Threshold effects of habitat fragmentation on fish diversity at landscapes scales

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Abstract. Habitat fragmentation involves habitat loss concomitant with changes in spatial configuration, confounding mechanistic drivers of biodiversity change associated with habitat disturbance. Studies attempting to isolate the effects of altered habitat configuration on associated communities have reported variable results. This variability may be explained in part by the fragmentation threshold hypothesis, which predicts that the effects of habitat configuration may only manifest at low levels of remnant habitat area. To separate the effects of habitat area and configuration on biodiversity, we surveyed fish communities in seagrass landscapes spanning a range of total seagrass area (2–74% cover within 16000-m² landscapes) and spatial configurations (1–75 discrete patches). We also measured variation in fine-scale seagrass variables, which are known to affect faunal community composition and may covary with landscape-scale features. We found that species richness decreased and the community structure shifted with increasing patch number within the landscape, but only when seagrass area was low (<25% cover). This pattern was driven by an absence of epibenthic species in low-seagrass-area, highly patchy landscapes. Additional tests corroborated that low movement rates among patches may underlie loss of vulnerable taxa. Fine-scale seagrass biomass was generally unimportant in predicting fish community composition. As such, we present empirical support for the fragmentation threshold hypothesis and we suggest that poor matrix quality and low dispersal ability for sensitive taxa in our system may explain why our results support the hypothesis, while previous empirical work has largely failed to match predictions.

Key words: community structure; habitat patch; movement; seagrass; species richness; species traits; species–area relationship.

Introduction

Habitat fragmentation is an aggregate process which involves both declines in total habitat area along with changes in spatial configuration (e.g., patch size, patch number, and patch isolation; Fahrig 2003). While habitat fragmentation in this broad sense is often associated with declines in biodiversity and decreased population fitness for many species (Saunders et al. 1991, Foley et al. 2005), the primary cause of these losses is not always clear. Because total habitat area changes concomitantly with changes in patch attributes, many studies confound area-based effects with those mediated through changes in habitat configuration or other forms of habitat degradation. This has led to debate surrounding the relative importance of habitat loss vs. changes in other habitat attributes in driving ecological change associated with habitat fragmentation (Lindenmayer and Fischer 2007, Fahrig 2013).

The positive relationship between habitat area and species richness is perhaps one of the most general and accepted patterns in ecology (Lomolino 2000). Therefore,

it is expected that habitat fragmentation should lead to a loss in biodiversity merely through the effects of decreasing habitat amount. In fact, numerous studies have supported the critical effects of habitat loss in mediating responses of diversity and population persistence to fragmentation (e.g., Summerville and Crist 2001). The more relevant question is then, with increasing habitat fragmentation, are there additional effects of habitat configuration or within-patch habitat quality on biodiversity that are separate from purely area-based responses? Determining if/when fragmentation matters for biodiversity will help prioritize conservation efforts, as recent studies have questioned the strong focus on changes in habitat configuration as a primary driver of habitat degradation (Fahrig 2013).

Relative to the effects of habitat area, evidence regarding the magnitude, and even direction, of the effects of habitat configuration (sometimes termed habitat fragmentation per se) on species richness and faunal abundance is much more equivocal (Fahrig 2003, Ewers and Didham 2006). Widely divergent species-level responses to habitat configuration indicate that traits like body size, trophic level, and movement ability may be key in determining species-specific sensitivities to fragmentation (Ewers and Didham 2006). Another reason why studies examining the effects of habitat

configuration have reported disparate results may be that the effects of configuration are contingent upon the cover of the focal habitat within the landscape. Studies employing simulation models have predicted that the effects of habitat patchiness on population persistence may only be detectable at low levels of remnant habitat area (usually below ~20–30% remnant habitat area; Fahrig 1997, 1998, Hanski and Ovaskainen 2000, Flather and Bevers 2002). These observations have led to the fragmentation threshold hypothesis, which may manifest as a statistical interaction between the effects of habitat area and configuration on species occurrence or diversity (Trzcinski et al. 1999).

Studies attempting to empirically quantify the independent effects of habitat configuration generally employ one of two approaches (McGarigal and Cushman 2002). The first approach involves experimental manipulation of habitat pattern, either through habitat removal (e.g., mowing grassland plots; Parker and Mac Nally 2002) or creation of new, artificial habitat (e.g., artificial seagrass units, ASUs; Johnson and Heck 2006). These experimental manipulations allow for a true separation of habitat configuration effects independent of habitat area, but are often limited in scale by logistical constraints. Specifically, most manipulative studies are conducted at relatively small spatial scales (~100 m^2 or less), are short in temporal duration, and are replicated across only a few levels of habitat area. This is particularly constraining in marine studies (Boström et al. 2006). The second approach involves observational tests of hypotheses based upon a priori selection of landscapes that vary in area and configuration. An advantage of this approach is the possibility to increase the scale and replication of the study, including a greater range in habitat area. Observational studies may offer the highest realism and generality because they are applied to real-word systems and are able to examine fragmentation at scales at which it occurs in nature (McGarigal and Cushman 2002). However, observational studies typically rely on space for time substitutions of landscapes along existing fragmentation gradients, which may introduce additional sources of variation if other habitat attributes covary with change in habitat configuration or area. For instance, local habitat quality/complexity may decline as habitat patchiness increases (e.g., Irlandi et al. 1995), making underlying drivers of organismal responses unclear. The few studies that have attempted to empirically identify the fragmentation threshold across a large range in habitat area, either through experimental or observational approaches, have largely failed to support the hypothesis (Trzcinski et al. 1999, Parker and Mac Nally 2002, Betts et al. 2006, Smith et al. 2011, but see Radford et al. 2005).

Our objective was to determine whether habitat configuration affected biodiversity and fish community structure within seagrass landscapes and whether its effect was mediated by total habitat area. We designed our study capitalizing on natural variation in seagrass landscape structure to a priori isolate the effects of seagrass habitat amount from differences in spatial pattern among landscapes. Specifically, we compared seagrass communities within landscapes that varied in habitat configuration (ranging from one contiguous patch of seagrass to many patches of seagrass; maximum of 75 patches), with extremes in patch number evaluated across a wide range of total seagrass area (~300–11,800 m^2, 2–74% cover). In addition to landscape-scale variation in habitat features, we also evaluated how within-patch characteristics (fine-scale variation in seagrass attributes: seagrass density, height, biomass) covaried with area and patch number. We then tested whether habitat area, habitat configuration, fine-scale seagrass variables, and/or their interaction were important in predicting fish diversity, density, and community structure. Finally, we assessed whether differences in species movement abilities among habitat patches could partially explain differential responses among taxa to changes in habitat configuration.

METHODS

Characterization of habitat area and habitat configuration

We sampled 21 isolated seagrass beds (hereafter referred to as landscapes) in Back Sound, North Carolina, USA (34°42′ N to 34°39′ N, 76°37′ W to 76°31′ W), a shallow (average depth ~2 m), back-barrier embayment (Appendix S1: Fig. S1). Seagrass landscapes in Back Sound range from contiguous to highly patchy (largely the result of physical processes associated with exposure; Fonseca and Bell 1998) and are dominated by eelgrass *Zostera marina* (Linnaeus, 1753) and shoal grass *Halodule wrightii* (Ascherson, 1868). We focused on seagrass landscapes that were isolated (by at least 100 m) from saltmarsh habitat, as interhabitat connectivity may also influence seagrass fish communities (Irlandi and Crawford 1997, Baillie et al. 2015), and that were reasonably isolated from other seagrass habitat (mean distance to the nearest seagrass patch = 27 m). We chose 200 × 80 m rectangles (16,000 m^2) as our focal landscape extent, as this matched the size and general shape of many isolated landscapes in our system. Furthermore, this extent likely encompasses the scale of potential short-term movements (days to months) of many of the most common fishes in our study (Szedlmayer and Able 1993, Irlandi and Crawford 1997, Miller and Able 2002, Potthoff and Allen 2003). Seagrass cover within each landscape was evaluated by digitizing orthorectified aerial photographs organized by the Albemarle Pamlico National Estuary Partnership (APNEP) and taken by the North Carolina Department of Transportation on 27 May 2013 using AcrGIS (Environmental Systems Research Institute, ArcGIS Desktop v 10.1, 2012, Redlands, CA, USA). We used seagrass surveys at randomly generated points conducted at five of the landscapes in August and October 2013 to ground-truth our seagrass maps. For the selected points, 98% were correctly categorized as seagrass (*n* = 22/22) or

bare substrate (n = 18/19). We intentionally chose landscapes that ranged from primarily contiguous to highly patchy across a range of total seagrass cover (range = 2–74%, or 260–11,764 m^2 beds). Total habitat area and metrics of habitat configuration were calculated in FragStats v4 based on rasterized maps of seagrass (2 × 2 m cell size) at each landscape (McGarigal et al. 2012). We calculated the total percent cover of seagrass within the landscape, the number of discrete seagrass patches, area-weighted mean patch size, and edge:area ratio (Appendix S1: Table S1).

For subsequent analyses, we focused on total seagrass area within the landscape as a measure of habitat amount, which is also typically correlated with area-weighted mean patch size (Fahrig 2003), as was the case in our study (Pearson $R = 0.95$, $P < 0.001$). As our quantitative metric of habitat configuration, we used number of discrete seagrass patches within the landscape. Number of patches was correlated with the edge:area ratio ($R = 0.63$, $P = 0.002$), another measure of habitat configuration. Furthermore, seagrass area and number of patches were uncorrelated ($R = 0.02$, $P = 0.9$), allowing us to independently assess the effects of seagrass area and habitat configuration on fish communities across our selected study landscapes.

Characterization of fine-scale seagrass characteristics

To assess how fine-scale attributes of the seagrass differed among landscapes varying in seagrass area and configuration, we collected 30 cm diameter cores from each landscape (n = 3–7 per landscape), removing the top 10 cm of sediment. We brought cores back to the laboratory for processing, where all seagrass was sorted by species. All shoots were enumerated and the heights of the first 20 shoots for each species were measured to the nearest millimeter. Above ground biomass was dried for 48 h at 60°C and dry mass was recorded for each species.

Relationships among habitat area, habitat configuration, and fine-scale seagrass characteristics

Fine-scale differences in seagrass variables across landscapes were evaluated based on mean aboveground biomass, mean shoot density, and mean shoot height averaged across all cores collected at a landscape for *Z. marina* and *H. wrightii*, separately. Whether any of these attributes varied with total seagrass area (log-transformed), habitat configuration (log number of seagrass patches), or their interaction was tested separately with a general linear model using the lm function in R (R Core Team 2015).

Characterization of fish assemblages

We sampled the fish community within each landscape with a 5 m wide otter trawl (15-m head rope, 2-cm mesh size, 0.6-cm cod end mesh) with no tickler chain, following Baillie et al. (2015). We completed two, 2-min tows (~100 m in length) at each landscape once in June and once in July of 2013 (four total trawls per landscape). Total travel distance for each tow was recorded based on measurements using a Garmin 72H handheld GPS unit (Garmin International, Olathe, Kansas, USA). All tows were conducted within 3 h of a diurnal high tide. The start location of each tow was haphazard, but we remained with the landscape boundaries during the tow and all tows sampled some seagrass habitat. During the tow, one observer sat at the back of the boat and recorded the total amount of time the trawl was over seagrass. The time spent within seagrass habitat was proportional to the total seagrass area within the landscape ($R = 0.91$). After each trawl, all fish were identified to species and enumerated.

We evaluated the fish assemblage within each landscape based on species richness (count of species sampled), total fish density (fish abundance/m^2), and community structure (i.e., species composition within the landscape). Prior to analyses, individuals within each species were summed across all four trawls at each landscape. We limited our analysis to seagrass-associated fishes and excluded pelagic species that may not have been using the seagrass habitat directly (e.g., *Anchoa mitchilli* [Valenciennes, 1848; bay anchovy] and *Selene vomer* [Linnaeus 1758; lookdown]).

Relationships among fish assemblage, habitat area, habitat configuration, and fine-scale seagrass characteristics

First, we tested the effects of habitat area, habitat configuration, and fine-scale seagrass characteristics on fish species richness and fish density. We used total seagrass biomass as our metric of fine-scale seagrass habitat quality, which was only weakly correlated with seagrass area (log-transformed, $R = 0.25$) and patch number (log-transformed, $R = 0.18$). Results of models were qualitatively similar regardless of the metric of fine-scale seagrass characteristic used (i.e., biomass, shoot density, shoot height by individual species or across both species). We regressed species richness or fish density onto seagrass area within the landscape, number of seagrass patches, total seagrass biomass, and all two-way interactions among the three variables using the lm function in R. Seagrass area and number of patches were log-transformed to improve normality and all variables were centered and scaled prior to analysis. We excluded one landscape that was an apparent outlier based on examination of model residuals; excluding this landscape did not qualitatively change the results but improved model fit. We used variance inflation factors (VIFs) to assess collinearity among the independent variables used in the multiple regression (Draper and Smith 1998, Zuur et al. 2010). None of the variables nor their interactions had VIFs above our conservative threshold of 3 (Appendix S1: Table S2), indicating low collinearity with little inflation of error around parameter estimates

(Zuur et al. 2010). The F tests of the significance of the independent variables were based on Type II Sum of Squares (SS), as our data were unbalanced and Type II SS preserves the principal of marginality when testing main effects (Langsrud 2003). We report η^2 (partial variation explained) for each independent variable as a measure of effect size, as it relates to the amount of unique variation in the response variable explained by each predictor variable.

Differences in fish community structure across landscapes were analyzed based on a Bray-Curtis similarity matrix of fish species observed at each landscape. A presence-absence transformation was applied to examine shifts in species composition across landscapes, as abundance-based metrics were dominated by *L. rhomboides*, which made up >85% of the 33,940 individuals collected. Species that were observed at one landscape only were excluded prior to community structure analysis. Whether community structure varied with seagrass area (log-transformed), number of seagrass patches (log-transformed), fine-scale seagrass biomass, or any two-way interaction among the variables was tested with a permutational analysis of variance (PERMANOVA) using Type II SS (PERMANOVA add-on to the PRIMER-E software; Anderson 2001). As a measure of effect size, we report the square-root of the estimate of components of variation ($\sqrt{ECV}$), which may be interpreted as percentage of Bray-Curtis dissimilarity attributed to each variable. We set a small negative $\sqrt{ECV}$ for seagrass biomass to zero under the assumption that it was a sample underestimate of a small or zero variance (Fletcher and Underwood 2002). A Nonmetric Multi-Dimensional Scaling plot was used to graphically display the data.

Next we examined whether variation in community structure among landscapes was attributed to differences in community dispersion (e.g., convergence or divergence among landscapes) and which species may be driving any differences. For these post-hoc tests, we grouped landscapes into four categories (high area and low patch number, high area and high patch number, low area and low patch number, low area and high patch number), as these tests are based on comparisons among groups. Landscapes were considered high area if total seagrass cover ≥25% (seagrass area ≥3,900 m^2), based on predicted thresholds in the fragmentation threshold hypothesis (Fahrig 1998, Flather and Bevers 2002), as well as corresponding to the median observed habitat area in the current study (3,908 m^2). High patch number landscapes were defined as those with greater than or equal to the median observed patch number across all landscapes (≥9 patches). We used a permutational test of multivariate dispersion (PERMDISP) to test whether differences in community structure among landscape types could be attributed to changes in the average within-group dispersion (betadisper function in vegan package; Anderson et al. 2006, Oksanen et al. 2014). A similarity percentages analysis (SIMPER) was used to identify which taxa were likely contributing to the difference among landscape groups (simper function in vegan package; Clarke 1993, Oksanen et al. 2014).

Fish movement and habitat configuration

As we found that certain species seemed to be sensitive to the effects of habitat configuration (see *Results*) and were notably absent from the low area, high patch number landscapes, we assessed whether varying movement rates between patches across taxa could be partly responsible for this pattern. We used mesocosm trials to assess the inter-patch movement rates of two groups of species, epibenthic species and benthopelagic species, aligning with general patterns in community structure we observed in the trawl data. We carried out the experiment in a laboratory setting equipped with three water tables (2.4 m long × 0.8 m wide) at the Institute of Marine Sciences, University of North Carolina at Chapel Hill, in Morehead City, North Carolina, USA. Within each water table, two ASUs (0.17 m^2) were placed at either end of the water table and secured under ~5 cm of sand, which evenly covered the bottom of the table. The ASUs were constructed with 30 cm high artificial blades at a density of ~470 blades/m^2. Filtered seawater from the adjacent Bogue Sound flowed continuously through the tanks and water depth was maintained at 17 cm (within the range of low-tide depth at our field sites). Fish used in the trials were collected from Back and Bogue Sounds and kept in separate holding tanks for 2 d prior to the start of a trial.

All trials were conducted between July and October 2014. Trials were run under dark conditions, lit by red light, as many estuarine species may move more at night (Sogard and Able 1994). For each trial, a tank was stocked with five individuals of one of five species. Two species were considered to be benthopelagic (generally floating/swimming above the bottom; *Lagodon rhomboides* [pinfish], *Orthopristis chrysoptera* [pigfish]) and three epibenthic (generally resting on the bottom or staying hidden with seagrass canopy; *Stephanolepis hispidus* [planehead filefish], *Opsanus tau* [oyster toadfish], and *Hypsoblennius hentz* [feather blenny]) based on general patterns in microhabitat use of each. After a 30-min acclimation period, trials were run for 3 h. Fish behavior was recorded with a GoPro camera (GoPro, Inc., San Mateo, CA, USA) placed at one end of the tank. A camera control (camera housing only) was placed at the other end to mimic the recording camera. A 10-min video segment from each trial was randomly selected. The number of inter-patch (between two ASUs) movements by all individuals during the 10-min period was recorded. To assess whether some fish were moving in and out of ASUs but not crossing all the way to the other ASU patch, we also counted the number of movements across patch boundaries (i.e., entry or emergence out of an ASU). Sixteen total trials were run ($n = 8$ per fish trait group).
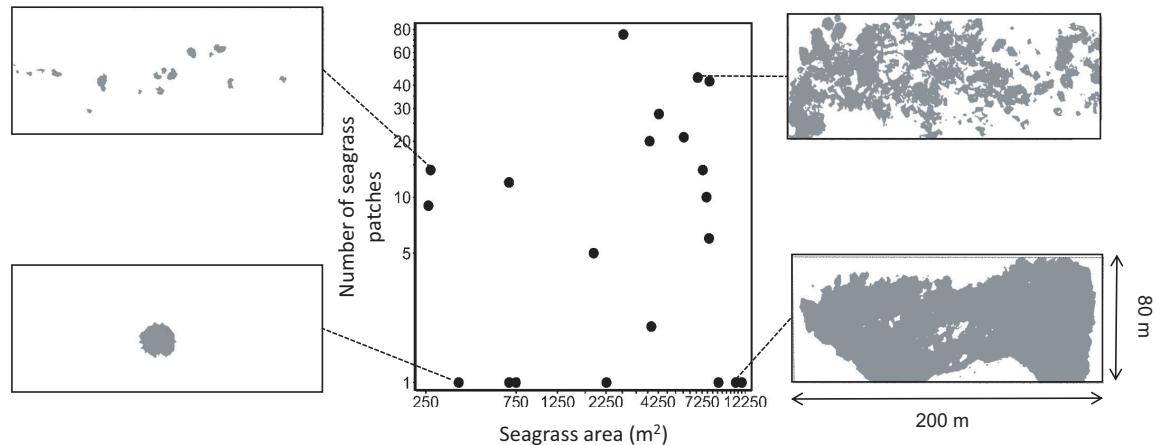
Fig. 1. Landscape attributes (total seagrass area and number of discrete seagrass patches) from 21 study landscapes. Four example maps of seagrass landscapes are shown and connected to the corresponding point on the biplot with dotted lines. Note that the *x*- and *y*-axes are plotted on a log-scale to be consistent with the statistical analysis, but axes values are back-transformed, representing raw data values to aid in interpretation.

Rates of inter-patch movement and seagrass entry/emergence from the mesocosm trials were compared among species and between the two a priori designated trait groups: epibenthic vs. benthopelagic species. The effects of trait group and species identity (nested within trait group) on the number of inter-patch movements was tested with a general linear model using the lm function in R. We ran a similar test with the number of movements across patch boundaries (entry/emergence rates) as the response. The number of inter-patch movements was square-root transformed and the number of movements across patch boundaries was log-transformed to improve normality.

Results

Habitat area, habitat configuration, and fine-scale seagrass characteristics

Our 21 study landscapes spanned a wide range of area-related (range in total seagrass area = 260–11,764 m², cover = 2–74%, area-weighted mean patch size = 29–11,764 m²) and habitat-configuration-related measures of landscape pattern (number of patches = 1–75 patches, edge:area = 0.06–0.98 m/m²; Appendix S1: Table S1). The 21 study landscapes were well distributed across both variables relating to independent effects of habitat area and habitat configuration, representing all combinations of high and low habitat area and high and low patch number (Fig. 1).

Fine-scale estimates of seagrass characteristics varied widely among study landscapes (Appendix S1: Fig. S2). Landscape-scale habitat variables (area and configuration) explained between 4% and 44% of the variance in fine-scale seagrass characteristics. We detected an interaction between habitat area and configuration on some of the fine-scale seagrass characteristics (*Z. marina* biomass and shoot density was lower and *H. wrightii*

shoot density was higher within low area, highly patchy landscapes; Appendix S1: Fig. S2).

Relationships among fish assemblage, habitat area, habitat configuration, and fine-scale seagrass characteristics

Variation in fish species richness and total fish density across landscapes was well-predicted by the habitat (landscape- and fine-scale) characteristics measured ($R^2 = 0.78$ and 0.76, respectively). In both cases, landscape-scale features (area and configuration) were much better predictors of the fish response variables than fine-scale seagrass biomass (Table 1). In the case of species richness, there was an interactive effect of habitat area and habitat configuration (area × configuration $F_{1,13} = 13.54$, $P = 0.002$; Table 1). This pattern was driven by a negative effect of patch number on species richness when area was low, but little effect on species richness when area was high (Fig. 2A). For fish density, there was a positive effect of habitat area ($F_{1,13} = 22.89$, $P = 0.0003$) and a negative effect of patch number ($F_{1,13} = 14.36$, $P = 0.002$; Fig. 2B). There was only a weak interaction between these two variables ($F_{1,13} = 3.84$, $P = 0.07$). In both models, the effects of fine-scale seagrass biomass on the fish community was relatively weak ($\eta^2 \leq 0.05$; $P \geq 0.1$ in all cases; Table 1).

Habitat (landscape- and fine-scale) characteristics explained 34.8% of the dissimilarity in fish community structure across study landscapes (Table 2, Fig 3). Similar to the results for effects on species richness, habitat area ($\sqrt{\text{ECV}} = 9.7$, $P = 0.0002$), habitat configuration ($\sqrt{\text{ECV}} = 8.4$, $P = 0.004$), and their interaction ($\sqrt{\text{ECV}} = 9.8$, $P = 0.008$) were the most important drivers of community structure across landscapes. Again, seagrass biomass and its interactions with area and configuration had only weak effects on fish community structure ($P \geq 0.2$ in all cases; Table 2). The shift in community structure among landscape types was not attributable to differences

TABLE 1. Results of multiple linear regression models of species richness and total fish density predicted by habitat (seagrass) area within the landscape, habitat configuration (number of seagrass patches), and fine-scale habitat quality (seagrass biomass).

Source of variation	df	Parameter estimate	η^2	Type II sum of squares	F	P
Fish species richness; $F_{6,13} = 7.80$, $P = 0.001$, $R^2 = 0.78$						
Seagrass area	**1**	**0.79**	**0.40**	**5.79**	**18.22**	**0.001**
Patch number	1	−0.18	<0.01	0.00	0.00	>0.9
Seagrass biomass	1	0.02	<0.01	0.07	0.22	0.6
Seagrass area × patch number	**1**	**0.69**	**0.30**	**4.30**	**13.54**	**0.003**
Seagrass area × seagrass biomass	1	0.08	<0.01	0.04	0.13	0.7
Patch number × seagrass biomass	1	0.06	<0.01	0.04	0.14	0.7
Residual	13		0.29	4.13		
Total fish density; $F_{6,13} = 6.87$, $P = 0.002$, $R^2 = 0.76$						
Seagrass area	**1**	**0.67**	**0.38**	**8.02**	**22.89**	**0.0003**
Patch number	**1**	**−0.48**	**0.24**	**5.03**	**14.36**	**0.002**
Seagrass biomass	1	0.31	0.05	1.05	3.00	0.1
Seagrass area × patch number	1	−0.38	0.06	1.35	3.84	0.07
Seagrass area × seagrass biomass	1	0.13	0.01	0.12	0.33	0.6
Patch number × seagrass biomass	1	−0.24	0.04	0.83	2.37	0.1
Residual	13		0.22	4.55		

Notes: Seagrass area and number of patches were log-transformed prior to analysis. df, degrees of freedom; η^2, proportion of variation in dependent variable explained by each predictor. Boldface type highlights variables that are statistically significant ($P < 0.05$).

in within-group (i.e., within landscape type) dispersion in community composition (PERMDISP, $F_{3,17} = 1.32$, $P = 0.3$). *Lagadon rhomboides*, *Orthopristis chrysoptera*, and *Paralichthys albiguttata* (gulf flounder) were ubiquitous across all landscapes (SIMPER, Appendix S1: Table S3). The difference among low area, high patch number landscapes and all other landscape types could be explained in part by the lack of *Syngnathus floridae* (dusky pipefish), *Opsanus tau*, and *Bairdiella chrysoura* (silver perch), which were common in the other landscape types.

Fish movement and habitat configuration

Mesocosm trials revealed strong differences in movement rates of fishes between ASUs related to fish trait group (Appendix S1: Fig. S3). The number of inter-patch movements differed between trait groups (lm, $F_{1,11} = 20.47$, $P < 0.001$), but not among species within trait groups ($F_{3,11} = 0.40$, $P = 0.8$). Inter-patch movement rates were an order of magnitude higher (49.4 ± 39.2 movements/min [mean ± SD]) for the benthopelagic species than the epibenthic species (2.9 ± 5.6 movements/min). The number of movements across the patch boundaries varied both between trait groups ($F_{1,11} = 49.42$, $P < 0.001$) and among species within a trait group ($F_{3,11} = 11.06$, $P = 0.001$). Mean patch entry/emergence rates were consistently higher for the benthopelagic species (68.4 ± 43.4 movements/min) than for the epibenthic species (13.5 ± 13.1 movements/min).

DISCUSSION

We found evidence that habitat configuration does affect biodiversity in natural landscapes, and the effects of configuration were dependent on the total habitat area within the landscape. Notably, the effects of habitat configuration were primarily manifest when total habitat area was low (<25% cover), where loss of fish species sensitive to increasing patch number below this area threshold resulted in shifts in community structure in the highly patchy landscapes. While other habitat attributes (e.g., fine-scale seagrass attributes) also varied with changes in landscape variables, habitat configuration in combination with habitat area appeared to be the primary drivers of differences in fish communities across landscapes. As such, our results empirically support the fragmentation threshold hypothesis predicted by modeling studies, and stand in contrast to the findings of previous empirical studies (Trzcinski et al. 1999, Parker and Mac Nally 2002, Betts et al. 2006, Ethier and Fahrig 2011), which have largely failed to support this hypothesis (but see Radford et al. 2005).

As we used an observational approach to test hypotheses, we could not completely isolate the effects of habitat configuration and area from other habitat features that covary along with these landscape-scale variables. This approach does, however, reveal how biodiversity varies naturally with fragmentation within seagrass landscapes. Furthermore, because we were able to capitalize on a natural fragmentation gradient, we avoided confounding other human disturbances with fragmentation, which can be problematic in many terrestrial systems (McGarigal and Cushman 2002). Nevertheless, we did find evidence that other habitat features vary along with habitat configuration and area along this natural gradient. Specifically, the correlation between fine-scale seagrass attributes with the landscape-scale variables might be one plausible mechanism explaining the observed
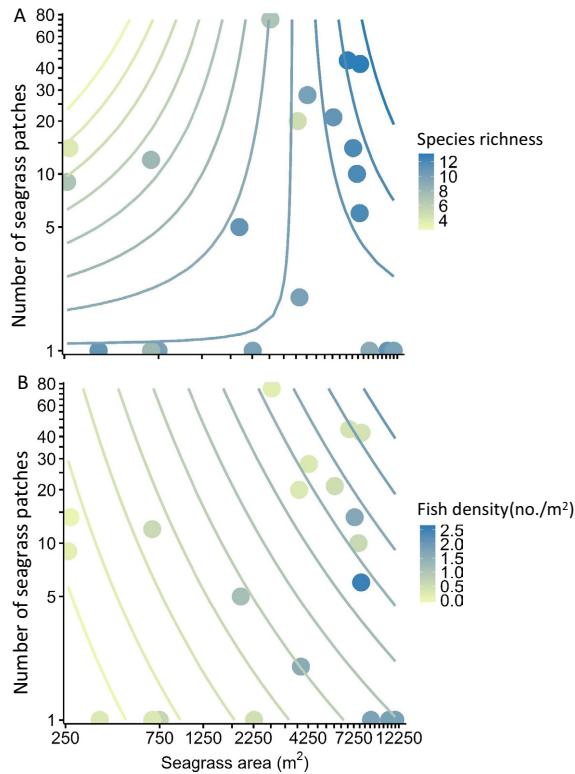
FIG. 2. Plots of the effects of seagrass area and habitat configuration on (A) fish species richness and (B) total fish density. Each point represents a landscape and the color of each point corresponds to the value of the response variable. Note that the x- and y-axes are plotted on a log-scale to be consistent with the statistical analysis, but axes values are back-transformed, representing raw data values to aid in interpretation. Contour lines on each plot show the model predictions for each response variable, holding seagrass biomass at its mean observed value. In panel (A), the changing curvature of the model prediction contours across the plot reflects the interaction between the two predictor variables in that the effects of habitat configuration on species richness vary with habitat area. Specifically, the model predicts a negative effect of patch number when seagrass area is low, no effect when seagrass area is moderate, and a slightly positive effect when seagrass area is high. In panel (B), the relatively consistently spaced and symmetrical contour lines reflect the independent effects of area and configuration on fish density, which increased with habitat area and decreased with patch number.

pattern (although these correlations were generally weak). The low-area, highly patchy seagrass landscapes were generally characterized by lower biomass and shoot density of *Z. marina* when compared to the other (higher area, less patchy) landscapes. *Zostera marina* may represent higher quality habitat for epifaunal organisms (invertebrates and small fishes) when compared to *H. wrightii* (Micheli et al. 2008), ultimately affecting prey densities for higher trophic level fishes. While the landscape-scale attributes appeared to be more important in predicting differences in fish community diversity and structure, we suspect that fine-scale seagrass characteristics may also play a role in driving the landscape-scale patterns. In addition to the fine-scale seagrass variables

we measured, it is likely that other factors vary along with fragmentation within these natural landscapes that contribute to the patterns of fish assemblage composition. Namely, hydrologic regime is known to be a primary driver of fragmentation in natural seagrass systems (Fonseca and Bell 1998). While we did not quantify differences in hydrologic exposure within our study, it is possible that high exposure represents another environmental filter driving diversity loss within the low area, highly patchy landscapes, especially since some of the lost taxa may be inferior swimmers. Future experimental work is needed to parse out the biotic drivers from the geophysical drivers of diversity loss along fragmentation gradients.

Previous studies examining the effects of habitat configuration (while controlling for area) in seagrass beds have found effects on a number of key population- and community-level processes. For example, Irlandi et al. (1995) found higher predation rates on an infaunal bivalve (*Mercinaria mercinaria*) within naturally patchy seagrass beds (two levels of patchiness, plots up to 440 m²) when compared to a contiguous bed, although seagrass shoot densities decreased as patchiness increased, confounding their interpretation of mechanistic drivers. Hovel (2003) found that patch size and isolation (along with fine-scale seagrass attributes) affected survival of juvenile crabs independently from seagrass cover while statistically controlling for covariation among the variables within 100-m² seagrass plots. Others have used manipulative experiments to test for the effects of habitat configuration on seagrass fauna. Johnson and Heck (2006) used ASUs to experimentally test for the effects of increasing edge:area ratios on densities and secondary production of faunal communities by comparing two levels of "fragmentation" at two different spatial scales (0.05 and 0.20 m²); the effects of each were highly variable across taxa and dependent on habitat context. Hovel and Lipcius (2001) used ASUs to control for variation in fine-scale seagrass attributes, and found that increasing patchiness had negative impacts on adult blue crab (*Callinectes sapidus*) and positive effects on juvenile blue crab survival, although they did not simultaneously examine effects of varying habitat area. Our results build on and expand these previous studies of habitat fragmentation in seagrass habitats by increasing the scale of the landscape examined (by at least two orders of magnitude, allowing us to match the scale at which fragmentation naturally occurs within this system) and increasing the resolution of the habitat area and configuration gradients examined, which may have allowed us to detect the fragmentation threshold in effects on faunal community structure.

Changes in habitat configuration of seagrass habitat within low-area landscapes appeared to be an important driver of the loss of sensitive fish taxa in our study. In particular, in the low-area, highly patchy landscapes, fish would need to use multiple habitat patches to access the same habitat amount as the higher seagrass area or

TABLE 2. Results of Permutational Analysis of Variance (PERMANOVA) testing the effects of habitat (seagrass) area within the landscape, habitat configuration (number of seagrass patches), and fine-scale habitat quality (seagrass biomass) on fish community structure.

Source of variation	df	$\sqrt{\text{ECV}}$	Type II sum of squares	Pseudo F	P
Seagrass area	**1**	**9.7**	**2,170.0**	**5.46**	**0.0002**
Patch number	**1**	**8.4**	**1,542.5**	**3.97**	**0.004**
Seagrass biomass	1	0.0	72.8	0.92	0.9
Seagrass area × patch number	**1**	**9.8**	**1,417.3**	**3.65**	**0.008**
Seagrass area × seagrass biomass	1	3.2	474.3	1.22	0.3
Patch number × seagrass biomass	1	3.7	615.6	1.59	0.2
Residual	14	19.7	5,433.8		

Notes: Seagrass area and number of patches were log-transformed prior to analysis. df, degrees of freedom; $\sqrt{\text{ECV}}$, square-root of estimated component of variation. Boldface type highlights variables that are statistically significant ($P < 0.05$).

more contiguous landscapes. Species that were absent from the low-area, highly patchy landscapes were generally smaller-bodied, epibenthic species, which may be inferior swimmers or have behavioral strategies (e.g., being tightly associated with seagrass structure) that result in a lower propensity to move out into the matrix or colonize new seagrass patches during the adult stage. The limited inter-patch dispersal abilities for these species were supported by our mesocosm experiments, which found epibenthic species to be less likely to move between simulated seagrass patches. While our mesocosm trials were run at a small spatial scale relative to inter-patch distances in the field, it is notable that epibenthic species only rarely emerged from the seagrass patch in which they were initially placed, which was in stark contrast with benthopelagic fishes. Recent work has highlighted emergence from structurally complex habitat as a significant predation gauntlet, even more so than the separate effects of using matrix habitat,

FIG. 3. Nonmetric multidimensional scaling plot representing similarity in community structure of the fish community among landscapes. Each point represents a landscape and landscapes that are closer in space have more similar communities. Point size corresponds to the amount of seagrass within the landscape the color of the point corresponds to the number of patches within the landscape. The black arrows show the direction of the (increasing) gradient for each habitat variable (area, configuration, and fine-scale seagrass biomass) with the length of the arrow being proportional to the correlation between each habitat variable and the ordination space.

following from the concentration of predators along habitat boundaries (sensu Martin et al. 2010). Therefore, the ability of fishes to connect/colonize patches within a fragmented landscape may be largely regulated by rates of emergence from seagrass, which is well defined at our mesocosm scale. Furthermore, dispersal of these epibenthic species at the larval stage may also be limited by their reproductive strategies. For example, *Syngnathus floridae*, like other Syngnathids, have direct developing young, which will greatly reduce dispersal potential relative to species with pelagic larval dispersal (Lourie and Vincent 2004). Similarly, *Opsanus tau*, which were also absent in the low area, highly patchy landscapes, lay demersal eggs and lack a pelagic larval stage (Gray and Winn 1961). Therefore, it seems likely that the poorer dispersal abilities (both larval and adult) for epibenthic species, are at least partially responsible for the loss of these species at the low area, highly patchy landscapes.

Contrasting attributes of our study system to those of previous studies may help reveal the types of systems where we would expect the fragmentation threshold hypothesis to hold. For example, matrix effects may in part explain why our results generally support the hypothesis, while other empirical studies have not. Specifically, matrix habitats, which are useable habitat (although often lower quality), may mitigate some of the negative effects of increased patch number or decreased patch size by facilitating inter-patch movements or increasing the effective habitat area (Ewers and Didham 2006). Many terrestrial studies of fragmentation focus on forest fragments embedded within matrices of secondary forest or agricultural fields (e.g., Gascon et al. 1999), which likely provide more shelter than a completely unvegetated environment. In our study, however, seagrass patches were embedded within an unvegetated, sand matrix, where predation risk is substantially higher and density of prey resources can be multiple orders-of-magnitude lower (Orth et al. 1984, Heck et al. 2003). The lack of suitable shelter for many species may preclude inter-patch movements or the use of sand as a secondary habitat. Therefore, seagrass patches embedded within sand matrices may be

more akin to the theorized habitat/non-habitat matrix and match the assumptions of some simulation modeling studies (e.g., Flather and Bevers 2002).

Our findings agree with the results of previous research that suggest conserving habitat area is paramount to curb biodiversity loss (Fahrig 1997), as landscapes with a high area of seagrass supported more species regardless of their habitat configuration. However, we also found strong evidence that habitat configuration can mediate biodiversity loss when habitat area in the landscape is low, which may be the result of both increasing patch number and within-patch quality. The effects of habitat configuration at low levels of habitat area may be particularly important in systems like ours that closely match the theoretical habitat/non-habitat matrix model and for species with low movement abilities (Fahrig 1998). In such cases, changes in habitat configuration can effectively add insult to injury in terms of biodiversity loss for landscapes where remnant habitat area is low.

LITERATURE CITED

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46.

Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. Multivariate dispersion as a measure of beta diversity. Ecology Letters 9:683–693.

Baillie, C., J. Fear, and F. J. Fodrie. 2015. Ecotone effects on seagrass and saltmarsh habitat use by juvenile nekton in a temperate estuary. Estuaries and Coasts 38:1414–1430.

Betts, M. G., G. J. Forbes, A. W. Diamond, and P. D. Taylor. 2006. Independent effects of fragmentation on forest songbirds: an organism-based approach. Ecological Applications 16:1076–1089.

Boström, C., E. L. Jackson, and C. A. Simenstad. 2006. Seagrass landscapes and their effects on associated fauna: a review. Estuarine, Coastal, and Shelf Science 68:383–403.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18:117–143.

Draper, N. R., and H. Smith. 1998. Applied regression analysis. Third edition. John Wiley and Sons, New York, New York, USA.

Ethier, K., and L. Fahrig. 2011. Positive effects of forest fragmentation, independent of forest amount, on bat abundance in eastern Ontario, Canada. Landscape Ecology 26:865–876.

Ewers, R. M., and R. K. Didham. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews 81:117–142.

Fahrig, L. 1997. Relative effects of habitat loss and fragmentation on population extinction. Journal of Wildlife Management 61:603–610.

Fahrig, L. 1998. When does fragmentation of breeding habitat affect population survival? Ecological Modelling 105:273–292.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Fahrig, L. 2013. Rethinking patch size and isolation effects: the habitat amount hypothesis. Journal of Biogeography 40: 1649–1663.

Flather, C. H., and M. Bevers. 2002. Patchy reaction-diffusion and population abundance: the relative importance of habitat amount and arrangement. American Naturalist 159:40–56.

Fletcher, D. J., and A. J. Underwood. 2002. How to cope with negative estimates of components of variance in ecological field studies. Journal of Experimental Marine Biology and Ecology 273:89–95.

Foley, J. A., et al. 2005. Global consequences of land use. Science 309:570–574.

Fonseca, M. S., and S. S. Bell. 1998. Influence of physical setting on seagrass landscapes near Beaufort, North Carolina, USA. Marine Ecology Progress Series 171:109–121.

Gascon, C., T. E. Lovejoy, R. O. Bierregaard Jr, J. R. Malcolm, P. C. Stouffer, H. L. Vasconcelos, W. F. Laurance, B. Zimmerman, M. Tocher, and S. Borges. 1999. Matrix habitat and species richness in tropical forest remnants. Biological Conservation 91:223–229.

Gray, G. A., and H. E. Winn. 1961. Reproductive ecology and sound production of the toadfish, *Opsanus tau*. Ecology 42:274–282.

Hanski, I., and O. Ovaskainen. 2000. The metapopulation capacity of a fragmented landscape. Nature 404:755–758.

Heck, K., G. Hays, and R. J. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series 253:123–136.

Hovel, K. A. 2003. Habitat fragmentation in marine landscapes: relative effects of habitat cover and configuration on juvenile crab survival in California and North Carolina seagrass beds. Biological Conservation 110:401–412.

Hovel, K. A., and R. N. Lipcius. 2001. Habitat fragmentation in a seagrass landscape: patch size and complexity control blue crab survival. Ecology 82:1814–1829.

Irlandi, E. A., and M. K. Crawford. 1997. Habitat linkages: the effect of intertidal saltmarshes and adjacent subtidal habitats on abundance, movement, and growth of an estuarine fish. Oecologia 110:222–230.

Irlandi, E. A., W. G. Ambrose, and B. A. Orlando. 1995. Landscape ecology and the marine environment: how spatial configuration of seagrass habitat influences growth and survival of the bay scallop. Oikos 72:307–313.

Johnson, M. W., and K. L. Heck Jr. 2006. Effects of habitat fragmentation per se on decapods and fishes inhabiting seagrass meadows in the northern Gulf of Mexico. Marine Ecology Progress Series 306:233–246.

Langsrud, Ø. 2003. ANOVA for unbalanced data: use type II instead of type III sums of squares. Statistics and Computing 13:163–167.

Lindenmayer, D. B., and J. Fischer. 2007. Tackling the habitat fragmentation panchreston. Trends in Ecology & Evolution 22:127–132.

Lomolino, M. V. 2000. Ecology's most general, yet protean pattern: the species-area relationship. Journal of Biogeography 27:17–26.

Lourie, S. A., and A. C. J. Vincent. 2004. A marine fish follows Wallace's line: the phylogeography of the three-spot seahorse (*Hippocampus trimaculatus*, Syngnathidae, Teleostei) in Southeast Asia. Journal of Biogeography 31:1975–1985.

Martin, C. W., F. J. Fodrie, K. L. Heck Jr, and J. Mattila. 2010. Differential habitat use and antipredator response of juvenile roach (*Rutilus rutilus*) to olfactory and visual cues from multiple predators. Oecologia 162:893–902.

McGarigal, K., and S. A. Cushman. 2002. Comparative evaluation of experimental approaches to the study of habitat fragmentation. Ecological Applications 12:335–345.

McGarigal, K., S. A. Cushman, and E. Ene. 2012. Fragstats v4: Spatial pattern analysis program for categorical and continuous maps. University of Massachusetts, Amherst, Massachusetts, USA. http://www.umass.edu/landeco/research/fragstats/fragstats.html

Micheli, F., M. J. Bishop, C. H. Peterson, and J. Rivera. 2008. Alteration of seagrass species composition and function over two decades. Ecological Monographs 78:225–244.

Miller, M. J., and K. W. Able. 2002. Movements and growth of tagged young-of-the-year Atlantic croaker (*Micropogonias undulatus* l.) in restored and reference marsh creeks in Delaware Bay, USA. Journal of Experimental Marine Biology and Ecology 267:15–33.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, G. L. O'Hara, P. Solymos, M. H. H. Stevens, and H. Wagner. 2014. vegan: community ecology package. http://CRAN.R-project.org/package=vegan

Orth, R., K. Heck, and J. van Montfrans. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 7:339–350.

Parker, M., and R. Mac Nally. 2002. Habitat loss and the habitat fragmentation threshold: an experimental evaluation of impacts on richness and total abundances using grassland invertebrates. Biological Conservation 105:217–229.

Potthoff, M. T., and D. M. Allen. 2003. Site fidelity, home range, and tidal migrations of juvenile pinfish, *Lagodon rhomboides*, in salt marsh creeks. Environmental Biology of Fishes 67:231–240.

R Core Team 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Radford, J. Q., A. F. Bennett, and G. J. Cheers. 2005. Landscape-level thresholds of habitat cover for woodland-dependent birds. Biological Conservation 124:317–337.

Saunders, D. A., R. J. Hobbs, and C. R. Margules. 1991. Biological consequences of ecosystem fragmentation: a review. Conservation Biology 5:18–32.

Smith, A. C., L. Fahrig, and C. M. Francis. 2011. Landscape size affects the relative importance of habitat amount, habitat fragmentation, and matrix quality on forest birds. Ecography 34:103–113.

Sogard, S., and K. Able. 1994. Diel variation in immigration of fishes and decapod crustaceans to artificial seagrass habitat. Estuaries 17:622–630.

Summerville, K. S., and T. O. Crist. 2001. Effects of experimental habitat fragmentation on patch use by butterflies and skippers (Lepidoptera). Ecology 82:1360–1370.

Szedlmayer, S. T., and K. W. Able. 1993. Ultrasonic telemetry of age-0 summer flounder, *Paralichthys dentatus*, movements in a southern New Jersey estuary. Copeia 1993:728–736.

Trzcinski, M. K., L. Fahrig, and G. Merriam. 1999. Independent effects of forest cover and fragmentation on the distribution of forest breeding birds. Ecological Applications 9:586–593.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3–14.

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