Habitat size modulates the influence of heterogeneity on species richness patterns in a model zooplankton community

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Abstract. Habitat heterogeneity is a primary mechanism influencing species richness. Despite the general expectation that increased heterogeneity should increase species richness, there is considerable variation in the observed relationship, including many studies that show negative effects of heterogeneity on species richness. One mechanism that can create such disparate results is the predicted trade-off between habitat area and heterogeneity, sometimes called the area-heterogeneity-trade-off (AHTO) hypothesis. The AHTO hypothesis predicts positive effects of heterogeneity on species richness in large habitats, but negative effects in small habitats. We examined the interplay between habitat size and habitat heterogeneity in experimental mesocosms that mimic freshwater ponds, and measured responses in a species-rich zooplankton community. We used the AHTO hypothesis and related mechanisms to make predictions about how heterogeneity would affect species richness and diversity in large compared to small habitats. We found that heterogeneity had a positive influence on species richness in large, but not small habitats, and that this likely resulted because habitat specialists were able to persist only when habitat size was sufficiently large, consistent with the predictions of the AHTO hypothesis. Our results emphasize the importance of considering context (e.g., habitat size in this case) when investigating the relative importance of ecological drivers of diversity, like heterogeneity.

Key words: gambusia affinis; habitat fragmentation; habitat loss; heterogeneity diversity relationship; homogenization; mesocosms.

Introduction

Habitat heterogeneity is a primary mechanism driving patterns of species richness; heterogeneous habitats typically support more species than homogeneous habitats (termed the heterogeneity–diversity relationship [HDR]; McIntosh 1991, Tews et al. 2004, Urban 2004, Kadmon and Allouche 2007, Hortal et al. 2009, Allouche et al. 2012, Seiferling et al. 2014, Stein et al. 2014). Further, the HDR is thought to at least partially underlie some of the other important drivers of variation in species richness, including the species–area relationship (Connor and McCoy 1979, Drakare et al. 2006), the productivity–diversity relationship (Tilman and Pacala 1993, Guégan et al. 1998, Rajaniemi 2003) and the latitudinal diversity gradient (Rohde 1992, Jetz et al. 2012). Conversely, a reduction of habitat heterogeneity due to anthropogenic activities has been implicated as a primary mechanism behind losses in biodiversity from local to global spatial scales (McKinney and Lockwood 1999, McGill et al. 2015).

Despite the typically observed positive relationship between habitat heterogeneity and species richness, there is considerable variability in the strength of the HDR, ranging from strongly positive to neutral, and even negative effects (Nilsson et al. 1988, Lundholm 2009, Bar-Massada and Wood 2014, Seiferling et al. 2014, Stein et al. 2014). While several factors appear to be correlated with variation in the HDR, the spatial scale of investigation appears to be particularly important (Lundholm 2009, Tamme et al. 2010, Stein et al. 2014). Researchers have suggested that at very small spatial scales, species might not be able to detect or respond to habitat heterogeneity, because the differences in habitat quality are too fine grained. When species cannot detect or respond to habitat quality differences, heterogeneity can have a negative effect on species richness (Lundholm 2009, Tamme et al. 2010, Gazol et al. 2013, Laanisto et al. 2013).

In addition to variation in effect size and direction that results from the spatial scale upon which habitat heterogeneity is expressed, the total size of a given habitat with a fixed area (e.g., a distinct island, habitat patch, or pond) can also influence the relative importance of heterogeneity within that habitat. When habitat area is

fixed, an increase in habitat heterogeneity will necessarily lead to a decline in the total amount of each habitat type as a geometric constraint, sometimes called the area-heterogeneity-trade-off (AHTO) hypothesis (Kadmon and Allouche 2007, Allouche et al. 2012). As a result, there could be reduced opportunities for species to coexist, because each distinct habitat patch is smaller for species that differentially specialize on habitat types. This can create an interaction whereby habitat size modulates the positive influence of habitat heterogeneity on biodiversity, possibly even leading to a hump-shaped relationship between habitat heterogeneity and species richness (Kadmon and Allouche 2007, Allouche and Kadmon 2009, Allouche et al. 2012).

In freshwater ecosystems, a major driver of invertebrate species abundance and composition are fishes (Brooks and Dodson 1965, Hall et al. 1970, Hurlbert et al. 1972, Vanni and Layne 1997). Fish can be viewed as a "biotic" environmental filter, because they are often heterogeneously distributed among ecosystems. Thus, their influence on invertebrate communities varies within and among aquatic environments. Specifically, fish differ from many other predators in that they are highly dispersal limited, and their presence varies greatly across landscapes. This can create a mosaic of fish-present and fish-free habitats in the landscape, each with a characteristic assemblage of invertebrates that favor these distinct habitat types (Wellborn et al. 1996, Vanni and Layne 1997, McPeek 1998, Knapp et al. 2001, Schilling et al. 2009). Even within a single ecosystem where fish are present, a number of features of the ecosystem can influence the vulnerability of prey species to fish predators. For example, many lakes and wetlands have areas that are too shallow for fish predators to enter, or have significant littoral margins (i.e., macrophytes) that limit the access of fish, and allow prey to have considerable within-ecosystem refuge from predation (Timms and Moss 1984, Burks et al. 2001, Meerhoff et al. 2007). This can create a heterogeneous mosaic of fish-present and fish-free habitats within a single lake or pond, influencing invertebrate abundance and composition, depending on their tolerance to fish predation. This within-habitat heterogeneity may indeed be one reason why there remains some enigma regarding the influence of fish predators (and other types of environmental filters) on invertebrate composition among freshwater ecosystems (Batzer 2013).

We devised an experiment to manipulate fish-mediated habitat heterogeneity and habitat size within a model community consisting of a diverse group of freshwater zooplankton (crustaceans and rotifers). Specifically, we established zooplankton communities in large (900 L) and small (300 L) mesocosms mimicking freshwater ponds and factored this with a manipulation of habitat heterogeneity based on fish presence/absence. We manipulated heterogeneity by dividing the mesocosms in half with a porous barrier and controlling the presence of mosquito fish (*Gambusia affinis*), which are important predators that alter zooplankton species richness and composition (Hurlbert and Mulla 1981). One treatment was heterogeneous, allowing fish access to one-half of the mesocosm, but not the other, and two treatments were homogeneous, either having no fish present, or allowing fish access to the entire mesocosms.

Environmental filters should lead to compositional differences (i.e., species' turnover) among the habitat types, because environmental filters dictate the types of species present in each habitat type, based on individual species' traits (HilleRisLambers et al. 2012, Mittelbach and Schemske 2015). However, the effect of environmental filters should be greater in larger compared to smaller areas, if species do not perceive (or respond to) habitat variability when habitat patches are small (e.g., grains of perception; Wiens 1989). Additionally, small habitat patches generally support fewer individuals and species compared to large habitat patches due to a sampling effect (Hill et al. 1994). We expected that species would not perceive differences in habitat type when the total area was small (a limit of environmental filtering; Allouche et al. 2012). We also expected that the density of individuals would be greater in large areas compared to small areas (a limit of stochastic extinctions; Allouche et al. 2012). With those expectations, heterogeneity should increase species richness more in large areas compared to small areas.

Methods

To examine how habitat size and habitat heterogeneity affect patterns of species richness, we used a diverse zooplankton (crustacean and rotifer) community that occurs in small freshwater ponds near St. Louis, Missouri, USA. Freshwater zooplankton richness and composition are known to be influenced by habitat area (Dodson 1991, Hessen et al. 2007, Schuler et al. 2015), as well as heterogeneity in a number of biotic and abiotic features of the environment, including fish predators (Dodson 1992, Shurin 2001). However, the interaction between heterogeneity and habitat size has not previously been examined in these communities.

In May 2013, we established 15 small (300 L) and 15 large (900 L) mesocosms in an old field at Washington University's Tyson Research Center (Eureka, Missouri, USA). Mesocosms were placed in the field according to a fully dispersed design with each treatment represented equally in each row and column across the mesocosm array, to avoid environmental biases that might be present in the field (e.g., shading, temperature). Mesocosms were systematically filled with water from a nearby well, ensuring that variation in the well conditions did not cause water quality differences among the treatments by filling each mesocosm half way and then starting from the beginning to fill each the rest of the way. We added inorganic nitrogen ($NaNO_3$) and phosphorus (Na_3PO_4) so that total dissolved nitrogen (TN) was 1600 µg/L and total dissolved phosphorus (TOP) was 100 µg/L (16 N:1 P). This quantity and ratio of nutrients has been shown

to maintain a diverse array of zooplankton species in freshwater mesocosms (Schuler et al. 2015). We repeated the nutrient addition after 45 d (assuming a ~5% loss rate/d) in order to ensure that sufficient nutrient levels were maintained to sustain algal growth for the duration of the experiment (Hall et al. 2005).

We inoculated a "regional pool" of zooplankton species into each mesocosm that were collected from eight environmentally heterogeneous ponds near St. Louis, Missouri, USA. Three ponds did not have fish, and five ponds had fish present at least during some period in the five years preceding the experiment; this ensured that our regional species pool would include species that were specialized to coexist with fish, and species that were fish intolerant. From each pond, we collected several liters of highly concentrated zooplankton and water by filtering pond water through an 80-μm zooplankton net. We inoculated each small mesocosm with 200 mL of filtered pond water (consisting of approximately 4,000 individuals) and inoculated each large mesocosm with 600 mL of filtered pond water (approximately 12,000 individuals) in order to maintain proportions of inoculation size relative to mesocosm size. To ensure potential representation of species that could have been rare or missing in initial inoculations, we repeated the inoculations 7 and 21 d following the initial inoculations. Finally, to limit additional dispersal beyond the manipulations of the experiment, each mesocosm was covered with a polyurethane mesh (0.85 mm holes) that served as a barrier to immigration by new zooplankton species (Howeth and Leibold 2010, Schuler et al. 2015).

For each mesocosm size, we established two homogeneous treatments (whole mesocosm with fish, whole mesocosm without fish) and one heterogeneous treatment (half the mesocosm with fish, half without), each with five replicates. To maintain proportionality among treatments in predation intensity, each small "fish" mesocosm received one adult female (*G. affinis*) and each large "fish" mesocosm received three adult female *G. affinis* 10 d after the initial inoculation of zooplankton; the "fishless" treatment was free of fish and most other major predators (e.g., insects) due to the polyurethane mesh covering. The small "heterogeneous" treatment received one adult female *G. affinis* and the large "heterogeneous" mesocosms each received three adult female fish that were confined to one side of a mesh net (1.6 mm diameter) placed across the center of each mesocosm, keeping the fish from swimming to the predator-free side (the mesh allowed zooplankton to swim freely throughout the mesocosm); this varied the heterogeneity, but not the mean, intensity of fish predation in the treatments. To control for any unintended effects of the mesh in the heterogeneous treatments, the mesh was installed in both fish and fishless homogenous mesocosms as with the heterogeneous treatment, but left partially separated from the bottom of the mesocosm, allowing fish to swim freely throughout the mesocosm.

All female *G. affinis* used in this experiment were captured from a single pond at Tyson Research Center and isolated for 20 d, so that most residual offspring of these live bearing fish would be born prior to the females being introduced into the experiment. Nonetheless, some of the females gave birth in the experimental mesocosms; those offspring were removed during daily observations, which only occurred during the first 30 d of the experiment, and were unlikely to have had any major effect on zooplankton communities.

Sampling and analyses

Mesocosms were sampled 90 d after the zooplankton were initially inoculated by filtering 36 L of water from small mesocosms (18 L × 2 samples) and 108 L of water from large mesocosms (18 L × 6 samples), which maintained a proportional sampling scheme among small and large mesocosms (1:3). Proportional sampling ensures an equal probability of detection when habitat size varies among sampling sites, because the amount of habitat area being sampled may affect the species' relative abundance distributions, due to processes like aggregation. Proportional sampling allows for the separation of sampling effects from other processes that influence the species–°area relationship and gives greater power to detect species turnover among habitat types (Schoereder et al. 2004). To demonstrate that sampling proportional to habitat size accounts for bias due to increased aggregation without exaggerating differences in species richness, we also report the number of species observed in an average sample (i.e., standardized, rather than proportional sampling) within each small and large mesocosm.

To capture any spatial variation and aggregation, each sample was taken from a variety of locations and depths within the mesocosms. In each small mesocosm, one (18-L) sample was taken from each side of the mesh divider, and three (18-L) samples were taken from each side of the mesh divider in the large mesocosms. Each sample (18 L) was filtered through an 80-μm zooplankton net, concentrated to 50 mL, and stained and preserved for future identification using Lugol's iodine. We counted the numbers of individuals and identified each to species in 20% of each of the 50-mL samples. To identify zooplankton, we used a Nikon 400 mm (Nikon Instruments, Melville, New York, USA) dissecting scope using standard protocols and keys (Balcer et al. 1984). Across all of the treatments, we identified 51 species of zooplankton, including 34 rotifer species, eight cladoceran species, six copepod species and one ostracod (Appendix S1). In one small homogeneous (fish) mesocosm, the fish died during the experiment, and that mesocosm was removed from the analyses. Additionally, the samples from one large homogeneous (fish) mesocosm were destroyed by too little Lugol's iodine being placed in each sample, and this mesocosm was removed from the analyses.

To understand if the effect of heterogeneity on species richness depended on habitat size, we estimated the

extrapolated number of species found in each mesocosm using Chao's (1984) nonparametric species estimator, which accounts for missed rare species based on the number of singletons and doubletons found. Chao's (1984) estimator allows for the comparison of species richness values even if sampling efficacy differs among habitat types, by estimating the number of potential missed species that results from under sampling (Gimaret-Carpentier et al. 1998, Chao et al. 2009, Colwell et al. 2012). To calculate estimated species richness, we used the estimateR function in the Vegan Package in R (Oksanen et al. 2010). If habitat size and heterogeneity interactively influence species richness, then we would expect that large heterogeneous mesocosms would support more species than the homogeneous mesocosms and the small heterogeneous mesocosms.

To test if an increase in species richness resulted from an increase in the density of individuals (i.e., a sampling effect; Carnicer et al. 2013, Schuler et al. 2015), we estimated the density of individuals (number of individuals per sample). Differences in the density of individuals would indicate that habitat heterogeneity or habitat size altered the density of individuals supported, changing the probability of species being present in some treatments (e.g., the more individuals effect; Srivastava and Lawton 1998).

To test for shifts in the relative abundance distribution in each treatment, we used Hurlbert's probability of interspecific encounter (PIE), which is a sample-size independent method of rarefaction, and gives an estimate of the evenness of a community (Hurlbert 1971). Probability of interspecific encounter defines the slope of the rarefaction curve at its base, and indicates whether species' relative abundances differ among sampling sites, or in this case, across treatments in our experimental mesocosms (Olszewski 2004, Dauby and Hardy 2012, Chase and Knight 2013). Probability of interspecific encounter is equitable to Simpson's index (where Simpson's index is D and PIE is $1 - D$). To fully represent diversity, we converted PIE to an "effective number of species" (ENS_{PIE}; $1/\sum_{i=1}^{S} p_i^2$), where S represents the number of species, and p_i is the proportion of the community comprised of species i (Jost 2006). ENS_{PIE} allows one to compare relative abundances among communities within a regional species pool (Dauby and Hardy 2012, Chase and Knight 2013). Additionally, ENS_{PIE} allows one to disentangle sampling effects (i.e., more individuals) from effects on species that would emerge, for example, if coexistence mechanisms vary among treatments (i.e., fish vs. fishless). If the treatments in our experimental mesocosms change species richness and ENS_{PIE} unidirectionally, then we can infer that coexistence mechanisms differ among those treatments. Alternatively, if species richness differs, but ENS_{PIE} does not, the differences in richness can be inferred to have resulted from changes in the total abundances of species, but not their relative abundances (i.e., a sampling effect). ENS_{PIE} was

calculated using the diversity function in the Vegan Package in R (Oksanen et al. 2010).

To test if differences in species richness resulted from the differential distribution of species among habitats in heterogeneous mesocosms, as might be predicted if predation acts as a "biotic" environmental filter, we calculated the differences in species diversity (ENS_{PIE}) between the two sides of the heterogeneous mesocosms. This reflects the magnitude by which species are intraspecifically aggregated among sides, and thus the amount of species turnover between the "fish" and "fishless" habitats in the heterogeneous mesocosms. The amount of aggregation of species in the two sides of the heterogeneous mesocosms was calculated by comparing the ENS_{PIE} values from each side of the heterogeneous mesocosms to the expected ENS_{PIE} value when all individuals from all samples in the mesocosm were pooled (i.e., an estimate of a component of β-diversity among habitat types; see Olszewski 2004). If there is no difference among the habitat types, the expected ENS_{PIE} value across the entire mesocosm will not differ from the ENS_{PIE} values from each habitat type (i.e., species are distributed randomly throughout the mesocosm).

We analyzed each of the response variables (i.e., species richness, ENS_{PIE}, etc.) using ANOVA, with habitat heterogeneity and mesocosm size as independent variables, followed by Tukey's honestly significant difference (HSD) post-hoc test for multiple comparisons.

RESULTS

The extrapolated species richness values were all slightly higher than the observed species richness (0.89 species higher on average), indicating some under-sampling of rare species. However, there were no differences in the outcomes of the analyses, or interpretation of analyses when using observed species richness, compared to extrapolated species richness. To avoid any potential biases, we only report the extrapolated species richness values. The extrapolated number of species per mesocosm was affected by mesocosm size, heterogeneity, and the interaction between mesocosm size and heterogeneity (Table 1A, Fig. 1). Tukey's post-hoc analysis showed that both large homogenous treatments (fish, $P = 0.001$; fishless, $P < 0.001$) contained fewer species than the large, heterogeneous mesocosms; however, no other differences among the treatments were observed. Proportional sampling did not exaggerate the effect of habitat size and heterogeneity on species richness. Our results did not differ if we compared the total number of species found in large and small mesocosms of each treatment (Table 1A) or if we compared the average number of species found per sample in large and small mesocosms in each treatment (Table 1B).

There were no differences in the density of individuals among any of the treatments (Table 1C). The ENS_{PIE} values per mesocosm showed that heterogeneity and mesocosm size significantly affected ENS_{PIE} (Table 1D,

TABLE 1. ANOVA tables for (A) the effects of habitat size and heterogeneity treatments on extrapolated species richness (Chao 1984) per mesocosm (Fig. 1); (B) the effects of habitat size and heterogeneity treatments on extrapolated species richness (Chao 1984) per sample (individuals/18L, figure not shown); (C) the effects of habitat size and heterogeneity treatments on the density of individuals per mesocosm in each of the treatments (figure not shown); and (D) the effects of habitat size and heterogeneity treatments on effective number of species (ENS_{PIE}) per mesocosm (Fig. 2).

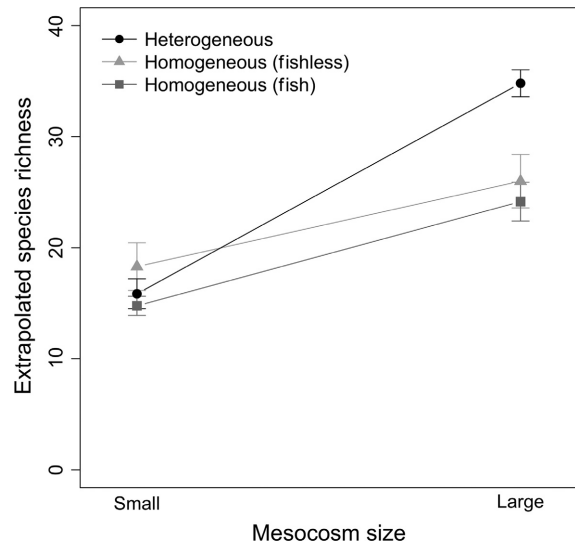| Treatment	df	F	P
(A)			
Size	1	88.2	<0.001
Heterogeneity	2	7.2	0.004
Size × heterogeneity	2	7.3	0.003
Residuals	22		
(B)			
Size	1	24.2	<0.001
Heterogeneity	2	3.7	0.039
Size × heterogeneity	2	4.1	0.031
Residuals	22		
(C)			
Size	1	3.1	0.091
Heterogeneity	2	0.7	0.489
Size × heterogeneity	2	1.6	0.232
Residuals	22		
(D)			
Size	1	3.4	0.08
Heterogeneity	2	5.3	0.013
Size × heterogeneity	2	1.0	0.386
Residuals	22		

FIG. 1. The effects of habitat size and heterogeneity treatments on extrapolated number of species per mesocosm. Habitat size, heterogeneity, and their interaction influence species richness (Table 1A).

DISCUSSION

In homogeneous mesocosms, the presence or absence of fish did not affect species richness or diversity (Figs. 1, 2). This likely occurred because zooplanktivorous fish act as a biotic environmental filter, and have differential effects on zooplankton taxonomic groups. Fish preferentially consume large-bodied cladocerans,
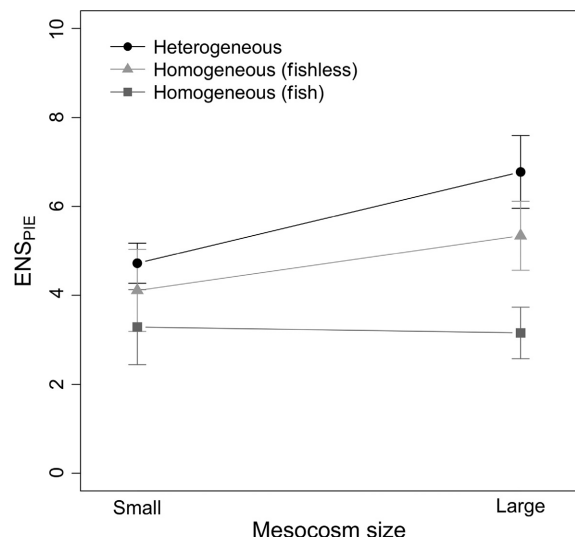
Fig. 2), but the interaction between heterogeneity and mesocosm size was not significant. The large heterogeneous mesocosms had greater ENS_{PIE} values than the large homogeneous (fish) mesocosms ($P = 0.029$), meaning that the relative abundance distribution became more evenly distributed, as shown by the Tukey's HSD post-hoc analysis; there were no other differences among the treatments.

The heterogeneous mesocosms had greater aggregation than the two homogeneous treatments (Fig. 3). Tukey's HSD post-hoc test for multiple comparisons revealed that heterogeneous mesocosms had significantly greater levels of species aggregation (higher species turnover) on each side of the mesocosms, compared to the homogeneous (fish) treatment ($P < 0.001$) and the homogeneous (fishless) treatment ($P < 0.001$), but there were no differences in aggregation between the two homogeneous treatments ($P = 0.7$). The size of the mesocosms did not affect species aggregation ($P = 0.30$). The number of individuals per sample (density of individuals) in the heterogeneous mesocosms was significantly affected by mesocosm size, but there were no differences in the density of individuals from the two sides of the mesocosms (fish and fishless), and the interaction between mesocosm size and the side of the mesocosm was not significant (Table 2, Fig. 4).

FIG. 2. The effects of habitat size and heterogeneity treatments on the effective number of species (ENS_{PIE}) for each mesocosm. Heterogeneity significantly affected ENS_{PIE}, mesocosm size was marginally significant, but the interaction between mesocosm size and heterogeneity was not significant (Table 1D).
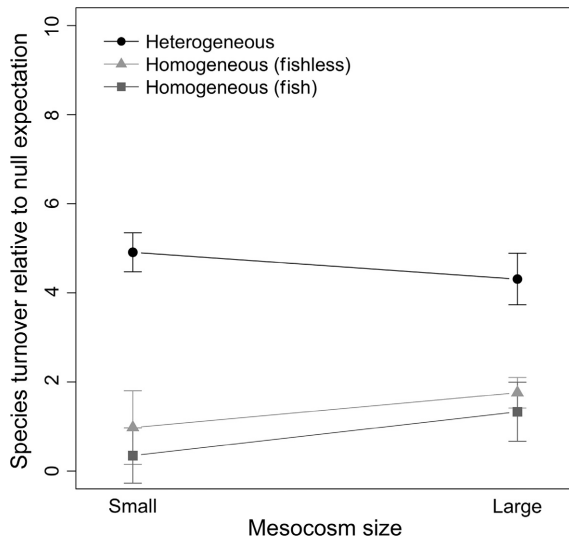
FIG. 3. The effects of habitat size and heterogeneity treatments on aggregation (species turnover). Aggregation was higher in both large and small heterogeneous mesocosms compared to homogeneous mesocosms (see *Results*).

which can lead to an increase in smaller rotifer species richness (Vanni and Layne 1997, Knapp et al. 2001). In this study, we found that populations of cladocerans, especially large species, were negatively affected by the presence of fish in the homogeneous mesocosms. The total abundance of cladocerans in mesocosms with fish was ~60% lower than in mesocosms without fish, and some of the largest species (e.g., *Daphnia pulex* and *Simocephalus vetulus*) were not found in the mesocosms with fish, but were present in the mesocosms without fish. Likewise, copepod abundance was ~80% lower in mesocosms with fish, compared to those without fish. The reduction of large cladocerans and copepods by fish likely indirectly increased the abundance of rotifers, which was 22% higher in mesocosms with fish. Thus, fish likely acted as a biotic environmental filter, altering species composition, but not species richness, by differentially feeding on large bodied cladocerans and copepods, allowing rotifers to increase in abundance and diversity.

By manipulating the distribution of fish in large and small mesocosms, we modified the level of heterogeneity experienced by zooplankton. We found that heterogeneity increased species richness and diversity in large, but not

TABLE 2. ANOVA table showing the effects of habitat size and habitat type (fish vs. fishless) on the density of individuals per sample (18 L) in heterogeneous mesocosms.

Treatment	df	F	P
Size	1	13.8	0.002
Habitat type	1	0.2	0.663
Size × habitat type	1	<0.1	0.923
Residuals	16		

small experimental mesocosms (Figs. 1, 2). The increase in species richness in large heterogeneous mesocosms relative to all other mesocosms resulted from a more even relative abundance distribution (as detected from the ENS_{PIE} values; Fig. 2), rather than any shifts in the total density of individuals (Table 1D). One explanation for this is that the presence and absence of fish within a single mesocosm provided an important "biotic" filter on which zooplankton species differentiated, allowing a more even distribution of species and higher overall richness in heterogeneous relative to homogeneous treatments. Indeed, when we examined species compositional variation among the sides of the heterogeneous mesocosms, this is exactly what we found; zooplankton species composition differed between habitat types (fish vs. fishless), as indicated by the increased aggregation (i.e., spatial differentiation) of species within heterogeneous relative to the homogeneous treatments (Fig. 4).

In contrast to the large heterogeneous mesocosms, zooplankton composition in the small heterogeneous mesocosms did not have higher richness (Fig. 1) or evenness (Fig. 2) relative to the small homogeneous mesocosms. One likely reason for this result is that despite the habitat differentiation of some of the more common species across the heterogeneous habitats, the smaller total area of fish and fishless habitat led to a reduction and loss of the rarest species from each habitat type; that is, a mechanism akin to the AHTO hypothesis (Kadmon and Allouche 2007, Allouche et al. 2012).

The AHTO hypothesis assumes that (1) species specialize on different habitat types; (2) landscapes with more habitat types thus have the potential for more species to co-occur than landscapes with fewer habitats; (3)
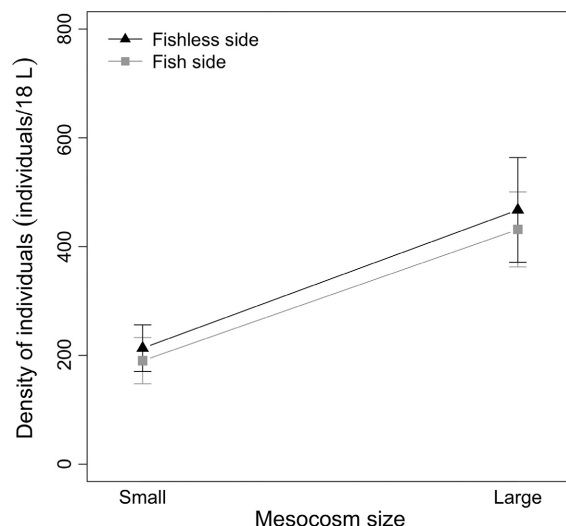

FIG. 4. The effects of habitat size on and habitat type (fish vs. fishless) on the density of individuals per sample in heterogeneous mesocosms. Large heterogeneous mesocosms supported a greater density of individuals (individuals per 18 L) than small heterogeneous mesocosms (Table 2).

the potential for more species to occur in a given habitat, and thus co-occur in heterogeneous landscapes, is only realized if there is sufficient area of each habitat type to maintain viable populations of the specialist species (Kadmon and Allouche 2007, Allouche et al. 2012). From these assumptions, the core prediction of the AHTO hypothesis is that habitat heterogeneity should lead to an overall increase in species richness only when differences among habitat types are detected by species, specialist species aggregate in the habitat type where their traits are best suited, and the total habitat area of each habitat type is large enough to maintain viable populations of habitat specialists.

Recent studies offer mixed results, with some in support, and some in refutation of the AHTO hypothesis (Hortal et al. 2009, 2013, Allouche et al. 2012, 2013a, b, Carnicer et al. 2013, Bar-Massada 2015, Chocron et al. 2015). One limitation of previous empirical studies is that they were observational, and thus unable to fully disentangle the fact that habitat size and habitat heterogeneity typically positively covary in natural environments (Connor and McCoy 1979, Hortal et al. 2009). In this context, our factorial manipulation of habitat size and habitat heterogeneity can more fully evaluate both the assumptions and predictions of the AHTO. Indeed, the findings of our study are at least superficially consistent with the key assumption of the AHTO hypothesis that habitat heterogeneity created conditions for species aggregations across habitat boundaries (Fig. 3), but that this only increased the evenness of species (Fig. 2) and the density of individuals (Fig. 4), and thus measured species richness (Fig. 1) in the larger mesocosms, but not the smaller mesocosms.

An alternative to the assumptions of the AHTO hypothesis that could lead to similar patterns where heterogeneity influences species evenness and richness in the large, but not small, mesocosms could have occurred if individual movements across the habitat boundaries were frequent enough so that habitat differentiation was not possible. That is, if zooplankton movements effectively "homogenized" the environment within the small mesocosms, such that the realized environmental variation (i.e., fish vs. fishless sides of the mesocosm) was nullified. We suspect, however, that at least in this case, this explanation is probably less likely because there was still a significant differentiation of zooplankton species composition from the fish to fishless sides, suggesting at least some degree of specialization among the heterogeneous habitats consistent with the AHTO hypothesis (Fig. 3).

Researchers have extensively discussed whether to include biotic filters as true environmental filters when considering species' assemblage rules (Gotelli et al. 2010, HilleRisLambers et al. 2012, Maire et al. 2012, Kraft et al. 2015, Maaß et al. 2015, Mittelbach and Schemske 2015). We argue that fish in freshwater ecosystems are a special case of a biotic environmental filter that act mechanistically the same as an abiotic environmental filter (see Kraft et al. 2015). This is because fish are often

heterogeneously distributed within and among ecosystems independent from environmental variation itself. The presence and distribution of fish within or among ponds creates a mosaic of fish-present and fish-free habitats, each with a characteristic assemblage of invertebrates that prefer these distinct habitat types (Brooks and Dodson 1965, Wellborn et al. 1996, Vanni and Layne 1997, McPeek 1998, Knapp et al. 2001, Schilling et al. 2009). This mosaic alters the coexistence of species that are tolerant to fish predation, and species that are intolerant to fish predation. In zooplankton, fish alter competition and coexistence by preferentially consuming large-bodied cladocerans (e.g., *Daphnia*) over small-bodied cladocerans and rotifers (Brooks and Dodson 1965, Vanni and Layne 1997). In our experiment, we found a similar shift in zooplankton composition from fish to fishless habitats (Fig. 3), either due to direct predation by fish on larger species, or indirectly if the larger species took refuge on the side without fish. The absence of these large bodied cladocerans on the fish side of the mesocosms in turn likely increased local algal resources for rotifers and small cladocerans that are tolerant of fish, and would normally be excluded.

Overall, our results show that there is an interaction between habitat size and heterogeneity in determining species richness. In our case, we suspect this occurred because there are geometric constraints in the amount of area of individual habitat types that can support habitat specialist species (as in the AHTO hypothesis; Kadmon and Allouche 2007, Allouche et al. 2012). However, there are other plausible explanations for why habitat size and habitat heterogeneity should interact, such that the diversity-enhancing effects of heterogeneity are increasingly nullified as habitat size decreases (e.g., organisms average across habitat heterogeneity, effectively responding to homogeneous conditions). Regardless of the specific underlying mechanism, an important implication of our result showing that the importance of habitat heterogeneity for species richness declines as habitat size declines, is that habitat loss could more strongly affect species loss relative to what might be expected if one were ignorant of such interactions. Even if habitat heterogeneity remains as habitats are lost and fragmented, the influence of the remaining heterogeneity on the maintenance of species richness might become constrained, such that fewer species can persist in smaller habitats than might have been expected from simple projections that do not consider such effects (He and Hubbell 2011, Halley et al. 2013).

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