000), in their analysis of potential sympatric speciation events on islands, proposed that a count of the number of genera with two or more endemic species provides a measure of the number of lineages that have diversified *in situ* by cladogenesis. Based on this alternative criterion of speciation, 31 of the 252 lakes with age estimates had evidence of cladogenesis. An analysis of the presence/absence of cladogenesis in these 252 lakes using a generalized linear model demonstrates strong and significant effects of latitude (GLM, estimate = -1.273, $P < 0.001$), area (GLM, estimate = 0.993, $P < 0.001$), and age (GLM, estimate = 1.562, $P < 0.0001$).

Subsequent analyses using a multiple missing data imputation allowed us to examine the effects of native fish species richness, as well as physical, chemical, and productivity variables on endemic fish in all 2,746 lakes. These analyses again revealed strong effects where older lakes, lakes of larger area, and lower latitude lakes had a greater probability of containing an endemic and having a greater number of endemics. However, no detectable effects of species richness, productivity, maximum depth, pH or temperature on endemism were found (Table 12).

Further analysis of an independently-assembled global database of endemic freshwater fish (Fish-SPRICH; Brosse et al. 2013) at the river-basin scale (not individual lakes) corroborates the patterns observed for lake fish. In river basins, endemic fish presence and endemic fish richness decreased significantly with latitude and increased significantly with area, and additional significant, positive effects were observed for both elevational range and native fish richness. The effect of basin age is unknown for this data set: Figure 29 and Table 14.

Much of the global endemicity of lake fish occurs in the African Great Lakes, where it is famously concentrated in a single family, the Cichlidae. Nevertheless, our results were robust to:

1) excluding the largest three or eight African Great Lakes from the data set (Table 20) and 2) excluding the >1,000 endemic fish in the family Cichlidae, whose mean latitudinal distribution is only 8.1° from the equator (Table 21). Moreover, an analysis comparing the geographic centers of distribution of non-endemic fish species to the mean latitudes of endemic fish shows that for 34 of 41 families, endemics are found at comparatively lower latitudes than non-endemics (exact binomial test, $P < 0.0001$; Figure 26; Table 22). The two families with the greatest number of endemic species, the Cichlidae (n = 745 named species) and Cyprinidae (n = 139), both display this low latitude bias in the distribution of endemics (Table 22).

Tropical lakes have more lineages (as measured by the total number of families or genera) than temperate lakes, but there is no evidence that the number of lineages affects whether a lake contains an endemic (Figure 30). Thus, the greater probably of a speciation event occurring in low-latitude lakes is not a function of more fish lineages in the tropics. Further, a comparison of temperate and tropical differences in the rate at which endemic species are being described suggests that the greater endemic species richness in tropical regions may actually underestimate latitudinal differences in diversification, since the description of new endemic fish is increasing much faster at tropical than extratropical latitudes (Figure 31).

Discussion

Lakes, like islands, provide model systems for studying evolution (Warren et al. 2015). Our analyses, along with many others (see introduction), demonstrate a positive effect of age and area on the probability of *in situ* speciation and the extent of diversification in island-like systems. Here, we provide addition evidence for a strong relationship between latitude and diversification that is independent of age and area effects. Our results show that the probability

of a speciation event and the extent of diversification increase with decreasing latitude, as judged by the distribution of endemicity in both lake and river fishes. Previous work on island endemics has used a similar approach to study how *in situ* speciation is affected by island size, age, and isolation (see Warren et al. 2015 for a recent review), to examine the incidence of sympatric speciation (Coyne and Price 2000), and the spatial scale of speciation on islands (Kisel and Barraclough 2010). However, to our knowledge, no island studies have directly examined how the probability of *in situ* speciation varies with latitude. Recently, Jetz and Fine (2012) and Belmaker and Jetz (2015) examined the influence of historical time, area, and present-day climate on global gradients in terrestrial vertebrate diversity by dividing the Earth into 32 evolutionarily distinct "bioregions". Like our lake analyses, they found strong, positive effects of bioregion age and area on endemic species richness. Unlike our study, Belmaker and Jetz (2015) conclude that diversification rates (estimated phylogenetically) appear to have a relatively minor influence on broad-scale patterns in species richness.

The observation that area is strongly predictive of endemism in lake fishes is consistent with findings in African cichlids (Wagner et al. 2014), *Anolis* lizards (Losos and Schluter 2000), poeciliid fish (Furness et al. 2016) and other taxa (e.g., Kisel and Barraclough 2010) suggesting that there is a minimum area for *in situ* speciation. We searched the literature for endemism in small lakes (surface areas < 50 km^2, below the minimum size used in our analysis) and found endemics in 74 of these water bodies, including those as small as 0.0028 km^2 (*Tilapia guinasana* in Lake Guinas, Namibia; Nxomani et al. 1999). Thus, *in situ* speciation can occur in small lakes, but despite the abundance of such lakes worldwide (>243,000 lakes between 0.1 and 50 km^2 in the Global Lakes and Wetlands Database; Lehner and Döll 2004), our finding of only 74 lakes (<50 km^2) with endemic fish suggests that these lakes have limited *in situ* speciation. Area may

increase the rate of speciation through larger population sizes, greater habitat heterogeneity (Kisel et al. 2011) and increased environmental stability through time. There are only a few old, deep, very large lakes worldwide and these also contain the extremes in the number of potential cladogenesis events in multiple genera (using Coyne and Price's (2000) criterion: Lake Malawi 38, Lake Tanganyika 31, Caspian Sea 10, Lake Baikal 9).

Molecular evidence suggests that the pace of divergence in extratropical lakes has been slow, even in those containing relatively large adaptive radiations such as the Caspian Sea and Lake Baikal. For example, the endemic Proto-Caspian gobies of the subfamily Benthophilinae are estimated to have originated 10 Mya from a common ancestor that diverged into multiple genera 4.29-6.25 Mya, with the most recent identifiable radiations occurring 1-2 Mya (Neilson and Stepien 2009). In Lake Baikal, the age of the root of its 33 species cottoid fish radiation is uncertain but is estimated as 1.2-6.5 Mya (Kontula et al. 2003). These deep-rooted divergence events contrast with the rapid divergence of hundreds of species of cichlids in the comparably young Lake Victoria, whose species diverged 15,000-100,000 years ago (Brawand et al. 2014). Another prime example of rapid ecological diversification in a tropical lake is the divergence of *Labeobarbus* fish in Lake Tana, Ethiopia. This lake has been continuously isolated from the rest of the Nile basin and dried completely within the last 25,000 years (De Graaf et al. 2007). It is hypothesized that a single riverine ancestor colonized Lake Tana and generated a 15 species endemic flock in 10,000-25,000 years (De Graaf et al. 2010), with clear ecological divergence among species, including piscivory, a rare trait in the family Cyprinidae (De Graaf et al. 2008).

Latitudinal Drivers of Speciation Beyond Age and Area

Our analysis of endemism in freshwater fish provides fresh insight into how age, area, and latitude are correlated with the probability of speciation and the extent of diversification.

Latitude, of course, does not directly affect speciation and diversification, but instead is correlated with mechanisms that may drive these processes. Several hypotheses have linked the latitudinal diversity gradient to faster diversification at lower latitudes (Mittelbach et al. 2007; Brown 2014; Fine 2015). For example, the evolutionary speed hypothesis (Rohde 1992; Allen and Gillooly 2006) postulates that molecular evolution (nucleotide substitution) is faster at higher temperatures, resulting in higher speciation rates at low latitudes. The biotic interactions hypothesis (Dobzhansky 1950; Schemske 2009; Schemske et al. 2009) proposes that the relatively benign and stable climate of the tropics leads to adaptation governed more by biotic than abiotic factors, resulting in faster speciation because of ongoing coevolution. Stable tropical climates may also result in lower extinction rates in the tropics, contributing to higher rates of diversification (e.g., Pyron 2014; Pulido-Santacruz and Weir 2016).

It has recently been suggested that speciation rates for some taxa are actually higher in temperate regions than in the tropics, because the relatively species-poor temperate zone provides greater opportunities for ecological divergence (e.g., more open niches) and therefore more rapid speciation (reviewed in Schluter 2016). There are well-documented cases of rapid diversification in temperate fishes, including species pairs within multiple families in postglacial lakes in the Northern Hemisphere (Taylor 1999, Schluter 2016), but few of these ecomorphs are formally recognized as species. The case can be made that some temperate-lake ecomorphs deserve species status (e.g., Knudsen et al. 2006; Harrod et al. 2010), but incipient speciation is suspected in many tropical lakes as well (e.g., Nxomani et al. 1999; Wilson et al. 2000; Herder et al. 2008). Our data in fact suggest that the description of tropical fish diversity likely lags that of the temperate zone (Figure 31).

Although species-poor environments can provide opportunities for ecological divergence and speciation, the flip-side of the coin is that over time species may become niches for other species; “Every species is potentially a resource on which some other species can in principle specialize or to which another species must adapt” (Vermeij 2005). Strong biotic interactions, coupled with relatively benign abiotic conditions, can create ecological opportunities that allow tropical organisms to explore a wider range of niche dimensions than their temperate counterparts, promoting greater species diversity. For example, fish that mainly consume plants or fruits are common in the tropics but rare elsewhere (Horn et al. 2011; Correa et al. 2015). Tropical fishes also display a bewildering array of adaptations rarely seen in temperate fishes, including scale-eating, eyeball-eating, parasite cleaning, and electrical communication (and predation). Some of these unusual traits have evolved independently in multiple families. For example, frugivory occurs in 17 Neotropical fish families (Correa et al. 2015) and scale-eating has evolved at least 19 times in tropical lineages (Martin and Wainwright 2013). In addition, adaptive evolution of visual receptors in African cichlids that occupy different light environments (Wagner et al. 2012) and electroreceptors for communication in African catfish (mormyrids) (Carlson et al. 2011) are thought to promote the extensive diversification of these groups.

We suggest that the more extensive diversification of fish (and other taxa) at low latitudes may be driven in part by ecological opportunities promoted by tropical climates and by the coevolution of strong species interactions. Endemism in lakes is not unique to fish and has been observed across a wide array of taxa including both benthic and planktonic crustaceans (Marijnissen et al. 2006; Boxshall and Defaye 2008; Väinölä et al. 2008; Von Rintelen et al. 2010; Lorenschat et al. 2014), molluscs (West and Michel 2000; Von Rintelen and Glaubrecht

2005; Albrecht et al. 2006; Von Rintelen and Glaubrecht 2006), and sponges (Meixner et al. 2007). Although freshwater fish follow a classic LDG, it has been suggested that the overall strength of the LDG is weaker in freshwater systems when compared with marine and terrestrial realms (Hillebrand 2004) and some freshwater groups appear to show an inverse latitudinal diversity gradient (Heino 2001). Comparisons of our findings in fish to other taxonomic groups may improve understanding of why diversification varies with latitude.

In conclusion, a global analysis controlling for the effects of lake age and area reveals a strong and independent effect of latitude on the probably of *in situ* speciation and on the extent of diversification in freshwater endemic fish. Mechanisms underlying the positive effects of age and area on diversification in island-like systems are well known (Warren et al. 2015), however, understanding why diversification may be enhanced at low-latitudes remains a challenge. We suggest that greater diversification in the tropics may be due to biological mechanisms that differ in kind and/or magnitude from those in temperate regions. The LDG may very well reflect a persistent difference in the selective forces across what is not merely a geographic arc but the principal climatic gradient on Earth.

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APPENDIX

Figure 21. Examples of endemic fish species from 11 different fish families: (*A*) Adrianichthyidae (*Oryzias nigrimas*), Lake Poso, Indonesia; (*B*) Characidae (*Moenkhausia pittieri*), Lake Valencia, Venezuela; (*C*) Cichlidae (*Haplochromis nyererei*), Lake Victoria; (*D*) Clariidae (*Bathyclarias foveolatus*), Lake Malawi; (*E*) Cyprinidae (*Carassius cuvieri*), Lake Biwa, Japan; (*F*) Gobiidae (*Benthophilus casachicus*), Caspian Sea; (*G*) Mastacembelidae (*Mastacembelus ellipsifer*), Lake Tanganyika; (*H*) Melanotaeniidae (*Melanotaenia lacustris*), Lake Kutubu, Papua New Guinea; (*I*) Mochokidae (*Synodontis grandiops*), Lake Tanganyika; (*J*) Poeciliidae (*Lamprichthys tanganicanus*), Lake Tanganyika; (*K*) Salmonidae (*Salmo letnica*), Lake Ohrid, Macedonia.

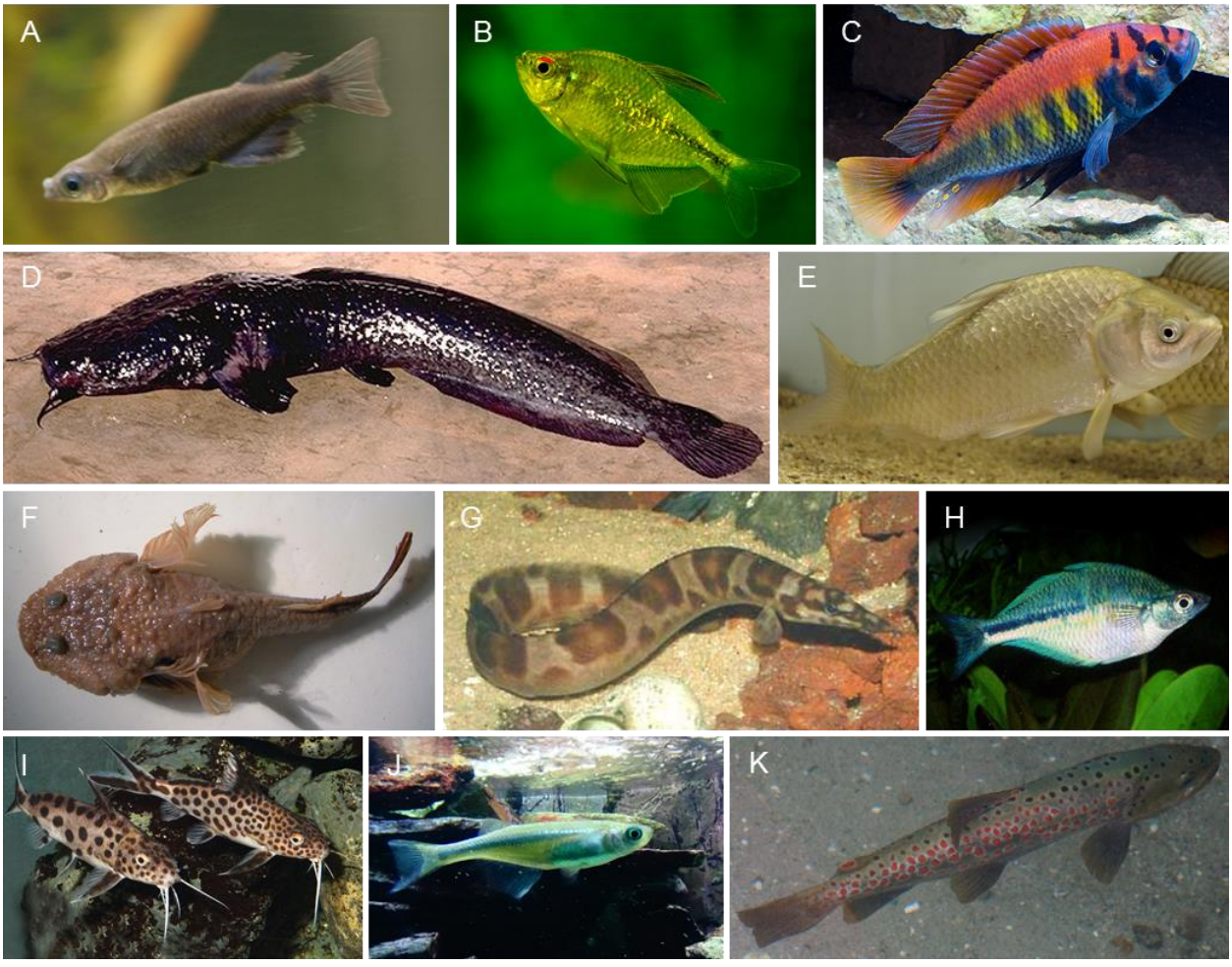

Figure 22. The distribution and richness of endemic fish species and subspecies (N = 1,933) in the 2,746 largest natural lakes in the world (surface area ≥ 50 km^2).

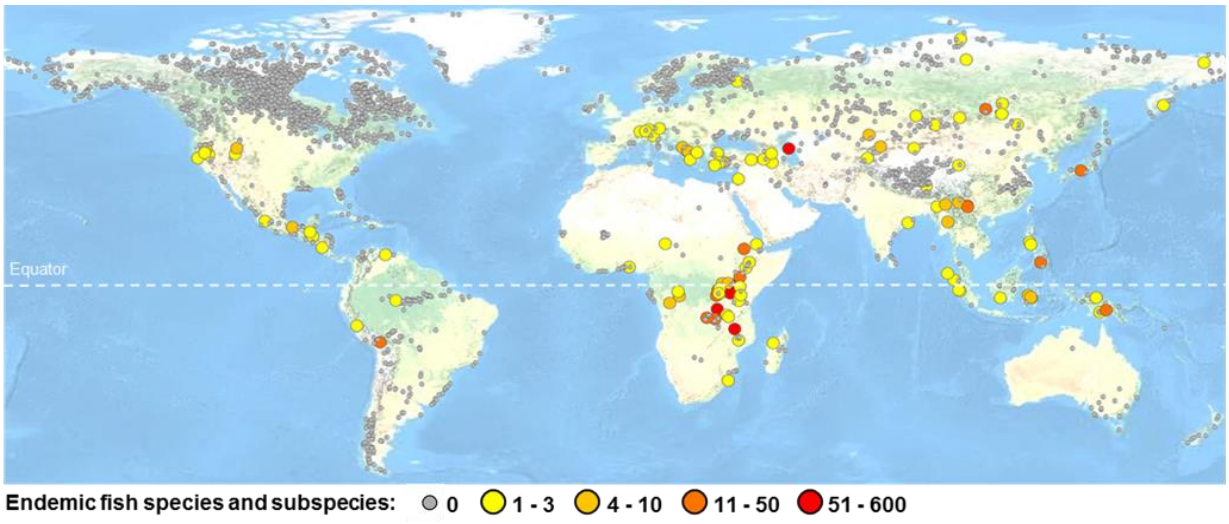

Figure 23. Relationship between biogeographic variables and the probability that an endemic species occurs in a lake (*A*) and the number of endemic fish species in a lake (*B*). Boxes represent the direction and magnitude of the standardized effect size of each variable with associated normal-based 95% confidence intervals. Asterisks (*) denote significant predictor variables: *$P < 0.01$, **$P < 0.001$, ***$P < 0.0001$.

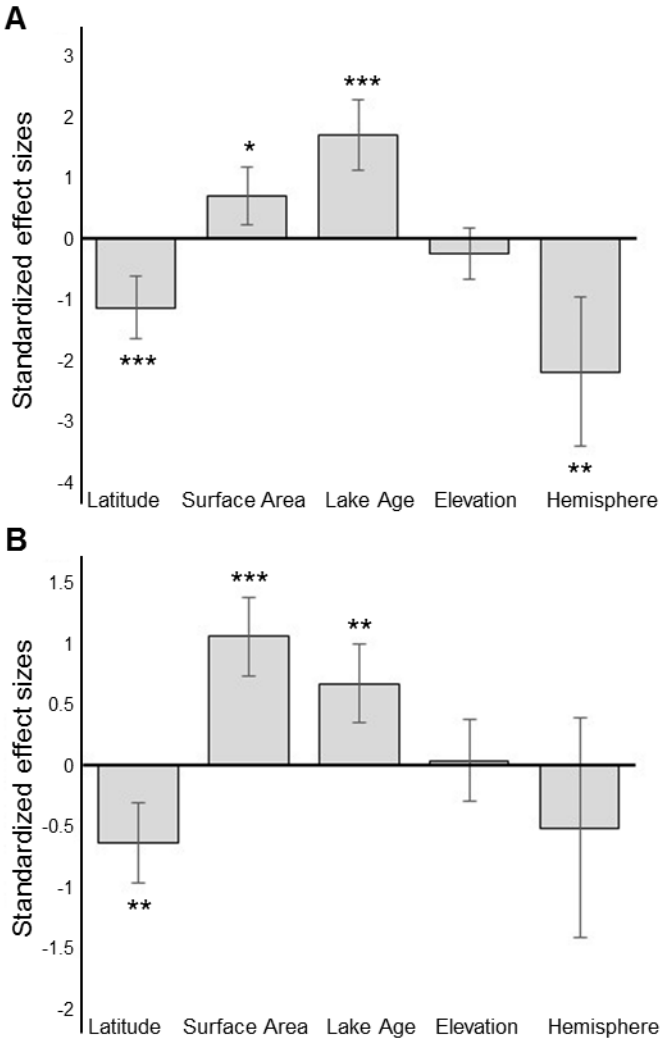

Figure 24. Probability that a lake (N = 252) contains one or more endemic fish species from a logistic regression that includes latitude, age, and surface area for lakes in the New World (*A*, 50 km^2; *B*, 500 km^2; and *C*, 5,000 km^2) and Old World (*D*, 50 km^2; *E*, 500 km^2; and *F*, 5,000 km^2). Shaded regions represent the 95% confidence bands around the best model fit (solid lines).

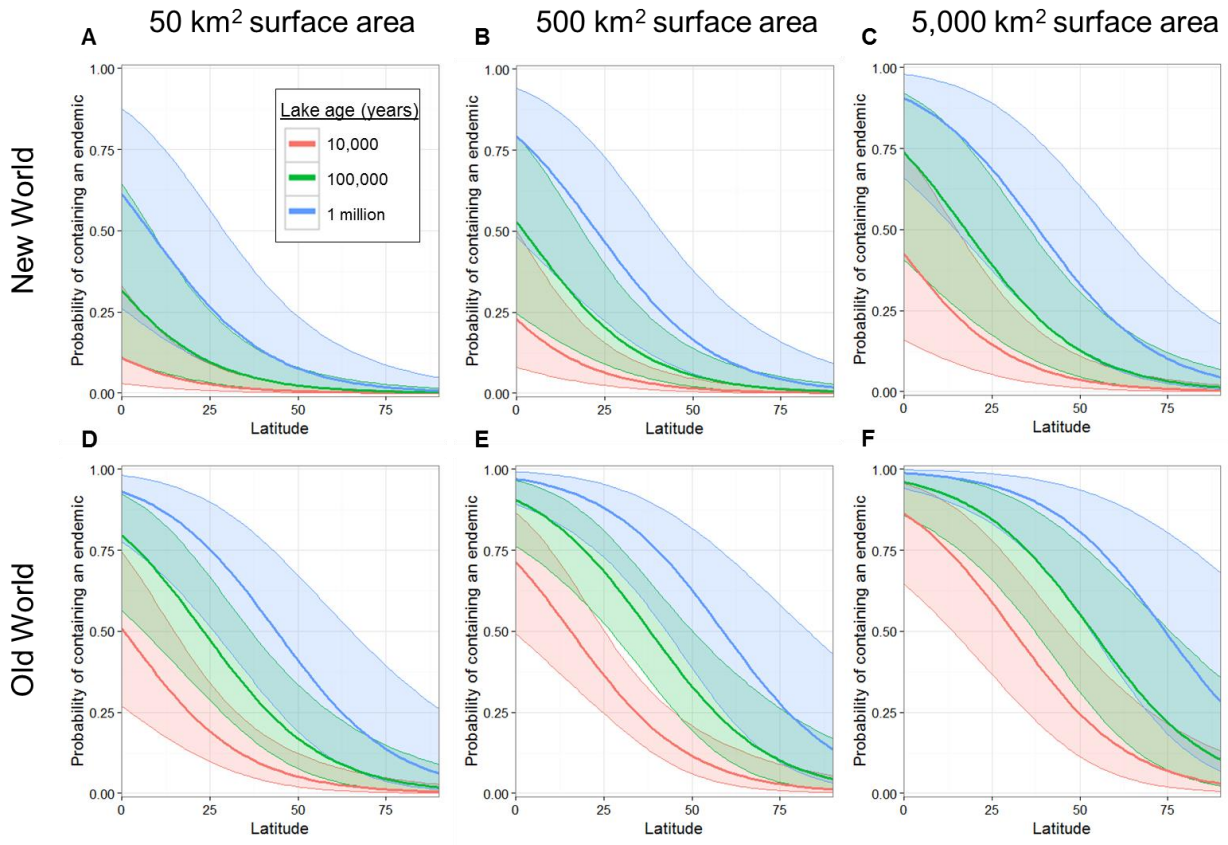

Figure 25. Heat map of the estimated number of endemic fish per lake (color scale) in tropical latitudes (*A*, 23.43° S – 23.43° N) and extratropical latitudes (*B*, > 23.43° S or N) as a function of lake age (x-axis) and surface area (y-axis) for lakes whose age is known and that contain at least one endemic fish.

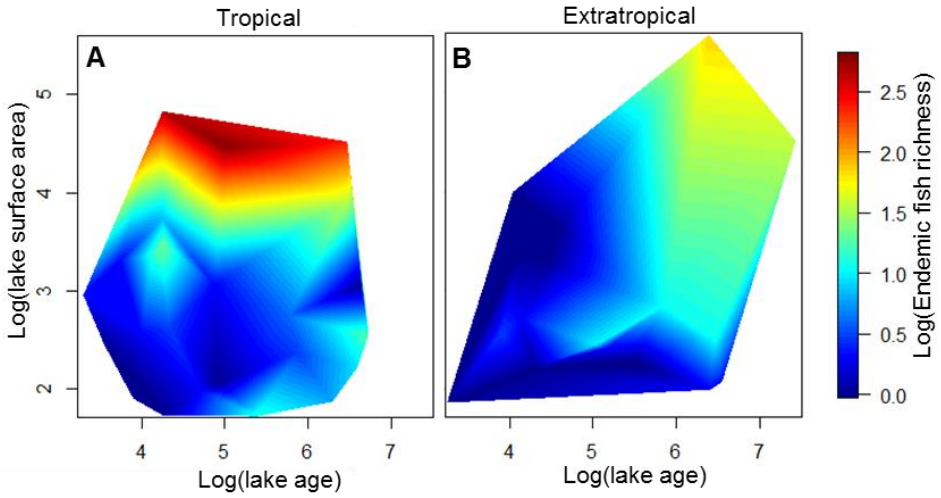

Figure 26. The degree latitude difference in the mean distribution of endemic fish (y-axis) from the latitudinal centers of distribution of the non-endemic species (x-axis) for each of 41 families. Across fish families, endemics are distributedfigur at lower latitudes than non-endemics (exact binomial test, $P < 0.0001$; below dotted line).

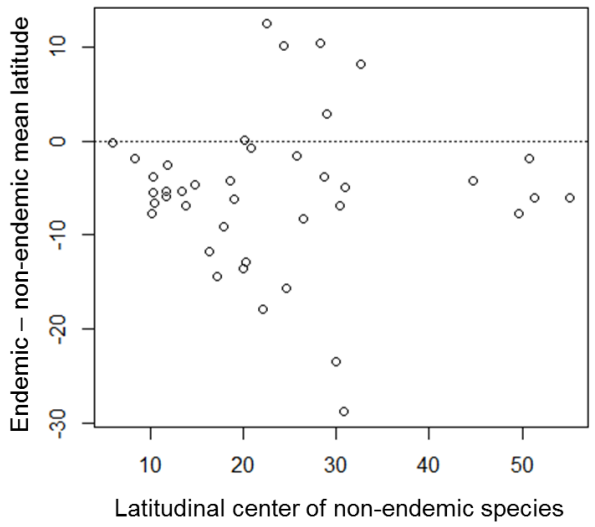

Figure 27. Scatterplots of lake latitude versus lake age (*A*) and lake latitude versus lake surface area (*B*) for lakes with available age data (N = 252), which are used to generate Figures 22-24.

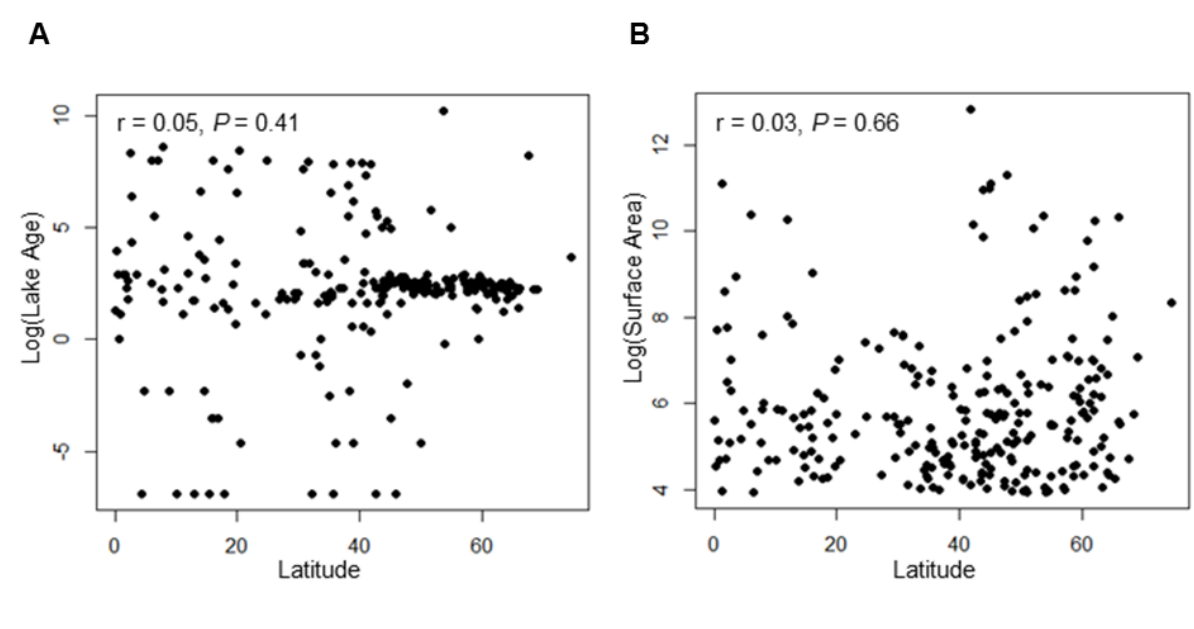

Figure 28. Histograms of the frequency of lakes by surface area (km^2; x-axis) and age (years; color) used in the logistic regression underlying Figure 22 separated into lakes with endemics at tropical (*A*, 23.43° S – 23.43° N) and extratropical latitudes (*B*, > 23.43° S or N) and lakes without endemics at tropical (*C*) and extratropical latitudes (*D*).

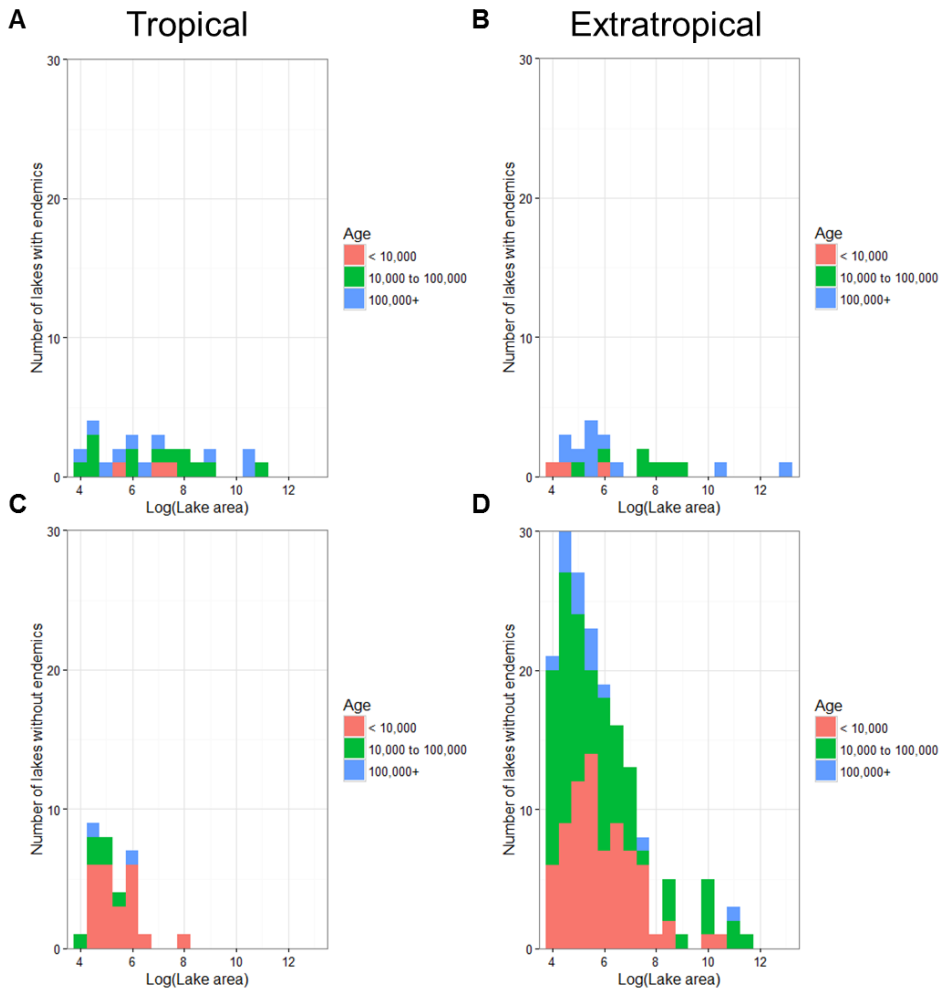

Figure 29. Probability that a river drainage basin contains at least one endemic fish species (i.e., a species unique to that drainage basin) across latitude. Data are from the Fish-SPRICH database. Old and New World modeled separately and plotted with 95% confidence bands around the model fits.

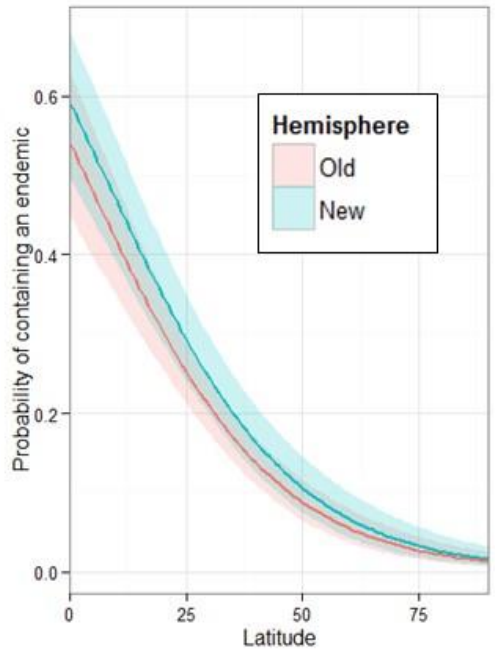

Figure 30. Scatterplot of lake latitude and the number of lineages (*A*, families and *B*, genera) contained in a single lake. Lakes are categorized by the presence (red) or absence (white) of at least one endemic fish. A generalized linear model predicting the presence of at least one endemic species or subspecies from the number of lineages in lake was insignificant using either families ($P = 0.942$) or genera ($P = 0.239$).

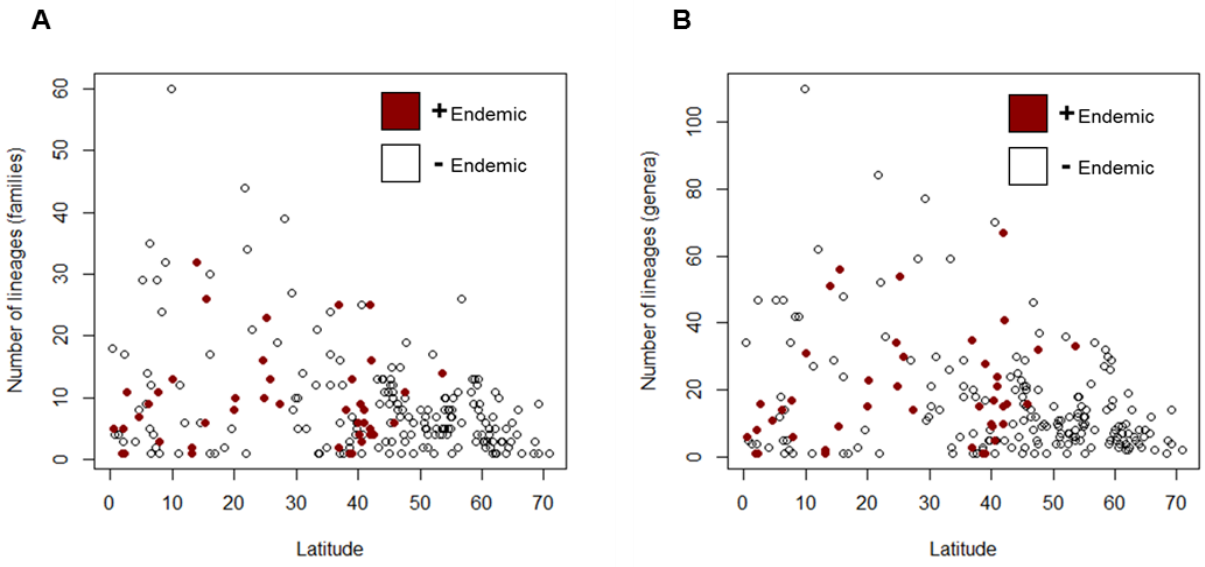

Figure 31. The rate of accumulation of newly described endemic fish species and subspecies over time from tropical (red, 23.43° S – 23.43° N) and extratropical (blue, > 23.43° S or N) lakes.

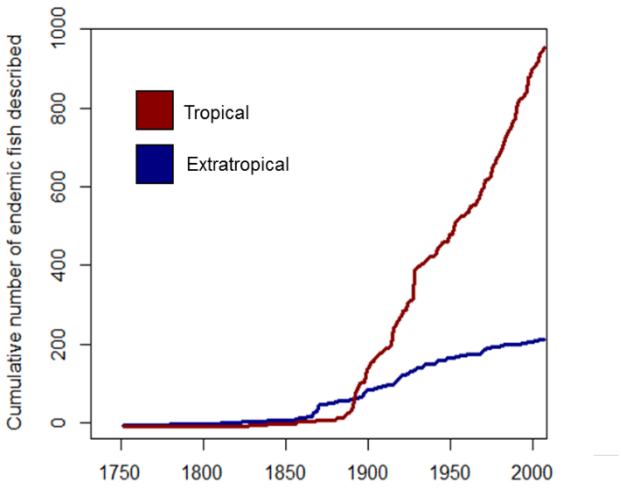

Table 12. Generalized linear model of the probability that a lake contains an endemic based on a missing data imputation of predictor variables for all 2,746 lakes.

Parameter	Standardized Estimate	Std. Error	t value	df	*P* value	FMI	λ
Intercept	-1.6929	0.4843	-3.4959	2152.9	0.0005	0.0817	0.0809
Latitude	-1.0464	0.2030	-5.1540	632.5	< 0.0001	0.3233	0.3211
Age	0.4113	0.2054	2.0023	353.0	0.0460	0.4647	0.4617
Surface Area	0.7328	0.0957	7.6578	1792.1	< 0.0001	0.1198	0.1188
Elevation	0.0901	0.1263	0.7140	921.3	0.4754	0.2456	0.2440
Hemisphere	-2.0031	0.3508	-5.7098	2205.4	< 0.0001	0.0762	0.0754
Maximum Depth	0.3795	0.2746	1.3822	503.6	0.1675	0.3750	0.3726
Mean pH	-0.0336	0.2265	-0.1483	431.4	0.8822	0.4127	0.4099
Surface Temperature	0.1220	0.3444	0.3542	368.0	0.7234	0.4536	0.4506
Native Richness	0.0642	0.2199	0.2919	417.4	0.7705	0.4209	0.4182
Chlorophyll *a*	0.0354	0.2347	0.1509	395.1	0.8801	0.4350	0.4321
Total P	0.1243	0.2468	0.5035	288.4	0.6150	0.5211	0.5178
Secchi Depth	0.1010	0.3345	0.3018	352.3	0.7630	0.4652	0.4622

Table 13. Generalized linear model of the number of endemics in a lake based on a missing data imputation of predictor variables for all 2,746 lakes.

Parameter	Standardized Estimate	Std. Error	t value	df	*P* value	FMI	λ
Intercept	0.1876	0.0259	7.2304	1668.8	< 0.0001	0.1335	0.1324
Latitude	-0.0834	0.0118	-7.0404	512.9	< 0.0001	0.3707	0.3683
Age	0.0265	0.0110	2.3979	365.0	0.0170	0.4557	0.4528
Surface Area	0.1138	0.0072	15.9034	2046.7	< 0.0001	0.0928	0.0919
Elevation	0.0110	0.0090	1.2299	1031.3	0.2190	0.2237	0.2222
Hemisphere	-0.0802	0.0155	-5.1582	1530.6	< 0.0001	0.1498	0.1487
Maximum Depth	0.0227	0.0144	1.5808	604.1	0.1144	0.3334	0.3312
Mean pH	-0.0023	0.0133	-0.1741	338.2	0.8619	0.4763	0.4732
Surface Temperature	0.0001	0.0165	0.0035	303.1	0.9972	0.5068	0.5035
Native Richness	0.0031	0.0125	0.2505	351.5	0.8023	0.4659	0.4628
Chlorophyll *a*	0.0054	0.0132	0.4119	384.8	0.6806	0.4418	0.4389
Total P	-0.0020	0.0136	-0.1483	344.1	0.8822	0.4716	0.4685
Secchi Depth	0.0137	0.0171	0.8007	471.6	0.4237	0.3907	0.3882

Table 14**.** Generalized linear model of the probability that a river basin contains an endemic fish. Data from the Fish-SPRICH database.

Parameter	Standardized Estimate	Std. Error	t value	*P* value
Intercept	-2.2042	0.1660	-13.2800	< 0.0001
Latitude	-0.9142	0.1212	-7.5410	< 0.0001
Area	0.9634	0.1558	6.1830	< 0.0001
Elevation Range	0.5096	0.1204	4.2310	< 0.0001
Hemisphere	0.1109	0.2134	0.5200	0.6030
Native Richness	0.9682	0.1558	6.2150	< 0.0001

Table 15. Generalized linear model predicting the number of endemic fish in a river basin. Data from the Fish-SPRICH database.

Parameter	Standardized Estimate	Std. Error	t value	*P* value
Intercept	0.3069	0.0267	11.4920	< 0.0001
Latitude	-0.1669	0.0226	-7.3950	< 0.0001
Area	0.1659	0.0277	5.9870	< 0.0001
Elevation Range	0.1543	0.0238	6.4790	< 0.0001
Hemisphere	0.0698	0.0444	1.5740	0.1160
Native Richness	0.3073	0.0264	11.6530	< 0.0001

Table 16. Generalized linear model of the probability of a lake containing an endemic fish with the three largest African Great Lakes removed

Parameter	Standardized Estimate	Std. Error	t value	*P* value
Intercept	-1.6325	0.2999	-5.4430	< 0.0001
Latitude	-1.0881	0.2352	-4.6270	< 0.0001
Age	1.6241	0.3119	5.2070	< 0.0001
Surface Area	0.6161	0.2319	2.6570	0.0079
Elevation	-0.2478	0.1900	-1.3050	0.1921
Hemisphere	-2.1155	0.5557	-3.8070	0.0001

Table 17. Generalized linear model of the number of endemic fish in lakes with endemics with the three largest African Great Lakes removed

Parameter	Standardized Estimate	Std. Error	t value	*P* value
Intercept	1.2922	0.1409	9.1730	< 0.0001
Latitude	-0.4631	0.1384	-3.3460	0.0019
Age	0.6492	0.1341	4.8400	< 0.0001
Surface Area	0.6017	0.1341	4.4880	0.0001
Elevation	0.0618	0.1371	0.4510	0.6548
Hemisphere	-0.4343	0.3630	-1.1960	0.2390

Table 18. Generalized linear model of the probability of a lake containing an endemic fish with the eight largest African Great Lakes removed

Parameter	Standardized Estimate	Std. Error	t value	*P* value
Intercept	-1.7302	0.3045	-5.6820	< 0.0001
Latitude	-0.9479	0.2367	-4.0040	< 0.0001
Age	1.6420	0.3126	5.2520	< 0.0001
Surface Area	0.5065	0.2388	2.1210	0.0339
Elevation	-0.2585	0.1916	-1.3490	0.1774
Hemisphere	-1.9885	0.5527	-3.5980	0.0003

Table 19. Generalized linear model of the number of endemic fish in lakes with endemics with the eight largest African Great Lakes removed

Parameter	Standardized Estimate	Std. Error	t value	*P* value
Intercept	1.1754	0.1539	7.6390	< 0.0001
Latitude	-0.3775	0.1545	-2.4430	0.0201
Age	0.6909	0.1443	4.7890	< 0.0001
Surface Area	0.5361	0.1526	3.5140	0.0013
Elevation	0.0574	0.1475	0.3890	0.6998
Hemisphere	-0.3796	0.3715	-1.0220	0.3143

Table 20. Generalized linear model of the probability of a lake containing an endemic fish with the family Cichlidae removed

Parameter	Standardized Estimate	Std. Error	t value	*P* value
Intercept	-1.8793	0.3163	-5.9420	< 0.0001
Latitude	-0.7173	0.2279	-3.1470	0.0017
Age	1.6599	0.3204	5.1800	< 0.0001
Surface Area	0.6772	0.2261	2.9960	0.0027
Elevation	0.0041	0.2050	0.0200	0.9842
Hemisphere	-1.7828	0.5508	-3.2370	0.0012

Table 21. Generalized linear model of the number of endemic fish in lakes with endemics with the family Cichlidae removed

Parameter	Standardized Estimate	Std. Error	t value	*P* value
Intercept	1.3210	0.1421	9.2980	< 0.0001
Latitude	-0.3233	0.1373	-2.3540	0.0243
Age	0.6905	0.1312	5.2630	< 0.0001
Surface Area	0.5592	0.1324	4.2220	0.0002
Elevation	-0.0517	0.1386	-0.3730	0.7117
Hemisphere	-0.3919	0.3543	-1.1060	0.2763

Table 22. Distributional centers of the non-endemic native species and endemic fish for the 47 families containing at least one endemic fish species or subspecies in the 2,746 largest lakes in the world.

Family	N Endemics	Native mean latitude	Endemic mean latitude	SD endemic latitude	SE endemic latitude
Abyssocottidae†	20	NA	53.63	0.0000	0.0000
Acipenseridae	1	43.05	53.63	NA	NA
Adrianichthyidae	7	30.75	2.00	0.2192	0.0829
Alestidae	4	10.31	4.80	1.4665	0.7332
Ambassidae	1	17.13	2.69	NA	NA
Amblycipitidae	1	28.70	24.85	NA	NA
Amphiliidae	5	5.91	5.77	3.4614	1.5480
Anabantidae	1	17.78	8.65	NA	NA
Apogonidae	1	13.78	6.95	NA	NA
Atherinidae	2	25.70	24.12	25.0740	17.7300
Bagridae	3	24.55	8.92	13.7971	7.9658
Blenniidae	1	28.19	38.56	NA	NA
Catostomidae	4	44.77	40.59	0.8919	0.4460
Channidae	1	20.75	19.99	NA	NA
Characidae	2	18.53	14.29	5.8124	4.1100
Cichlidae	745	13.38	8.02	4.5260	0.1658
Clariidae	14	11.83	9.31	3.5347	0.9447
Claroteidae	11	11.70	5.75	1.7149	0.5170
Clupeidae	29	24.37	34.52	14.7794	2.7445
Cobitidae	5	32.60	40.84	1.7625	0.7882
Comephoridae†	2	NA	53.63	0.0000	0.0000
Cottidae	3	51.37	45.28	7.2877	4.2076
Cottocomephoridae†	9	NA	53.47	0.4733	0.1578
Cyprinidae	139	30.37	23.56	14.6478	1.2424
Cyprinodontidae	24	26.41	18.16	7.7887	1.5899
Eleotridae	3	11.67	6.39	0.0000	0.0000
Erethistidae†	1	NA	25.15	NA	NA
Gasterosteidae	1	50.76	48.97	NA	NA
Gobiidae	45	22.44	34.96	13.6885	2.0406
Goodeidae	1	20.04	20.21	NA	NA
Latidae	2	10.45	3.87	3.1113	2.2000
Loricariidae	1	14.77	10.18	NA	NA
Mastacembelidae	16	20.21	7.31	3.6872	0.9218
Melanotaeniidae	2	16.26	4.51	2.6658	1.8850
Mochokidae	19	10.21	6.45	1.8098	0.4152
Mormyridae	3	8.29	6.44	4.4514	2.5700
Nemacheilidae	7	30.95	26.06	17.1837	6.4948
Nothobranchiidae†	2	NA	11.23	3.1396	2.2200
Petromyzontidae	1	49.60	41.85	NA	NA
Plotosidae	1	29.90	6.39	NA	NA
Poeciliidae	9	18.90	12.72	7.6463	2.5488
Salmonidae	37	55.16	49.14	9.2755	1.5249
Siluridae	3	28.92	31.78	6.0044	3.4667
Telmatherinidae†	7	NA	2.54	0.1096	0.0414
Terapontidae	1	19.90	6.39	NA	NA
Tetraodontidae	1	22.10	4.22	NA	NA
Zenarchopteridae	3	10.05	2.40	0.4430	0.2558

† Family not used for comparison due to insufficient data or true absence of non-endemic native distribution.

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