COASTAL RECREATIONAL FISHING LICENSE

FINAL PERFORMANCE REPORT

Recipient: University of North Carolina at Chapel Hill (PI: J. Fodrie)

Grant Award #: 5118

Grant Title: Effects of landscape setting on the function of NC seagrass meadows as essential fish habitat

Grant Award Period: 7/01/13-12/31/16

Performance Reporting Period: 7/01/16-12/31/16

Project Costs:

Expenditures for the Project (including last 6 mo):

Category	Expenditures
Personnel	$140,668.39 ($2,992.00)
Fringe	$26,957.39 ($268.99)
Travel	$2,559.34
Equipment	$0.00
Supplies	$13,093.12 ($722.11)
Construction	$0.00
Contractual	$79.83
Other	$5,550.00 ($4,475.00)
Total Direct	$188,908.07 ($8,458.10)
Indirect	$28,336.33 ($1,269.71)
TOTAL	$217,244.40 ($9,726.81)

Total Cumulative Expenditures: $217,244.40
Total Remaining Balance: $121.60

Description of Work**:**

We proposed a series of studies that together would allow us to determine how landscape setting (e.g., seagrass patch size, patch connectivity, and micro-scale patch characteristic such as shoot density) affects secondary production within North Carolina sounds and estuaries.

Specifically, we said we would:

(1) identify major landscape types of seagrass habitat using fine scale aerial mapping at select study sites across multiple seasons – supported by standard ground-truthing approaches;

(2) quantify abundance of fishes among landscape types based on monthly trawl and seine sampling;

(3) Determine resource (prey) availability among landscapes based on seine and core sampling;

(4) use tethering experiments (small crabs as prey) to measure the foraging rates of higher consumers among landscape types;

(5) analyze tissue samples of select fishes (e.g., spot, flounders, gag, snappers) to quantify the condition of individuals among landscapes; and

(6) assess the effects of competition (inter- and intra-specific) within various landscape settings on condition and foraging opportunities for select species (e.g., gulf and summer flounder, blue crabs).

Project Status/Work Accomplished:

In addition to our targeted work/experiments to address these questions, we have continued to conduct monthly trawl samples – begun in 2010 - in twelve different sites, covering four distinct landscape types: continuous seagrass beds, fragmented seagrass beds, seagrass beds adjacent to saltmarsh, and un-vegetated mud flats. Below, we detail how we have addressed each primary task (N=6) included in our proposal. In several cases, we have included draft of published manuscripts that describe our approach and accomplishments.

Task 1: Identify major landscape types of seagrass habitat using fine scale aerial mapping at select study sites across multiple seasons – supported by standard ground-truthing approaches.

A description of all seagrass landscapes we identified are included in the report sections below describing our efforts to document fish abundance, prey abundance, predation rates (tethering), and food-web structure. We did conduct extensive, fine-scale ground-truthing of seagrass maps generated from aerial imagery at >20 sites, and detail that work under **Task 2** in the section on fragmentation effects on fish community composition. Shortly after this project begun, however, we learned that Don Field and Amy Urin at NOAA were developing improved automated processing algorithms to convert aerial imagery in to fine-scale seagass cover maps, and were therefore conducting fine-scale ground-truthing already. Therefore, we decided it would be redundant to focus a major effort on ground-truthing at the scale we originally proposed.

We also examined how fine-scale seagrass meadow structure impacts the reproductive ecology of eelgrass, and present our findings here.

Livernois, MC, JH Grabowski, AK Poray, TC Gouhier, AR Hughes, KF O'Brien, LA Yeager, and ***FJ Fodrie*** *(submitted to Aquatic Botany) Effects of habitat fragmentation on Zostera marina seed distribution.*

Abstract

Habitat fragmentation is a process which can alter the spatial configuration and reduce the overall area of a habitat. This generally results in a degradation of habitat functioning. Fragmentation of seagrass (*Zostera marina*) beds has become increasingly common, and it may threaten the valuable ecosystem services they provide. Sexual reproduction through flowering and seed dispersal could contribute to the species' potential resiliency by reducing its vulnerability to fragmentation. We investigated whether the proportion and density of flowering *Z. marina* shoots, and subsequently the density and distribution of seeds, differed between fragmented and continuous beds. Our results revealed that while flowering effort did not differ between the two bed types, seed density was significantly reduced in fragmented versus continuous beds. Further, seed distributions were altered in fragmented beds when compared to continuous beds, both within and directly outside the bed's boundaries. Seagrass patch size positively influenced seed density, with lower seed densities in small patches. Fragmented beds consistently contained fewer seeds per-unit-area than continuous beds, regardless of bed seagrass area and flowering effort. Collectively, these results emphasize the vulnerability of *Z. marina* to habitat fragmentation by demonstrating a negative effect on seed density and an impact on seed distribution, which likely reduces the potential advantages of sexual reproduction for bed growth and resiliency to perturbations.

1. Introduction

Habitat fragmentation is the process by which a continuous habitat is transformed into increasingly smaller, more numerous, and more isolated patches, resulting in a mixed landscape of structured habitat and unstructured matrix area that functions differently than the original continuous habitat (Wilcove et al., 1986). Fragmentation, which often occurs concomitantly with habitat loss, generally has negative effects on biodiversity, genetic diversity and population growth (Fahrig, 2003, Haddad et al., 2015). This process occurs in terrestrial, aquatic and marine communities. A marine species that is particularly vulnerable to fragmentation is the widespread submerged marine angiosperm, *Zostera marina* (eelgrass), commonly found in coastal regions and in estuaries ranging from temperate to near arctic waters in both the Atlantic and Pacific.

The productive and structurally complex habitat created by *Z. marina* offers a multitude of ecosystem services, such as providing nursery and foraging grounds, promoting nutrient cycling, increasing local sedimentation rates, and protecting against coastal erosion (Thayer et al., 1978; Thayer & Phillips, 1977). Though fragmentation of *Z. marina* can occur through natural disturbances such as wave energy, grazing, and wasting disease, anthropogenic forces have caused an estimated decline of 29% of

seagrass area globally via fragmentation and outright bed loss (Waycott et al., 2009). Through reduced water quality, mechanical damage, and other indirect impacts to coastal waters, human disturbances have resulted in a degradation of the critical ecosystem services seagrasses provide (Short & Willy-Echeverria, 1996; Orth et al., 2006).

Fragmentation of *Z. marina* beds results in bare, unvegetated spaces that must be colonized via clonal or sexual reproduction. *Z. marina* reproduces through vegetative growth by rhizome elongation, as well as sexually by the dispersal of seeds for germination of new seedlings (Orth et al., 1994). Though beds rely heavily on vegetative reproduction for maintenance and survival, sexual reproduction through seed dispersal is thought to contribute substantially to genetic diversity, patch development, and recovery of disturbed areas (Greve at al., 2005; Orth et al., 2006). Monoecious flowering shoots emerge in the late winter, and develop until ambient water temperatures reach approximately 20°C in the summer, signaling a release of the seeds (Moore & Short, 2006). The seeds have a hard outer coating, exhibit a distinct dormancy period, and remain viable in the transient seed bank for approximately 12 months (Orth et al., 2000).

Z. marina seeds are likely limited to primarily short-distance dispersal strategies (Orth et al., 1994), contributing to the maintenance and development of the parent bed. When seeds are released from the flower, they are negatively buoyant and drop quickly to the sediment surface (Harwell & Orth, 2002). Seeds have been shown to disperse <14 m on the bare sediment surface after release (Orth et al., 1994), but this distance is highly variable and can be dependent on local micro-topography (Luckenbach & Orth, 1999). Approximately 5-13% of seeds are released with a small air bubble, and can float in the water column for at least 40 minutes with the potential to travel up to 200m before falling to the benthos (Churchill et al., 1985). Long-distance dispersal by 'rafting' of detached flowering shoots is thought to account for only a small proportion, <6%, of the seeds produced by a single meadow (Hosokowa et al., 2015). Therefore, most seeds likely remain within or near their parental source bed. Abiotic factors such as wind and currents generally drive the dispersal of seeds after their initial release, but biotic mechanisms such as crab predation can also affect the distribution and density of seeds within meadows (Fishman & Orth, 1996, Infantes et al., 2016).

Throughout much of its range, the dominant life history strategy of *Z. marina* follows a biannual flowering pattern. However, some beds in particularly stressful environments (i.e., high summer temperatures, ice scour, heavy storms) exhibit an annual mode of increased flowering followed by extensive die-off after the flowering season (Jarvis et al., 2012). In fully annual populations, the standing crop is produced almost entirely from seeds each year, requiring the production of an extensive seed bank to ensure persistence (Jarvis & Moore, 2010; Jarvis et al., 2014). Both perennial and annual cycles have been observed in *Z. marina* beds in North Carolina, near the southernmost limit of the species' geographic distribution, where summer water temperatures reaching over 30°C surpass the species' thermal tolerance (Jarvis et al., 2012). Understanding the response of sexual reproduction and seed bank dynamics to fragmentation in North Carolina would be particularly informative considering the stressful summer temperatures and seasonal above ground die-backs the species endures in this region. Prolonged exposure to high temperatures has been shown to result in losses of *Z. marina* shoot density and increased mortality (Bintz et al., 2003;

Nejrup & Pedersen, 2008), which could result in fragmentation of a continuous meadow over time. Sexual reproduction may mitigate these seasonal losses of biomass, as well as provide critical genetic diversity that enhances the species' ability to respond to disturbances (Ehlers et al., 2008; Hughes & Stachowicz, 2004). However, Reusch (2003) found that seed production and pollination potential of Z. *marina* was negatively affected by fragmentation and manipulation of shoot densities in the parent population.

The purpose of this study was to determine whether sexual reproduction differs between continuous and fragmented beds at the lower latitudinal limit of *Z. marina's* range. Field surveys during and after the flowering season were designed to quantify and compare flowering effort and subsequent seed bank composition across both landscape types. We hypothesized that with the stress of high temperatures in this region exacerbating seasonal diebacks, potential increased flowering effort in fragmented beds may allow them to expand existing patches and colonize bare spaces through the production and retention of seeds. Additionally, we expected to record differences in the settlement, density, and distribution of seeds between fragmented and continuous landscapes. This study explores the potential vulnerability or resiliency of *Z. marina* to habitat fragmentation, and describes the impacts of bed-wide characteristics on seed bank dynamics.

2. Methods

2.1 Study area

The seagrass beds included in this study were located in Back Sound, North Carolina, a shallow coastal estuarine system with an average water depth of 2m and a semi-diurnal tidal range of 0.7m (Fig. 1A.). Salinities fluctuate from 24 to 36 ‰, and yearly water temperatures range from approximately 4 to 30 ^{o}C (Kenworthy et al., 1982; NOAA Weather Station BFTN7, 2014). Seagrass beds in Back Sound are often composed of mixed stands of *Z. marina* and *Halodule wrightii* (shoalgrass). *H. wrightii* is abundant in coastal regions along the southeastern U.S. Atlantic coast, with its northern range limit in North Carolina, and its presence potentially influences *Z. marina* seed bank dynamics.

A total of 12 beds were selected based on a priori visual inspection of landscape configuration, 5 of which appeared continuous and 7 of which were fragmented, (See Table 1 for site descriptions). Separate landscapes were defined as a bed of seagrass separated from other seagrass habitat by an unvegetated distance of at least 25 m. Landscape size ranged from 10,918.31 to 108,840.81 m^2 for continuous beds and from 4,335.63 to 31,464.99 m^2 for fragmented beds. Bed types were defined as continuous if the percentage of seagrass area contained within the bed's largest patch (largest patch index, LPI) was greater than 80%, or fragmented if the LPI was less than 75%. The average LPI was 93.85% ± 2.89% (mean ± SE) for continuous beds and 44.72% ± 7.38% for fragmented beds. On average, continuous and fragmented beds contained 19.8 ± 15.15 and 52.29 ± 12.30 discrete seagrass patches, respectively. Fragmentation metrics such as LPI and patch number were determined using the program FRAGSTATS v.4 (McGarigal et al., 2012). As a proxy for potential wave energy, average fetch (from the cardinal directions) was calculated as 2697 ± 239 m for continuous beds and 2477 ± 319 m for fragmented beds.

2.2 Aerial photograph analysis

In order to quantify landscape characteristics (area, percent cover, etc.) at each site, orthorectified aerial photographs of the study area were obtained and imported into ArcGIS for analysis. The photos were taken by the North Carolina Department of Transportation on May 26, 2013, and were organized by the Albemarle-Pamlico National Estuary Partnership (APNEP). Each individual site was digitized by manually outlining visible seagrass within the extent of the bed, excluding any bare spaces between or within patches. Seagrass area was calculated as the additive areas of all seagrass polygons present within the landscape. Core locations were recorded from a handheld GPS at the time of sampling, the coordinates of which were later entered into ArcGIS and overlaid atop the aerial photographs using the same coordinate system. Excluding cores from bare sand, the area (m^2) of the seagrass patch each core was collected from was quantified by manually outlining the extent of the patch.

2.3 Flowering effort

To quantify the average shoot density of *Z. marina* in each bed as well as the ratio of flowering to vegetative shoots, six 0.063 m^2 quadrats were haphazardly placed over vegetated substrate, and all seagrass shoots present within each quadrat were removed by the roots. *Z. marina* flowering and vegetative shoots were separated and counted in the lab. Sampling occurred in May 2014, when the flowering season was determined to be at its approximate peak based on observations of flowers in the region, and when water temperatures reached the optimal range for flowering, 20-21°C (Moore & Short, 2006).

2.4 Sediment coring for seeds

To sample the distribution and density of seeds at specified positions within each bed, 10-cm diameter sediment cores were taken to a depth of approximately 10 cm, as *Z. marina* seeds are generally buried no deeper below the sediment surface (Morita et al., 2007). Sediment core samples were collected in July 2014, after the flowering season had ended and sufficient time had passed for all seeds to settle. In continuous beds, two transects ran from the center of the bed to the edge. The first transect direction was selected haphazardly, with the second being approximately perpendicular to the first. In each transect, one core sample was taken at the starting point, located at the approximate center of the bed; a second core sample was collected halfway between the center and the edge of the bed, the location of which differed for each bed based on its size; and a third core sample was taken at the edge of the bed (Fig. 1B.).

In fragmented beds, one core sample was taken within each of two different vegetated patches near the center of the bed; within each of two vegetated patches along the edge of the bed; within each of two bare, unvegetated areas in the interior region of the bed; and in each of two bare areas along the edge of the bed (i.e., 8 cores per bed; Fig. 1C.). In both continuous and fragmented beds, two additional transects were used to sample directly outside of the bed. These transects ran perpendicular to the edge of the bed, and one core sample in each transect was collected at the following distances away from the edge of the bed: 0, 2.5, 5, 7.5, 10, and 15 meters (Fig. 1B.,C.).

Each core was wet-sieved in the field in 400-micron mesh bags to wash away sediment. Remaining coring contents were taken to the lab where they were frozen until processed, which involved individually examining them under a dissecting microscope. Any seeds, whether they were fully intact or the casing of an already germinated or dead seed, were identified and counted. *Z. marina* and *H. wrightii* shoots in each core were also counted.

2.5 Data analysis

2.5.1 Site characteristics

To determine whether the bed types contained similar overall area and percent cover of seagrass, two-sample t-tests were utilized to compare those variables between fragmented and continuous beds. Bed areas were log-transformed to avoid violating the assumption of normality; no transformations were necessary for percent cover. A generalized linear mixed effects model (Bolker et al., 2009) with a poisson error distribution was used to determine whether the density of vegetative *Z. marina* shoots differed between fragmented and continuous beds. In this analysis, bed type (fragmented or continuous) was treated as a fixed effect and site (bed) as a random effect. This test was repeated to compare the density of *H. wrightii* shoots between bed types. For these and all subsequent generalized linear models, statistical significance was assessed via Wald chi-squared tests using type II sum of squares to account for the slightly unbalanced nature of the data.

2.5.2 Flowering effort

The proportion of flowering *Z. marina* shoots was determined by dividing the number of flowering shoots by the total number of *Z. marina* shoots in each quadrat. We used a generalized linear mixed effects model with a binomial error distribution to determine whether flowering proportion differed between bed types. In this analysis, bed type was treated as a fixed effect and site (bed) as a random effect. Similarly, we used a generalized linear mixed effects model with a poisson error distribution to determine whether density of flowering *Z. marina* shoots (number of shoots per 0.25 m^2) differed between bed types, with bed type serving as the fixed effect and site as a random effect.

2.5.3 Sediment coring for seeds

Next, a generalized linear mixed effects model (poisson distribution) was performed using data from only vegetated areas within all sites to compare seed counts from distinct positions between both bed types. This model incorporated seed count per core sample as the response variable, bed type and position within the bed (center or edge) as fixed explanatory effects, and position nested within site as the random explanatory effects. A separate generalized linear mixed effects model (poisson distribution) was performed using data from fragmented sites only to compare seed counts from different positions within the bed, as well as among core types, vegetated patch or bare sand. This model incorporated seed count as the response, position within the bed (center or edge) and type of core (patch or sand) as fixed explanatory effects, as well as site and position nested within site as the random explanatory effects.

To determine the relationship between seed density and distance away from the bed edge, we fit separate generalized linear models for each bed type. The quasipoisson error distribution was utilized to account for overdispersion of the count data. Similar analyses were performed to identify the relationship between *Z. marina* and *H. wrightii* shoot densities within each sediment core, as well as between each of those species and the associated number of *Z. marina* seeds.

We used a generalized linear model (quasipoisson distribution) to characterize the relationship between *Z. marina* seed count per core and the size (area) of the seagrass patch the core was taken from. Patch areas were log-transformed due to non-normality and high variability, as the sizes ranged from <5 m^2 to >60000 m^2.

We used generalized linear models (quasipoisson distribution) to quantify the relationship between flowering *Z. marina* shoots per 0.25 m^2 and seagrass bed area, as well as the number of *Z. marina* seeds per 0.25 m^2 and bed area. Bed areas were log-transformed due to skew and high variability. To investigate the relationship between the average density of flowering *Z. marina* shoots to the average density of *Z. marina* seeds within both fragmented and continuous beds, a linear regression was performed. The residuals were then extracted for each data point (each site), representing the difference between the observed values and those predicted by the linear regression. Next, the residuals of fragmented and continuous beds were compared via a two-sample t-test.

For all statistical analyses, the open-source statistical software R was utilized (R Development Core Team, 2008), and analyses were conducted using the 'lme4' and 'car' packages (Bates et al., 2015; Fox & Weisberg, 2011).

3. Results

3.1 Site characteristics

Overall area of seagrass in fragmented beds (8144 ± 1461 m^2, mean ± SE) was lower than continuous beds (27747 ± 10502 m^2, P = 0.04). Percent cover of seagrass was higher in continuous beds (80% ± 7.9%) than fragmented beds (42% ± 3%, P < 0.01). The density of vegetative *Z. marina* shoots did not differ significantly between fragmented (438.4 ± 34.8 shoots per 0.25 m^2) and continuous beds (478.1 ± 30.1 shoots per 0.25 m^2, P = 0.53). Similarly, *H. wrightii* shoot density was not significantly different between fragmented (480.2 ± 75.3 shoots per 0.25 m^2) and continuous beds (775.2 ± 87.6 shoots per 0.25 m^2, P = 0.15).

3.2 Flowering effort

Flowering effort did not differ between the two bed types. The average proportion of *Z. marina* flowering shoots to total *Z. marina* shoots was not significantly different between continuous (<0.1 ± 0.1) and fragmented beds (0.1 ± 0.1; Fig. 2A., P = 0.65). The average density of *Z. marina* flowering shoots was also similar between bed types, with continuous beds containing 51.5 ± 8.2 flowering shoots per 0.25 m^2 and fragmented beds containing 60.9 ± 10.6 flowering shoots per 0.25 m^2, on average (Fig. 2B., P = 0.92).

3.3 Sediment coring for seeds

Differences in seed density and distribution were observed between fragmented and continuous beds. There were fewer seeds in vegetated areas within fragmented beds (166.1 ± 28.2 per 0.25 m^2) than in continuous beds (336.0 ± 41.4 per 0.25 m^2) on average (Fig. 3A., $P = 0.01$). There was no significant effect of position within the bed, center or edge, on seed density in vegetated areas in both bed types (Fig. 3A., $P = 0.16$). Within fragmented beds, there were fewer seeds in bare sand between seagrass patches (63.7 ± 16.1 per 0.25 m^2) than in vegetated areas (166.1 ± 28.2 per 0.25 m^2) on average (Fig. 3B., $P < 0.01$).

Patterns of seed density outside of the bed edge differed between fragmented and continuous beds. There was no significant difference in seed density among any positions along the 15-meter transects outside of fragmented beds (Fig. 4B., (β (model parameter estimate) = -0.03) $P = 0.37$). Conversely, outside of continuous beds, there was a negative relationship between seed density and distance from the bed edge, indicating higher seed counts at the 0m mark compared to the remainder of the transect (Fig. 4A., ($\beta = -0.09$) $P < 0.01$).

When considering the other species of seagrass in this system, *H. wrightii*, multiple patterns emerged. No significant relationship was found between *H. wrightii* shoot count per 0.25 m^2 and *Z. marina* shoot per 0.25 m^2 (Fig. 5A., ($\beta = -0.04$) $P = 0.08$). There was, however, a positive relationship between *Z. marina* shoot count per 0.25 m^2 and the number of *Z. marina* seeds per 0.25 m^2 (Fig. 5B., ($\beta = 0.05$) $P = 0.02$). Conversely, the relationship between *H. wrightii* shoot count per 0.25 m^2 and *Z. marina* seed count per 0.25 m^2 was negative (Fig. 5C., ($\beta = -0.02$) $P = 0.02$).

A positive relationship was observed between *Z. marina* seed density per 0.25 m^2 and the size (m^2) of the seagrass patch the cores were taken from (Fig. 6, ($\beta = 0.23$) $P < 0.01$). There was no significant relationship between seagrass bed area (m^2) and *Z. marina* flowering shoot density (Fig. 7A., ($\beta = -0.31$) $P = 0.34$). There was also no significant relationship between seagrass bed area (m^2) and *Z. marina* seed density per 0.25 m^2 (Fig. 7B., ($\beta = 0.47$) $P = 0.09$). However, there was a difference between fragmented and continuous beds in the residual values extracted from the linear regression comparing average density of flowering *Z. marina* shoots to average density of *Z. marina* seeds. The residuals were positive on average for continuous beds, meaning they fell above the regression line, and the opposite was true for fragmented beds, indicating that fragmented beds contained fewer seeds than continuous beds with similar flowering shoot densities (Fig. 7C., $P < 0.01$).

4. Discussion

Our results indicate that although flowering effort did not differ between fragmented and continuous *Z. marina* meadows, seed distributions and densities were significantly affected by fragmentation. In beds where the eelgrass was fragmented, seed density in vegetated areas was lower than within continuous beds, and seeds were at their lowest densities in bare areas that were devoid of any adult plants. These results illustrate the vulnerability of *Z. marina* to fragmentation, as a reduced seed bank in fragmented beds could impede patch development and colonization of bare areas.

Habitat fragmentation did not have a detectable effect on the effort that *Z. marina* expended on flowering, with the average proportion and density of flowering shoots

differing between bed types by only 0.02 flowering shoots/total shoots and 9.5 flowering shoots per 0.25 m^2, respectively (Fig. 2). Though not observed in this study, flowering effort can increase in response to high temperature stress (De Cock, 1981, Potouroglou et al., 2014), heightened wave activity, and increasing water depth (Fonseca & Bell, 1998), three major sources of stress or disturbance that can drive fragmentation. Fonseca and Bell (1998) found that seagrass bed landscape characteristics such as bed coverage and shape (perimeter to area ratio) were strongly correlated with wave exposure and current speed in Back Sound, indicating the fragmented beds in our study may exist in regions experiencing stronger hydrodynamic forcing than the continuous beds. However, the flowering effort of both bed types, though they exist in the highest range of the species' tolerable temperatures and in a variable estuarine system, did not appear to be affected differently by these environmental factors.

Eelgrass meadows have the capacity to significantly reduce current velocities, and patch edges are especially important in controlling current flow (Fonseca et al., 1982) For example, Peterson et al. (2004) found current velocities in high density continuous eelgrass can be reduced up to 60% at 0.25 m into the bed. This influence of eelgrass beds on local hydrodynamics may be a critical factor in controlling the dispersal of seeds after their release, as the density of seeds found in vegetated areas within our fragmented beds was significantly lower than within the continuous beds (Fig. 3A.). This suggests that seeds produced in fragmented beds may experience stronger or more variable currents that remove them from vegetated areas more readily than those released within continuous beds, where current velocities may be lower or more consistent. Therefore, the slowing and directing of currents by the eelgrass itself, in addition to the bed's location within the estuary (as discussed previously, Fonseca & Bell, 1998), may drive differences in hydrodynamics, and thus seed distribution, between fragmented and continuous beds.

Higher seed densities were found at the outer edge of continuous beds as compared to farther away from the bed (Fig. 4A.). Thus, continuous beds appear to be retaining a significant amount of the seeds produced near the bed edge. In contrast, seeds were found at statistically equal densities from 0 to 15 meters away from the edge of fragmented beds (Fig. 4B.). The even distribution of seeds outside of these fragmented beds lends support to the hypothesis that they may be experiencing more dynamic and stronger flow regimes than they would at the continuous sites. Since short-distance dispersal accounts for a large proportion (i.e., >90%) of the settled seeds within and near a bed (Hosokawa et al., 2015), the majority of these seeds found outside of each bed were likely produced there, not from a different bed in the region.

The lowest seed densities across both meadow types occurred in the bare spaces within fragmented beds (Fig. 3B.). While it is unclear what density of seeds is required to successfully colonize a bare region, it is possible that the observed densities are low enough to be limiting eelgrass survival in those areas. For example, Jarvis and Moore (2010) determined that following a 2005 large-scale mortality event of *Z. marina* in the York River (VA, USA), seed germination accounted for the majority of recovery the following year. However, the available seed bank density in that region was reduced greatly to a maximum density of 12.5 ± 6 seeds per 0.25 m^2 in 2006. A subsequent die-off in 2006 resulted in an almost complete lack of recovery the following year, suggesting that seed availability was perhaps not high enough to support recolonization.

Therefore, the reduced density of seeds in inter-patch bare spaces (63.7 ± 16.1 per 0.25 m^2) compared to vegetated areas within fragmented beds observed in this study may be similarly limiting the colonization potential of seedlings. Although that density is nearly 5-times higher than the density reported in the York River, germination success of *Z. marina* seeds is variable, and can be as low as <5% in natural systems (Orth et al., 2003), suggesting that generally low seed densities may result in minimal to no seedling production. Environmental conditions in those sandy, exposed areas may not have been conducive to germination, perhaps explaining why adult plants were not already present. Any seedlings produced in bare spaces would likely exist as single shoots, or in very small low-density patches, which may not survive to the following year (Ramage & Schiel, 1999; Worm & Reusch, 2000). For instance, *Z. marina* patches containing <32 shoots have been found to experience intense mortality and rapid turnover (Olesen & Sand-Jensen, 1994), so the colonization potential of a few seedlings in inter-patch bare spaces is likely quite low.

The size of a seagrass patch positively influenced seed density, with larger patches generally containing higher densities of seeds (Fig. 6). However, there was high variability in seed densities within large patches, while small patches generally contained low seed densities. This “wedge-shaped” pattern in the data indicates that seed density is partially influenced by patch size, but there are other unmeasured factors controlling the distribution and density of seeds in fragmented landscapes, especially in large patches (Cade et al., 1999). This result highlights the importance of investigating habitat fragmentation from both a patch and landscape scale. The effects of habitat patch size may be influential on variables such as seed density and distribution, but other large-scale factors such as hydrodynamics and habitat configuration are also critically important in understanding those patterns.

Despite similar patterns in flowering effort and seed density compared to their overall vegetated area (Fig. 7A.,B.), fragmented beds consistently contained fewer seeds than continuous beds. This finding indicates that flowering effort, regardless of seagrass bed area, is generally not a strong predictor of future seed-bank densities (Fig. 7C.). Differences in total landscape seagrass area and cover did vary among fragmented and continuous seagrass beds, which likely influenced the total production of seeds. However, our results suggest that the overall availability of flowering shoots in a bed does not definitively determine the number of seeds retained in the sediment seed bank. Though we did not examine pollination success, it is possible that flowering shoots in isolated patches within fragmented beds could be experiencing pollen limitation, and thus reduced seed production per shoot. This pattern has been observed in terrestrial plants (Cunningham, 2000; Knapp et al., 2001), and Reusch (2003) detected a similar negative effect of fragmentation on *Z. marina* reproductive output. Therefore, depressed pollination, separate from flowering effort, may have influenced the seed production of fragmented beds, followed by potential physical factors affecting the retention of those seeds after they were released.

A negative relationship emerged between *H. wrightii* shoot density and *Z. marina* seed density (Fig. 5C.). Though we did not detect a relationship between *H. wrightii* and *Z. marina* shoot densities (Fig. 5A.), environmental factors likely influence the distribution and densities of these two species in Back Sound, one of the few regions on the East coast where they co-exist. For example, *H. wrightii* can survive in shallower

waters than *Z. marina,* and can even tolerate aerial exposure at low tide (Thayer et al., 1984). In areas where environmental conditions are poor for *Z. marina*, *H. wrightii* may thrive, and vice versa. Additionally, while *Z. marina* is abundant in the cooler months through the winter and spring, it senesces in the summer heat while *H. wrightii* remains year-round. Our results suggest that if *Z. marina* seeds are dispersed to a region with high *H. wrightii* density, they do not get entangled and settle in the structure *H. wrightii* provides. This lack of a beneficial interaction between the two species may have influenced the low observed *Z. marina* seed densities in areas with high *H. wrightii* density, in addition to the abiotic factors driving the distribution of each species in the estuary. Further investigation is required to understand how the interaction of these species may affect *Z. marina* sexual reproduction across a range of environmental conditions.

The negative effect of habitat fragmentation on seed production and retention observed in this system aligns with many similar studies in terrestrial plant communities (Aguilar et al., 2006). Habitat fragmentation has far-reaching effects on not only the reproductive success of plant communities (Aizen & Feinsinger, 1994, Steffan-Dewenter & Tscharntke, 1999), but it can also result in a reduction of biodiversity and an alteration of interactions with associated faunal communities (Debinski & Holt, 2000, Yeager et al., 2016). However, these effects are highly variable in seagrass ecosystems, and the influence of habitat fragmentation on flowering dynamics and seed dispersal is poorly understood (Boström et al., 2006).

Reduced seed densities in fragmented *Z. marina* beds could impede patch development and colonization of barren areas. These findings have implications for the conservation and management of this critically valuable habitat. With an estimated 29% global loss in the historical abundance of seagrasses (Waycott et al., 2009), growth and maintenance of meadows is of utmost importance. In *Z. marina*'s southernmost limit in North Carolina, where summer water temperatures already reach the species' thermal tolerance, any future increases in temperature stress or wave activity may pose significant threats to the species' persistence (Carr et al., 2012). Sexual reproduction can provide an alternative to vegetative growth under these stressful conditions, but it is unclear if barren areas will be colonized by seedlings without active restoration efforts. Our results indicate how eelgrass seed production may be affected by habitat fragmentation, which has implications for not only the health and persistence of the species itself, but also its role as a critical estuarine habitat that provides several valuable ecosystem services.

Acknowledgements

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Table 1. Descriptions of individual sites, including site name, type (fragmented or continuous), overall bed area (a polygon encompassing all vegetated and bare space within the bed (m^2)), area of seagrass within the site (m^2), seagrass percent cover (%), average vegetative *Z. marina* per 0.25 m^2 (± 1 SE), average flowering *Z. marina* per 0.25 m^2 (± 1 SE), average vegetative *H. wrightii* per 0.25 m^2 (± 1 SE), average *Z. marina* seeds per 0.25 m^2 (within vegetated substrate only (± 1 SE)), average fetch (meters, calculated from N, S, E, and W orientations), number of discrete seagrass patches, and largest patch index (% of area contained within the largest patch).

Site	**Type**	**Bed Area**	**Seagrass Area**	**Percent Cover**	**Vegetative *Z. marina***	**Flowering *Z. marina***	***H. wrightii***	***Z. marina* Seeds**	**Fetch**	**Patch #**	**LPI**
C1	Cont	14566.66	13899.38	95.42	292 ± 15	20 ± 5	1093 ± 246	319 ± 58	3071.24	2	90.58
C2	Cont	59802.79	44362.67	74.18	471 ± 79	33 ± 16	978 ± 143	329 ± 92	2873.62	5	98.77
C3	Cont	11401.35	11323.11	99.31	463 ± 31	22 ± 5	886 ± 160	138 ± 100	2945.17	1	100
C4	Cont	10918.31	8353.58	76.51	601 ± 51	121 ± 13	217 ± 66	504 ± 106	1729.66	11	95.61
C5	Cont	108840.81	60801.03	55.86	565 ± 69	61 ± 9	701 ± 132	319 ± 56	3298.18	80	84.28
F1	Frag	23718.44	6260.04	26.39	427 ± 68	45 ± 3	523 ± 133	104 ± 68	3036.36	119	38.46
F2	Frag	31464.99	13007.67	41.34	326 ± 72	13 ± 3	590 ± 100	106 ± 28	1999.12	43	61.48
F3	Frag	27810.62	11676.76	41.99	409 ± 69	28 ± 8	28 ± 8	88 ± 27	3095.24	39	48.56
F4	Frag	12652.67	5231.86	41.35	621 ± 85	136 ± 26	243 ± 67	358 ± 87	1914.57	49	24.25
F5	Frag	22157.81	9900.56	44.68	330 ± 99	37 ± 13	971 ± 212	191 ± 84	3128.94	58	19.35
F6	Frag	4335.63	1994.40	46.00	312 ± 58	20 ± 5	894 ± 286	48 ± 16	3162.26	13	46.43
F7	Frag	17012.03	8938.02	52.54	645 ± 104	148 ± 41	113 ± 48	279 ± 53	1007.47	45	74.50

Figure 1: Description of study area and sediment coring methods. (A.) Map of study area, Back Sound, North Carolina, with individual study sites marked. Depiction of sediment coring methods in continuous (B.) and fragmented (C.) beds, with example core positions represented. Within-grass cores are depicted as solid white circles, while within-sand cores are depicted as circle-crosses.

Figure 2: Flowering effort of *Z. marina* within continuous and fragmented beds. (A.) Comparison of the average proportion of flowering *Z. marina* shoots to vegetative *Z. marina* shoots between continuous and fragmented beds ($P = 0.65$). (B). Comparison of the average density of *Z. marina* shoots per 0.25 m^2 between continuous and fragmented beds ($P = 0.92$). Error bars represent ± 1 SE.

Figure 3: Effect of habitat fragmentation on within-bed *Z. marina* seed densities. (A.) Average seed densities across fragmented and continuous beds, from cores in vegetated areas exclusively ($P = 0.01$). Average seed densities at specific positions within the bed, center or edge, compared across bed types ($P = 0.16$). (B.) Average seed density compared between vegetated and bare areas within fragmented beds ($P < 0.01$). Comparison of average seed densities at specific positions within the bed, center or edge, within fragmented beds ($P = 0.27$). Error bars represent ± 1 SE.

Figure 4: Influence of habitat fragmentation on *Z. marina* seed density outside of the bed edge. Seed density along a transect beginning at the outer edge of the bed, with increasing distance from continuous beds (A., $P < 0.01$), and fragmented beds (B., $P = 0.37$). Generalized linear regression line (quasipoisson error distribution) represented in panel A.

Figure 5: Interaction between *H. wrightii* and *Z. marina*. (A.) Comparison of *H. wrightii* shoot count per 0.25 m^2 and *Z. marina* shoot count per 0.25 m^2 ($P = 0.08$). (B.) Positive relationship between *Z. marina* seed count per 0.25 m^2 and *Z. marina* shoot count per 0.25 m^2 ($P = 0.02$). (C.) Negative relationship between *Z. marina* seed count per 0.25 m^2 and *H. wrightii* shoot count per 0.25 m^2 ($P = 0.02$). Panels B and C include generalized linear regression line (quasipoisson error distribution).

Figure 6: *Z. marina* seed density across varying seagrass patch sizes. Positive relationship between *Z. marina* seed density per 0.25 m^2 and seagrass patch size (m^2 log transformed, $P < 0.01$), with the generalized linear regression line (quasipoisson error distribution) represented.

Figure 7: Effect of seagrass area and *Z. marina* flowering effort on *Z. marina* seed densities. (A.) Flowering *Z. marina* shoot density across seagrass bed areas (m^2, log transformed, ($P = 0.34$). (B.) Comparison of seagrass bed area (m^2, log transformed) and *Z. marina* seed density per 0.25 m^2 ($P = 0.09$). (C.) Linear regression comparing flowering *Z. marina* shoot density and seed density per 0.25 m^2, with a significant difference in the residuals of fragmented and continuous beds ($P < 0.01$).

Figure 1.

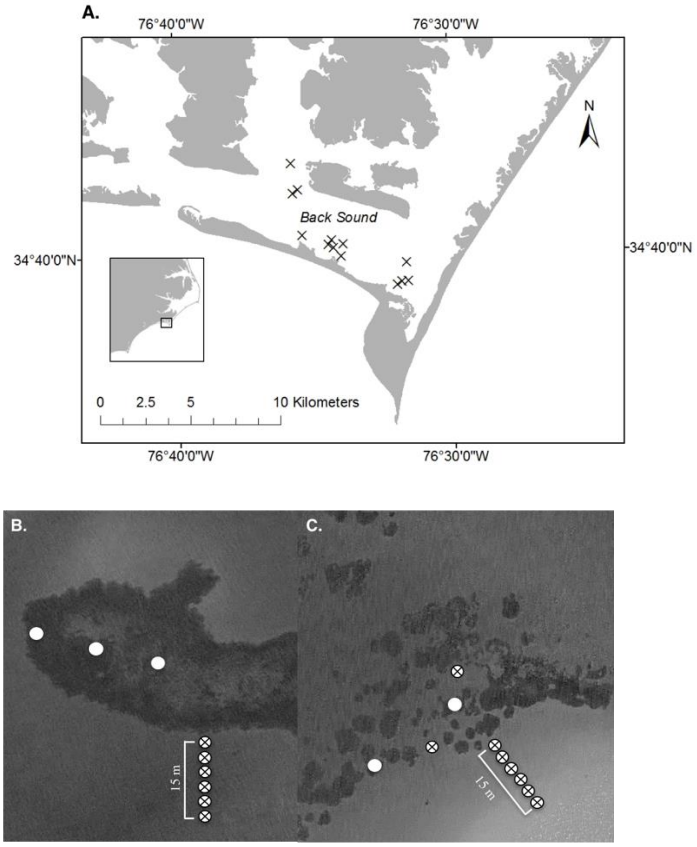

A.
76°40'0"W
76°30'0"W
N
Back Sound
34°40'0"N
34°40'0"N
0 2.5 5 10 Kilometers
76°40'0"W
76°30'0"W
B.
15 m
C.
15 m

Figure 2.

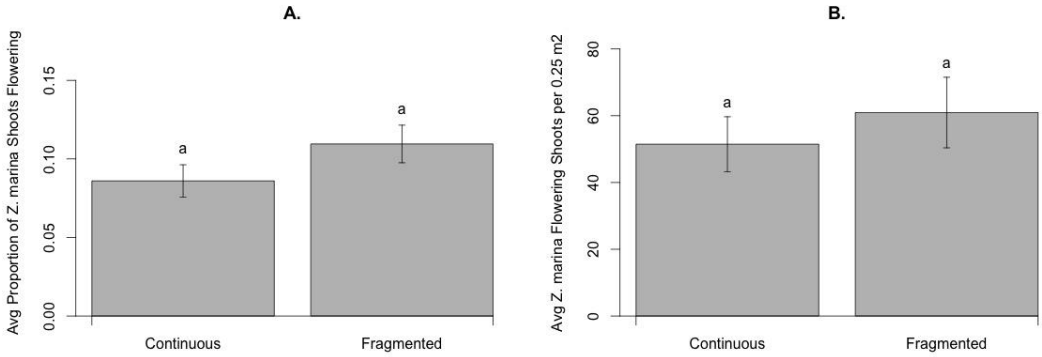

A.
Avg Proportion of Z. marina Shoots Flowering
0.00
0.05
0.10
0.15
a
a
Continuous
Fragmented
B.
Avg Z. marina Flowering Shoots per 0.25 m2
0
20
40
60
80
a
a
Continuous
Fragmented

Figure 3.

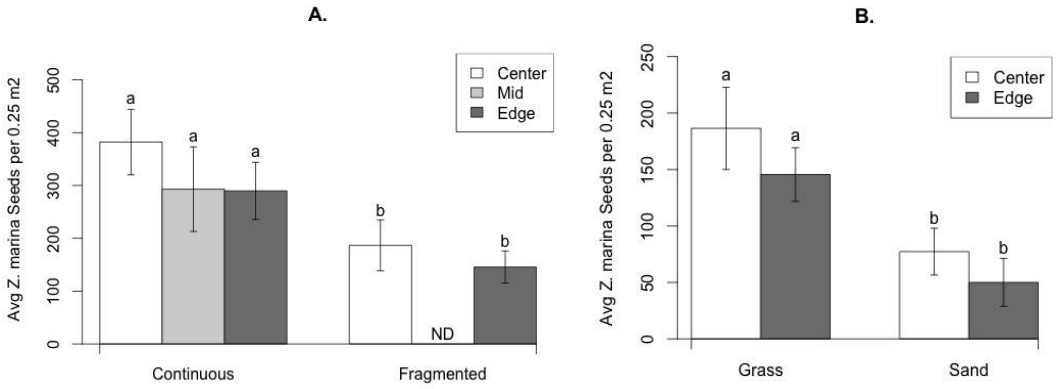

A.
Avg Z. marina Seeds per 0.25 m2
0
100
200
300
400
500
Center
Mid
Edge
a
a
a
b
ND
b
Continuous
Fragmented
B.
Avg Z. marina Seeds per 0.25 m2
0
50
100
150
200
250
Center
Edge
a
a
b
b
Grass
Sand

Figure 4.

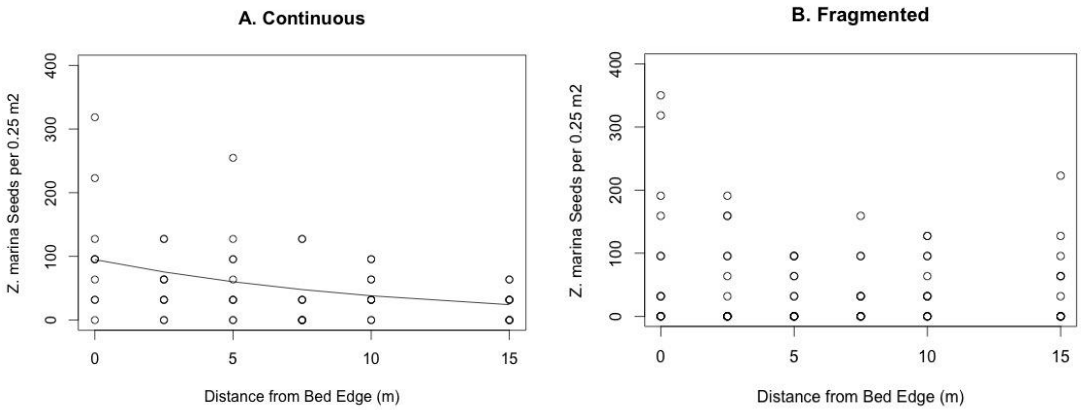

A. Continuous
B. Fragmented
Z. marina Seeds per 0.25 m2
0
100
200
300
400
0
5
10
15
Distance from Bed Edge (m)

Figure 5.

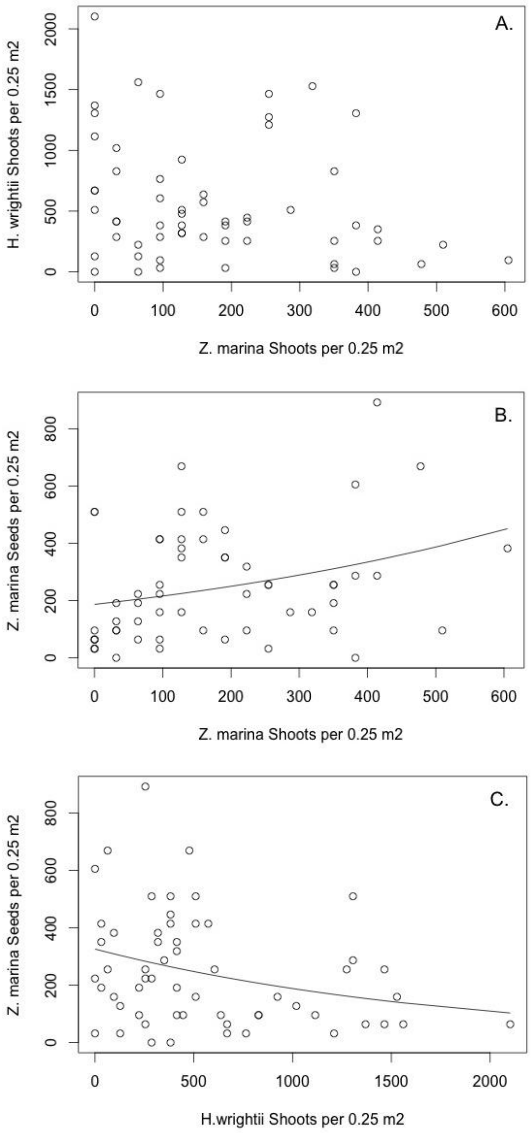

A.
H. wrightii Shoots per 0.25 m2
0
500
1000
1500
2000
0
100
200
300
400
500
600
Z. marina Shoots per 0.25 m2
B.
Z. marina Seeds per 0.25 m2
0
200
400
600
800
0
100
200
300
400
500
600
Z. marina Shoots per 0.25 m2
C.
Z. marina Seeds per 0.25 m2
0
200
400
600
800
0
500
1000
1500
2000
H.wrightii Shoots per 0.25 m2

Figure 6.

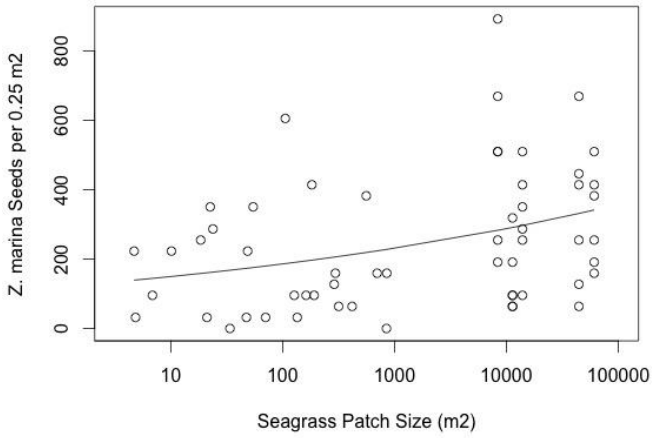
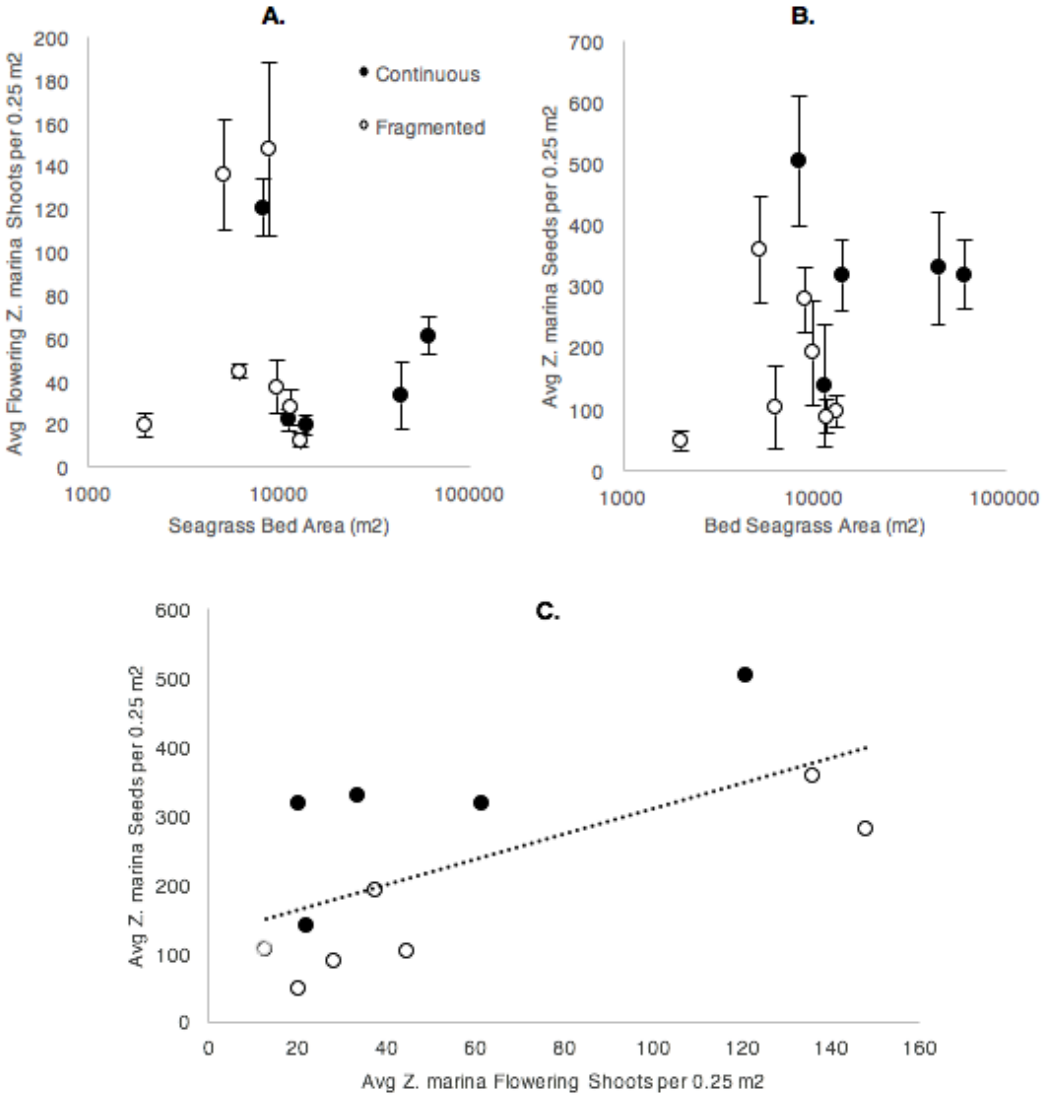

Z. marina Seeds per 0.25 m2
0
200
400
600
800
10
100
1000
10000
100000
Seagrass Patch Size (m2)

Figure 7.


A.
Avg Flowering Z. marina Shoots per 0.25 m2
Seagrass Bed Area (m2)
Continuous
Fragmented
B.
Avg Z. marina Seeds per 0.25 m2
Bed Seagrass Area (m2)
C.
Avg Z. marina Seeds per 0.25 m2
Avg Z. marina Flowering Shoots per 0.25 m2

Task 2: Quantify abundance of fishes among landscape types based on monthly trawl and seine sampling.

We present our findings in two sections – the first of which explores the effects of ecotones on the role of seagrass as fish habitat, and the second of which explores the effects of fragmentation on the role of seagrass as fish habitat.

Baillie, CJ, JM Fear, and FJ Fodrie (2015) Ecotone effects on seagrass and saltmarsh habitat-use by juvenile fishes in a temperate estuary. Estuaries and Coasts 38: 1414-1430.

ABSTRACT

While considered important juvenile fish habitats individually, both seagrass and saltmarsh are often highly connected with other subtidal and intertidal habitats. As a result, juvenile fishes and crustaceans may utilize multiple habitats across tidal, diel or seasonal cycles in a manner that makes inter-habitat proximity an important driver of fish distribution and community composition. In this context, we examined the importance of seagrass (*Zostera marina* and *Halodule wrightii*) and saltmarsh (*Spartina alterniflora*) habitat characteristics in driving fish and crustacean catch rates and community composition in a temperate, polyhaline-euhaline, estuary. We found that habitats with highly connected seagrass and saltmarsh vegetation exhibited higher average catch rates of many recreationally and commercially valuable fish and crustacean species, as well as overall fish catch rates and Shannon diversity (H), than habitats composed of either seagrass or saltmarsh habitat alone. Fish-habitat associations varied temporally, showing strong seasonal trends which were potentially indicative of temporal shifts in relative habitat value. Catch rates of numerous recreationally and commercially targeted species were correlated with patch-scale variables, particularly seagrass canopy height, water temperature and depth, however, regression analysis indicated that habitat type was more powerful in predicting overall nekton catch rates and Shannon diversity (H). We conclude that emergent properties (i.e., those operating at 10s – 100s m) are important drivers of fish distributions among and within habitats. Considering the spatial and temporal scales at which humans are encroaching on estuarine ecosystems, our findings highlight the need for investigating organism-habitat associations at expanded spatial scales, as well as the need to adopt fishery and coastal management plans that consider habitat characteristics at multiple spatial scales to account for inter-habitat connectivity.

INTRODUCTION

Estuarine landscapes are generally composed of spatially heterogeneous but functionally connected habitats types. The abundance and distribution of organisms within a given habitat is thought to be inextricably linked to habitat structure at multiple spatial scales (Wiens, 1989; Bell *et al.*, 1991; Levin, 1992). At small spatial scales (<10 m^2), numerous studies have demonstrated the effects of within-patch habitat structural complexity, such as seagrass shoot density, epiphytic algae cover and canopy height, on catch rates of marine organisms inhabiting these habitats (Orth & Heck, 1980; Bell & Westoby, 1986b; Worthington *et al.*, 1991; Irlandi, 1994). For example, Hovel *et al.*

(2002) found that seagrass shoot biomass and relative wave exposure were the environmental factors exerting the greatest influence on species densities within seagrass beds in a temperate estuary. At broader scales, landscape ecology - functionally defined as the relationship between ecological function and spatial patterns - has received increased attention as scientists and managers have recognized the importance of environmental variables at expanded spatial scales on organism-habitat associations and the capacity for human activities to fragment, degrade or destroy marine habitats (Hovel *et al.*, 2002; Grober-Dunsmore *et al.*, 2004; Tanner, 2006; Dorenbosch *et al.*, 2007).

Investigating organism-habitat associations at multiple spatial scales is crucial for ecosystem-based management plans given that multi-scale approaches are likely necessary to adequately develop holistic understanding of fishery species' niches (Sandel & Smith, 2009, but see Chittaro, 2004; Yeager *et al*., 2011). Studies focusing on landscape-scale processes have demonstrated the important role of habitat heterogeneity across 10s – 100s of meters in maintaining species diversity and augmenting species abundance through modification of predator-prey interactions and impacts on growth rates as a result of different bioenergetic benefit-cost ratios associated with resource acquisition (Parrish, 1989; Danielson, 1991; Irlandi *et al.*, 1995). For instance, fishes moving between mangrove and adjacent seagrass habitat will incur lower predation and smaller energetic expenditure than fishes moving across unstructured benthic habitat separating mangrove forests and seagrass meadows (Sheaves, 2005). Indeed, studies in both terrestrial and marine environments have underscored the influence of habitat adjacency and configuration on community composition and organism movement (Belisle & Desrochers, 2002; Robertson & Radford, 2009; Ryan *et al.*, 2012). Consequently, species distributions are often fundamentally different as a result of habitat context and, as such, resource management would benefit from a more comprehensive understanding of how species abundance and nekton assemblage structure are impacted by the composition and configuration of habitat mosaics. To date, the significance of landscape and habitat-scale factors in structuring fish assemblages has been well documented in tropical ecosystems (Dorenbosch et al., 2004; Grober-Dunsmore et al., 2007; Grober-Dunsmore et al., 2009), but less is known about how factors at broader scales influence faunal assemblages in temperate estuarine systems (but see Hovel et al., 2002).

Seagrass meadows, saltmarshes, tidal creeks, mudflats, and oyster reefs are the dominant shallow-water benthic habitats in temperature estuaries. Structurally complex estuarine habitats are generally recognized as important for many of the world's valuable fishery species largely because of their potential to increase the survival and growth of fishes and crustaceans during vulnerable early life stages compared to unstructured bottom (Boesch & Turner, 1984; Heck & Thoman, 1984; Hemminga & Duarte, 2000; Beck et al., 2001; Stunz et al., 2001; Minello et al., 2003), although these effects are likely species dependent and spatially variable (Phelan *et al.*, 2000; Elliott & Hemingway, 2008). Factors at multiple spatial scales; however, mediate the value of benthic habitats as nurseries for estuarine-dependent fishes. At both patch- and landscape-scales, factors such as prey availability, foraging efficiency, and refuge from predators can vary widely according to species morphology and life-history characteristics (Heck & Thoman, 1984; Werner & Gilliam, 1984; Levin, 1994; Camp et

al., 2011; Froeschke & Stunz, 2012; Tait & Hovel, 2012). For instance, Yeager et al. (2011) found in sub-tropical systems that amount of seagrass at both patch- and landscape-scales were positively correlated with fish abundance within restored patch reefs; however, amount of seagrass at the landscape-scale was the most important variable influencing differences in fish abundance. As many of the bottlenecks that are thought to regulate fish population size occur during early life stages, it is particularly critical to determine what constitutes high-value habitat for juveniles of fishery targeted species (Kennedy et al., 2008).

Seagrass and saltmarsh habitats are often highly connected with each other or alternative estuarine habitats. As a result, individuals or species may utilize multiple habitats across tidal, diel or seasonal cycles in a manner that makes habitat connectivity an important driver of fish distributions and community assemblages. Habitats that include ecotones, or habitat transition zones, may have appreciably different ecological processes and communities than interior habitat as some species and life stages may rely on edge habitat while others may preferentially utilize interior habitat (Yahner, 1988; Fagan *et al.*, 1999). For instance, pinfish (*Lagodon rhomboides*) were shown to be more than twice as abundant in intertidal marshes with adjacent seagrass beds than marsh adjacent to unvegetated bottom (Irlandi & Crawford, 1997). Similarly, the presence of seagrass between oyster reef and saltmarsh habitat was shown to strongly influence macroinvertebrate abundance on oyster reefs due to seagrass acting as a nighttime corridor for predatory blue crabs (*Callinectes sapidus*) between marsh and reef patches (Micheli & Peterson, 1999). These findings highlight the role of habitat composition and configuration in evaluating the functional role of seagrass-saltmarsh habitats for fishes and crustaceans.

The main objective of the present study was to examine how saltmarsh and seagrass habitat setting and abiotic characteristics influence juvenile fish abundance and community composition in a temperate estuary. A further purpose was to investigate whether structuring factors at patch or habitat-scale had greater influence on fish abundance and community composition. Specifically, we asked the following questions regarding habitat utilization in seagrass-saltmarsh ecotone habitats: (1) how does habitat composition effect nekton community characteristics and catch rates of individual nekton species (2) what are the biotic (seagrass shoot density, canopy height) and abiotic (temperature, salinity, depth) factors that correlate with the observed catch rates of key species within and among habitats and (3) are study wide habitat effects on species catch rates and community composition temporally and spatially variable?

METHODS

Study area

We surveyed fish communities within 2 sites in Back and Core Sounds, North Carolina, USA (Figure 1). The first site, Middle Marsh, was located within the Rachel Carson component of the North Carolina National Estuarine Research Reserve (RCNERR), in Back Sound near Beaufort Inlet (Figure 1). The second site, henceforth referred to as Core Sound, is approximately 10 km east of Middle Marsh in the southern extent of Core Sound, a 35 km water body oriented in a northeast-southwest direction (Figure 1). These locations were selected because they represent a range of environmental conditions (wave exposure and fetch direction, salinity, vegetation

patchiness, etc.) and contained our target habitats with multiple ecotone alternatives represented for seagrass and saltmarsh areas. Within each site, expanding on the work by Irlandi & Crawford (1997), we identified multiple habitat types: (1) mudflat (MF), unvegetated sandy bottom absent of appreciable shell or drift algae spatially isolated from any vegetated habitat by greater than 200 meters; (2) seagrass meadow (SG), which were comprised of eelgrass *Zostera marina*, shoal grass *Halodule wrightii*, or of mixed composition, adjacent to unvegetated bottom and separated from any saltmarsh habitat by at least 200 meters; (3) seagrass-saltmarsh interface (I) the interface between fringing *Spartina alternaflora* saltmarsh and an immediately adjacent seagrass meadow, consisting of *Z. marina*, *H. wrightii*, or of mixed composition, and not situated within a tidal creek; and (4) saltmarsh creek (SM), *S. alternaflora* bordered tidal creek with unvegetated sand or mud bottom absent of appreciable shell or drift algae. At the Core Sound site, we also sampled: (5) vegetated saltmarsh creek (MX), *S. alternaflora* bordered tidal creek with *Z. marina*, *H. wrightii*, or mixed seagrass composition bottom. For both SM and MX habitats, creeks were approximately 75 to 125 meters wide and sampling was conducted at least 100 meters from the creek mouth. For each habitat type, we identified 3 replicate stations yielding 12 sampling stations (4 habitats * 3 replicate stations) in Middle Marsh and 15 sampling stations (5 habitats * 3 replicate stations) in Core Sound.

Sampling Methods

To determine the relative catch rates of fishes and decapod crustaceans within and among habitats, we conducted monthly surveys during daylight hours between July and November 2010, May and November of 2011 and May and June of 2012. This corresponded with the periods when most winter and spring spawned fishes inhabit shallow-water estuarine habitats. Organisms were collected in all habitats using a 5-m otter trawl (15-m head rope, 2.0-cm body mesh; 0.6-cm cod end mesh; 0.3 x 0.7m doors) with a 4-seam balloon design, with floating and lead lines but without a tickler chain. Otter trawling permits sampling of large areas and was shown to be an effective method of quantifying catch rates of both abundant and rare fishes in North Carolina seagrass beds (Petrik & Levin, 2000). Owing to the reduced catch efficiency of otter trawls over saltmarsh vegetation (Zimmerman *et al.*, 1985), sampling in saltmarsh creeks (SM and MX habitats) was conducted immediately adjacent to the edge of saltmarsh vegetation. Furthermore, we attempted to mitigate gear performance issues associated with erratic trawl flight by towing at speeds shown to maintain trawl mouth diameter and contact with bottom, as well as by visually monitoring the trawl during tows for indications of erratic behavior (Wathne, 1977; Gibbs & Matthews, 1982). At each station during each month, two trawls lasting 1.89 ± 0.02 (mean ± 1 SE) minutes were conducted behind a small (~7m) research vessel at a speed of 3.33 ± 0.02 kilometers hour^{-1}. Sampling was conducted within two hours of high tide with typical water depths > 1.25 m (range 0.5-3.5 m). All organisms captured (Table 1) were enumerated and weighed to the nearest 1 g and released. Any unidentified specimens were euthanized by means of rapid cooling (IACUC ID 10-133.0-B) and returned to the lab for meristic identification by at least two technicians.

During each tow, we recorded salinity (‰), temperature (°C) and depth (m) using a Brix handheld analog refractometer, a Dwyer W-10 digital thermometer, and a

sounding rod marked in 0.25-m increments, respectively. Depth measurements were taken at the inception of each tow and, due to the absence of appreciable sills or slopes at tow locations, were representative of the depth across the entire tow path. At each sampling station, mean seagrass shoot density was measured in June, 2012 by averaging shoot counts from 3 randomly placed 0.01 m^2 quadrats. Within the same quadrats, we also measured the lengths (m) of up to 10 (if present) individual seagrass plants to determine average canopy height. Habitat characteristics and sampling effort are listed in Table 2. Overall, 741 individual, 111 ± 1-m long tows were conducted covering a total linear distance of 82,710 m.

Data Analyses.

Catch data were standardized to catch-per-unit-effort (fishes 100 m-towed^{-1}) and used for all subsequent analyses. Although the primary focus of our research was to examine the influence of habitat and ecotone types on fish community composition and species catch rates, patch-level biotic factors, abiotic factors, site and temporal factors were also considered in our analyses to gauge the relative importance of factors at multiple spatial scales on the abundance of fishes and crustaceans. In our ensemble analysis, the effect of habitat/ecotone (MF, SG, I, MX, SM), site (Middle Marsh, Core Sound), and month (May, June, July, August, September, October, November) on overall catch rates, Shannon diversity index (H) and catch rates of numerically dominant and economically important species were analyzed separately with linear mixed effects models for each response variable (e.g. catch rates of each species). We used R (R Core Team, 2011) and *nlme* (Pinheiro *et al.*, 2007) to perform linear mixed effects analyses for the relationship between response variables and independent variables. For our analysis of the effect of habitat type on catch rates and diversity, we entered habitat and site measured over time as fixed effects. Station was entered as a random effect nested within site. To investigate the temporal variability of catch rates among habitat types, habitat and month were entered as fixed effects and station was entered as a random effect. To address the potential biases associated with temporal autocorrelation in times series data, our mixed effected models incorporated an autocorrelation structure with a continuous time covariate, function corCAR1 (Box *et al.*, 2013). *Post-hoc* multiple comparison analyses for fixed effects was conducted using the *multcomp* procedure which conducts simultaneous tests and confidence intervals for linear mixed effects models while mitigating issues associated with multiplicity by employing exact multivariate *t*-distribution or asymptotic multivariate normal distribution (Hothorn *et al.*, 2008; Bretz *et al.*, 2010). Regression analyses were used to test for relationships between biotic (mean *Z. marina* density, mean *H. wrightii* density, mean canopy height) or abiotic (salinity, depth, temperature) factors and overall catch rates, Shannon diversity index (H), and catch rates of numerically dominant and commercially or recreationally targeted species. Analyses of the relationship between catch rates and seagrass characteristics was limited to the month of June 2012. Data were tested for normality with Shapiro-Wilk test prior to regression analyses. Normally distributed data were analyzed using Pearson correlation and those that failed to meet the assumption of normality were analyzed using Spearman's rank order correlation.

We used regression tree analyses to evaluate which factors were most powerful in determining overall nekton catch rates and Shannon diversity index (H). Regression

trees explain variation of a single response variable using combinations of explanatory variables to repeatedly split data into increasingly homogenous groups and are increasingly being used as a valuable tool for analyzing ecological data (De'ath & Fabricius, 2000; Sheaves, 2006). In particular, regression trees have been shown to be an effective method for predicting species richness in shallow-water seascapes (Pittman *et al.*, 2007). Trees were grown using recursive partitioning and over fitted trees were pruned using k-fold cross-validation. Cross-validation estimates the predicted error for trees of each size and the tree with the lowest cross-validation predicted error is considered the optimal tree. Regression tree analyses were of particular importance in determining the spatial scale (and associated factors) which had the greatest effect on fish catch rates and species richness patterns: i.e., do patch scale, habitat scale, or regional scale (site) factors have the greatest influence on assemblage characteristics? The regression tree analyses for both total catch rates and Shannon index (run separately) considered seven independent variables: year, month, site (Middle Marsh or Core Sound), habitat, temperature, salinity and depth. Temperature, salinity and depth measurements were incorporated as individual measurements taken at the time of each tow. Separate regression tree analyses were conducted for June 2012 data catch data only that included seagrass characteristics variables. All regression tree analyses were run using R (R Core Team, 2011) and *rpart* (Therneau *et al.*, 2010).

RESULTS

A total of 139,663 fishes and decapod crustaceans representing at least 71 species were captured during the course of this study. Pinfish (*Lagodon rhombiodes*) were by far the numerically dominant species, comprising 82% of all individuals captured. The ten most abundant species accounted for greater than 98% of our total catch (Table 2). There was a significant interaction between month and habitat on total fish catch rates ($F_{4,728} = 7.48$, $P < 0.0001$). In the late spring and early summer (May, June, July pooled among years), all vegetated habitats had relatively high catch rates of nekton, however seagrass (SG) showed an increasing trend in total catch rates while total catch rates in saltmarsh (SM) habitat decreased significantly between May and June before plateauing until the fall (Fig. 2). In late summer (August, September) fish catch rates began to decline in MF, SG and I habitats, while catch rates in MX and SM habitats remained relatively stable (Fig. 2). Fish catch rates were relatively low across all habitats by October in both sampling years (Fig. 2).

Total catch rates were found to be significantly different among habitats ($F_{4,715} = 43.43$, $P < 0.0001$, Fig. 3, Tables 3 & 5). All vegetated habitats had significantly higher fish catch rates than mudflat sites by nearly an order of magnitude (Fig. 3). Overall fish catch rates were significantly higher at I, MX and SG habitats, with an average of 348.1, 276.2 and 202.4 fish 100m towed^{-1} respectively, than at SM and MF habitats, which averaged 95.2 and 11.7 fish 100m towed^{-1} respectively (Fig. 3). Among the habitats of highest catch rates, I habitat stations had significantly higher fish catch rates than SG habitat stations, however neither was significantly different from MX habitat stations (Fig. 3). There was also a significant effect of habitat on mean Shannon diversity index (H) ($F_{4,715} = 84.16$, $P < 0.0001$, Table 5). Mean diversity (H) was significantly higher at MX, I, and SG stations, with an average of 0.73, 0.66 and 0.64, respectively, than at SM stations (Tukey's HSD, $P < 0.05$), which had an average Shannon diversity index of

0.45, suggesting that habitats containing saltmarsh vegetation alone are characterized by lower diversity than habitats containing seagrass – either isolated or connected to saltmarsh habitat. Average Shannon diversity at MF sites was significantly lower than all other habitats with an average value of 0.14 (Tukey's HSD, $P < 0.05$).

Habitat type significantly influenced the catch rates of many numerically dominant and economically valuable species. Lane snapper catch rates were significantly higher at SG stations than at any other habitat ($F_{4,715} = 6.02$, $P < 0.0001$, Fig. 4 H, Table 3), by greater than an order of magnitude. Gag grouper catch rates were significantly higher at I habitat stations, by 390%, than any other habitat sampled ($F_{4,715} = 14.86$, $P < 0.0001$, Fig. 4 I, Table 3). Penaeid shrimp, gray snapper, and speckled trout catch rates were all significantly higher, by 240%, 338%, and 200%, respectively, at MX stations that at any other habitat type (Fig. 4 B, G & K, Table 3). Unvegetated saltmarsh creek (SM) and mudflat (MF) habitats were not characterized by significantly higher catch rates of any numerically dominant or economically important fish or crustacean species.

As expected, overall catch rates varied significantly throughout the year ($F_{1,736} = 45.48$, $P < 0.0001$, Table 4), with highest catch rates in June, when fish catch rates were 2071% higher than catch rates in November, the month with lowest catch rates. Pinfish catch rates strongly tracked overall monthly CPUE due to their numerical dominance, but we noted appreciable seasonal variability among other numerically dominant and economically valuable species (Table 4, Fig. 4 A). Highest catch rates of pigfish, spot, penaeid shrimp, blue crab, gag grouper, sheepshead and flounders were observed in late spring and early summer (May, June, July) months (Table 4). Gray snapper, lane snapper, spotted sea trout and red drum catch rates were highest in late summer and early fall (September, October, November) months (Table 4). Average diversity (H) was also significantly different among months, with highest average diversity observed in July and the lowest average diversity observed in November ($F_{1,736} = 49.36$, $P = <0.0001$, Table 4). Despite seasonal patterns, habitat remained a consistently important driver of catch rates across months.

Mean *Z. marina density, H. wrightii* density and seagrass canopy height were all positively correlated with both overall nekton catch rate and Shannon diversity index (H) in June 2012 (Table 6). Among numerically dominant and targeted species, mean canopy height was the biotic factor which influences the catch rates of the greatest number of numerically dominant and economically important species; blue crab, penaeid shrimp, pinfish, spot, gag grouper, pigfish and flounder catch rates were all positively correlated with mean canopy height in June 2012 (Table 5). We found a significant, positive correlation between *Z. marina* shoot density and catch rates of pinfish, pigfish and flounders (Table 5). Only one species, pinfish, exhibited a positive relationship between catch rates and *H. wrightii* shoot density (Table 5). Catch rates of spotted sea trout, lane snapper, and gray snapper were too low in June 2012 to permit analysis of correlation with seagrass characteristics. Temperature was found to be positively correlated with the catch rates of sheepshead, pinfish, spot, gag grouper, pigfish, flounders, overall catch rates and Shannon diversity index, and negatively correlated with catch rates of red drum (Table 5). Salinity was correlated with catch rates of sheepshead, pinfish, spot, pigfish, flounders, overall catch rates and Shannon diversity index, all of which tended to have higher abundances in euhaline than

polyhaline conditions. Blue crab, penaeid shrimp, pinfish, pigfish, spot, gray snapper, lane snapper, flounders and overall catch rates as well as Shannon diversity index were negatively correlated with depth (Table 5).

Regression tree analyses were employed to determine the relative importance of spatial (site, habitat), temporal (year, month) and abiotic (depth, salinity, temperature) variables in determining overall nekton catch rates and Shannon diversity index (H). Regression tree analysis revealed that habitat type was the most powerful factor influencing overall fish and crustacean catch rate, partitioning SG, I, and MX into one node with significantly higher organism catch rates (275.3 fishes 100m towed^{-1}) than the node containing MF and SM (54.3 fishes 100m towed^{-1}) (Fig. 5). Further partitioning of the lower catch rate node revealed that habitat was again the most powerful explanatory factor influencing overall catch rates; SM habitat having significantly higher fish and crustacean catch rates (95.2 fishes 100m towed^{-1}) than MF habitat (11.7 fishes 100m towed^{-1}) (Fig. 5). Regression tree analysis revealed habitat type was also the most powerful predictor of Shannon diversity index. In contrast with overall catch rate patterns, SM habitat was grouped with all other vegetated habitats (mean H = 0.60) and MF (mean H = 0.14) occupied a node by itself (Fig. 6). Within the lower diversity, MF node, the most powerful splitting factor was year, with 2010 (mean H = 0.27) having significantly higher Shannon diversity than 2011 or 2012 (mean H = 0.07) (Fig. 6). Within the higher diversity, vegetated habitat node, the most powerful splitting factor was month, with May, June, July, August, and September occupying a higher diversity (mean H = 0.75) node, and October and November occupying a lower diversity (mean H = 0.28) node.

DISCUSSION

Regression tree analyses revealed that habitat type (with distinct ecotones) was an overarching factor influencing assemblages of fishes and crustaceans in our study region. While seagrass and saltmarsh have commonly been investigated separately as potential nursery habitats, the setting (i.e., inter-habitat adjacency and configuration) of these habitats affected the catch rates of several ecologically dominant or economically valuable species. If these patterns correlate with higher survival and ultimately greater fish productivity, conservation efforts that overlook the broader “landscape” context within which a seagrass bed or saltmarsh is situated may fail to maximize their efficacy as essential fish habitat. Habitats composed of either seagrass (SG) or emergent saltmarsh vegetation (SM) both exhibited higher catch rates of fishes and crustaceans than unstructured bottoms (MF). That said, ecotone habitats comprised of abutting seagrass and saltmarsh vegetation (I and MX) exhibited higher catch rates of many fisheries species than habitats that lacked seagrass-saltmarsh boundaries. For instance, the presence of seagrass within tidal creeks (MX) sites was correlated with significantly higher overall catch rates, diversity (H), and catch rates of fishery targeted species than in marsh creek sites without seagrass (SM). Notably, these results contrast previous experimental studies that determined complex estuarine habitats such as seagrass, oyster reef, and saltmarsh were functionally redundant as juvenile fish habitat. In particular, experimentally restored oyster reefs ($\leq$ 15 m^2) placed adjacent to vegetated habitats did not augment juvenile fish catch rates over vegetated landscapes without reef habitats (Grabowski *et al.*, 2005; Geraldi *et al.*, 2009). Our data, collected

within much larger habitat patches (> 100s m^2), suggest that structured estuarine habitats are not necessarily functionally redundant when adjacent to each other. Rather, heterogeneous habitats with both seagrass and saltmarsh habitat outperformed more homogeneous habitats (even those that offer juvenile fish structure) as juvenile habitats. A potential explanation for this difference among studies is that previous oyster-reef restoration projects have occurred at patch scales (10-15 m^2), while our survey study considered habitat context at much larger scales (100s m^2).

Although saltmarsh platforms are only accessible to fishes during a portion of the tidal cycle, species accessing saltmarsh habitat consume greater quantities of food and greater prey diversity than species without access to saltmarsh habitat (West & Zedler, 2000; Hollingsworth & Connolly, 2006). Fishes must balance the benefit of additional prey resources with refuge needs during saltmarsh emergence at low tide when predation intensity is high in tidal creeks as a result of concentration of predators and prey (Kneib, 1984, 1987). As such, in habitat with a seagrass and saltmarsh ecotone, organisms may exploit the resources of both habitats without the need to traverse bare subtidal substrate, where predation risk may be elevated (Micheli & Peterson, 1999). Hence, habitats with proximate seagrass and saltmarsh vegetation may reduce mortality and enhance growth for resident, mobile nekton (Irlandi & Crawford, 1997). At the population and assemblage levels, these fitness advantages could translate in to elevated species catch rates as we observed in this study for a diverse assemblage of ecologically and economically important species. It is important to acknowledge that our findings represent diurnal patterns of habitat use and that these patterns may vary appreciably for species that move within and among habitats nocturnally (Hammerschlag & Serafy, 2010).

Our results suggest that local habitat-scale attributes or processes may contribute to elevated catch rates of fishes and crustaceans, Shannon diversity indices, and catch rates of fishery target species in habitats with adjacent seagrass and saltmarsh vegetation. Specifically that ecotone type in similar settings (i.e. saltmarsh-seagrass vs. saltmarsh-sand within tidal creeks) may influence species abundance, community structure and productivity. In our study, marsh creeks with seagrass (MX) had significantly higher catch rates of numerous commercially and recreationally important fish and crustacean species compared to marsh creek stations without seagrass (SM). Additionally, we found significantly higher total nekton catch rate and diversity in tidal creeks containing seagrass, indicative of inter-habitat synergies. At stations with seagrass beds adjacent to fringing saltmarsh (I) total fish catch rate and catch rates of flounder species, gag grouper and pinfish were significantly greater than at isolated seagrass bed stations (SG), but the inverse was true for lane snapper and pigfish, a result potentially indicative of differences in species specific mortality, cross-habitat subsidies or movement at ecotones (Fagan *et al.*, 1999). It is important to acknowledge that towed nets are likely subject to variable sampling efficiency across habitats which may have influenced our results and, as such, application of these results requires consideration of such limitations.

Our work also demonstrates the potential for regulation of fish-habitat linkages across multiple spatial scales. Among vegetated habitats, for instance, stations with greater canopy height tended to have significantly higher diversity, overall fish catch rates, and higher catch rates of many economically valuable fish and crustacean

species. While it has been theorized that increased canopy heights result in greater microhabitat and higher food availability (Connolly & Butler, 1996), previous empirical studies have not necessarily supported this premise (Bell & Westoby, 1986a; Connolly, 1994). For the month we sampled seagrass characteristics (June 2012), our data suggests that canopy height was an important factor influencing nekton catch rates, but further work is needed to examine whether this effect is temporally variable as well as the mechanism driving this positive relationship in our system. Seagrass shoot density influenced catch rates of fewer species of interest than expected in our study, as increasing structural complexity is generally observed to positively correlate with species catch rates at smaller spatial scales (Bell & Westoby, 1986a), although we recognize our tows integrated fish distributions over 100s of m^2. We did note that *Z. marina* shoot counts in our surveys were significantly positively correlated with catch rates of pinfish, pigfish and flounder which may benefit from reduced predation and/or greater prey availability in more structurally complex habitats. Other studies in North Carolina and the Chesapeake Bay have also found that juvenile flounder occupy shallow water *Z. marina* beds, particularly during spring and summer months when these habitats attract their prey (Adams, 1976b, a; Lascara, 1981). Pinfish catch rates were also significantly positively correlated with *H. wrightii* shoot density, and due to their numerical dominance likely resulted in overall catch rate being correlated with increased shoot density of both seagrass species. Stoner (1982) found that pinfish consumption of amphipods was higher in *H. wrightii* than other seagrass species. Furthermore, Crowder and Cooper (1982) postulated that prey densities should be highest at intermediate levels of habitat complexity where the balance between opportunities to forage and refuge from predators is maximized. In this vein, pinfish feeding was negatively impacted by high seagrass biomass, which is a characteristic of dense *Z. marina* beds, indicating that *H. wrightii* dominated grass beds may provide refuge without hindering foraging ability.

Over broad seasonal/regional scales, a majority of numerically dominant and economically valuable species catch rates were positively correlated with temperature. As many of these species (e.g. pinfish, spot, pigfish, flounder) spawn on the continental shelf and have peak larval immigrate into estuaries during in the early months of the year, the positive correlation with temperature may reflect increased catchability as individuals grew during the spring and summer and before moving to deeper water habitats as waters cool in the fall (Warlen & Burke, 1990). Nearshore and estuarine spawners, such as blue crabs and penaeid shrimp species, exhibited higher catch rates earlier in the year than continental shelf spawners (van Montfrans *et al.*, 1995). Previous work has shown that transformational stage snapper ingress much later in the year at Beaufort Inlet, between July and October, peaking in September, matching our observations that snapper catch rates peak in the early fall (Tzeng *et al.*, 2003). Red drum and spotted sea trout spawn near inlets to estuaries in the late summer and early fall before dispersing to lower salinity habitats, which also matches our findings that peak abundances of these occur in the fall months, resulting in a significant negative correlation between red drum catch rates and temperature (Stewart & Scharf, 2008). The negative correlation between depth and catch rates of many numerically dominant or economically valuable species, overall catch rates and diversity may have been the result of increased risk of predation in deeper areas (Ruiz *et al.*, 1993; Miltner *et al.*,

1995). For example, mortality rates of tethered daggerblade grass shrimp, killifish and juvenile blue crabs was significantly higher in the Chesapeake Bay estuary in water depths over 70 cm, where large predators such as spot, large blue crabs and Atlantic croaker are more abundant (Ruiz *et al.*, 1993). It is plausible that reduced catch rates in deeper waters may have been influenced by reduced gear efficiency with increasing depth but efforts were made to mitigate factors associated with the erratic trawl characteristics responsible for variability in towed net performance (see Methods Section).

Although patch-level characteristics, particularly fine-scale plant structure, were shown to be significantly correlated with catch rates of numerous recreationally and commercially important species, regression tree analyses for June 2012 (not shown) revealed that habitat type was a more powerful predictor of overall catch rates and diversity than factors at patch-level scales. Over the course of our entire study, habitat type proved to be a more powerful predictor of overall catch rate and diversity than any abiotic factor or site differences (Middle Marsh vs. Core Sound), underscoring that habitat scale effect persist across regional scales (>10s of kilometers). Considering the spatial and temporal scale at which human encroachment on marine environments continues to take place, future application of landscape ecology approaches to investigate marine organism-habitat associations will likely be critical for successful ecosystem-based management. It is important to acknowledge that our findings represent an assessment of nekton abundance during the higher end of the tidal range and that community composition and species abundances may be appreciably different at the lower end of the tidal range. Higher densities of certain estuarine have been observed at low tide due to the aggregating effect of receding waters, however, other fish species may recede to adjacent deeper channels resulting in lower abundances, or in the case of intertidal areas, complete absence of a fishes at low tide (Hettler Jr, 1989; Blaber *et al.*, 1995; Marshall & Elliott, 1998; Morrison *et al.*, 2002).

Our findings highlight the importance of heterogeneity at both patch and ecotone scales in structuring faunal communities in seagrass and saltmarsh habitat in temperate, high-salinity estuarine waters. Additionally, they underscore the need to consider marine habitats as mosaics of interconnected habitats that interact dynamically as a function of their broader context, rather than discreet entities, and may have critical implications for management and conservation efforts, especially as managers begin to incorporate ecosystem based approaches into fisheries management plans and designation of protected areas (Beck *et al.*, 2001). That habitat type was a more powerful predictor of juvenile species catch rate, total catch rate and diversity than patch-level characteristics suggests that relative habitat value for juvenile fishes and crustaceans is likely influenced by processes at the scale of 100s of meters and that greater focus on the mechanisms underlying emergent habitat effects is merited. We suggest that effective coastal management requires consideration of the matrix of neighboring habitats as well as ecotone characteristics in habitat suitability on a nearly species-by-species basis (Tanner, 2006). Central to the arguments surrounding the nursery role hypothesis debate is whether elevated juvenile abundances translate to increased production of adults, a methodologically difficult issue to address, but, until such a time as fisheries research can definitively answer this question, prioritizing areas of high juvenile abundance for protection and restoration seems a sensible approach

(Beck *et al.*, 2001). Furthermore, as estuarine habitats continue to be imperiled by human intervention, there is a very real need for increased attention to how species catch rates may be further impacted by habitat loss and fragmentation (Lotze *et al.*, 2006).

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Table 1. Species that comprised greater than or equal to 0.01% of overall catch across all habitats listed in descending percent abundance (out of 139,633 total fishes captured).

Species Name	Common Name	% of Total Catch
Lagodon rhomboides	Pinfish	81.98
Leiostomus xanthurus	Spot	5.63
Orthopritis chrysoptera	Pigfish	3.84
Farfantepenaeus spp.	Penaeid Shrimp	2.35
Gerridae spp.	Mojarra	1.55
Callinectes sapidus	Blue Crab	0.97
Paralichthys spp.	Flounder	0.86
Archosargus probatocephalus	Sheepshead	0.41
Bairdiella chrysoura	Silver Perch	0.34
Stephanolepis hispidus	Planehead Filefish	0.29
Dilpdus holbrooki	Spottail Pinfish	0.25
Tozeuma carolinense	Arrow Shrimp	0.16
Opsanus tau	Oyster Toadfish	0.16
Palaemonetes spp.	Common Shore Shrimp	0.15
Anchoa spp.	Anchovy	0.14
Fundulus spp.	Killifish	0.12
Sygnathus spp.	Pipefish	0.10
Mycteroperca microlepis	Gag Grouper	0.08
Symphurus spp.	Tonguefish	0.06
Synodus foetens	Inshore Lizardfish	0.06
Lutjanus griseus	Grey Snapper	0.04
Chilomycterus schoepfi	Striped Burrfish	0.04
Chaetodipterus faber	Spadefish	0.03
Libinia spp.	Spider Crab	0.03
Blenniidae	Blenny	0.03
Sesarma reticulatum	Marsh Crab	0.03
Cynoscion nebulosus	Speckled Sea Trout	0.02
Sciaenops ocellatus	Red Drum	0.02
Sphoeroides maculatus	Norther Puffer	0.02
Lutjanus synagris	Lane Snapper	0.02
Citharichthys macrops	Spotted Whiff	0.02
Lolliguncula brevis	Brief Squid	0.01
Dasyatis americana	Southern Stingray	0.01

Table 2. Biotic and abiotic environmental variables measured in each habitat during each tow. n = total number of tows conducted in each habitat.

	Mudflat (n = 162)			Seagrass (n = 165)			Interface (n = 164)			Mixed (n = 81)			Salt Marsh (n = 169)		
	$\bar{x}$(± 1 SE)	Min	Max	$\bar{x}$(± 1 SE)	Min	Max	$\bar{x}$(± 1 SE)	Min	Max	$\bar{x}$(± 1 SE)	Min	Max	$\bar{x}$(± 1 SE)	Min	Max
Temperature (°C)	24.4 (0.4)	13.4	31.4	23.7 (0.4)	11.7	30.2	23.7 (0.4)	11.5	30.7	22.6 (0.8)	9.7	31.1	23.2 (0.5)	10.0	32.9
Salinity (‰)	32.8 (0.3)	22.0	40.0	32.6 (0.4)	24.3	41.0	32.4 (0.3)	23.0	40.0	31.1 (0.5)	21.0	38.0	31.8 (0.3)	20.0	40.0
Depth (m)	1.4 (0.0)	0.5	3.5	1.3 (0.0)	0.5	3.1	1.1 (0.0)	0.5	2.2	1.0 (0.0)	0.5	1.5	1.3 (0.1)	0.5	2.0
Halodule Density (shoots .01m^{-2})	0.0 (0.0)	0.0	0.0	80.0 (3.9)	26.5	179.3	116.0 (9.0)	0.0	304.3	110.0 (1.8)	86.5	124.8	24.3 (3.0)	0.0	103.5
Zostera Density (shoots .01m^{-2})	0.0 (0.0)	0.0	0.0	17.1 (1.3)	1.0	52.5	16.1 (1.3)	0.0	50.8	0.0 (0.0)	0.0	0.0	0.0 (0.0)	0.0	0.0
Canopy Height (cm)	0.0 (0.0)	0.0	0.0	16.5 (0.3)	11.8	22.8	30.1 (1.6)	11.9	68.3	19.2 (0.2)	17.6	21.8	5.9 (0.7)	0.0	21.0

Table 3. Average number of fishes caught 100m towed^{-1} (± 1 SE) for numerically dominant species, economically valuable species and all species pooled, as well as mean Shannon index value by habitat.

		Habitat				
Species	Common Name	Mudflat $\bar{x}$(±1SE)	Seagrass $\bar{x}$(±1SE)	Interface $\bar{x}$(±1SE)	Mixed $\bar{x}$(±1SE)	Salt Marsh $\bar{x}$(±1SE)
Lagodon rhomboides	Pinfish	8.6 (2.3)	168.9 (14.7)	309.3 (33.2)	218.4 (28.2)	56.9 (8.1)
Leiostomus xanthurus	Spot	1.2 (0.6)	8.0 (1.7)	8.3 (3.1)	12.6 (2.7)	20.6 (4.2)
Orthopritis chrysoptera	Pigfish	0.3 (0.1)	15.6 (2.1)	10.0 (1.2)	5.5 (1.5)	1.9 (0.5)
Farfantepenaeus spp.	Penaeid Shrimp	0.0 (0.0)	0.6 (0.0)	4.1 (0.7)	17.7 (4.3)	5.2 (1.7)
Callinectes sapidus	Blue Crab	0.2 (0.0)	1.3 (0.3)	2.4 (0.5)	3.2 (0.6)	2.3 (0.5)
Paralichthys spp.	Flounder	0.1 (0.0)	1.2 (0.1)	3.1 (0.4)	2.9 (0.4)	0.9 (0.2)
Archosargus probatocephalus	Sheepshead	0.1 (0.0)	0.4 (0.0)	1.2 (0.3)	2.6 (0.7)	0.4 (0.1)
Mycteroperca microlepis	Gag Grouper	0.0 (0.0)	0.1 (0.0)	0.5 (0.1)	0.0 (0.0)	0.0 (0.0)
Lutjanus griseus	Grey Snapper	0.0 (0.0)	0.1 (0.0)	0.1 (0.0)	0.4 (0.1)	0.0 (0.0)
Lutjanus synagris	Lane Snapper	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Cynoscion nebulosus	Speckled Sea Trout	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.1)	0.1 (0.0)
Sciaenops ocellatus	Red Drum	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.1)	0.1 (0.0)
Overall Fish Abundance		11.7 (3.2)	202.4 (16.3)	348.2 (34.6)	276.3 (32.2)	95.2 (11.6)
Shannon Index		0.14 (0.02)	0.64 (0.03)	0.66 (0.03)	0.73 (0.04)	0.45 (0.03)

Table 4. Average catch 100 m towed^{-1} (± 1 SE) for numerically dominant species, economically valuable species and pooled species by Month. Shannon diversity index (H) values are also reported as averages standardized to 100-m tows. n = Total number of tows between 2010-2012.

Species	Common Name	May (n = 81) $\bar{x}$(±1SE)	June (n = 130) $\bar{x}$(±1SE)	July (n = 103) $\bar{x}$(±1SE)	August (n = 137) $\bar{x}$(±1SE)	September (n = 68) $\bar{x}$(±1SE)	October (n = 115) $\bar{x}$(±1SE)	November (n = 107) $\bar{x}$(±1SE)
Lagodon rhomboides	Pinfish	127.1 (16.0)	265.5 (39.5)	226.3 (25.6)	170.6 (20.7)	137.1 (22.5)	48.5 (8.9)	8.1 (2.1)
Leiostomus xanthurus	Spot	51.2 (9.7)	9.3 (1.8)	10.5 (2.6)	4.2 (0.7)	3.8 (0.7)	0.6 (0.2)	0.2 (0.1)
Orthopritis chrysoptera	Pigfish	4.3 (1.7)	11.5 (1.8)	16.8 (2.7)	7.2 (1.0)	5.0 (1.0)	1.2 (.3)	0.0 (0.0)
Farfantepenaeus sp.	Penaeid Shrimp	10.6 (3.2)	4.1 (1.0)	7.1 (3.2)	2.7 (0.7)	6.5 (2.0)	0.5 (0.1)	0.9 (0.2)
Callinectes sapidus	Blue Crab	4.6 (1.0)	2.2 (0.6)	2.1 (0.4)	1.2 (0.2)	1.2 (0.3)	0.5 (0.1)	0.9 (0.2)
Paralichthys spp	Flounder spp.	2.3 (0.4)	2.7 (0.4)	2.1 (0.4)	1.3 (0.2)	1.4 (0.3)	0.5 (0.1)	0.6 (0.1)
Archosargus probatocephalus	Sheepshead	0.1 (0.0)	0.8 (0.2)	1.6 (0.5)	1.4 (0.4)	0.9 (0.2)	0.1 (0.0)	0.7 (0.0)
Mycteroperca microlepis	Gag Grouper	0.0 (0.0)	0.2 (0.1)	0.3 (0.1)	0.2 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Lutjanus griseus	Grey Snapper	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)	0.3 (0.1)	0.2 (0.1)	0.0 (0.0)
Lutjanus synagris	Lane Snapper	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.1)	0.0 (0.0)
Cynoscion nebulosus	Speckled Sea Trout	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.1)	0.1 (0.0)	0.0 (0.0)
Sciaenops ocellatus	Red Drum	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.1 (0.1)
Overall Fish Abundance		205.3 (85.6)	302.0 (191.7)	275.1 (103.1)	197.9 (92.4)	167.6 (66.9)	57.8 (30.4)	13.9 (13.1)
Shannon Index		0.68 (0.20)	0.54 (0.17)	0.69 (0.2)	0.64 (0.15)	0.53 (0.14)	0.34 (0.10)	0.13 (0.07)

Table 5. Results from analysis of variance on linear mixed effects models for numerically dominant and economically valuable species as well as overall fish abundances and Shannon diversity index (H). Degrees of freedom, F-value and p-value are reported for fixed effects.

	Habitat			Site			Habitat x Site		
Species	DF (num, den)	F	p	DF (num, den)	F	p	DF (num, den)	F	p
Archosargus probatocephalus	4, 715	12.24	< 0.0001	1, 718	5.18	0.0161	3, 631	1.77	0.1523
Callinectes sapidus	4, 715	7.14	< 0.0001	1, 718	5.85	0.0158	3, 631	10.94	< 0.0001
Cynoscion nebulosus	4, 715	9.65	< 0.0001	1, 718	8.88	0.0030	3, 631	3.64	0.0126
Farfantepenaeus spp.	4, 715	16.36	< 0.0001	1, 718	14.32	0.0002	3, 631	7.03	0.0001
Lagodon rhomboides	4, 715	40.73	< 0.0001	1, 718	6.62	0.0103	3, 631	3.95	0.0083
Leiostomus xanthurus	4, 715	7.85	< 0.0001	1, 718	1.52	0.2176	3, 631	12.82	< 0.0001
Lutjanus griseus	4, 715	10.26	< 0.0001	1, 718	11.1	0.0009	3, 631	0.63	0.5930
L. synagris	4, 715	6.02	0.0001	1, 718	0.03	0.8520	3, 631	1.80	0.1466
Mycteroperca microlepis	4, 715	14.86	< 0.0001	1, 718	2.56	0.1103	3, 631	3.29	0.0202
Orthopristic chrysoptera	4, 715	29.45	< 0.0001	1, 718	3.87	0.0495	3, 631	8.22	< 0.0001
Paralichthys spp.	4, 715	28.46	< 0.0001	1, 718	4.78	0.0291	3, 631	1.37	0.2514
Sciaenops ocellatus	4, 715	6.25	0.0001	1, 718	7.14	0.0077	3, 631	4.80	0.0026
Overall Fish Abundance	4, 715	43.43	< 0.0001	1, 718	9.56	0.0021	3, 631	5.83	0.0006
Shannon Index	4, 715	84.16	< 0.0001	1, 718	23.24	<0.0001	3, 631	21.63	< 0.0001

Table 6. Results of Spearman's Rank Order correlation (Spearman's rho, ρ) on the relationship between biotic and abiotic factors and abundance of numerically dominant and economically valuable fishes and crustaceans, overall fish abundance and Shannon diversity index (H). Seagrass characteristic analyses were limited to June 2012 data. ns = non-significant, NA = insufficient data, *p < 0.01, **p < 0.001, ***p <0.0001.

	Factor					
	$\bar{x}$ *Zostera* Density	$\bar{x}$ *Halodule* Density	$\bar{x}$ Canopy Height	Temperature	Salinity	Depth
Species						
Archosargus probatocephalus	ns	ns	ns	0.266***	0.114*	ns
Callinectes sapidus	ns	ns	0.505***	ns	ns	-0.226***
Cynoscion nebulosus	NA	NA	NA	ns	ns	ns
Farfantepenaeus spp.	ns	ns	0.519***	ns	ns	-0.200***
Lagodon rhomboides	0.443***	0.453***	0.744***	0.323***	0.154**	-0.301***
Leiostomus xanthurus	ns	ns	0.321*	0.255***	0.177***	-0.280***
Lutjanus griseus	NA	NA	NA	ns	ns	-0.140***
L. synagris	NA	NA	NA	ns	ns	-0.148***
Mycteroperca microlepis	ns	ns	0.470***	0.166***	ns	ns
Orthopristic chrysoptera	0.597***	ns	0.482***	0.453***	0.130*	ns
Paralichthys spp.	0.313*	ns	0.686***	0.157***	0.131*	-0.141**
Sciaenops ocellatus	ns	ns	ns	-0.177***	ns	ns
Overall Fish Abundance	0.435***	0.417***	0.722***	0.270***	0.122*	-0.310***
Shannon Index	0.486***	0.303*	0.702***	0.355***	0.171***	-0.326***

Figure 1. Study locations (left graph): Middle Marsh (top right graph), located in Back Sound, and southern Core Sound (bottom right graph), USA. Symbols indicate trawl locations, with filled triangles indicating mudflat (MF), open triangles representing isolated seagrass meadows (SG), filled circles indicating seagrass-saltmarsh interface (I), filled squares indicating vegetated saltmarsh creeks (MX), and open circles indicating unvegetated saltmarsh creeks (SM).

Figure 2. Seasonality of overall catch rate of fishes during 2010-2012 by habitat. Data are shown as means ± 1 standard error.

Figure 3. Average catch rate of fishes and invertebrates across habitats. MF = mudflat, SG = seagrass meadow, I= seagrass-saltmarsh interface. MX = saltmarsh creek with seagrass, SM = saltmarsh creek without seagrass. Data are shown as means ±1 standard error.

Figure 4. Average catch rate of numerically dominant and economically valuable juvenile (A) sheepshead, (B) blue crab, (C) spotted sea trout, (D) flounder, (E) pinfish, (F) spot, (G) gray snapper, (H) lane snapper, (I) gag grouper, (J) penaeid shrimp (K) pigfish and (L) red drum among landscapes. MF = mudflat, SG = seagrass meadow, I= seagrass-saltmarsh interface. MX = saltmarsh creek with seagrass, SM = saltmarsh creek without seagrass. Colored bars are Middle Marsh sites and open bars are Core Sound sites. Data are shown as means ± 1 standard error. Different letters indicate significant differences from linear mixed effects models (Tukey, $p < 0.01$) among habitats (with Middle Marsh and Core Sound data pooled).

Figure 5. Regression tree for overall fish and crustacean abundance. Separate branches indicate statistically significant differences at $p < 0.05$.

Figure 6. Regression tree for Shannon Diversity Index (H). Separate branches within the tree indicate statistically significant differences at $p < 0.05$.

Figure 1.

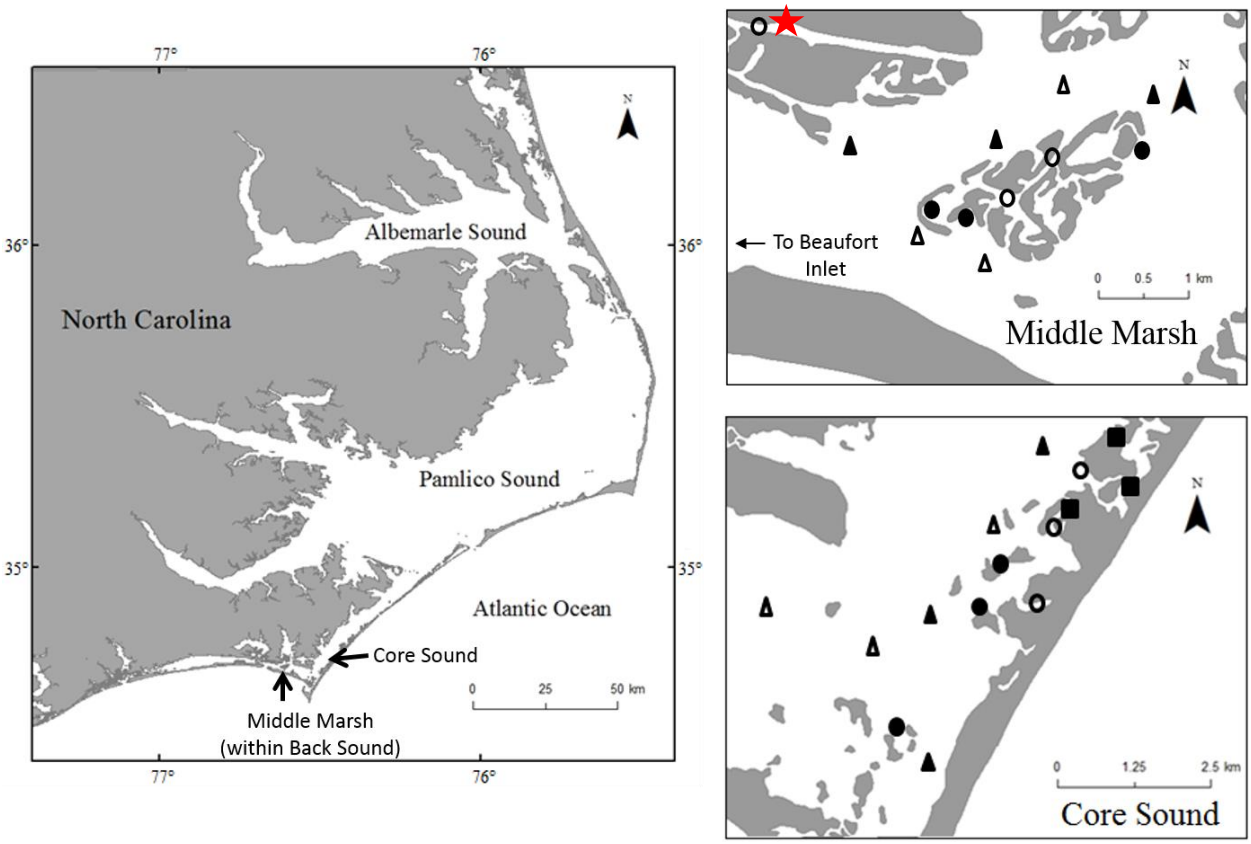

77°
76°
36°
35°
N
Albemarle Sound
North Carolina
Pamlico Sound
Atlantic Ocean
Core Sound
0 25 50 km
Middle Marsh
(within Back Sound)
To Beaufort
Inlet
0 0.5 1 km
Middle Marsh
0 1.25 2.5 km
Core Sound

Figure 2.

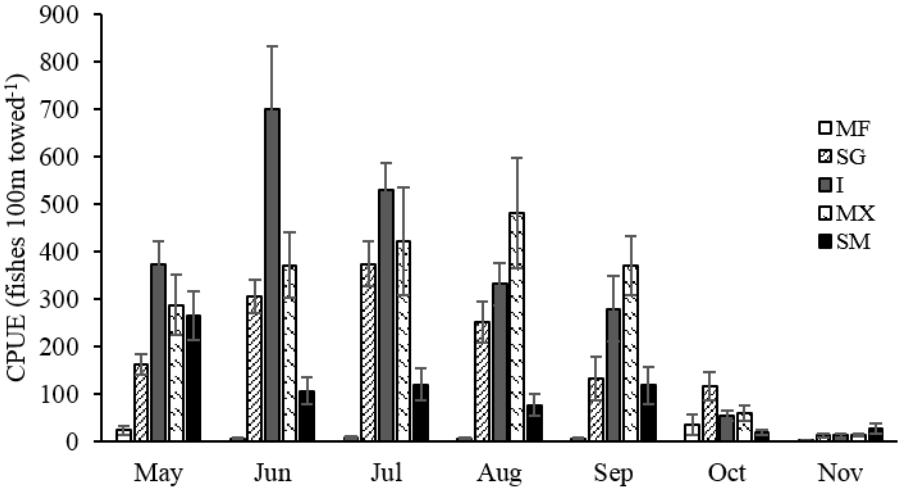

900
800
700
600
500
400
300
200
100
0
CPUE (fishes 100m towed-1)
MF
SG
I
MX
SM
May
Jun
Jul
Aug
Sep
Oct
Nov

Figure 3.

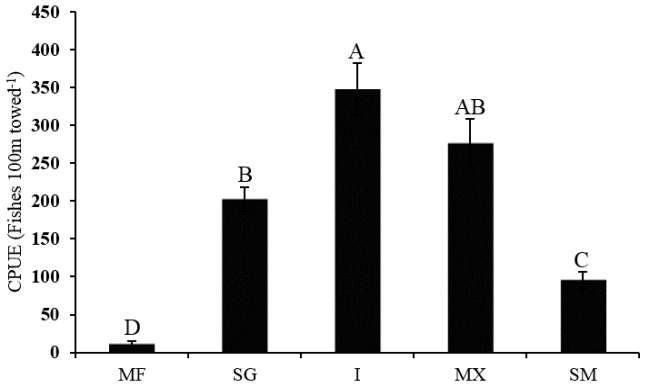
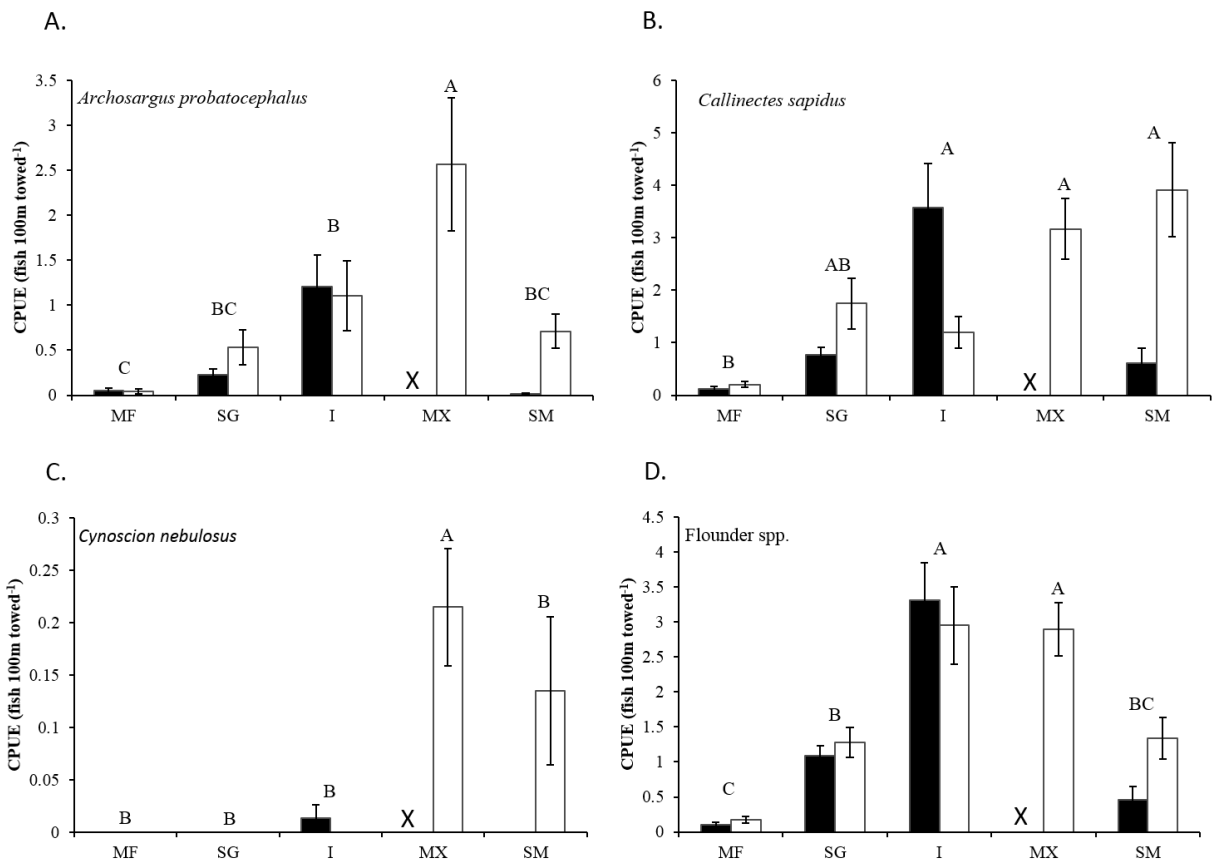
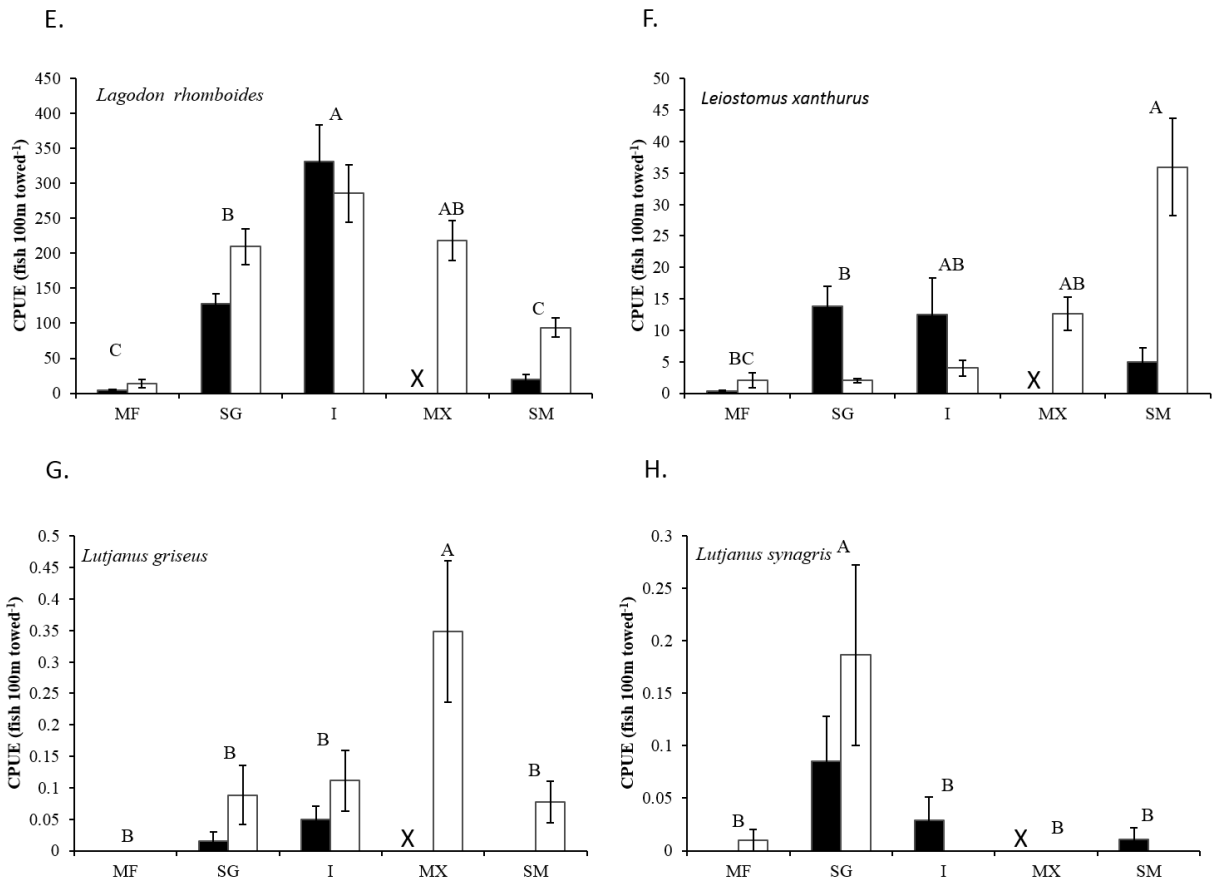

CPUE (Fishes 100m towed-1)
450
400
350
300
250
200
150
100
50
0
D
B
A
AB
C
MF
SG
I
MX
SM

Figure 4.


A.
Archosargus probatocephalus
CPUE (fish 100m towed-1)
3.5
3
2.5
2
1.5
1
0.5
0
C
BC
B
X
A
BC
MF
SG
I
MX
SM
B.
Callinectes sapidus
CPUE (fish 100m towed-1)
6
5
4
3
2
1
0
B
AB
A
X
A
A
MF
SG
I
MX
SM
C.
Cynoscion nebulosus
CPUE (fish 100m towed-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
B
B
B
X
A
B
MF
SG
I
MX
SM
D.
Flounder spp.
CPUE (fish 100m towed-1)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
C
B
A
X
A
BC
MF
SG
I
MX
SM


E.
Lagodon rhomboides
CPUE (fish 100m towed-1)
450
400
350
300
250
200
150
100
50
0
C
B
A
X
AB
C
MF
SG
I
MX
SM
F.
Leiostomus xanthurus
CPUE (fish 100m towed-1)
50
45
40
35
30
25
20
15
10
5
0
BC
B
AB
X
AB
A
MF
SG
I
MX
SM
G.
Lutjanus griseus
CPUE (fish 100m towed-1)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
B
B
B
X
A
B
MF
SG
I
MX
SM
H.
Lutjanus synagris
CPUE (fish 100m towed-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
B
A
B
X
B
B
MF
SG
I
MX
SM

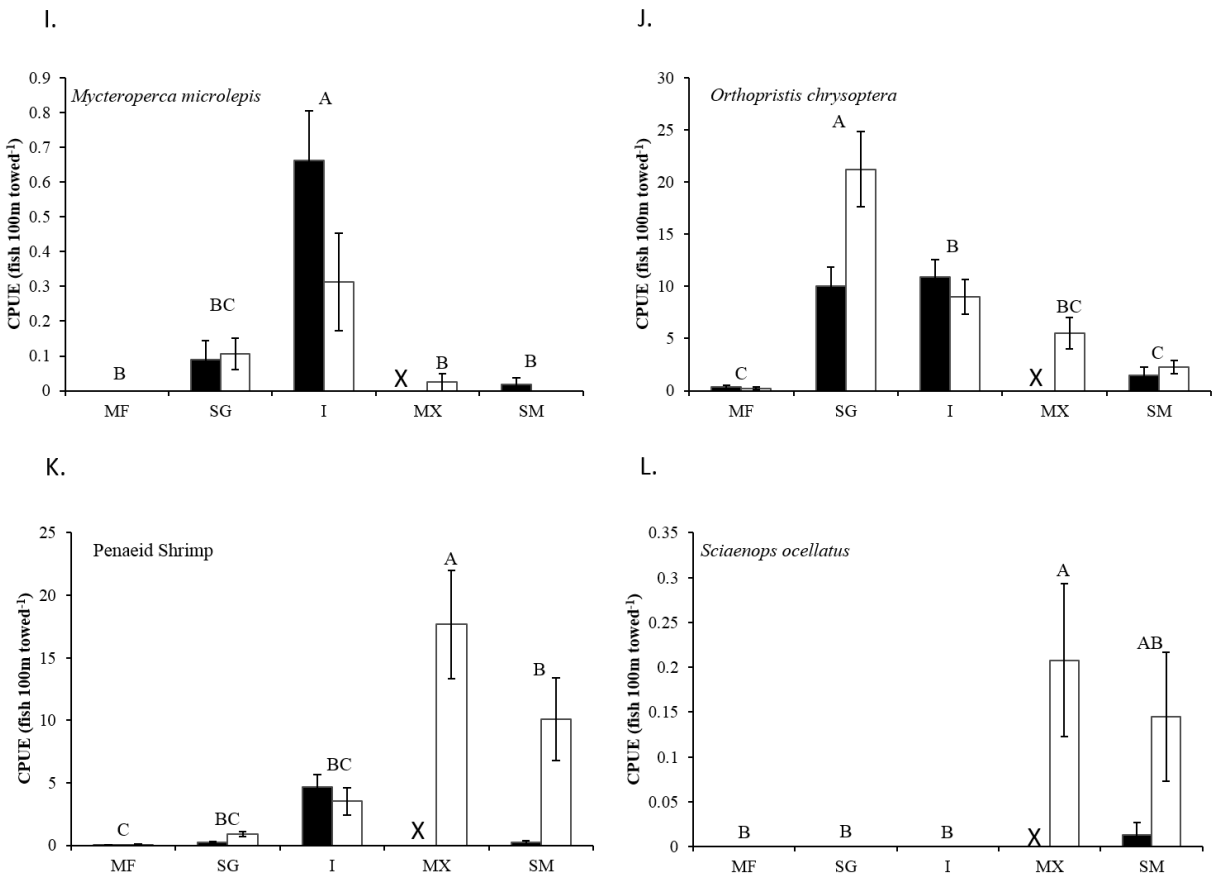

I.
Mycteroperca microlepis
CPUE (fish 100m towed-1)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
B
BC
A
X
B
B
MF
SG
I
MX
SM
J.
Orthopristis chrysoptera
CPUE (fish 100m towed-1)
30
25
20
15
10
5
0
C
A
B
X
BC
C
MF
SG
I
MX
SM
K.
Penaeid Shrimp
CPUE (fish 100m towed-1)
25
20
15
10
5
0
C
BC
BC
X
A
B
MF
SG
I
MX
SM
L.
Sciaenops ocellatus
CPUE (fish 100m towed-1)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
B
B
B
X
A
AB
MF
SG
I
MX
SM

Figure 5.

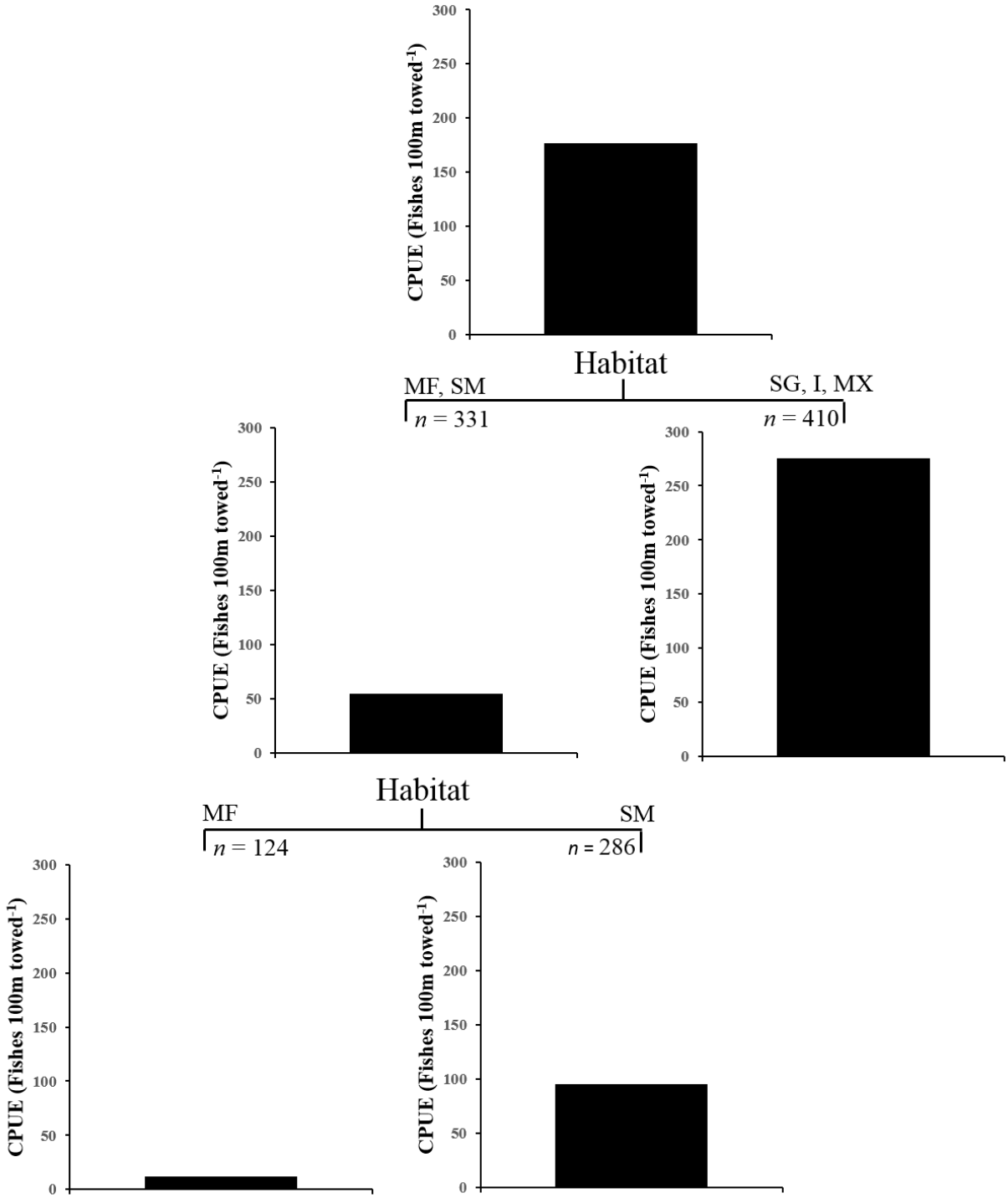
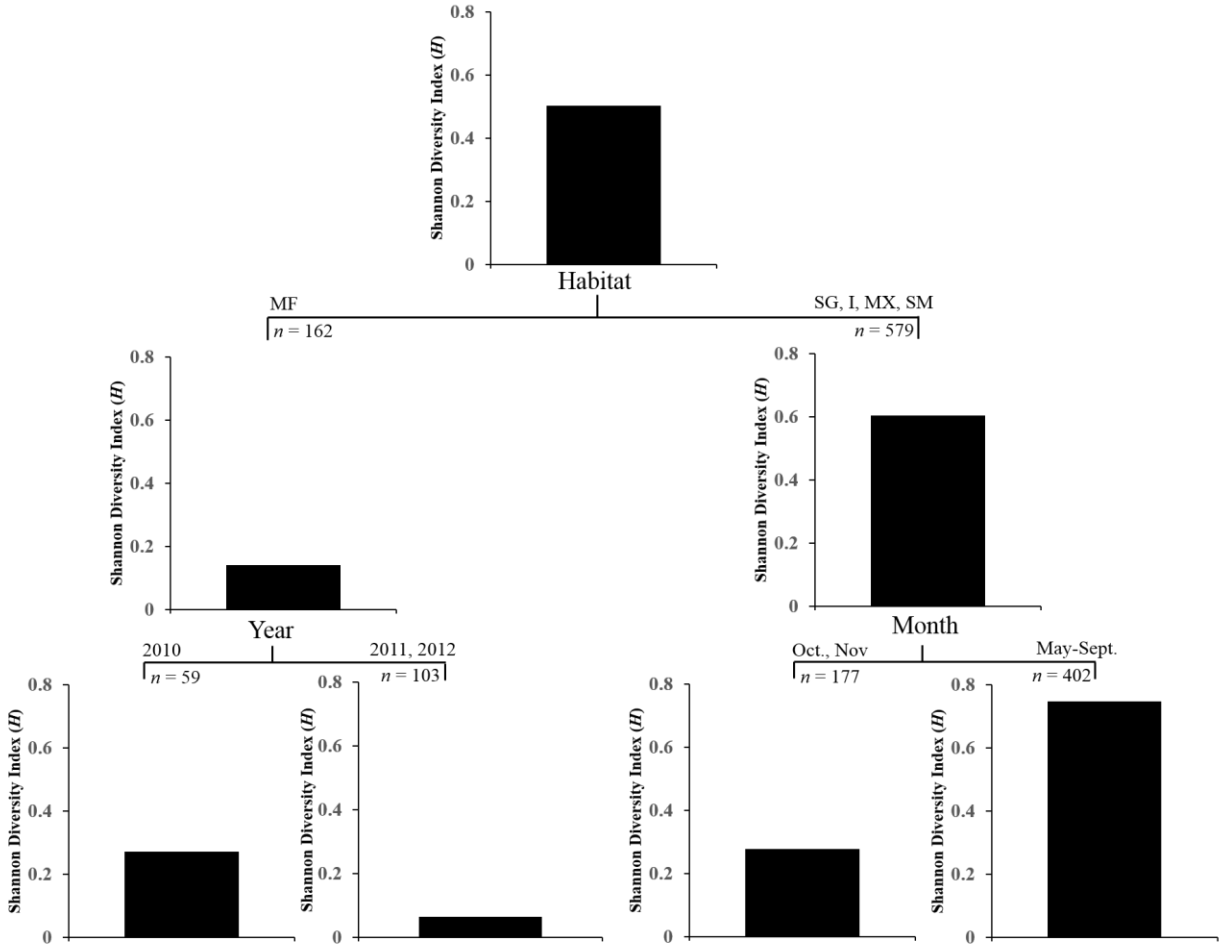

CPUE (Fishes 100m towed-1)
300
250
200
150
100
50
0
Habitat
MF, SM
n = 331
SG, I, MX
n = 410
Habitat
MF
n = 124
SM
n = 286

Figure 6.


Shannon Diversity Index (H)
0.8
0.6
0.4
0.2
0
Habitat
MF
n = 162
SG, I, MX, SM
n = 579
Year
2010
n = 59
2011, 2012
n = 103
Month
Oct., Nov
n = 177
May-Sept.
n = 402

Yeager, LA, DA Keller, TR Burns, A Pool, and FJ Fodrie (2016) Threshold effects of habitat fragmentation on fish diversity at landscapes scales. Ecology 97: 2157–2166.

Abstract.
Habitat fragmentation involves habitat loss concomitant with changes in spatial configuration, confounding mechanistic effects of habitat disturbance on biodiversity. 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 16,000 m^2 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 co-vary 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 more-sedentary species in low seagrass area, highly patchy landscapes. Additional tests corroborated that low movement or emergence rates among patches may underlie loss of vulnerable taxa. Fine-scale seagrass biomass was generally unimportant in predicting aspect of fish community composition. As such, we present empirical support for the fragmentation threshold hypothesis and 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.

INTRODUCTION

Habitat fragmentation is an aggregate process that 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 versus 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 remaining 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, Ewer and Didham 2006). Widely divergent species-level responses to habitat configuration have suggested 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 manifest at low levels of remnant habitat area (usually below ~20-30% remnant habitat area; Fahrig 1997, Fahrig 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). The inconsistent effects of habitat configuration on biodiversity have led to variable predictions regarding the cumulative impacts of habitat disturbance, and more research is needed to determine what attributes of habitat change or specific-species traits drive sensitivity to habitat configuration (Villard and Metzger 2014).

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 (~100m^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 that rely on 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 (McGargial and Cushman 2002). However, observation 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 co-vary 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 = 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) co-varied 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 the observed patterns.

METHODS

Characterization of habitat area and habitat configuration

We sampled 21 isolated seagrass beds (heareafter 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 (Fig. S1). Seagrass landscapes in Back Sound range from highly-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 m by 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 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 May 27, 2013 using AcrGIS v 10.1. 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 260-11,764m^2 beds). Total habitat area and metrics of habitat configuration were calculated in FragStats v4 based on rasterized maps of seagrass (2 m x 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 (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 ($R = 0.95$, $P < 0.001$). 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 height of the first 20 shoots for each species was measured to the nearest mm. Above ground biomass was dried for 48 h at 60 °C and dry mass was recorded for each species.

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

Fine-scale differences in seagrass variables across landscapes were evaluated based on mean above-ground 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 (4 total trawls per landscape). Total travel distance for each tow was recorded based on measurements using a Garmin™ 72H handheld GPS unit. 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 (Pearson R = 0.91). After each trawl, all fishes were identified to species and enumerated.

We evaluated the fish assemblage based on species richness (count of species within the landscape), total fish density (fish abundance/m^2), and community structure (i.e., species composition and relative abundance). 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)]. Prior to analysis, individuals within each species were summed across all four trawls at each landscape.

Relationship between 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 the 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. All variables were centered and scaled and seagrass area and number of patches were log-transformed prior to analysis to improve normality. 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 VIF's above our conservative threshold of 3 (Table S3), 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 and the sum of η^2 values across all variables equals the total model R^2.

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, adonis function in the vegan package; Anderson 2001, Oksanen et al.

2014). A non-metric Multi-Dimensional Scaling plot was used to graphically display the data.

Next we examined whether variation in community structure among landscapes were attributed to variability in community composition (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-low patch number, high area-high patch number, low area-low patch number, low area-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). 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).

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 x 0.8 m wide) at the Institute of Marine Sciences, University of North Carolina at Chapel Hill, in Morehead City, NC, USA. Within each water table, two artificial seagrass units (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). Fishes used in the trials were collected from Back and Bogue Sounds and kept in separate holding tanks for 2 days prior to the start of a trial.

All trials were conducted between July-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 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).

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 = 260-11,764m^2, cover = 2-74%, area-weighted mean patch size = 29-11,764 m^2) and habitat configuration-related measures of landscape pattern (number of patches = 1-75 patches, edge:area = 0.06-0.98 m/m^2; Table S1). The 21 study landscapes were well-distributed across both variables relating to independent effects of habitat area and habitat configuration, with 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 (Fig S2). Landscape-scale habitat variables (area and configuration) explained between 4 to 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, high patch number landscapes; Fig S2).

Relationship between 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 the fine-scale seagrass characteristics (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 positive effect of area on species richness when patch number was high, but little effect on species richness

when patch number was low (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 ($\eta^2 \leq 0.04$; $P > 0.1$ in all cases; Table 1).

Habitat (landscape- and fine-scale) characteristics explained 46% of the variation in fish community structure across study landscapes (Table 2). Similar to the results of effects on species richness and fish density, habitat area and habitat configuration were the most important variables driving difference in community structure across landscapes ($R^2 = 0.11$, $P = 0.03$ and $R^2 = 0.14$, $P = 0.01$, respectively). Again, seagrass biomass, and its interaction with area and configuration, had only a weak effect on fish community structure ($R^2 \leq 0.08$ and $P \geq 0.1$ in all cases; Table 2). The shift in community structure among landscape types was not attributable to differences 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, Table S2). The difference among low area-high patch number landscapes and all other landscapes 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 landscapes.

Fish movement and habitat configuration

Mesocosm trials revealed strong differences in movement rates of fishes between ASUs related to fish trait group (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 (mean = 49.4 ± 39.2 movements/min) for the benthopelagic species than the epibenthic species (mean = 2.9 ± 5.6). 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 lower for epibenthic species (13.5 ± 13.1 movements/min) than for the benthopelagic species (68.4 ± 43.4).

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 co-vary 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 pattern (although these correlations we generally low). 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 fragmented) landscapes. *Zostera marina* may represent higher quality habitat for epifuanal organisms (invertebrates and small fishes) when compared to *H. wrightii* (Micheli et al. 2008), ultimately affecting prey densities for invertivorous 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 were another mechanism contributing to 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 a gradient of fragmentation.

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 (2 levels of patchiness, plots up to 440 m^2) 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 affected survival independently from seagrass cover while statistically controlling for covariation among the variables (along with fine-scale seagrass attributes) within 100 m^2 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 production of faunal communities by comparing two levels of "fragmentation" at two different spatial scales (0.20 and 0.05 m^2); 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 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 mescosm experiments, which found epibenthic species to be less likely to move between simulated seagrass patches. While our mesocosm trials were run at a small 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, 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 seagrass emergence rates, 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-high fragmentation 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 the 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. For example, 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 & Ridham 2006). Specifically, many terrestrial studies of fragmentation focus on forest

fragments embedded within matrices of secondary forest or agricultural fields, which likely provide more shelter than a completely unvegetated environment (e.g., Gascon et al. 1999). 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, in particular, 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 modelling 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.

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Table 1. Results of General Linear 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). 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. Bolded variables have P-values statistically significant at α= 0.05.

Source of variation	df	Parameter Estimate	η^2	Type II Sum of Squares	F-statistic	*P*-value
1. Fish species richness; F = 7.80, P = 0.001, R^2 = 0.78						
Seagrass area	**1**	**0.79**	**0.30**	**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**	**0.23**	**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		0.22	4.13		
2. Total fish density; F = 6.87, P = 0.002, R^2 = 0.76						
Seagrass area	**1**	**0.67**	**0.42**	**8.02**	**22.89**	**0.0003**
Patch number	**1**	**-0.48**	**0.26**	**5.03**	**14.36**	**0.002**
Seagrass biomass	1	0.31	0.06	1.05	3.00	0.1
Seagrass area* patch number	1	-0.38	0.07	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		0.24	4.55		

Table 2. Results of Permutational Analysis of Variance 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. Seagrass area and number of patches were log-transformed prior to analysis. df = degrees of freedom. Variables with P-values statistically significant at α= 0.05 are bolded.

Source of variation	df	R^2	Type II Sum of Squares	Pseudo F-statistic	*P*-value
Seagrass area	**1**	**0.11**	**0.13**	**2.82**	**0.03**
Patch number	**1**	**0.14**	**0.17**	**3.57**	**0.01**
Seagrass biomass	1	0.03	0.03	0.66	0.7
Seagrass area* patch number	1	0.05	0.07	1.36	0.3
Seagrass area*seagrass biomass	1	0.08	0.10	2.00	0.1
Patch number*seagrass biomass	1	0.07	0.08	1.73	0.1
Residual	14	0.54	0.67		

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 bi-plot 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.

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.

Fig. 3. Non-metric 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.

Fig. 1.

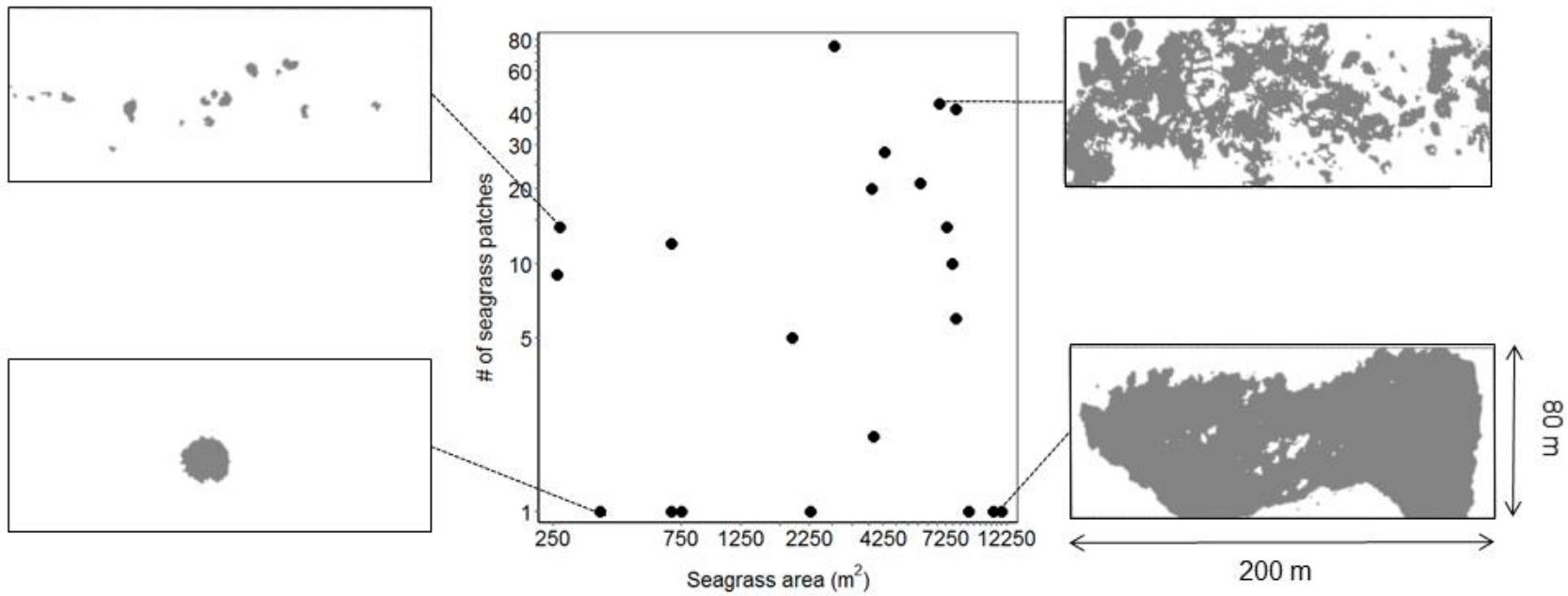

80
60
40
30
20
10
5
1
# of seagrass patches
250
750
1250
2250
4250
7250
12250
Seagrass area (m^2)
80 m
200 m

Fig. 2.

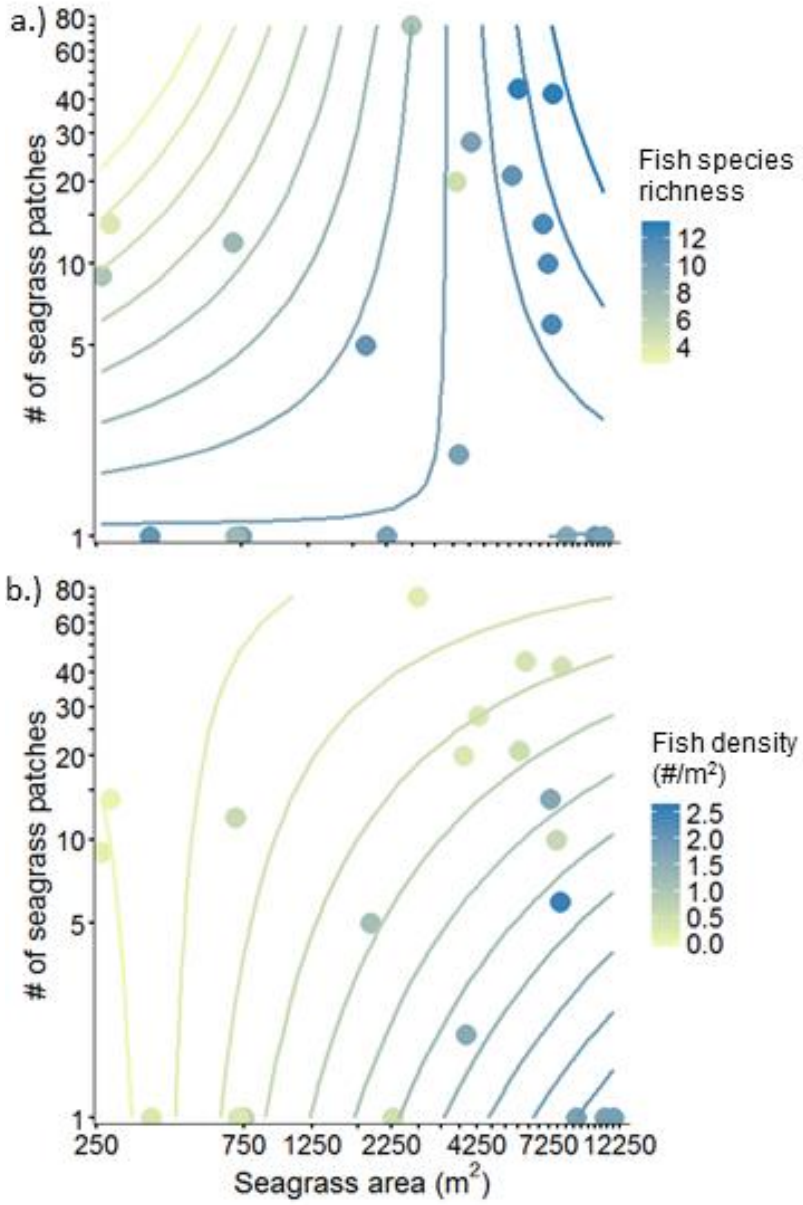
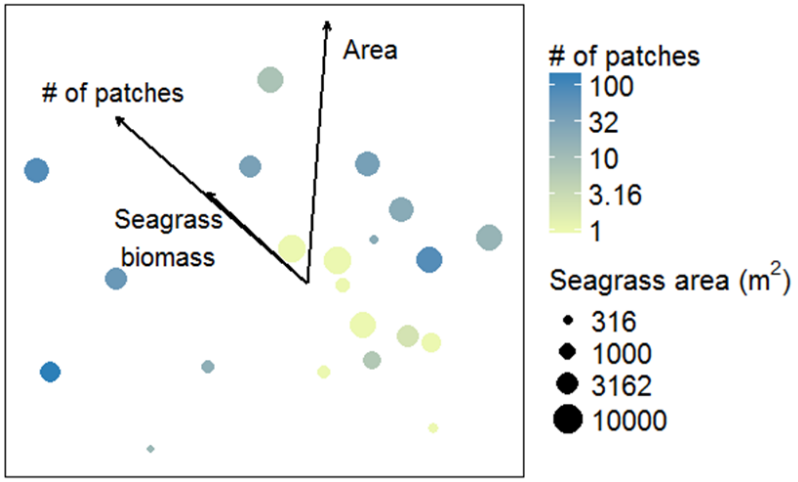

a.)
# of seagrass patches
80
60
40
30
20
10
5
1
Fish species richness
12
10
8
6
4
b.)
# of seagrass patches
80
60
40
30
20
10
5
1
Fish density (#/m²)
2.5
2.0
1.5
1.0
0.5
0.0
250
750
1250
2250
4250
7250
12250
Seagrass area (m²)

Fig. 3


Area
# of patches
Seagrass
biomass
# of patches
100
32
10
3.16
1
Seagrass area (m2)
316
1000
3162
10000

Task 3: Determine resource (prey) availability among landscapes based on seine and core sampling.

We present our major findings in a single section.

Geyer, JK, LA Yeager, and FJ Fodrie (submitted to Ecography) Differential trait sensitivities to habitat fragmentation across spatial scales shape local community structure processes.

ABSTRACT

Understanding the processes that shape the structure of local communities is the central goal of community ecology. The structure of local communities is believed to be the function of interactions between species traits and environmental variation across scales. However, more empirical data related to specific scale-dependent responses are needed. We examined the effects of environmental variation associated with habitat fragmentation of seagrass habitat measured from microhabitat to landscape scales in controlling the overall community structure of benthic fauna. Additionally, we tested the value of species traits in predicting species-specific responses to habitat fragmentation across scales. While univariate measures of faunal communities (e.g., total biomass, abundance, and species richness) were only well-predicted by microhabitat variables, overall community structure was controlled by environmental variation at the microhabitat, patch, and landscape scales. Furthermore, fourth corner analysis revealed that species traits explained as much variation in species-specific responses as species identity. We therefore present quantitative evidence supporting hierarchal models of community assembly which predict that interactions between species traits and environmental variation across scales ultimately drive local community composition. Additionally, variable responses of individual traits to changing environmental conditions associated with habitat fragmentation and temperate versus tropical foundation species suggest that community assembly processes will be altered under continuing environmental change.

INTRODUCTION

Community assembly is thought to be determined by a set of multi-step processes by which species are selected from a regional pool by a series both abiotic and biotic factors (Cornell and Lawton 1992, Keddy 1992). For example, environmental stress gradients may serve to preclude species with sensitive trait values, while biotic interactions like competition and predation may then further affect species persistence as well as relative abundance (Wiens 1989, Hillebrand et al. 2008, Cavender-Bares et al. 2009, de Bello et al. 2013). Inherent within this understanding of community assembly is the tenant that these processes are hierarchical in nature with their relative importance being manifest at different spatial scales (McGill 2010, de Bello et al. 2009, Cavender-Bares et al. 2009). Specifically, it may be expected that biotic interactions will drive species abundances at small spatial scales (Araújo and Rozenfeld 2013) while abiotic

factors will control community composition at large spatial scales (Jackson et al. 2001). Thus, a multi-scale approach to models of community are needed to capture the complex, non-random mechanisms by which local community structure is determined.

Trait-based approaches to analyzing community structure are valuable in predicting species responses to habitat variables. These approaches capture the ecological role and habitat requirements of a given species within a discrete environment (Diaz and Cabido 2001), while also revealing underlying mechanisms of coexistence (Pacala and Tilman 1994, Tilman 1994, Chesson 2000). These trait-based approaches link variations in community structure with environmental factors, therefore improving biodiversity predictions (Sydenham et al. 2014, de Bello et al. 2013). Assessing functional diversity within a landscape has become an increasingly important tool in understanding the mechanisms governing community structure.

Community assembly mechanisms may become altered under environmental change if fluctuating abiotic and biotic conditions modify selective pressure on species based on their traits. Specifically, habitat disturbances, like fragmentation, may alter habitat suitability for species by reducing habitat area and habitat connectivity (Fahrig 2003) while changing abiotic conditions (Laurance 1991). For example, forest edges in fragmented landscapes have substantially different microclimates due to increased light penetration, higher wind turbulence and greater desiccation which have been shown to drive different species composition along these edge habitats (Laurance 1991, 1997, Ferreira and Laurance 1997, Lovejoy et al., 1986, Sizer 1992, Kapos et al. 1997). Similarly, habitat fragmentation has been shown to decrease reproductive success by lowering dispersal capabilities (Andren 1994, Hovel and Regan 2008) and altering food availability at fine spatial scales (Irlandi et al. 1995, Irlandi 1997). In this way, habitat fragmentation may alter biodiversity by modifying selection mechanisms across scales, ultimately resulting in shifts in community structure and biodiversity.

Seagrass ecosystems represent an ideal model system to understand the effects of habitat fragmentation on community assembly because they exhibit large gradients in natural and anthropogenic fragmentation. Previous work on fragmentation with seagrass habitats has generally focused on either landscape-scale or patch-scale responses, revealing that fragmentation may alter predator-prey dynamics along patch edges (Kareiva 1987) or decrease overall species diversity at landscape-scales (Saunders et al. 1991, Foley et al. 2005, Yeager et al. 2016). Because fragmentation alters environmental conditions across scales, it is often difficult to identify casual mechanisms driving community shifts from single scale studies. In this study, we focused on responses of benthic fauna to habitat fragmentation, specifically examining effects of landscape, patch and microhabitat environmental gradients on species abundance, diversity, and community structure. Additionally, we employed recently developed fourth-corner models to evaluate the predictive value of species traits in determining responses to environmental variables across scales to inform models of community assembly.

We expected to find that environmental variables across scales would be an important determinant of benthic faunal community structure, with higher animal abundance and species richness associated with higher structural complexity, larger seagrass patch size and higher area. Furthermore, we expected that species would exhibit variable responses to environmental measurements across scales based on their traits. Specifically, we predicted that traits related to dispersal and reproductive mode would mediate responses to landscape-scale environmental gradients while traits that mediate microhabitat use and biotic interactions (e.g., diet) would show the strongest interactions with patch and micro-habitat scale variables. Finally, we predicted that body size and mobility would also be important determinants of the scale of organismal response to the environmental gradient, with larger and more mobile species response to environmental variation at coarser scales.

METHODS

Study Sites

We sampled 21 sites within seagrass habitats throughout Back Sound, North Carolina, USA, in the Rachel Carson National Estuarine Research Reserve (34°42' N to 34°39' N, 76°37' W to 76°31' W). Back Sound is a shallow (average depth = 2m), well-mixed estuarine system with salinities ranging from 24 to 36 ppt (Kenworthy et al. 1982). This estuarine system lies at a critical interface between temperate zones, thereby facilitating codominance of both the temperate species, *Zostera marina* (eelgrass) and the sub-tropical species, *Halodule wrightii* (shoalgrass). The seagrass sites were distributed across 21 focal seagrass beds defined by 200m x 80m rectangles (matching common isolated bed size and shape within our system). These beds were previously selected to represent independent gradients in both total seagrass cover (Table S1) and landscape patchiness (1-75 individual patches; Yeager et al. 2016).

Seagrass core sampling and laboratory processing

Core samples were taken from each sampling site; the cores measured 30cm in diameter, and captured the above ground seagrass habitat as well as the topmost 10 cm of the sediment surface. All cores were taken within 2 h of low tide and the GPS location of each core was marked with a Garmin 72H handheld unit (Garmin International, Olathe, Kansas, USA). Water depth was measured in situ at each site at the time of sampling to the nearest 10 cm.

Core samples were taken back to the lab and screened using a 500μm sieve; benthic fauna were sorted from larger debris and seagrass tissue and transferred to a 70% ETOH solution for storage. These organisms were then identified to the lowest taxon of certainty, enumerated, dried (48 h at 60 °C), and weighed to the nearest 0.0001 g. Pinfish (*Lagodon rhomboides*) was excluded from further analysis because they are not tightly-associated with the benthos and were likely not well-sampled with cores. In addition, only polychaetes with in-tact anterior appendages were identified and counted in abundance measurements to prevent overestimation of abundance and because these are requisite features for accurate identification.

Seagrass tissue from the cores as also separated and rinsed with clean freshwater. Seagrass was separated by species (*Z. marina* and *H. wrightii*), all shoots were enumerated, and the first 20 shoots from each species was measured to assess maximum canopy height (rounded to the nearest mm). Seagrass was then sorted according to above and below-ground biomass; the above-ground biomass was dried at 60 °C for 48 h and the dry mass/biomass of each species was measured to the nearest 0.01 g. These data were then summarized as above-ground biomass, seagrass shoot density, and mean shoot height per seagrass species per core.

Characterization of environmental variables across scales

We quantified environmental variables that we predicted would be important in mediating benthic community structure at three spatial scales: microhabitat, patch and landscape. To assess microhabitat conditions, we evaluated fine-scale seagrass characteristics of seagrass sampled within the cores and water depth at each core locations. Because seagrass biomass, shoot density, and shoot height for each species were correlated within species across cores (Table S4), we used seagrass biomass of each species (*Z. marina* and *H. wrightii*) separately as a composite metric fine-scale habitat complexity.

Seagrass habitat was mapped within each seagrass bed based on orthorectified aerial photographs as described in Yeager et al. (2016). For our patch-scale environmental variables, we used GPS locations of individual cores to locate the individual seagrass patch from which it was sampled and estimated seagrass patch size in m^2 in ArcGIS v 10.1.

To assess landscape-scale environmental condition, we assessed both total seagrass habitat area within the landscape (isolated seagrass bed) as well as number of discrete seagrass patches with in the landscape as a measure of habitat fragmentation. Total seagrass area and number of seagrass patches were calculated using FragStats (v 4). Finally, we used pairwise Pearson correlations between environmental variables to check for collinearity in variables within and across scales.

Characterization of species traits

We identified six traits we hypothesized would be important in meditating a species response to the environment and its functional role in the ecosystem; these traits included: primary trophic mode, microhabitat use, reproductive mode, larval development, mobility, and maximum body size (Table 4). We assigned species trait values by compiling data from both peer-reviewed literature and web-based identification guides (Appendix, Table S2). For species with little or no available information, trait values were estimated using class or genus level information. To evaluate possible associations between multiple nominal trait values (e.g. reproductive mode, larval development), we calculated Cramér's V, which ranges between 0 and 1. A value of 0 means that there is no correlation between trait values, while a value of 1 reveals complete association.

Relative influence of environmental variables on univariate measure of faunal communities

First, we examined the relative influence of environmental variables at multiple spatial scales (microhabitat, patch and landscape) in predicting total faunal abundance, total faunal biomass, and total species richness from our seagrass cores. We regressed each faunal variable onto the selected environmental variables using the lm function in R (R Core Team 2016). As a measure of effect size, we also report η^2 values which quantify the unique variation in a given response variable explained by an individual environmental variable. All environmental variables were scaled and log-transformed prior to analyses for all statistical tests to improve normality. To test for potentially confounding effects of collinearity among environmental predictor on model results, we quantified variance inflation factors (VIFs). All VIF were below our conservative threshold of 4 and thus are not considered problematic (Field 2000).

Relative influence of environmental variables on faunal community structure

Next, we sought to examine whether benthic community structure varied predictably along environmental gradients across scales. Bray-curtis dissimilarity among pairs of sites was calculated based on square-root transformed species abundances. We ran distance-based linear models (Primer Permanova add-on DISTLM; Legendre and Anderson 1999, McArdle and Anderson 2001) to examine whether differences in community structure were driven by environmental variables. We created a NMDS (non-metric multidimensional scaling) plot to visualize shifts in in benthic community structure across sites and correlations between ordination space and environmental variables

Linking species abundance to environmental variables and species functional traits

Finally, we evaluated whether species traits were important in predicting faunal responses to environmental gradients. We employed a recently developed method for fourth corner analysis (Brown et al. 2014) where species abundance is modelled as a function of environmental variables, species traits, and their interaction. The response variable is the species abundance x site matrix and the predictors are and environment x site matrix and a species x trait matrix. In our fourth corner analysis, we used generalized linear models with species abundance modelled with a Poisson distribution. We used a model selection technique based on cross validation and a least absolute shrinkage and selection operator (LASSO) penalty to determine which trait x environment interactions to include in the final model following (Hastie et al. 2009) using code provided by Brown et al. (2014) in R. We selected the model that minimized the LASSO penalty parameter and cross-validation was based on leaving out 10% of study sites over 50 replicate runs to select the parameter. We report average percent deviance explained (R^2_{test}) in the 10% hold-out sample averaged across the 50 replicate runs as a measure of effect size.

Finally, to determine how much of species-to-species variation in abundance across sites was due to trait responses to environmental parameters,

we compared the “trait*env” model (described above) with a model fitting individual species responses to environmental variation across site (as in SDM; “spp*env” model) following Brown et al. (2014). Lastly, we fit a hybrid model which retained the trait*environment terms in the model, but also include a species*environment term to account for additional variation in species abundance across sites not explained by variation in traits (“trait*env + spp*env”). Again we compared the predictive R^2_{test} values across models to evaluate model performance.

RESULTS

Environmental context of seagrass sites

Study sites exhibited wide variation in environmental variables measured with landscapes ranging from contiguous to highly patchy and microhabitats dominated entirely by *Z. marina*, *H. wrightii*, or a mix of the two (Table 1). Correlations among environmental variable retained for analyses were low (absolute value ≤0.40) expect for patch and landscape-scale seagrass area, which were moderately correlated (0.68) (S3). Low VIFs for all environmental variables indicate that these moderate and low correlations should not be problematic for interpreting multiple regression results, however (S4).

Relative influence of environmental variables on univariate measure of faunal communities

Environmental variables across scales explained between 15 to 23 % of the total variance in univariate measures of faunal communities (faunal abundance, faunal biomass, and species richness), with seagrass biomass at the microhabitat scale being the only significant predictor of variation across sites (Table 2). *Z. marina* biomass was the most important predictor of faunal abundance (η^2=0.09), faunal biomass (η^2=0.12) and species richness (η^2=0.07). *H. wrightii* biomass was significant predictor of faunal biomass (η^2=0.04), but not faunal abundance or species richness (η^2=0.01 and η^2<0.01, respectively). Our other estimates of environmental context, like depth, patch area, number of patches, and landscape area were not important in predicting these response variables (Table 2, Figure 2).

Relative influence of environmental variables on faunal community structure

While only microhabitat variables were significant predictors of univariate measures of faunal communities, environmental variables measured across all three scales were important in predicting overall community structure (explaining 20% of total variation in community structure across sites; Table 3). Fragmentation at the landscape scale emerged as the most important driver of variation in community structure across sites (total variation explained = 0.07), followed by seagrass patch area (0.05) and *Z. marina* biomass at the microhabitat scale (0.05). Depth, seagrass area at the landscape scale and *H. wrightii* biomass contributed to 4% or less of the explained variance in faunal community structure (Table 3). Communities associated with highly fragmented landscapes were most distinct from those within deeper microhabitats; as were

communities associated with high area landscapes distinct from those with high *H. wrightii* biomass microhabitats. Patch area and microhabitat-scale *Z. marina* biomass tended to have similar effects on community structure (Figure 3).

Role of species traits in mediating environment-community structure patterns

Species traits, environmental variation, and their interaction explained 26% of the variation in organismal abundance across sites within the 10% hold-out samples. Interestingly, we found evidence that all traits were sensitive to environmental variation at all three spatial scales considered (microhabitat, patch, and landscape) (Figure 4, Table S6). However, this pattern was often the result of individual trait values of the same trait responding at different scales. While some traits showed moderate collinearity across species (e.g, Cramer's V for reproductive mode and trophic mode = 0.63; Table S5), even moderately-correlated traits showed variable responses to environmental variation.

Larval dispersal mode showed sensitivity to environmental variation at the microhabitat and landscape scales. Organisms with planktonic larvae (relative species that brood their young) we more abundant in high area, low fragmentation landscapes, as well in deeper microhabitats with high *H. wrightii* biomass. Benthic fauna that display internal fertilization show moderate negative response to both *H. wrightii* biomass and patch-scale seagrass area as compared to broadcast spawners. Intuitively, microhabitat use was most strongly predicted by microhabitat variables, with infaunal species (those that live within the sediment) showing preference for shallow sites with less *Z. marina* biomass. As predicted, more mobile species responded most strongly to coarse-scale environmental variation, with their abundance being positively related to landscape seagrass area. Sedentary species responded to microhabitat variables, being more abundant at shallow sites. Similar to predictions related to mobility, species with larger body sizes responded most strongly to landscape-scale variables. Fragmentation has a strong negative effect and landscape seagrass area a weak negative effect on large-bodied species. Conversely, small-bodied species responded most strongly to microhabitat variables, be more abundant in shallow sites, low *Z. marina* biomass sites. Trophic mode traits responded to environmental variation at all three scales. Herbivores were more abundant in shallow sites and high seagrass area landscapes. Omnivores and parasites showed only weak responses to measured environmental variables. Suspension feeders were most abundant in fragmented landscapes, while interface feeders we more abundant within large seagrass patch and high seagrass area landscapes. Deposit feeders were more in shallow sites with higher Z. marina biomass and within more contiguous landscapes. Carnivores should to strongest response to landscape-scale seagrass area, being less abundant in high seagrass area beds.

The trait*env model performed slightly better than the spp*env model (R^2_{test} = 0.25), indicating species traits are a similar (or better) predictor of individual responses to environmental variation than species identity alone. The trait*env+spp*env (R^2_{test} = 0.26) model performed similarly to the trait*env model,

again indicating that species traits alone are able to predict as much variation in organismal abundance as species identity.

DISCUSSION

Our work reveals the influence of environmental variation at multiple spatial scales on benthic community assembly for seagrass fauna. While univariate measures of community structure (e.g., total biomass, species richness) were only predicted by microhabitat-scale environmental variables, overall community composition was controlled by variables measured at the landscape, patch and microhabitat scales. Incorporating community information on species identity and relative abundance was therefore key in elucidating the role of coarse-scale environmental variation in driving community structure. Furthermore, results of the fourth corner analysis revealed that species traits were as useful as species identity in predicting the distribution of individuals across sites, suggesting that species traits were the primary driver of species-specific patterns in abundance. Together, these results support models of hierarchical community assembly whereby environmental variation at multiple scales acts upon species traits to control the persistence and relative abundance of species locally. Although the mediating effect of scale on processes regulating community structure has been established, there remains little empirical evidence linking the two.

While we predicted that some traits would be most strongly affected by environmental variation at a single scale (e.g., that dispersal and reproductive mode would response to landscape-scale variation only), we instead found that every trait category we considered was affected by environmental variation at two or three scales. These multi-scale responses of species based on traits to environmental variables reveal the numerous ways by which fragmentation may impact community assembly. For example, interactions between trophic mode and water depth, landscape-scale fragmentation, and landscape-scale seagrass area seemed to have strong effects on individual abundance for this trait. For herbivores, higher seagrass area within the landscape may confer to higher total food availability if they consume seagrass or epiphytes directly. However, herbivores may also be responding to the negative effect of landscape seagrass area on carnivores. These benthic carnivores and omnivores may in turn be avoiding high area seagrass landscapes since they harbor higher abundances of higher-order predators (Yeager et al. 2016). For example, many of the omnivores in our study were juvenile blue crabs (*Callinectes sapidus*) which have been previously shown to prefer smaller seagrass patches to avoid adult blue crabs which are highly cannibalistic (Hovel and Regan 2008).

Differences in flow regime between patchy and more highly continuous seagrass sites may drive the distribution of suspension-feeding and deposit-feeding organisms. Patchier seagrass landscapes are associated with higher flow regimes (Fonseca and Bell 1998), with reduced seagrass density often conferring reduced sediment stabilization (Ginsburg and Lowenstam 1958, Scoffin 1970, Orth, 1977, Fonseca and Fisher 1986) concomitant with increased sediment transport and resuspension (Ward et al. 1984, Fonseca and Fisher

1986, Fonseca and Cahalan 1992). Therefore, suspension-feeding organisms which filter plankton and organic matter from the water-column may select for patchy habitats because higher flow confers greater food availability. Conversely, deposit feeders, which feed on settled organic matter, may show the opposite habitat preference because slower water velocities increased deposition of organic matter onto the benthos. Differences in flow rates among fragmented and contiguous seagrass beds may also explain the strong effect of landscape fragmentation on dispersal mode of organisms. Specifically, lower flow rates within larger, contiguous seagrass landscapes may facilitate the settlement of planktonic larval (Almasi et al. 1987) with coarse particles filtering through edge habitats, and fine particles accumulating in the interior (Fonseca et al. 1982, Ackerman and Okubo 1993). At the microhabitat scale, the positive effect of *H. wrightii* biomass on species with planktonic dispersal could also be driven by decreased water velocities associated with higher seagrass structural complexity. The importance of interactions between dispersal mode and environmental variation at fine and coarse spatial scales matches predictions that dispersal processes may be important in controlling the arrival organisms to both a general landscape area and then secondarily to given microhabitats (McGill 2000).

As we predicted, more mobile species and larger-bodied species preferred sites with higher cover of seagrass at the landscape scale. As these species likely use habitat over larger areas relative to smaller-bodied and more sedentary species, making it intuitive that they prefer higher area landscapes and responded at largest spatial scale considered. As expected, microhabitat use responded more strongly to microhabitat variation, with infaunal (burrowing) organisms preferring seagrass beds with less shoot biomass relative to epifaunal species; These results should be interpreted relative to range of environmental variation at our selected study sites, however, as all sites were located within habitat with at least some seagrass. Specifically, a negative species response to *Z. marina* biomass does not mean that infaunal communities do not rely on the structure of these biogenic habitats—rather, these communities show preference for relatively less biomass within seagrass habitat which maybe be to avoid overcrowding for these burrowers by roots and rhizomes.

The observed effects of environmental variation across scales on benthic community composition observed herein will help inform predictions of community responses to environmental change. For example, we observed that environmental changes associated with habitat fragmentation across scales (i.e., increasing patch number, decreasing patch size, shifts in fine-scale seagrass composition) were important in driving shifts in faunal community structure. Previous studies documenting fragmentation effects on seagrass community composition have generally focused on environmental effects on community structure at one or two scales (e.g., microhabitat and patch). Differential responses to fragmentation effects across scales based on species traits suggest that habitat fragmentation results may alter community structure by shifting selective pressures on species traits during multiple community assembly stages.

Our observation that *Z. marina* biomass is a stronger predictor of community structure than *H. wrightii* suggests that the role of microhabitat

variables in driving seagrass community composition as well as total community abundance, biomass and species richness may be weakened under global change. Globally, increasing temperatures may force range shifts of many seagrass species (Short and Neckles 1998). As North Carolina sits at a biogeographic boundary between sub-tropical and temperate ecoregions, effects of increasing global temperature on overall community structure will likely be severe here. While we didn't measure the effects of temperature on benthic seagrass fauna directly, the strong, differential responses of benthic fauna to seagrass species identity suggest that temperature-driven shifts in seagrass bed composition will have cascading effects on associated fauna. Specifically, it is predicted that increasing summer heat stress will result in more severe die-offs of *Z. marina* (Carr et al. 2012), potentially allowing *H. wrightii* to become the dominate seagrass species in the region. Our observed results of differences in benthic species abundance, diversity, and community composition associated with *Z. marina* dominated vs. *H. wrightii* dominated seagrass beds support previous studies documenting distinct benthic communities associated with these two seagrass habitat types (Micheli et al. 2008). Furthermore, our observation that different traits were important in mediating the response of species to *Z. marina* or *H. wrightii* biomass indicates that climate change will alter the traits important in predicting community assembly near this range boundary.

While we found that species traits are useful variables in analyzing shifts in community structure, there are important limitations and analytical considerations when applying our results across systems. First, we used body length as a measure of body size as a result of data availability, but depending on the ecological process considered, biomass may a be more relevant measures of ecological interactions or ecosystem functions and differences in body morphology across taxa will obviously yield very different length-biomass relationships. For example, body size annelid worms were typically classified as large-bodied due to their long body lengths, but length along may not be representative of true space requirements or vulnerability to gape-limited predators. However, our sensitivity analyses revealed that interactions between body-size and environmental variables were robust to the inclusion of worms. Additionally, as we considered maximum body size for each species, this trait does not capture intraspecific variation across sites. Trophic guild similarly fails to capture variation in diet among individuals across sites which could explain even greater variation in community composition across sites than that explained used only species levels estimates as we did.

Our study contributes to growing empirical support for hierarchal community assembly models. In particular, the utility of species traits in explaining variation in species-specific responses to environmental variation is encouraging for creating more generalizable community assembly models and future comparisons across systems. Similarly, our work builds on limited empirical results from multi-scale studies while identifying individual trait-environment effects across scales using a novel fourth-corner approach. While we were able to explain a significant amount of variation in community structure in response to environmental variation measured from the landscape to

microhabitat scales, it is likely that environmental factors operating at even larger scales (e.g., estuary scale to continental) are also important in driving community assembly processes mediated through large-scale dispersal and abiotic tolerances of species. Refining trait-based community assembly models based on environmental variation across scales will be critical in predicting biodiversity responses to continued environmental change.

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Table 1. Summary of Environmental variables

Environmental variable	Description	Mean	SD	Min	Max
Depth (m)	Depth of the water column	0.56	0.22	0	0.9
Z. marina biomass (g DW)	Above-ground biomass per 10cm core	1.63	1.46	0	5.2
H. wrightii biomass (g DW)	Above-ground biomass per 10cm core	2.07	1.73	0	10.04
Patch area (m^2)	Individual patch areas per site, calculated in FRAGSTATS v 4	3543.23	3773.03	0.82	11745.96
Number of seagrass patches	Number of patches per site, calculated in FRAGSTATS v 4	12.24	17.02	1	75
Landscape seagrass area (m^2)	Total seagrass area per site, calculated in FRAGSTATS v 4	4476.60	3547.58	260	11764

Table 2. Functional trait classification for all invertebrate species classified along 5 axes

Category	Possible Trait Values
primary trophic mode	suspension feeding, deposit feeding, interface, carnivore, omnivore, parasite, herbivore
microhabitat use	epifaunal, infaunal
reproductive mode	internal, external
larval development	direct, planktonic
post-settlement mobility	mobile, sedentary, sessile
body size	range = 5.08-1219.2 mm, median = 50.8 mm

Table 3. Results of general linear models of the effects environmental variables measured at three spatial scales (microhabitat, patch, and landscape) on total faunal abundance, total faunal biomass, and total species richness from seagrass cores. η^2 = the unique variation in the response variable explained by each environmental variable. Environmental variables with a statistically significant effect on the response at α = 0.05 have are asterixed.

Environmental variable	Sum Sq	Df	F-value	P-value	η^2
Response variable: Log faunal abundance; R^2 = 0.22, F = 3.53, P = 0.04					
Log depth	0.192	1	2.02	0.2	0.02
Log Z. marina biomass*	0.779*	1*	8.22*	0.005*	0.09*
Log H. wrightii biomass	0.102	1	1.08	0.3	0.01
Log patch area	0.010	1	0.10	0.7	0.00
Log number of patches	0.038	1	0.41	0.5	0.00
Log landscape seagrass area	0.244	1	2.58	0.1	0.03
Residuals	7.109	75			0.78
Response variable: Log faunal biomass; R^2 = 0.23, F = 3.68, P = 0.003					
Log depth	0.151	1	0.57	0.5	0.01
Log Z. marina biomass*	3.014*	1*	11.37*	0.001*	0.12*
Log H. wrightii biomass*	1.145*	1*	4.32*	0.04*	0.04*
Log patch area	0.198	1	0.75	0.4	0.01
Log number of patches	0.240	1	0.90	0.3	0.01
Log landscape seagrass area	0.199	1	0.75	0.4	0.01
Residuals	19.88	75			0.77
Response variable: Species richness; R^2 = 0.15, F = 2.16, P = 0.06					
Log depth	16.29	1	0.76	0.4	0.01
Log Z. marina biomass*	130.24*	1*	6.11*	0.02*	0.07*
Log H. wrightii biomass	5.32	1	0.25	0.6	<0.01

Log patch area	18.43	1	0.86	0.4	0.01
Log number of patches	14.62	1	0.69	0.4	0.01
Log landscape seagrass area	22.16	1	1.04	0.3	0.01
Residuals	16.29	1	0.76	0.4	0.85

Table 4. Results of marginal tests from distance-based linear models for the effects environmental variables measured at three spatial scales (microhabitat, patch, and landscape) on differences in community structure (measures as Bray-Curtis dissimilarity) across sites. Prop. Var. explained is the total variation explained by each environmental variable not taking into account other environmental variables in the model and thus sums to greater than the total model R^2.

Environmental variable	Sum Sq	Pseudo F-value	P-value	Prop. Var. explained
Total model R^2 =0.20				
Log depth	10962.0	3.51	0.001	0.04
Log Z. marina biomass	12031.0	3.87	0.001	0.05
Log H. wrightii biomass	6773.9	2.13	0.009	0.03
Log patch area	12542.0	4.05	0.001	0.05
Log number of patches	17951.0	5.92	0.001	0.07
Log landscape seagrass area	6891.4	2.17	0.01	0.03

Figure 1. Site map; all cores were collected in replicate within sampling sites.

Figure 2. Univariate plots modeling the effects of environmental variables measured at three spatial scales (microhabitat, patch, and landscape) on total faunal abundance, total faunal biomass, and total species richness from seagrass cores.

Figure 3. Non-metric multidimensional scaling plot representing similarity in community structure of the faunal community among seagrass cores. Each point represents a core and cores that are closer in space have more similar communities. The black arrows show the direction of the (increasing) gradient for each environmental variable with the length of the arrow being proportional to the correlation between each environmental variable and the ordination space. All environmental variables were log-transformed prior to analysis.

Figure 4. Species abundance is modelled as a function of environmental variables, species traits, and their interaction. This is based on species abundance, and LASSO penalty with minimum predictive error. Note all environmental variables were log-transformed and scaled prior to analysis. R2 = 0.26

Figure 1.

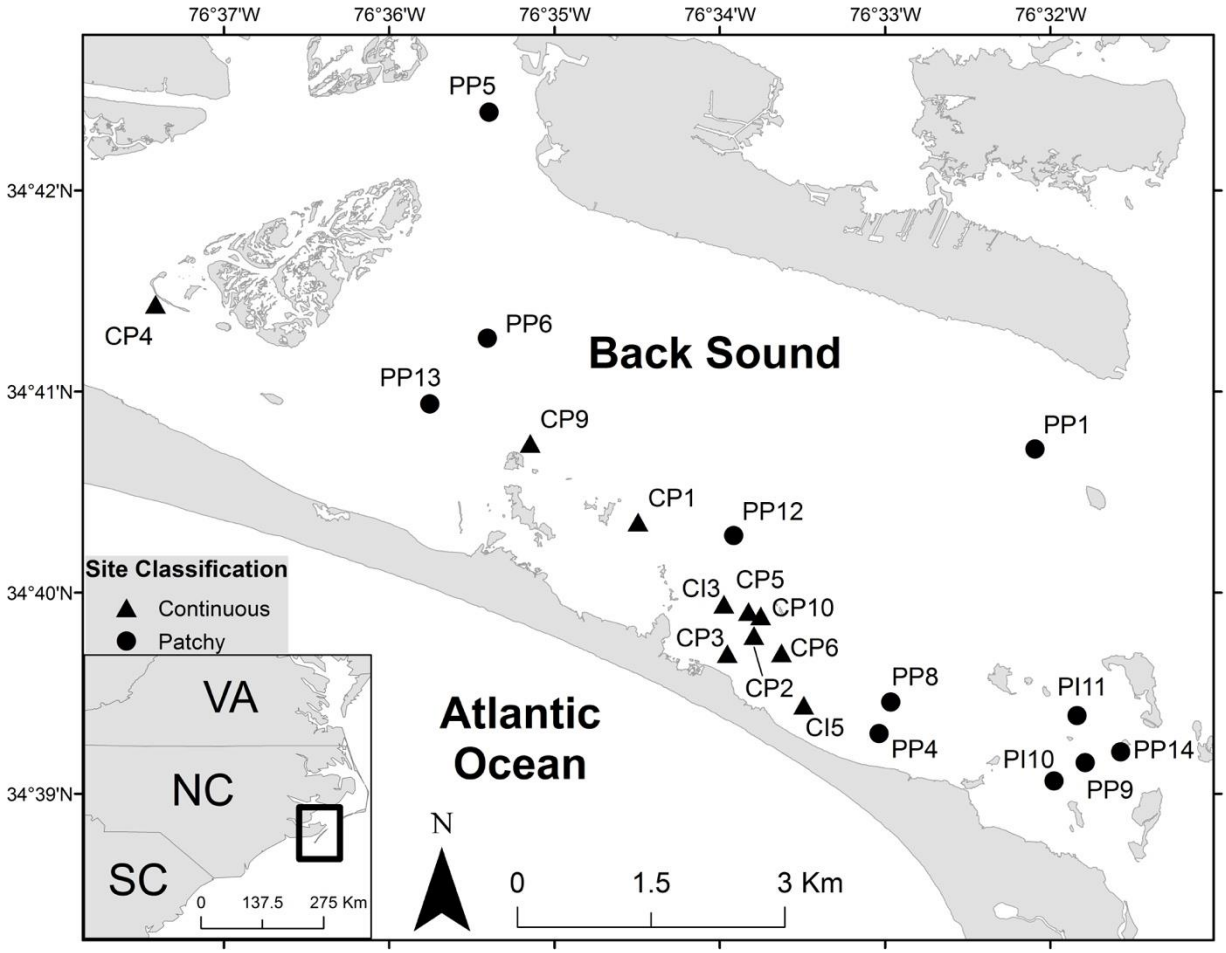

76°37'W
76°36'W
76°35'W
76°34'W
76°33'W
76°32'W
34°42'N
34°41'N
34°40'N
34°39'N
PP5
CP4
PP6
Back Sound
PP13
CP9
PP1
CP1
PP12
Site Classification
Continuous
Patchy
CI3
CP5
CP10
CP3
CP6
CP2
PP8
PI11
CI5
PP4
PI10
PP14
PP9
Atlantic
Ocean
VA
NC
SC
0
137.5
275 Km
N
0
1.5
3 Km

Figure 2.

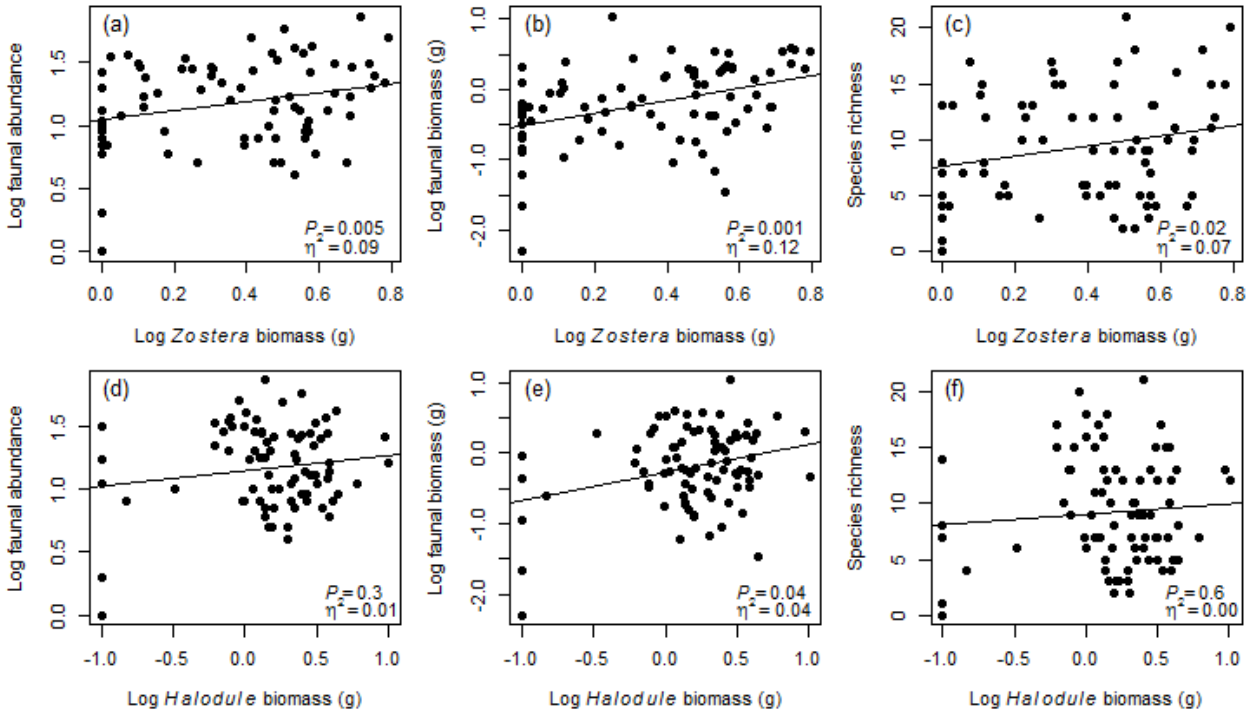

(a)
Log faunal abundance
Log Zostera biomass (g)
P = 0.005
η² = 0.09
(b)
Log faunal biomass (g)
Log Zostera biomass (g)
P = 0.001
η² = 0.12
(c)
Species richness
Log Zostera biomass (g)
P = 0.02
η² = 0.07
(d)
Log faunal abundance
Log Halodule biomass (g)
P = 0.3
η² = 0.01
(e)
Log faunal biomass (g)
Log Halodule biomass (g)
P = 0.04
η² = 0.04
(f)
Species richness
Log Halodule biomass (g)
P = 0.6
η² = 0.00

Figure 3.

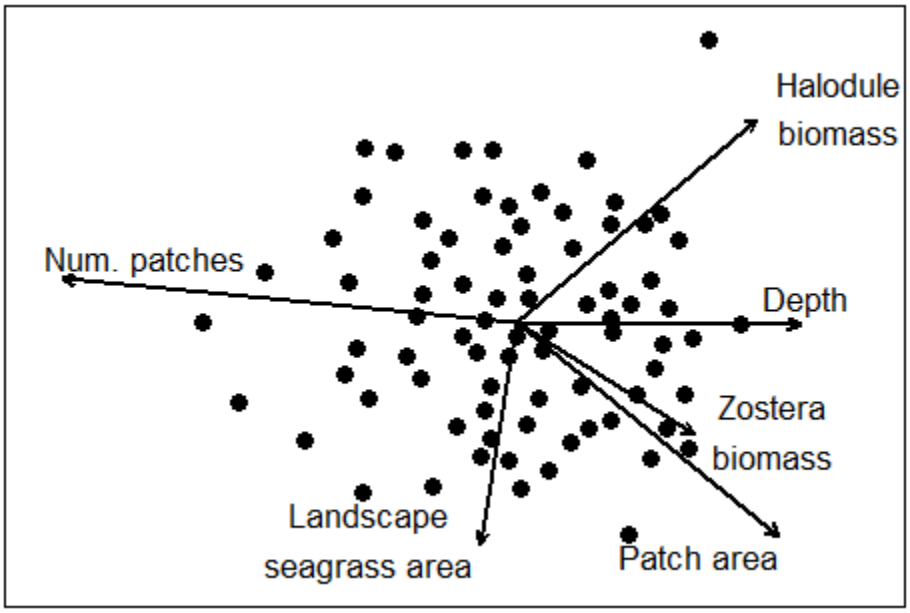
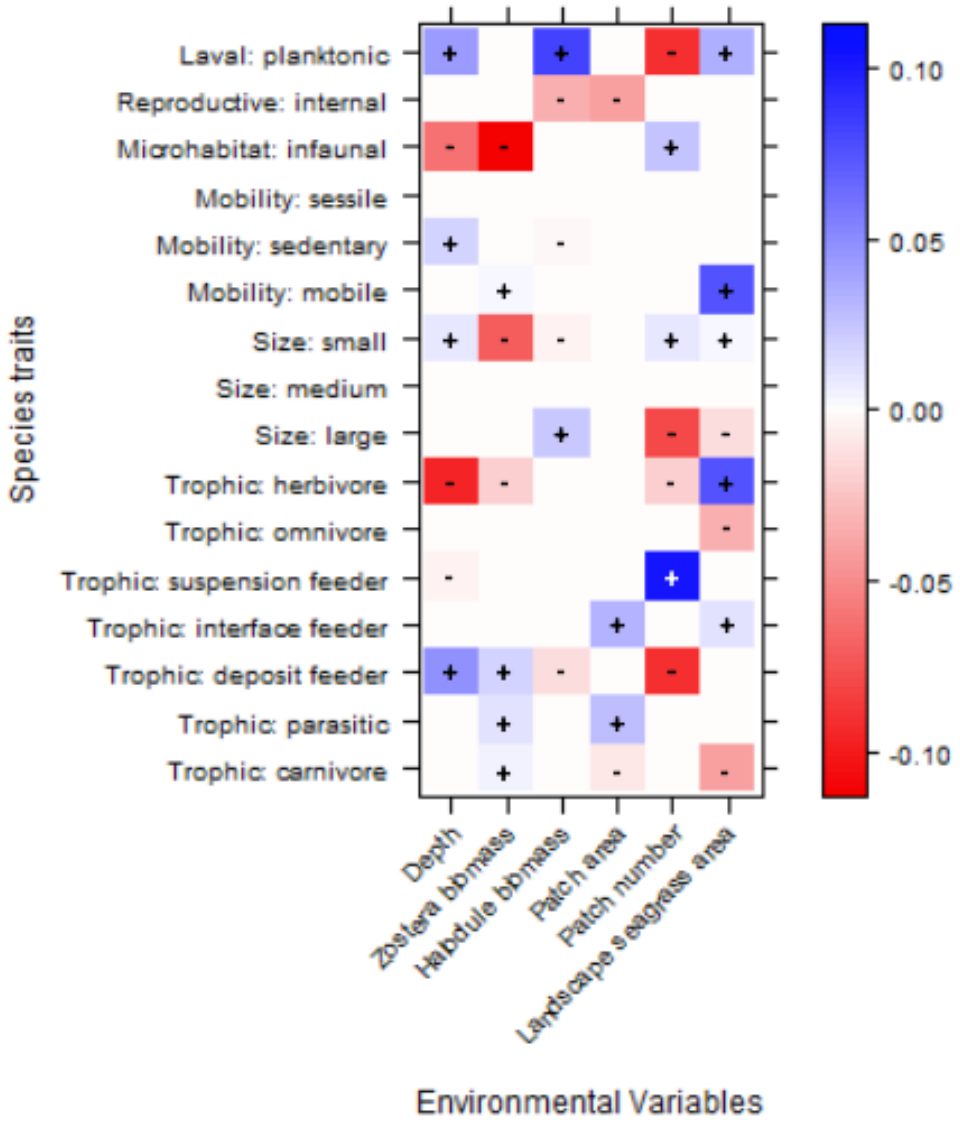

Halodule
biomass
Num. patches
Depth
Zostera
biomass
Landscape
seagrass area
Patch area

Figure 4.


Laval: planktonic
Reproductive: internal
Microhabitat: infaunal
Mobility: sessile
Mobility: sedentary
Mobility: mobile
Size: small
Size: medium
Size: large
Trophic: herbivore
Trophic: omnivore
Trophic: suspension feeder
Trophic: interface feeder
Trophic: deposit feeder
Trophic: parasitic
Trophic: carnivore
Species traits
Depth
Zostera bbmass
Halodule bbmass
Patch area
Patch number
Landscape seagrass area
Environmental Variables
0.10
0.05
0.00
-0.05
-0.10

Task 4: Use tethering experiments (small crabs as prey) to measure the foraging rates of higher consumers among landscape types.

We present our findings in a single section.

*Mahoney, R, MD Kenworthy, JK Geyer, and FJ Fodrie (planned as submission to Journal of Experimental Marine Biology and Ecology) Prey abundance, predator distribution, and relative predation intensity reveal limited edge effects within a temperate seagrass habitat.**

**the Discussion of this section represents an under-revision draft version of the manuscript.*

Abstract:

Edge effects may be important drivers of community dynamics across marine habitat mosaics. Past research has consistently suggested that within temperate and sub-tropical seagrass habitats, organisms inhabiting meadows edges experience lower survivorship, presumably following from increased predation. However, these survivorship trends have not consistently translated to differences in faunal densities between edge and interior regions of seagrass meadows. We evaluated the evidence of edge effects on predation upon two dominant members of temperate eelgrass (*Zostera marina*) communities: blue crab (*Callinectes sapidus*) and pinfish, *Lagodon rhomboids*). We considered three lines of data to arbitrate the null hypothesis that edge has no impact on predation on blue crabs or pinfish: (1) catch-per-unit-effort (CPUE) of blue crabs and pinfish within edge (<1 m from unstructured sandflat boundaries) versus interior (> 3 m from unstructured sandflat boundaries) regions of eelgrass meadows; (2) distribution of acoustically tagged red drum (*Sciaenops ocellatus*), a recognized predator of both blue crabs and pinfish, within eelgrass meadows (at 1-m bin resolution); and (3) mortality of tethered blue crabs and pinfish within edge versus interior regions of eelgrass meadows. Additionally, tethering trials considered the potential interactions between local habitat complexity and edge effects via manipulations of seagrass shoot density. We found no statistically detectable difference in catch rates of blue crab or pinfish in edge versus interior habitats, and red drum detection frequencies were statistically indistinguishable moving from the seagrass-sandflat boundary toward the meadow interior. Despite these findings, we did record statistically and ecologically significant edge effects on predation rates of tethered blue crabs and pinfish. Counter to previous work, we found that blue crabs survived >2.5x longer, and pinfish survived 2x longer, along the meadow edge relative to seagrass interior. Furthermore, the strength of these predation-related edge effects was most notable for blue crabs within plots with higher shoot density, while the opposite pattern was true for pinfish. These findings are, in part, consistent with the dichotomy apparent in the seagrass literature with respect to edge effects on faunal density and survivorship, but also provide new detail on how habitat edges

may affect the population ecology of larger bodied, more mobile prey species that have not received as much attention in previous studies (i.e., higher survivorship possible along edges).

Introduction

Estuarine landscapes are comprised of mosaics of interconnected habitats such as mudflats, salt marshes, shellfish reefs, mangrove forests, and seagrass meadows. Along the boundaries between these habitats, edge effects may manifest as differences in the density, biomass, settlement, growth, or survivorship of flora and fauna between the interior versus outer margin (i.e., edge) of a habitat patch (Jelbart et al. 2006, Johnson and Heck 2006, Murcia 1995, Ries et al. 2004). In marine habitats, these differences may arise following from gradients in flow (e.g., food or larval supply; Carroll et al. 2012) or predator accessibility (Smith et al. 2011) along an axis moving from the habitat boundary in to the interior. Despite widespread appreciation for the potential importance of edge effects on population fitness or community dynamics, the width of functional edges within habitat patches are notoriously hard to define and are likely taxa and habitat specific. Moreover, evidence regarding the direction (i.e., negative, positive) of impact of edges on resident organisms is equivocal, with many examples of fitness being depressed (Shulman 1985 and Amortegui-Torres et al. 2013), enhanced (Baltz et al. 1993 and Peterson and Turner 1994), or insensitive (Hindell and Jenkins 2005 (Biomass)) with regard to proximity to habitat boundaries.

When focusing within individual estuarine habitats; however, more consistent edge effects may emerge. Seagrass, for example, is one of the major structurally complex, biogenic habitats in estuarine environments, and has received considerable attention in studies assessing edge effects (Bostrom et al. 2006). Seagrass serves as a useful model system in this context due to the diverse patch orientations observed among meadows (Bostrom et al. 2006, Yeager et al. 2016), clear boundaries between seagrass and unstructured sandflat habitats, as well as the presence of abundant and species-rich faunal communities (Thayer 1984). In particular, the dynamics of predator-prey interactions along habitat edges, with subsequent effects on faunal density, have been scrutinized across a range of seagrass species and predator-prey combinations (Table 1). We reviewed the literature and identified six studies that explicitly compared survivorship of small prey species (bivalves, crustaceans, and small fishes) in edge versus interior regions of seagrass meadows. In every case, survivorship of these taxa was depressed along seagrass edges, presumably due to elevated predation threats (e.g., Carroll et al. 2012; Table 1). Notably, these differences in survivorship between edge versus interior habitats do not appear to reliably translate in to elevated bivalve, crustacean, or fish densities in interior regions of seagrass habitat relative to seagrass edges (e.g., Bell et al. 2001 versus Warry et al. 2009). Approximately two-thirds of the 17 published studies actually documented higher densities along edge regions of seagrass habitat relative to seagrass interiors (Table 1).

Several factors may contribute to this disconnect. Elevated settlement (Carroll et al. 2012) or growth rates (Bologna and heck 2002) within edge regions of seagrass habitat may offset relationships between survivorship and density. Additionally, many seagrass-associated species are mobile (Table 1), and movement between edge and interior regions within habitat patches may swamp gradients in predation pressure, attenuating the effects of spatially structured survivorship on resultant prey density patterns. Furthermore, most previous seagrass-related studies have examined survivorship of small epifauna (bivalves, mesograzer crustaceans) preyed upon by crabs and fishes that range in size between 5-15 cm (carapace width or total length). Notably, these predatory crabs and fishes are also subject to predation by even larger fishes, birds, reptiles, and mammals that may forage differentially between edge and interior regions of habitat patches. For instance, tiger shark tracking has shown that these large mobile predators prefer seagrass edge microhabitats (Heithaus et. al 2006). Indeed, large (>50 cm) mobile predators are often conceptualized as putative edge specialists within estuarine habitat mosaics (Wirsing et al. 2007), although quantitative data on the distribution of these animals is markedly rare at landscape scales. Thus, understanding how these higher-order predators affect the survivorship and distribution of mesopredators (5-15-cm crabs and fishes) could inform why previous research has documented an obvious disconnect between the survivorship and abundance of even smaller (1-5 cm bivalves, crustaceans, and fishes) seagrass-associated fauna along edge-to-interior gradients (sensu Table 1).

Within temperate U.S. East Coast and Gulf of Mexico estuaries, red drum (*Sciaenops ocellatus*) are often anecdotally depicted as edge predators along seagrass, oyster reef, and saltmarsh habitats. Red drum are also major predators on blue crab (*Callinectes sapidus*) and pinfish (*Lagodon rhomboids*), which themselves function as dominant mesopredators within seagrass communities (CITATION). Notably, blue crab (Hovel and Lipcius 2002) and pinfish (Hovel et al. 2002) densities have been reported as depressed along seagrass meadow edges, relative to interior microhabitats, potentially corroborating the hypotheses that higher-order predators such as red drum exert elevated mortality of mesopredators along seagrass edges. Therefore, we examined the potential for differences in predator-prey interactions among these species between edge and interior regions of seagrass meadows to expand the functional groups (e.g., larger body size, greater mobility) represented in tests of edge effects, and potentially contribute toward the reconciliation of an existing paradox evident in previous edge studies regarding seagrass-associated faunal survivorship and density patterns. Our approach was to evaluate evidence for edge effects in this model system using three complimentary lines of data: relative density of blue crabs and pinfish between edge and interior regions of seagrass meadows; distribution of red drum between edge and interior regions of seagrass meadows; and mortality of blue crabs and pinfish between edge and interior regions of seagrass meadows.

Methods

Study Sites
Our investigation of seagrass edge effects on predator-prey dynamics took place in Back Sound, NC (N34º40, W76º34). We leveraged two existing studies, comprising two study sites, to complete our experiments. First, density (catch rates) and mortality (loss rates of tethered individuals) of blue crabs and pinfish were monitored within a 5,600 m^2 seagrass meadow at Jack's Island along the southern rim of Back Sound. These density and mortality measurements were collected June-July, 2015, in connection with the global-scale Zostera Experimental Network study (sensu, Duffy et al. 2015). Second, the distribution of acoustically tagged red drum was evaluated July-August, 2011, across a 31,000 m^2 seagrass meadow and adjacent sandflat in Middle Marsh, along the western side of Back Sound (Fodrie et al. 2015). We exploited Middle Marsh for the tracking component of our study because of its semi-enclosed nature (useful in maximizing the detection range/frequency of fishes). Although we were not able to conduct all aspect of our study within the same seagrass meadow, the sites at Jack's Island and Middle Marsh are of comparable depth, tidal regime, salinity, seagrass shoot density/height, and faunal abundance - including blue crab and pinfish catch rates (Baillie et al. 2015). Therefore, we expect results from each site to be representative of the other in the broader context of how predation might affect blue crab and pinfish densities within temperate seagrass meadows.

Blue crab and pinfish densities between seagrass edge and interior
We recorded catch rates in Gee-style minnow traps (41-cm long, 22-cm wide, 0.3-cm galvanized mesh-wire cylinders, with funneled openings standardized to 4-cm diameter) as a proxy for juvenile blue crab and pinfish densities within the edge and interior regions of the seagrass meadow at Jack's Island. Catch rates were evaluated across five trials, with 24 minnow traps deployed during each cycle. Within each trial, we placed 12 minnow traps 0-1 m from the seagrass-sandflat boundary (i.e., "edge"), and 12 minnow traps >3 m from the seagrass-sandflat boundary (i.e., "interior"). These distances correspond with edge/interior classifications in previous seagrass studies (those cited in Table 1). We used a combination of baited (N=6; using 6-8 pieces of dried dog food; Able et al. 2015) and unbaited (N=6) traps during each deployment in both edge and interior regions of the seagrass meadow to sample individuals that may be attracted to the addition of food+structure (baited trap), or structure alone (trap only). Minnow traps soaked for 24 hour, after which we identified and enumerated all captured crustaceans and fishes. Catch-per-unit-effort (CPUE; individuals $trap^{-1}$) was determined for both blue crabs and pinfish for each of the 120 total trap deployments.

Red drum distribution between seagrass edge and interior
We used a combination of high-resolution aerial images and acoustic tagging to chart the movement activity of red drum in relation to distance from a sandflat-seagrass boundary in Middle Marsh as part of a broader red drum tracking study

(e.g., Fodrie et al. 2015 and methods therein). Briefly, satellite images (<1m) of Middle Marsh taken on July 15, 2011, were obtained through National Oceanic and Atmospheric Administration databases (Geo-Eye), and seagrass and sandflat habitats were subsequently mapped as distinct polygon features in ArcMap 10.0.

In July, 2011, we collected eight red drum (47.1 ± 2.7 cm total length, mean ± 1 SE) via hook-and-line from Middle Marsh. Coded acoustic tags (LOTEK Wireless MM-MR-11-28) were surgically implanted following Dresser and Kneib (2007), and these fish were released 24-h post-implant surgery at the center of our study site in Middle Marsh, where they were then allowed to move freely. The LOTEK tags emitted a pulsed chirp, unique to each fish, every 5 seconds that we used to chart each individual's position with respect to the seagrass-sandflat boundary.

We used a LOTEK MAP 600 Acoustic Telemetry System to detect the signals emitted from the tagged red drum. Our system consisted of eight georeferenced hydrophones, each cabled to a central processing unit. Hydrophones were positioned to allow "line of sight" (acoustic) for $\geq$3 of the listening stations along the entire seagrass-sandflat boundary (375 m in length), and to a distance of >40 m in to the interior of both the seagrass meadow and the adjacent sandflat. When signals from a LOTEK transmitter were detected by $\geq$3 of the hydrophones, we triangulated that fish's position with sub-meter accuracy. Detection accuracy was checked daily using beacon tags placed at known, georeferenced positions within the array (Fodrie et al. 2015). All detection locations where mapped in ArcMap 10.0 to determine the position of fish, throughout the entire study period, in relation to distance from the seagrass-sandflat boundary. We treated individual red drum as the unit of replication. For each fish, we binned position data with breaks at every meter from 0-40 m, both within the seagrass and the sandflat habitats, separately. We determined the spatial coverage (m^2) of each of those 1-m wide bins moving from the seagrass-sandflat boundary toward the habitat interior by creating new polygon features in ArcMap 10.0. We then calculated the detection density (detections m^{-2}) at each distance (e.g., 0-1 m, 1-2 m, 2-3 m, etc.) for each fish within seagrass and sandflat habitats, separately.

Predation on blue crab and pinfish between seagrass edge and interior

We utilized tethering experiments as a proxy of predator-driven mortality of blue crabs and pinfish within edge and interior regions of the seagrass meadow at Jack's Island. Our design consisted of 21 "edge" (0-1 m from seagrass-sandflat boundary) and 21 'interior" (>3 m from seagrass-sandflat boundary) plots, with each plot defined by two 1x1m subplots separated from each other by 0.5 m (but with the entirety of each plot being at the suitable distance for edge/interior designations). Each of the 42 total plots were separated from one another by >2 m. For both the edge and interior treatments, seagrass shoot density was reduced by 50% in a third of the plots, seagrass shoot density was reduced by 80% in another third of the plots, and seagrass shoot density was left at ambient in the final third of plots (all randomly assigned). This resulted in a 2x3

experimental design in which meadow location and shoot density were fully crossed. Reduction of shoot densities was achieved by deploying a 1x1-m quadrant with a 10x10 grid (with each grid cell = 0.01 m^2). We then removed all seagrass in 50 or 80 of the cells for the 50% and 80% reduction treatments, respectively. The resultant shoot densities were as follows: ambient treatments had a mean of 570 shoots m^{-2}50% reduction treatments had a mean of 283 shoots m^{-2}, and 80% reduction treatments had a mean of 124 shoots m^{-2}.

We deployed 126 tethered blue crabs (5.2 ± 0.1 cm carapace width) and 168 tethered pinfish (5.1 ± 0.1 cm total length) in each plot over three and four trials, respectively. We ran one less trial with blue crabs due to the availability of specimens within our preferred size range during our experimental window. During each trial, a tethered blue crabs was randomly assigned to one of the subplots within each plot, while a tethered pinfish was placed in the remaining subplot (i.e., 42 juvenile blue crabs and 42 pinfish were deployed in each trial). Each tethering device consisted of a lawn staple as an anchor placed in the center of a subplot, connected to a 30-cm long section of 3.6-kg clear monofilament fishing line. For blue crabs, the free end of the monofilament was glued to the center of the crab's carapace after making a lasso around the crab's body. Blue crabs had each of their claws glued shut using Loctite super glue gel to prevent them from cutting the tether. Pinfish were tethered through the soft tissue immediately behind their lower jaw bone by piercing this tissue, threading the line through the piercing, and the tying an overhand knot in the line. As a method check, we tethered >40 individual blue crabs and >20 individual pinfish in laboratory tanks outfitted with artificial seagrass. Over a 4-day period, none of the tethered animals became free, tethered pinfish did not behave noticeably different that untethered pinfish also in the tank, and tethered animals did not become entangled in artificial seagrass blades.

Blue crabs and pinfish were deploying in our field experiment ~3 hours before daytime, high tides. Following deployment, each tethered animal was checked after 1 hour, 2 hours, 3 hours, and 24 hours to assess loss rates (presumably via predation). Individual blue crabs or pinfish missing at the 1-, 2-, 3-, and 24-hour checks were randomly assigned a survival time ranging between 0-1, 1-2, 2-3, and 3-24 hours, respectively. Any animal remaining on its tether after 24 hours was assigned a survival time of 24 hours, and then immediately released.

Statistical approaches

To assess whether catch rates of blue crabs and pinfish varied across a seagrass meadow, we ran two-way ANOVAs, separately for each species, with location (edge v interior) and trap presentation (unbaited v baited) as factors on CPUE data from minnow traps (individuals $trap^{-1}$). CPUE data passed tests of normality and homoscedasticity (F-max test) for each main effect for both species. To assess the distribution of red drum across a seagrass-sandflat ecotone, we ran one-way ANOVAs, separately for acoustic positioning data from the two distinct habitat types. We used distance from the seagrass-sandflat boundary (in 1-m bins) as the experimental factor, and detection density within

each distance bin, averaged among fish, as the response variable (detections m^{-2} $fish^{-1}$). For both seagrass and sandflat, the red drum position data appeared normally distributed and passed the parametric assumption of homoscedasticity (Levene's test). Finally, two-way ANOVAs were performed to assess the main and interactive effects of meadow location (edge v interior) and local shoot density (ambient v 50% reduced v 80% reduced) on predation rates of tethered blue crabs. Time to consumption (removal from tether) of each blue crab among treatment combinations was used as the response variable. Time-to-consumption data passed tests of normality and homoscedasticity (F-max test) for blue crabs, but only the normality assumption for pinfish. Log (x+1) transformations failed to homogenize variance levels between treatments (edge v interior) for pinfish data. Therefore, we proceeded with non-parametric analogues to assess the effects of meadow location (Mann-Whitney U) and shoot density (Kruskal-Wallis) on time to consumption for tethered pinfish. Statistical significance was set at $\alpha = 0.05$ for all analyses. We conducted all analyses in StatView 5.0.1.

Results

Blue crab and pinfish densities between seagrass edge and interior

Pinfish (63.2% of total catch) and blue crabs (15.2%) dominated our catches, in which overall we captured 809 individual animals representing 20 species. For blue crab CPUEs, we found no statistically significant interaction between ($F=0.262$, $df=1$, $P=0.610$), or main effects of, location within the seagrass meadow ($F<0.001$, $df=1$, $P=0.993$) and trap presentation ($F=1.501$, $df=1$, $P=0.223$). Indeed, we uniformly captured ~1.5-1.75 blue crabs $trap^{-1}$ across all treatment combinations (Fig. 2a). Similarly, we found no statistically significant interaction between meadow location and trap presentation on the CPUEs of pinfish ($F=0.010$, $df=1$, $P=0.921$), and there was no statistically reliable main effect of meadow location on pinfish CPUEs ($F=0.273$, $df=1$, $P=0.602$). We do acknowledge, however, that in both baited and unbaited traps, there were statistically non-significant trends of higher pinfish CPUEs along the seagrass meadow edge (~4-9 pinfish $trap^{-1}$) relative to the interior (~2-7 pinfish $trap^{-1}$) (Fig. 2b). Unlike blue crabs, we did document a statistically significant effect of trap presentation on pinfish CPUEs ($F=20.179$, $df=1$, $P<0.001$). Along both the meadow edge, as well as in the meadow interior, we caught ~25-50% more pinfish in baited traps than in unbaited traps (Fig. 2b).

Red drum distribution between seagrass edge and interior

For both the seagrass meadow ($F=0.126$, $df=39$, $P=0.999$) and sandflat ($F=0.445$, $df=39$, $P=0.994$), red drum detection densities were statistically indistinguishable moving across transects from the seagrass-sandflat boundary towards the interior of each habitat. Broadly, we recorded 0.02-0.08 detections m^{-2} $fish^{-1}$ across the entire seagrass meadow (Fig. 3). Although the data suggested a modest peak in detection densities at 10-15 m inside the seagrass meadow, our study was characterized by high individual variability among red

drum, indicating no clear gradient in the use of edge versus interior regions of seagrass meadows of this predator at the population level. Compared to seagrass habitat, detection densities were uniformly low across the sandflat (Fig. 3; >0.02 detections m^{-2} $fish^{-1}$). We do note that within 4 m of the seagrass-sandflat boundary, the mean detection density of red drum on the sandflat roughly doubled to ~0.04 detections m^{-2} $fish^{-1}$), although this trend was also characterized by high inter-individual variability (Fig. 3).

Predation on blue crab and pinfish between seagrass edge and interior
For predation rates on blue crabs (time to consumption), we found a statistically significant interaction between location within the seagrass meadow and shoot density (F=3.448, df=2, P=0.036). While time to consumption generally decreased with decreasing shoot density, there was little difference in time to consumption between edge and interior treatments when seagrass shoot density had been reduced by 50% (time to consumption was ~14 hours regardless of meadow location) or 80% (time to consumption was ~10 hours regardless of meadow location) (Fig. 4a). Conversely, mean time to consumption of tethered blue crabs was >2.5x longer in edge plots (~20 hours) relative to interior plots (~7.5 hours) where seagrass shoot density remained at ambient levels (Fig. 4a). Overall, only 12% of blue crabs tethered along the meadow edge were consumed within 3 hours, while 40% of the blue crabs tethered within the seagrass interior were consumed during that period. Time to consumption of pinfish was consistently ~2 hours across shoot densities (averaged between seagrass locations within the meadow; Fig. 4b), and not strongly affected by our experimental manipulation (removal) of patch-level biogenic structure (H=5.533, df=2, P=0.063; with no clear differences in mean time to consumption across treatments). Pinfish survival was statistically different, however, between edge and interior treatments (Z=-2.243, P=0.024). Across all individuals, 70% of pinfish tethered along the seagrass edge were consumed within 3 hours, while 94% of pinfish tethered in the seagrass interior were consumed over that interval. Whereas location within the seagrass bed appeared to have the largest effect on blue crab survival at ambient shoot densities, the opposite was true for pinfish. Time to consumption for pinfish was roughly equivalent between meadow locations at ambient shoot densities (~4.5 hours), but was 2x longer in edge versus interior plots in both the 50% and 80% shoot reduction treatments (i.e., ~4.5 hours compared to ~2.25 hours) (Fig 4b).

Discussion

A seagrass meadow edge is often defined as the area of seagrass that is $\leq$1 meter from the sandflat-seagrass boundary (Table 1). To different faunal species, the edge and interior of seagrass meadows offer different benefits and risks, making it difficult to determine which area the different species prefer. Here, we measured blue crab and pinfish survival rates, blue crab and pinfish catch rates, and red drum detection frequencies looking for differences from the edge to the interior of a seagrass meadow. The blue crabs and pinfish had higher survival

rates on the edge of the seagrass meadow and showed no difference in catch rates from the edge to the interior of the seagrass meadow. Despite the red drum being a major predator of the blue crabs and pinfish the detections showed it having no edge or interior preference in the seagrass meadow. Below we consider possible drivers that may, in part, explain why these relationships for the blue crabs and pinfish are present.

Blue crab and pinfish catch rates

Different densities of organisms within a given habitat often reflect the trade offs between risk of predation and food availability (Kneib 1987). However, blue crabs and pinfish are not showing a difference in catch rates from the edge to the interior of the seagrass meadow. Existing studies have received mixed results for whether faunal densities are highest on the edge or the interior of the seagrass meadow. One possible explanation for these mixed results may be the sampling gear used. In some existing studies, instantaneous sampling gears (e.g. cylindrical suction rings) have been used compared to sedentary sampling gears (e.g. minnow traps). Sedentary sampling gears will allow highly mobile organisms (e.g. blue crabs and pinfish) to encounter the sampling gear more often possibly making it difficult to detect any difference in catch rates. With our use of sedentary minnow traps, this could explain why we were unable to see a similar difference in catch rates and survival rates from the edge to the interior of the seagrass meadow.

Blue crab and pinfish survivorships and red drum detections

Existing predation literature has only pointed to predation being higher on the edges of seagrass meadows, most likely an explanation of the predators feeding behaviors (Table 1). In contrast, neither the blue crabs nor the pinfish demonstrated higher predation on the edges, but had higher survival rates on the edge compared to the interior of the seagrass meadow. One possible explanation for these results may be due to the larger body size of our experimental organisms. In two-thirds of existing predation studies looking at seagrass edge effects they looked at organisms that range from 10-48mm. Our mean for both the blue crabs and pinfish is above this size range. The increased size of our blue crabs and pinfish suggests they are a higher up predator compared to the smaller organisms used in other studies. The larger predators, like the blue crabs and pinfish in our study, could be driving the relationship of decreased survivorship on the edge for the smaller organisms used in other studies. In an estuary blue crabs and pinfish only experience predation pressure from a few different species. With blue crabs being a highly cannibalistic species, we assumed that they might be playing a role in the survival rates of the blue crabs in our study (Hines and Ruiz 1995). Little is known about adult blue crab movements over seagrass meadows, making it difficult to know if adult blue crabs were driving the survival rates observed for the blue crabs. The red drum detections did not demonstrate the red drum preferring the edge or the interior of the seagrass meadow. However, the red drum did show a response to the seagrass-sandflat boundary. From 4m into the sandflat to the seagrass sandflat

boundary the detections doubled compared to the rest of the sandflat. Stunz et. al 2002 supports our finding that red drum are not perceiving an edge within a seagrass meadow, but red drum do see the seagrass-sandflat boundary and are possibly perceiving other boundaries in different habitats.

Shoot density interactions

Shoot densities interacted with the position (edge or interior) within the seagrass meadow for survival rates of blue crabs and pinfish. Blue crabs showed the greatest shoot density interaction with the ambient shoot densities on the edge of the seagrass meadow. This interaction maybe due to the predators of the blue crabs (e.g. other blue crabs). Many fauna tend to select habitats with higher structural complexity to avoid predation (Bell and Westoby 1986). If the predator blue crabs are at a higher abundance in the ambient shoot densities in the interior of the seagrass meadow they could be driving the survivorship trends. In contract, the pinfish are showing the greatest shoot density interaction with the reduced shoot densities on the edge of the seagrass meadow. This interaction suggests that the predator of the pinfish is a highly mobile, visual predator.

Caveats

Two caveats of this study were tethering artifacts and two different study sites. Tethering of organisms to look at survival rates within a seagrass meadow has been assessed in several previous studies (Hovel and Lupcious 2002 and McIvor and Odum 1988). With laboratory studies done by us and others (Pile et al. 1996) it has showed that tethering can be a useful and unbiased way of looking at survival rates compared among treatments. The embayment in Middle Marsh was used for the red drum detections because of the close proximity of different habitats (eg. Salt marsh, seagrass, oyster reefs, etc.) used for other parts of research. In addition, it is a partially enclosed embayment and this would allows for a higher probability of detecting the fish entering the system over Jack's Island, which is a more open system. Jack's Island and Middle Marsh are around 4500 m apart so they experience similar tidal regimes, temperatures, salinity, and water clarity. The similarity between the two areas allows us to be confident in comparing them to each other.

Conclusions

Studies on edge effects are abundant in the published literature, but an overall pattern has still not emerged (Table 1). With so many different variables such as predator behaviors, prey abundances and survival rates, settlement, temporal variability, and adjacent habitats it is difficult to find an overall trend between different species. In this study, we examined edge effects for survivorship and catch rates for two major prey species (blue crabs and pinfish) and a major predators (red drum) detection frequencies over a seagrass meadow. In contrary to existing seagrass predation studies pointing to the edge being a more dangerous place our study points to the larger more mobile blue crabs and pinfish demonstrating higher survivorship at the edge of the seagrass meadow, with seagrass shoot densities also being an influencing variable. Blue crab

survival was highest in ambient shoot densities on the edge and in contrast, pinfish survival was highest in reduced shoot densities on the edge. This relationship is suggesting a predator-prey interaction taking place with the different shoot densities. However, catch rates were similar for the edge to the interior of the seagrass meadow, possibly due to our organisms being more mobile than other studied organisms. The red drum did not prefer the edge or the interior of the seagrass meadow, but did recognize the seagrass-sandflat boundary. Our study suggests that edge effects for blue crab and pinfish survivorship and catch rates may depend on a mix of deterministic processes (e.g. predator-prey interactions, shoot densities, level of organismal mobility). Future research on edge effects for faunal survivorship rates and catch rates should focus more on understanding where and why the larger mobile predators (e.g. adult blue crabs) are to better understand the results of the smaller less mobile fauna.

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Table 1. Summary table of studies examining potential edge effects on the density and survival of seagrass-associated fauna.

Study	Seagrass	Location	Taxa	Edge Distance	Response Variable	Conclusion
Hovel and Lipcius 2002	Z. marina	Virgina (USA)	Crustaceans	< 1 m	Density	Edge < Interior
Bell et al. 2001	H. wrightii, T. testudinum	Florida (USA)	Polychaetes	<1 m	Density	Edge < Interior
Johnson and Heck 2006	H. wrightii, T. testudinum	Florida, Alabama (USA)	Crustaceans	<1 m	Density	Edge < Interior
Hovel et al. 2002	H. wrightii, T. testudinum	North Carolian (USA)	Fishes	Undefined	Density	Edge < Interior
Carroll et al. 2012	ASU	New York (USA)	Bivales	<1 m	Density	Edge > Interior
Macreadie et al. 2010	ASU	South Australia (AUS)	Fishes	<0.5 m	Density	Edge > Interior
Macreadie et al. 2010	ASU	South Australia (AUS)	Crustaceans	<0.5 m	Density	Edge > Interior
Smith et al. 2008	H. nigricaulis	South Australia (AUS)	Fishes	<1 m	Density	Edge > Interior
Warry et al. 2009	H. nigricaulis	Port Phillip Bay (AUS)	Many taxa	0 m	Density	Edge > Interior
Bologna and Heck 1999	T. testudinum	Florida (USA)	Bivales	<1 m	Density	Edge > Interior
Eggleston et al. 1998	Z. marina, H. wrightii	North Carolian (USA)	Crustaceans	Undefined	Density	Edge > Interior
Eggleston et al. 1999	ASU	North Carolian (USA)	Crustaceans	Undefined	Density	Edge > Interior
Bologna and Heck 2002	T. testudinum	Florida (USA)	Multiple Invert Taxa	<0.5 m	Density	Edge ><= Interior
Jelbart et al. 2006	Z. capricorni	Sydney (AUS)	Fishes	<4 m	Density	Edge ≤ Interior
Bostrom et al. 2006	Multiple Species	Global	Multiple Invert taxa	Undefined	Density	Edge ≥ Interior
Tanner 2005	Zostera	South Austrailia (AUS)	Multiple Invert taxa	<1 m	Density	Edge ≥ Interior
Smith et al. 2011	H. nigricaulis	South Australia (AUS)	Fish	<1 m	Denstiy	Edge >< Interior
Carroll et al. 2012	ASU	New York (USA)	Bivales	<1 m	Surviorship	Edge < Interior
Peterson et al. 2001	Z. marina	Maine, Florida (USA)	Crustaceans	<2 m	Surviorship	Edge < Interior
Gorman et al. 2009	Z. marina	Newfoundland (CAN)	Fish	0 m	Surviorship	Edge < Interior
Hovel and Lipcius 2002	Z. marina	Virgina (USA)	Crustaceans	<1 m	Surviorship	Edge < Interior
Smith et al. 2011	H. nigricaulis	South Australia (AUS)	Fish	<1 m	Surviorship	Edge < Interior
Bologna and Heck 1999	T. testudinum	Florida (USA)	Bivales	<1 m	Surviorship	Edge < Interior

Artificial sea grass (ASU) articifical seagrass unit

Figure 1. Aerial imagery of Jack's Island (A) and Middle Marsh (B) seagrass study sites in Back Sound, North Carolina. Included in the Jack's Island imagery is the spatial orientation of tethering plots to assess the effects of location with meadow and seagrass shoot density on predation on juvenile blue crabs and pinfish.

Figure 2. Catch Per Unit Effort (CPUE; mean + 1SE) of Blue Crabs (A) and Pinfish (B) in baited and unbaited minnow traps. Catch rates of both species were recorded along the seagrass edge (<1 m from seagrass-sandflat boundary) and within the meadow interior (>3 m inside the seagrass-sandflat boundary).

Figure 3. Density of red drum detections, averaged across 8 acoustically tagged individuals (detections m^{-2} $fish^{-1}$), at 1-m intervals, from the seagrass-sandflat boundary (centered along the x-axis) to 40 m inside each of these two habitats.

Figure 4. Time to consumption of tethered blue crabs (A) and pinfish (B) as a function of location with a seagrass meadow and seagrass shoot density. Tethered animals were deployed along the seagrass edge (<1 m from seagrass-sandflat boundary) and within the meadow interior (>3 m inside the seagrass-sandflat boundary). Within these two meadow locations, tethers were placed in plots that either remained at ambient shoot densities, or in which shoot densities were reduced by 50% or 80%. We determined time to consumption via serial checks of tethered individuals at 1, 2, 3, and 24 hours.

Figure 1.

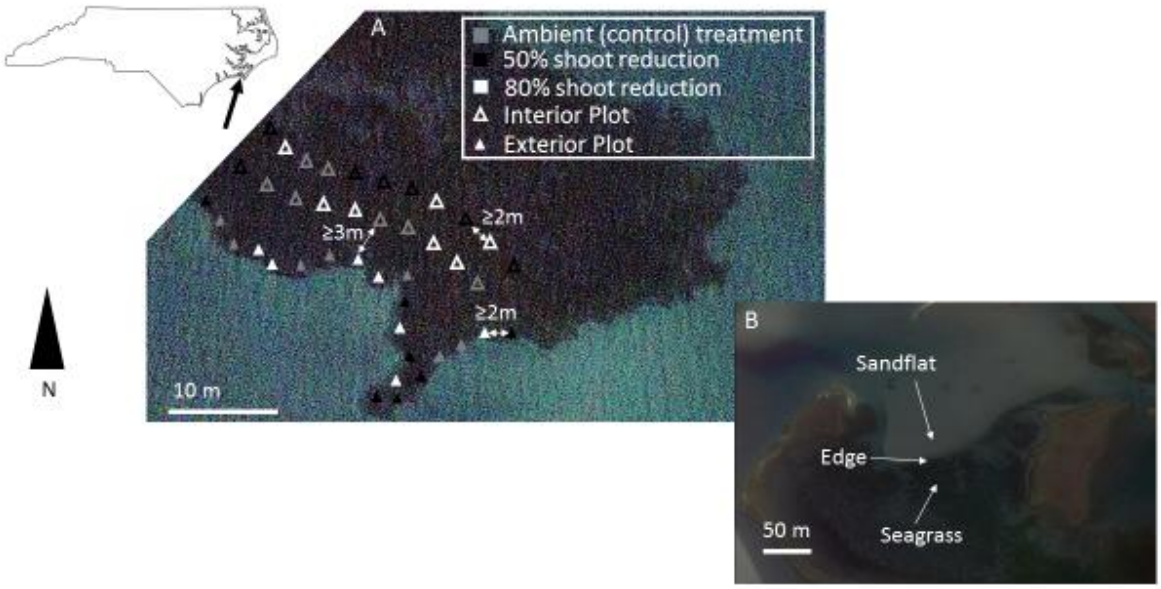

A
Ambient (control) treatment
50% shoot reduction
80% shoot reduction
Interior Plot
Exterior Plot
≥3m
≥2m
≥2m
N
10 m
B
Sandflat
Edge
Seagrass
50 m

Figure 2.

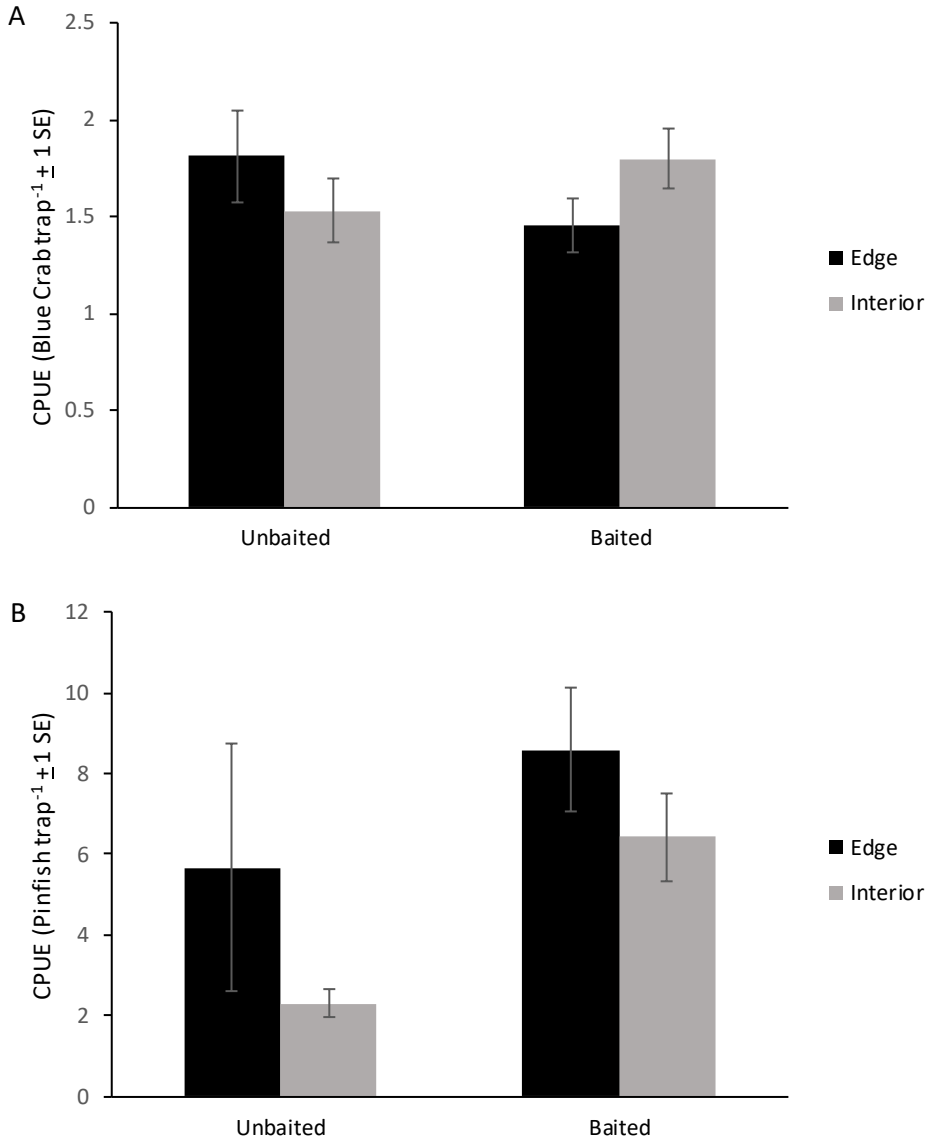

A
2.5
2
1.5
1
0.5
0
CPUE (Blue Crab trap-1 ± 1 SE)
Unbaited
Baited
Edge
Interior
B
12
10
8
6
4
2
0
CPUE (Pinfish trap-1 ± 1 SE)
Unbaited
Baited
Edge
Interior

Figure 3.

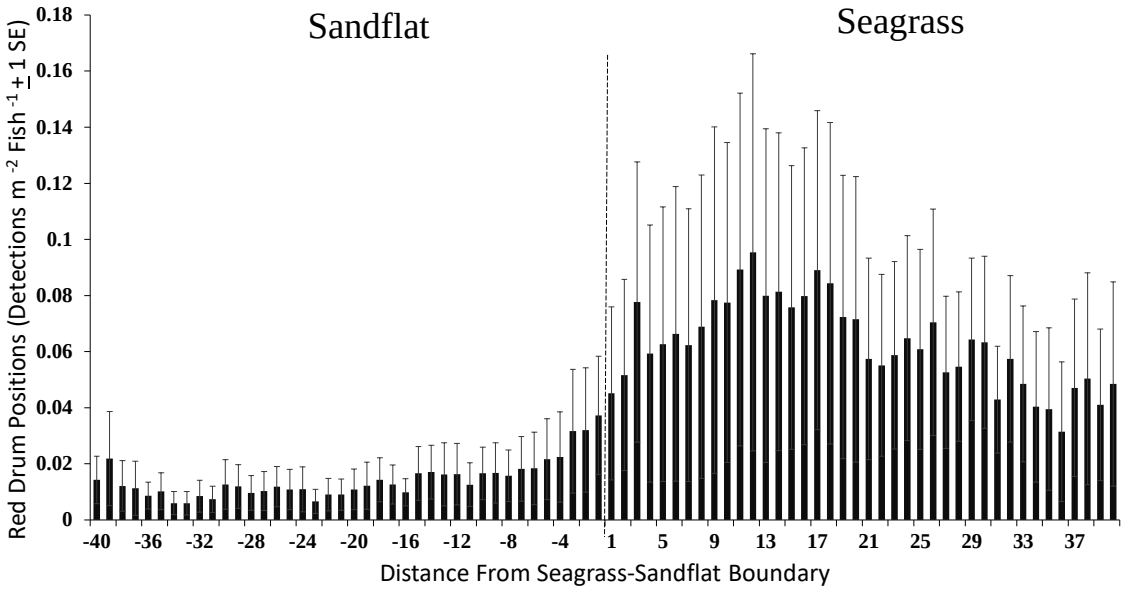
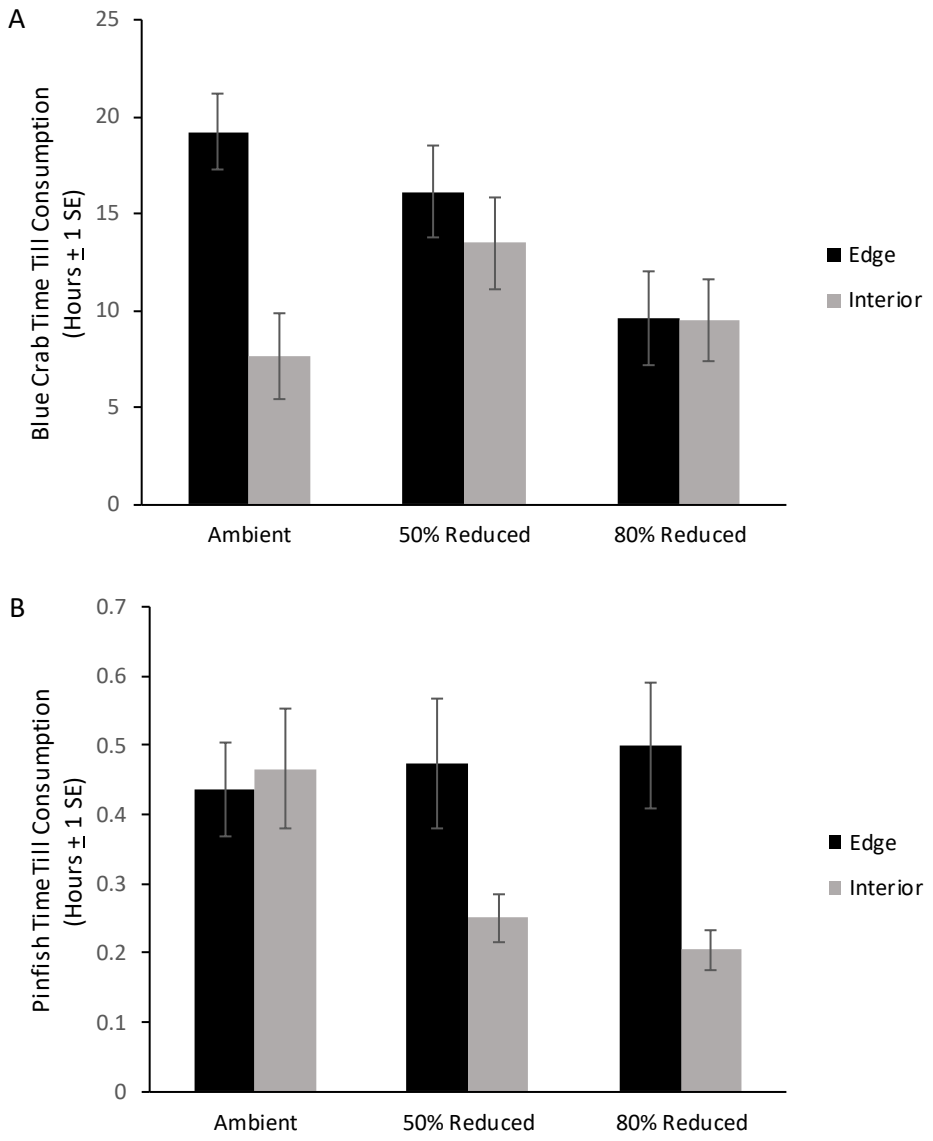

Sandflat
Seagrass
Red Drum Positions (Detections m -2 Fish -1 + 1 SE)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-40
-36
-32
-28
-24
-20
-16
-12
-8
-4
1
5
9
13
17
21
25
29
33
37
Distance From Seagrass-Sandflat Boundary

Figure 4.


A
Blue Crab Time Till Consumption (Hours ± 1 SE)
25
20
15
10
5
0
Ambient
50% Reduced
80% Reduced
Edge
Interior
B
Pinfish Time Till Consumption (Hours ± 1 SE)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Ambient
50% Reduced
80% Reduced
Edge
Interior

Task 5: Analyze tissue samples of select fishes (e.g., spot, flounders, gag, snappers) to quantify the condition of individuals among landscapes.

We present our findings in a single section.

ABSTRACT

Seagrass beds in North Carolina that are adjacent to saltmarsh support higher densities of fishes than either habitat in isolation, but the mechanism driving this apparent habitat enhancement is not yet clear. Food web subsidies from saltmarsh to seagrass food webs are one potential driver of this increased food web productivity. The importance of saltmarsh basal resources is difficult to track with traditional food web methods like direct diet observations. We set out to investigate the efficacy of stable isotopes in detecting evidence of salt marsh resource pools in seagrass food webs by running natural abundance stable isotope analysis, including $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, on a suite of seagrass producers and consumers. We found evidence of some effect of proximity to salt marsh on the isotopic signatures of seagrass itself in sites near marsh. This effect was not detectable at higher trophic levels, however. This preliminary results may suggest that saltmarsh production in not a dominant energy sources in adjacent seagrass food web, and that higher resolution food web methods (e.g., fatty acid analysis) may be necessary to discriminate weaker subsidies if they exist.

INTRODUCTION

Nearshore, vegetated habitats (e.g., seagrass, saltmarsh) are known to be highly valuable in terms of fisheries production, but studies often report wide variation in value among seemingly similar habitat areas. For example, recent research in North Carolina on the effects of landscape context of nearshore ecosystems has revealed that seagrass beds near marsh appear to be highly productive habitats for fishes when compared to either habitat in isolation (Ballie et al., 2015). Specifically, abundance of juveniles of many commercially valuable species like flounder (*Paralichthys* spp.), Gag grouper (*Mycteroperca microlepis*), Sheepshead (*Archosargus probatocephalus*), and Gray snapper (*Lutjanus griseus*) are 2x to 6x greater in seagrass beds adjacent to marshes than in seagrass beds isolated from marshes or marshes bordered by unvegetated bottom. These patterns invite the questions of what processes may be underlying these observed productivity hotspots. Preliminary work in these systems has not supported the importance of top-down control in driving differences in the abundance of fish mesopredators (Yeager et al., unpublished data).

Another hypothesis related to drivers of higher fish densities is that increased productivity of potential food sources is highest in these mixed seagrass/marsh areas. Fishes using seagrass beds adjacent to marsh could be making opportunistic foraging forays into marsh or are benefiting from detrital or prey flux from the marsh into adjacent seagrass beds. Many species like pinfish (which are important prey for higher-order predators) are known to make tidally-driven movements between seagrass and saltmarsh habitats, likely to feed (Irlandi 1997, Potthoff and Allen 2003). Diet analysis based on stomach contents

from these different habitat types is useful in determining prey identity. However, it is often difficult to determine resource pools or energy flow pathways from stomach content analyses alone. For example, stomach content analysis may reveal that flounder consume pinfish in both seagrass habitat that is isolated and seagrass that is connected to saltmarsh habitat. However, we would not be able to determine if that pinfish was feeding on resources ultimately derived from saltmarsh or seagrass production.

Stable isotope analysis has proved to be a highly-useful tool for food-web ecologists and is particularly informative in helping to distinguish among various basal resource pools (Post et al. 2002, Layman et al. 2012). Isotopic ratios of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ may all be applied in food-web studies and can each provide unique information about the diet source of a consumer. For example, $\delta^{13}C$ often varies among producers with different photosynthetic pathways (e.g., C_3, C_4 plants, algae). The $\delta^{15}N$ increases with trophic transfers and is therefore highest in predators. Finally, while $\delta^{13}C$ and $\delta^{15}N$ are the most commonly applied in food web studies, $\delta^{34}S$ may be even more useful in distinguishing among marine producers and typically varies more than $\delta^{13}C$ (Connolly et al. 2004). By combining analyses of multiple stable isotope ratios, the power to distinguish among producers increases. Based on published seagrass and saltmarsh isotopic values in other systems [e.g., the coasts of the Gulf of Mexico (Sullivan and Moncreiff 1990, Moncreiff and Sullivan 2001), Georgia (Peterson et al. 1985) Massachusetts (Galván et al. 2011), and Australia (Connelly et al. 2004, Melville and Connolly 2003)], we suspect that analysis of all three elements will be necessary to distinguish among various pools of primary production in North Carolina.

Our objective was to test whether stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) are effective tools for elucidating food-web connections between seagrass and saltmarsh habitats in North Carolina, with the hopes that this work will provide the foundation for more detailed studies into trophic connectivity and energy flow pathways in this system.

METHODS

During June and July of 2014 and 2015, we collected primary producers, primary consumers, and higher order consumers from seagrass and saltmarsh habitats in Back Sound, North Carolina. We compared seagrass beds that were both adjacent to saltmarsh (< 50 m from the marsh edge) and isolated from saltmarsh habitat (> 100 m from the marsh edge), including beds that were either contiguous or patchy (fragmented) within each. In total we sampled 12 seagrass beds for stable isotopes (3 replicates within each combination of marsh adjacency and fragmentation category, Figure 1).

Figure 1. Locations of 12 study sites within Back Sound, North Carolina. CI = contiguous, isolated form marsh sites, CN = contiguous, near marsh sites, PI = patchy, isolated from marsh sites, PN = patchy, near marsh sites.

Seagrass and saltmarsh samples from each site were collected by hand. Seston (zooplankton, phytoplankton and suspended particulates) was sampled by filtering ~2L of water on a 47 mm GF/F filter from each site. Microphytpbenthos was collected by carefully scraping the top ~5 mm of sediment into a small plastic bag. Small epibenthic invertebrates and fishes were collected using push nets and larger mobile species were collected with 5 m otter trawls at each site.

Once back in the lab, seagrass samples were carefully rinsed with DI water and epiphytes were scraped off the blades for separate analysis. Besides small gobies (which were analyzed whole), a small piece of dorsal muscle tissue was dissected for analysis. For blue crabs, muscle tissue from within the claw was removed. For mollusks, soft tissue was removed from the shell. All other animals were analyzed whole. After rinsing all samples with DI water, they were dried in the drying over at 60º C for 48-72 h. Samples were then ground to a fine powder and homogenized with a mortar and pestle. Samples were weighed into tin capsules (weight based on sample type and C/N/S concentration). In some cases when individuals were very small (e.g.,

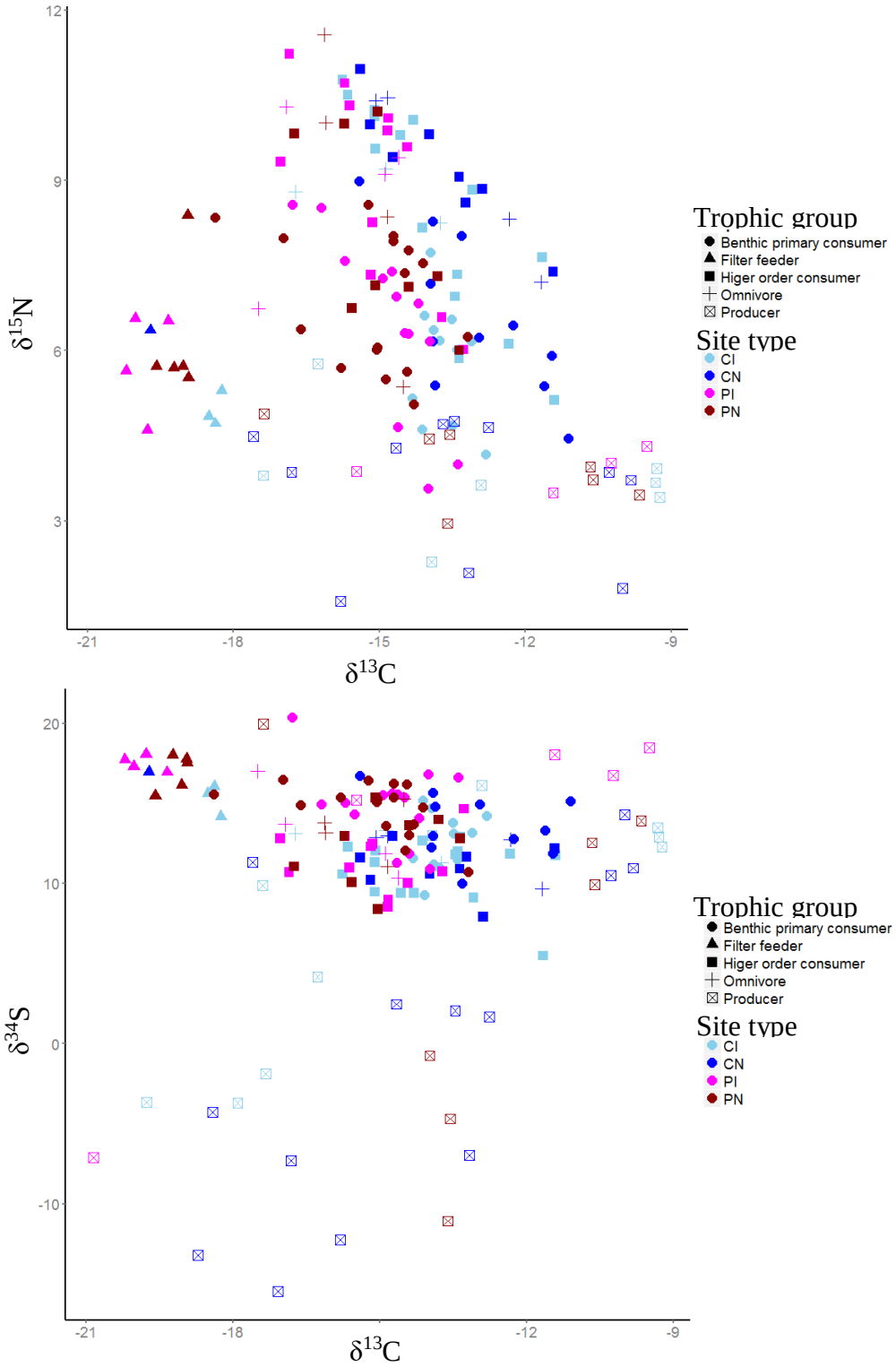


Figure 2. Isotopic signatures of all samples run for isotopic food web analyses plotted in bi-plot space. The shape of the point corresponds to trophic group of the organism and the color of the point corresponds to the type of the sites. See Figure 1 legend for site codes.

grass shrimp), multiple individuals were pooled to meet the minimum sample size for analysis. Two samples were run for each specimen, one for $\delta^{13}C$ and $\delta^{15}N$ and another for $\delta^{34}S$. For samples with a high amount of inorganic carbon (sediment, epiphytes, crustaceans), an additional sample was run for $\delta^{13}C$ after the sample was acidified with HCl. All samples were sent to the UC Davis Stable Isotope Facility for analyses.

To visualize the data we plotted samples in stable isotope bi-plot space ($\delta^{13}C$ vs. $\delta^{15}N$ and $\delta^{13}C$ vs. $\delta^{34}S$). We also compared values for each isotope across site types within taxa to see if there was an effect of proximity to marsh, fragmentation, or their interaction. We ran separate multiple regression analyses for primary producers, seagrasses, and higher order consumers to represent a range of trophic levels which might be effected.

Results and Discussion

We analyzed 340 samples for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ across 39 taxa (Figure 2). These included 9 primary producers, 15 benthic primary consumers, 6 omnivores, 2 filter feeders, and 7 higher order consumers. Seston samples still have to be re-run due to lower elemental concentrations, so we included literature values (Peterson et al. 1999) on plots for this group for reference.

For primary producers, $\delta^{13}C$ was most enriched (less negative) for seagrasses, intermediate for saltmarsh and seagrass epiphytes, and the most depleted (more negative) for microphytobenthos. $\delta^{13}C$ did not differ consistently across sites types when all producers were analyzed together ($P > 0.3$ in all cases; Figure 3). Seagrass $\delta^{13}C$ in sites near saltmarsh (CN and PN) was more depleted than the isolated sites (CI and PI; df = 1,20, $F = 4.48$, $P = 0.03$), being closer to the observed signatures of saltmarsh carbon. Seagrass $\delta^{13}C$ was also higher in the contiguous seagrass beds than in the patchy beds (df = 1,20, $F = 5.68$, $P = 0.03$). For $\delta^{15}N$ there was not clear separation among producer groups, although some groups (e.g., saltmarsh and saltmarsh microphtyobenthos) were more variable among samples. $\delta^{15}N$ did not differ overall across site types ($P > 0.08$ in all cases). However, for seagrasses, there were higher $\delta^{15}N$ values in patchy sites (df = 1,20, $F = 7.67$, $P = 0.03$) and sites isolated from marsh (df = 1,20, $F = 7.27$, $P = 0.01$). The $\delta^{34}S$ axis provided additional discrimination between seagrass and seagrass epiphytes from the microphytobenthos and saltmarsh carbon pools. The $\delta^{34}S$ values of primary producers differed systematically among site types, (df = 1,37, $F = 5.33$, $P = 0.01$), being higher in sites isolated from marshes. This appeared to be driven by lower $\delta^{34}S$ in seagrass and seagrass microphytobenthos at the near marsh sites. Analysis of seagrass alone supported this in that $\delta^{34}S$ was more enriched in sites isolated from marsh (df = 1,20, $F = 18.36$, $P = 0.003$). The shift in seagrass signatures along all three isotopic axes to be closer to the signatures of saltmarsh suggest some remineralization of saltmarsh tissue that is then taken up by seagrass locally.

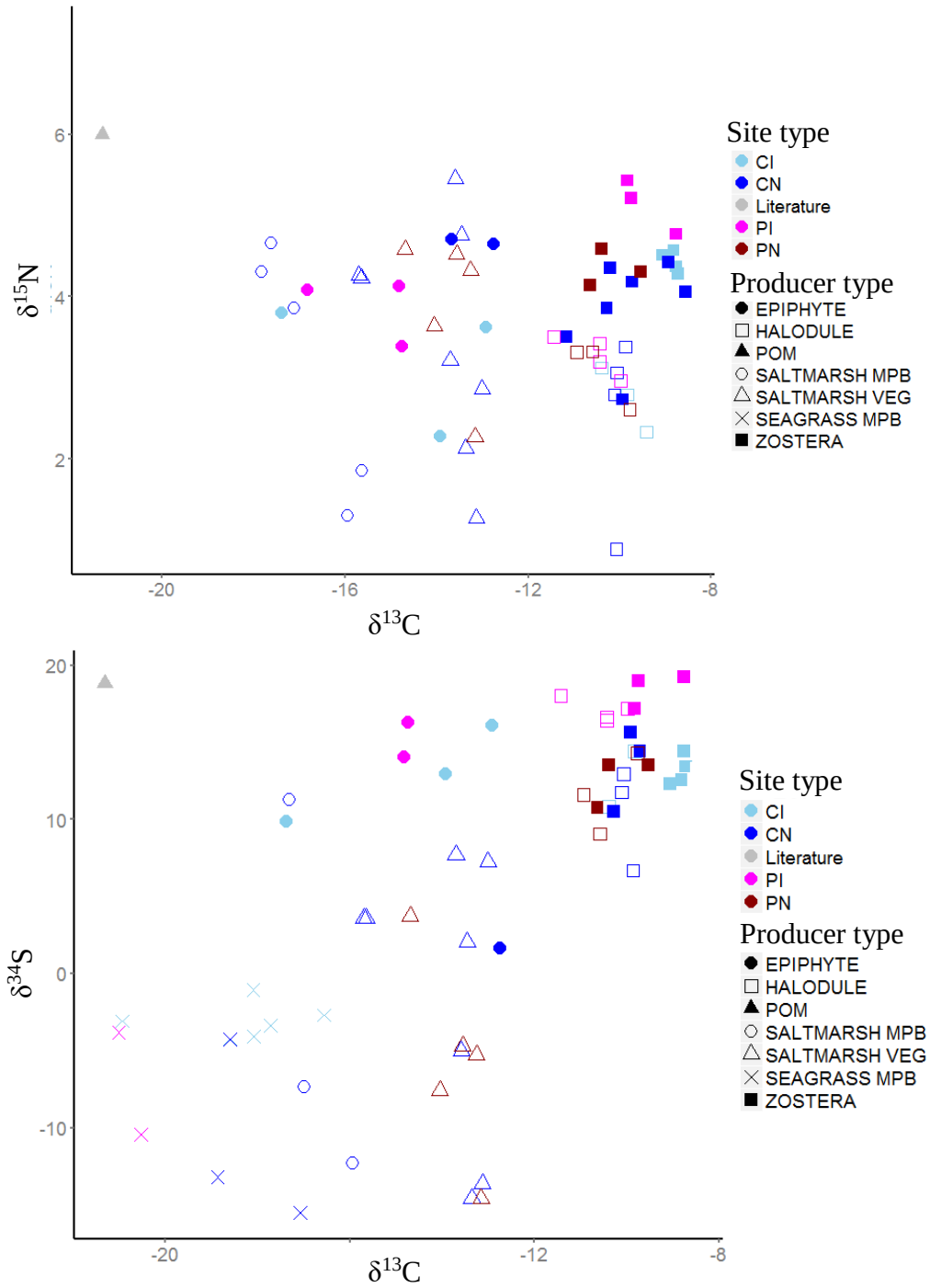


Figure 3. Isotopic signatures of primary producers run for isotopic food web analyses plotted in bi-plot space. The shape of the point corresponds to type of producer and the color of the point corresponds to the type of the sites. See Figure 1 legend for site codes.

For shrimps (*Tozeuma carolinense*, *Palaeomonetes* spp., Penaeidae, and *Hippolyte* spp.) there were taxa-specific differences in isotopic niche space (Figure 4). Signatures of the shrimps suggest that their diet is composed of seagrass, seagrass epiphytes or microphytobenthos. Along the $\delta^{13}C$ axis there was a distinct difference between patchy and contiguous sites (df = 1,41, F = 22.04, P <0.0001), matching the pattern of more enriched values in the contiguous sites for epiphytes. $\delta^{15}N$ values were more enriched in the near marsh sites (df = 1,41, F = 11.97, P <0.003), perhaps reflecting a heavier reliance on *Zostera marina*, as opposed to *Halodule wirghtii*, which is more abundant in these sites. There was no clear trend across sites for $\delta^{34}S$ for the shrimps. The lack of shift in $\delta^{34}S$ may indicate that incorporation of saltmarsh carbon is not an important source of diet differentiation across sites for shrimps.

For fish consumers [pinfish (*Lagadon rhomboides*), pigfish (*Orthopristis chrysoptera*), spot (*Leiostomus xanthurus*), and Gulf flounder (*Paralichthys albiguttata*)], the $\delta^{13}C$ signatures were variable within species and intermediate to the range of values observed for producers, likely reflecting the integration of multiple carbon sources in these higher order consumers (Figure 5). There was a shift towards more enriched $\delta^{13}C$ signatures for the near marsh sites (df = 1,91, F = 21.78, P < 0.0001). This may reflect a higher incorporation of seagrass carbon in the diets of fishes at these sites, but does not match what we would expect if saltmarsh carbon was more important. The $\delta^{15}N$ signatures were higher than the producers, consistent with what we would expect if they are feeding at 1-3 trophic levels higher than the producers. However, we didn't see any differences in $\delta^{15}N$ among species (df = 1,91, F = 2.07, P = 0.1). This was counter to our expectations as we expected to see that Gulf flounder would have the highest $\delta^{15}N$ values because they are pscivourous and were frequently observed with fish in their stomachs (LA Yeager, unpublished data). There were no differences in $\delta^{15}N$ with proximity to marsh or degree of fragmentation (P > 0.05 in both cases). There were no obvious shifts in $\delta^{34}S$ among site types, again suggesting that saltmarsh carbon does not likely represent an important contribution to fish diets.

CONCLUSIONS

The use of $\delta^{34}S$, in conjunction with $\delta^{13}C$ and $\delta^{15}N$, provided additional power to discriminate among primary producer in seagrass-saltmarsh food webs. Specifically, saltmarsh separated most strongly from seagrass along the $\delta^{34}S$ axis as opposed to the $\delta^{13}C$ and $\delta^{15}N$ axes. While we were able to detect some differences in the isotopic signatures of seagrass primary producers dependent on their proximity to marsh, these differences were not detectable at higher trophic levels. This loss of saltmarsh signal in consumers may indicate that saltmarsh is not an important basal resource pool for seagrass fish consumers, even in sites in close proximity. However, while the use of $\delta^{34}S$ seemed to be important in distinguishing marsh and seagrass sources pools, the analyses herein is likely not sensitive relatively small differences in diet contribution across sites. Identifying all potential basal source pools will likely require the use of more

sophisticated mixing models to parse out the relative contributions of multiple sources or new, higher-resolution tracers like fatty acid analysis or compound specific isotope work.

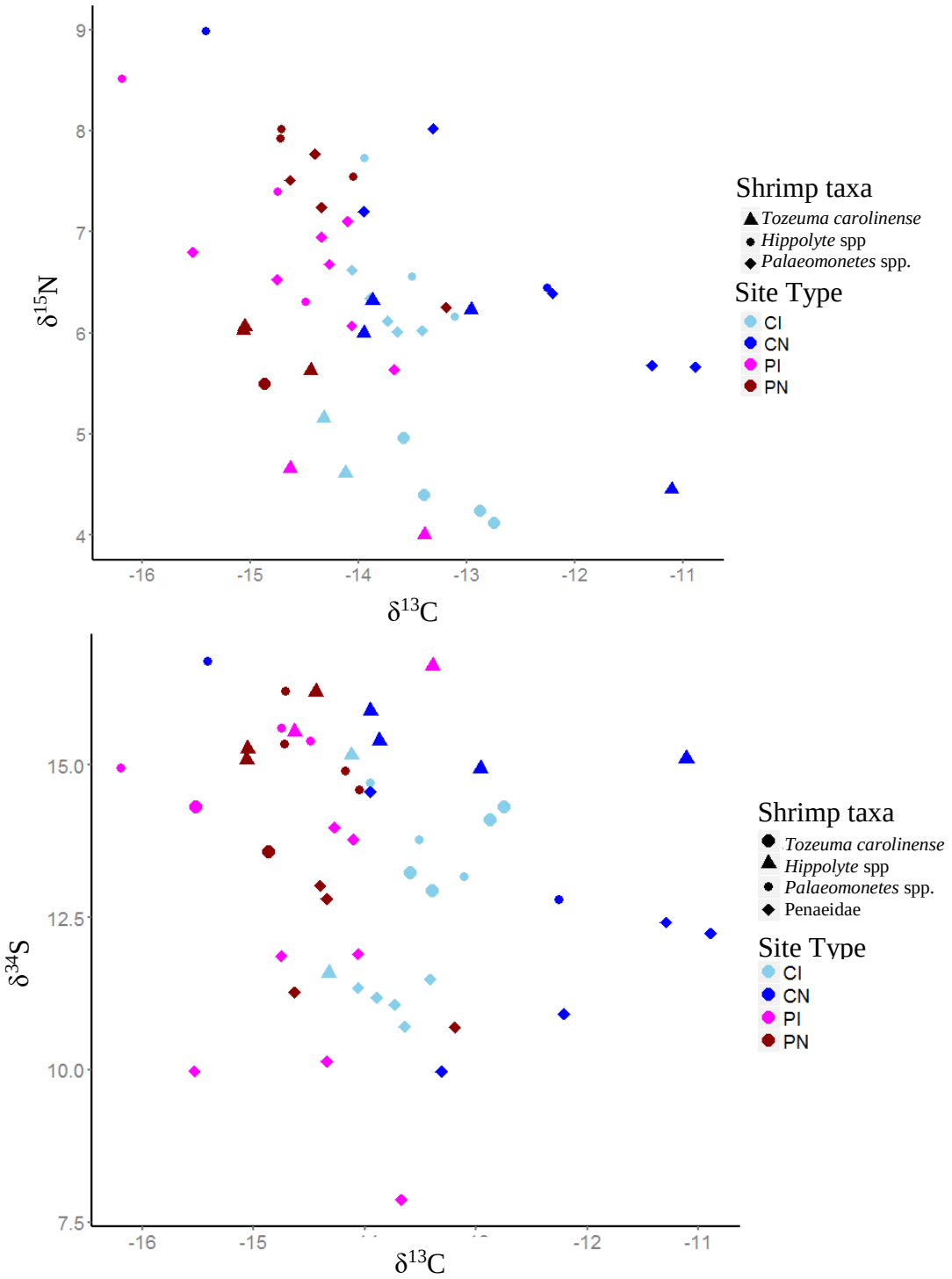


Figure 4. Isotopic signatures of shrimp consumers run for isotopic food web analyses plotted in bi-plot space. The shape of the point corresponds to shrimp taxa and the color of the point corresponds to the type of the site. See Figure 1 legend for site

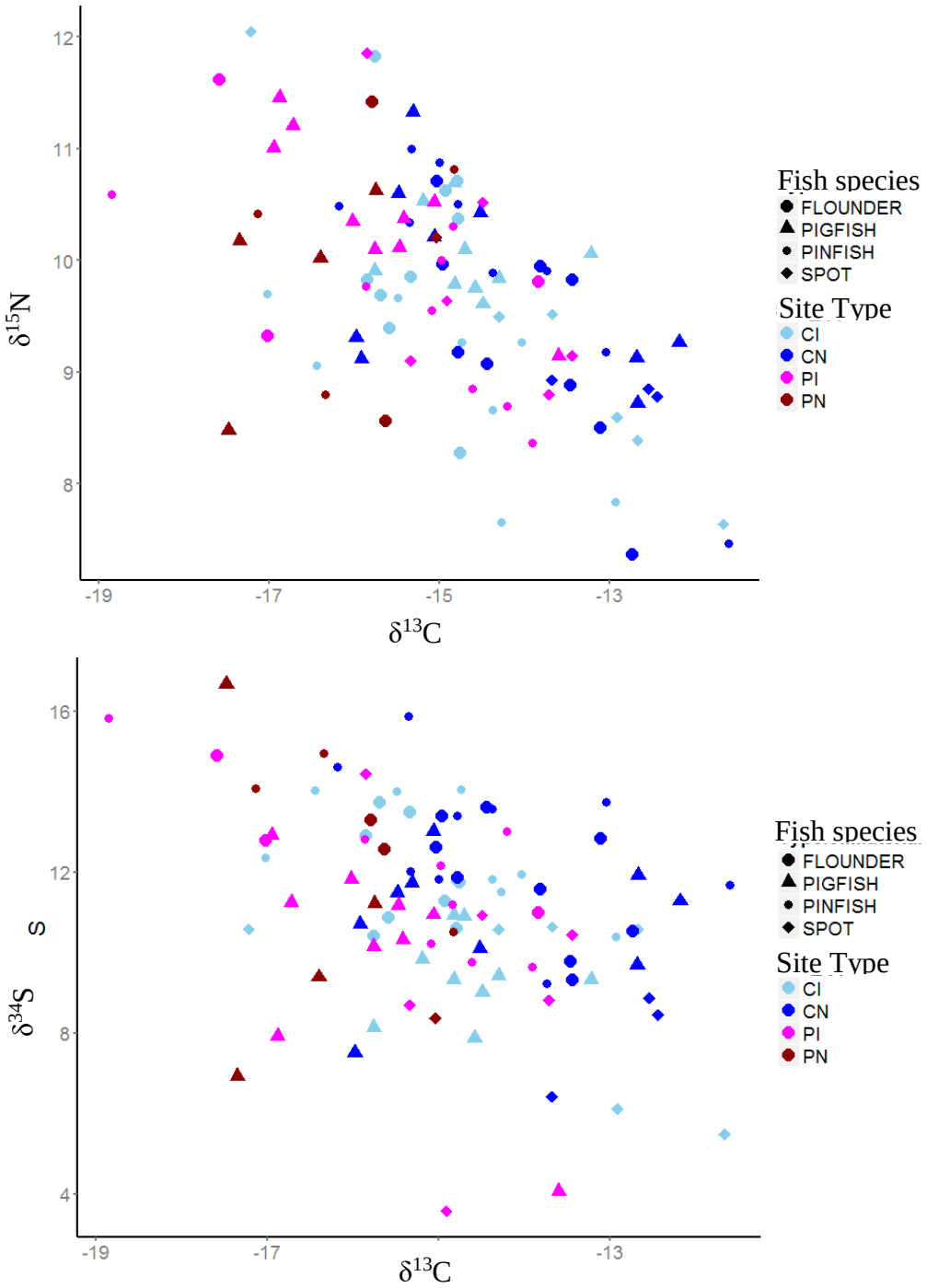


Figure 5. Isotopic signatures of fish consumers run for isotopic food web analyses plotted in bi-plot space. The shape of the point corresponds to the fish species and the color of the point corresponds to the type of the site where the fish was collected. Pinfish = *Lagadon rhomboids*; Pigfish = *Orthopristis chrysoptera*; Spot = *Leiostomus xanthurus*; Flounder = *Paralichthys albiguttata.* See Figure 1 legend for site codes.

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Sullivan M.J. and Moncreiff, C.A. 1990. Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analyses. Marine Ecology Progress Series 62: 149–159.

Task 6: Assess the effects of competition within various landscape settings on condition and foraging opportunities for select species (e.g., gulf and summer flounder).

We present our findings in two sections – the first of which explores the effects of fragmentation on the role of seagrass as fish habitat (vis-à-vis the diets of key taxa), and the second of which explores the effects of marsh-seagrass connectivity on the role of seagrass as fish habitat (again, vis-à-vis the diets of key taxa). The first section, on fragmentation, represents the independent research project of UNC-CH undergraduate student Danielle Douchette. The second section, on marsh-seagrass connectivity, represents the independent research project of UNC-CH undergraduate student Matthew Dwindell.

Assessing the impacts of seagrass habitat fragmentation on fish diets in Back Sound, North Carolina, USA

Abstract

This study investigated the impacts of seagrass bed fragmentation on fish diet for four species present in the seagrass habitat in Back Sound, North Carolina. The species studied include pigfish (*Orthopristis chrysoptera*), pinfish (*Lagodon rhombiodes*), spot (*Leiostomus xanthurus*) and flounder (*Paralichthys spp.*). It was found that flounder, the highest trophic level species, has greater resource consumption success in continuous seagrass beds, while the three lower trophic level species; pigfish, pinfish and spot; had greater resource consumption success in fragmented seagrass beds. These results have implications for the maintenance and restoration of critical seagrass bed habitats.

Introduction

Habitat fragmentation is a process in which continuous areas of habitat are divided into smaller, more isolated patches. Fragmentation of habitat results in loss of habitat and alteration of habitat configuration aspects such as habitat patch size, proportion of habitat edge to interior, and distance between habitat patches (Hovel 2003; Bell et al. 2001; Healey and Hovel 2004). Among other factors, these changes in habitat configuration influence the survival, distribution, community composition, abundance and behavior of fauna within the fragmented habitat (Healey & Hovel 2004; Bell et al. 2001). In terrestrial ecosystems, habitat fragmentation is known to be a key driver of biodiversity loss (Layman et al. 2007). Habitat fragmentation has been well studied in terrestrial ecosystems, but its effects in marine ecosystems remain largely unknown. Seagrass beds are excellent marine habitats in which to study the impacts of fragmentation because they occur in a variety of habitat configurations and habitat patch sizes can vary greatly within a small area, which minimizes spatial differences between patch sizes under study (Irlandi et al. 1995). One linear kilometer of seagrass landscape can contain patches ranging in size from less than $1m^2$ to more than

10,000m^2 (Hovel 2003). Seagrass beds are also highly productive ecosystems that serve as habitat for diverse and abundant coastal fauna (Hovel & Lipicus 2001; Hovel et al. 2002).

Worldwide, seagrass habitat covers 177,000 km^2 and its ecosystem services were estimated in 1997 to provide almost $3.8 trillion per year (Boström et al. 2006). These ecosystem services include: adding complexity to shallow soft-bottom systems; providing organisms with habitat, refuge and food; improving water clarity; stabilizing sediments; and serving as nursery habitat for commercially and recreationally important finfish and shellfish (Healey & Hovel 2004). Despite their ecological importance, seagrass habitats are increasingly at risk of anthropogenic loss and fragmentation. About 12,000 km^2 of seagrass habitat was lost in the decade from 1985-1995, much of it as a result of human activity (Boström et al. 2006).

Seagrass distribution and abundance patterns are driven by many natural and anthropogenic factors. Storms, waves, currents, herbivory, disease and bioturbation can all naturally influence distribution patterns of seagrass and cause fragmentation, dividing seagrass patches into areas ranging from less than one square meter to as large as several thousand square meters (Boström et al. 2006; Healey & Hovel 2004; Hovel 2003). Fragmentation can also be directly or indirectly caused by human activity. Eutrophication, sedimentation from coastal development, propeller damage from boating and damage from fishing gear have contributed to declines in seagrass area and fragmentation of seagrass habitat (Hovel & Lipicus 2001; Healey & Hovel 2004).

In North Carolina, seagrass beds are dominated by shoalgrass (*Halodule wrightii*) and eelgrass (*Zostera marina*) and may also contain widgeongrass (*Ruppia maritima*) and macroalgae (Fonseca & Bell 1998; Hovel et al. 2002). For the purposes of this study, only shoalgrass and eelgrass were considered in seagrasss surveys, but no differentiation was made between these seagrass species either for the purposes of determining habitat configuration or resource consumption. In Core and Back Sounds, Carteret County, USA, seagrass beds along the coast cover about 7000 hectacres over about one linear kilometer (Fonseca & Bell 1998).

To determine whether resource consumption differs between fragmented and continuous seagrass beds, this study conducted gut content analysis on four different species of fish that are native to North Carolina and are abundant in seagrass habitats in Back Sound. The species chosen for analysis were pigfish (*Orthopristis chrysoptera*), pinfish (*Lagodon rhombiodes*), spot (*Leiostomus xanthurus*) and flounder (*Paralichthys spp.*). Gut content analysis of these species was used to compare frequency of stomach emptiness, prey abundance, prey species richness and prey biomass between species and habitat types in order to determine whether fragmentation drives predation or diet changes in these species.

Methods

Fish were collected by trawl from July 14-22, 2014. Trawling for habitat fragmentation analysis was completed at six separate sites, three that were considered continuous and three that were considered fragmented. The continuous sites were defined as having >95% of the seagrass in one continuous patch while the fragmented sites were defined as having 19% - 85% of the seagrass in one patch. The continuous sites that were trawled had approximately 100% seagrass coverage within the trawled area while the fragmented sites were estimated to contain about 25-50% seagrass coverage within the trawled area. Seagrass coverage for each site was determined by completing seagrass surveys along five random 50m transects at each site trawled. Every two meters along the transects, percent coverage of shoalgrass and eelgrass was estimated to the nearest 5%.

Each site was trawled twice and trawls were run for two minutes at approximately 3 km/hr. Salinity, temperature, and depth were also measured at each site. During all analyzed trawls, salinity remained about 34 ppt while temperature ranged from 26.5-28.4 °C and depth varied from 0.5-1m.

Fish collected during trawling were immediately frozen and were processed between September 5th and November 7th of 2014. The wet weight and standard length of each fish was measured before the gut contents were removed from each stomach and analyzed under a microscope. Tissue samples were also taken from each fish for further stable isotope analysis. Each prey species was identified to the lowest possible taxonomic level and the volume and weight of each prey item was measured. Stomach contents were preserved in vials with 70% Ethyl alcohol.

The data obtained from the fish stomach contents was analyzed using JMP. A binomial regression was used to compare frequencies of empty stomachs to stomachs that contained prey items between species and habitat treatments. Two-way ANOVA was used to compare total numbers of prey individuals, total prey biomass, and prey species richness between species and treatment. MANOVA was used to compare the numbers and biomasses of each species of prey found in the stomachs of fishes between treatments.

Results

177 fish and 775 distinct prey items were found in our analysis. Table 1 provides a break down of the numbers of fish analyzed by habitat treatment and species.

As shown in Figure 2, flounder and spot tended to show a higher percentage of empty stomachs in both fragmented and continuous seagrass habitats. This is expected for flounder because they are piscivorous and occupy a higher trophic level than the three other species studied (Arrington et al. 2002). The trend of high percentages of empty stomachs in spot may be due to the low levels of replication of spot in both habitats. Some individual sites had no spot for analysis and only nine total spot were analyzed for the continuous sites while 19 were used for analysis for the fragmented sites. These low levels of repetition were not enough to produce conclusive results. In general, flounder and pigfish

had greater percentages of empty stomachs in fragmented habitats while pinfish and spot had greater percentages of empty stomachs in continuous habitats. This may indicate that predation impacts of fragmentation are species specific or may vary based on food web and trophic interactions.

The relative abundances of each prey species for each species and habitat type is reflected in Figure 3. General trends show that flounder consumed more shrimp in continuous seagrass beds than fragmented beds. It is also shown that pinfish consumed more egg masses in fragmented seagrass beds, but this trend is likely driven by high egg mass consumption by a few individuals rather than by trends in consumption for the entire pinfish population. In general, amphipods and copepods contributed greatly to the prey abundances of the three lower trophic level consumers. Total prey abundance comparisons across habitat type and species are shown in Figure 4. Pigfish, pinfish and spot showed trends of greater abundance of prey consumed in fragmented habitats, but these results are not statistically significant. Flounder showed the opposite trend and had a statistically significant result of greater total prey abundance consumed in continuous habitats.

Analysis of prey species richness showed variable trends in the number of different prey species consumed in each habitat type. Most trends were not statistically significant, although pinfish were found to have consumed significantly more prey species in fragmented habitats, as shown in Figure 5. Contributions of individual prey species to the prey biomass consumed are shown in Figure 6. Fish was the largest contributor to prey biomass consumed by flounder. Seagrass was an important contributor to the biomass consumed by the lower trophic level fish. Although amphipods and copepods were abundant prey species, they had minimal biomass. The scales used to measure prey item weight were in fact not sensitive enough to detect the weight of these two prey items so they were each attributed an arbitrary biomass value of 0.001g per individual. Total biomass consumption trends, shown in Figure 7, show greater biomass consumption in fragmented habitats for pigfish, pinfish and spot. However, greater biomass was consumed in continuous habitats by flounder. All total biomass results were statistically significant.

Discussion

The results of this study show that the predation and diet effects of seagrass habitat fragmentation are highly variable between species and trophic levels. Fragmentation of habitat seems to benefit lower trophic level fishes such as pinfish, pigfish and spot as they were shown to consume greater biomass and greater prey abundance within fragmented habitats. This may be because these consumers are more generalist and utilize seagrass bed edges for prey acquisition. However, flounder are shown to have acquired more prey biomass and to have consumed a greater abundance of prey within continuous habitats.

These results have implications for seagrass conservation and restoration. Both continuous and fragmented habitats are important for different fish species and trophic levels, so both edge areas and continuous seagrass beds should be

maintained. Restoration and maintenance of seagrass habitats must be highly site specific to account for the variable habitat needs of local biota (Bell et al. 2001).

Further study may help to elucidate the relationship between fish predation and resource consumption and seagrass fragmentation. This area of study would benefit from prey abundance surveys within both continuous and fragmented seagrass habitats, which might help researchers determine whether prey consumption within these habitats is determined by prey availability or foraging success. Investigation of other ecological and physical factors that could be contributing to resource consumption, such as hydrology or seagrass species or shoot density should also be undertaken.

It would also be useful to determine how fish move around each landscape in order to better understand how they utilize the habitat. In addition, studies should be conducted over larger temporal and spatial scales to determine how these factors might influence resource consumption.

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Table 1: Number of fish used in analysis by habitat and treatment

	Habitat Treatment	
Species	*Continuous*	*Fragmented*
All Species	96	81
Flounder	16	5
Pigfish	33	28
Pinfish	38	29
Spot	9	19

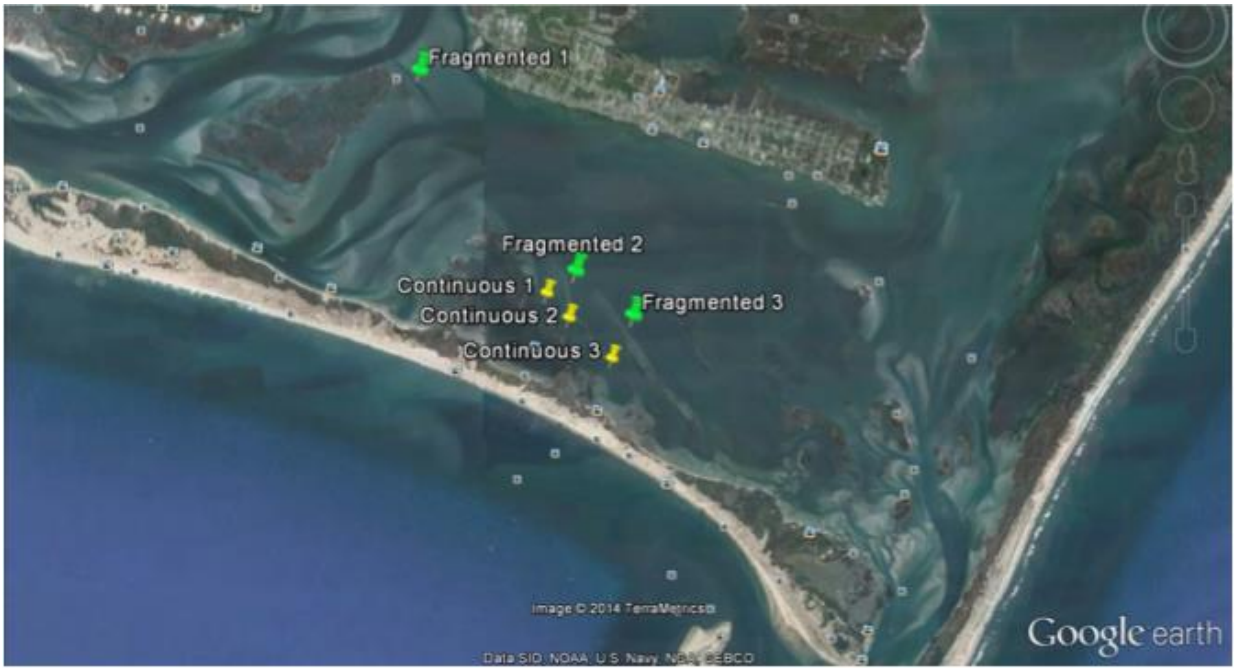

Figure 1: This map shows the areas in which trawls were conducted for the fish used in this study. The initial location of each trawl was determined by GPS. All six trawling locations are located in Back Sound, NC, between Harker's Island and Shackelford Banks. The three fragmented sites are indicated with green pins while the three continuous sites are indicated with yellow pins.

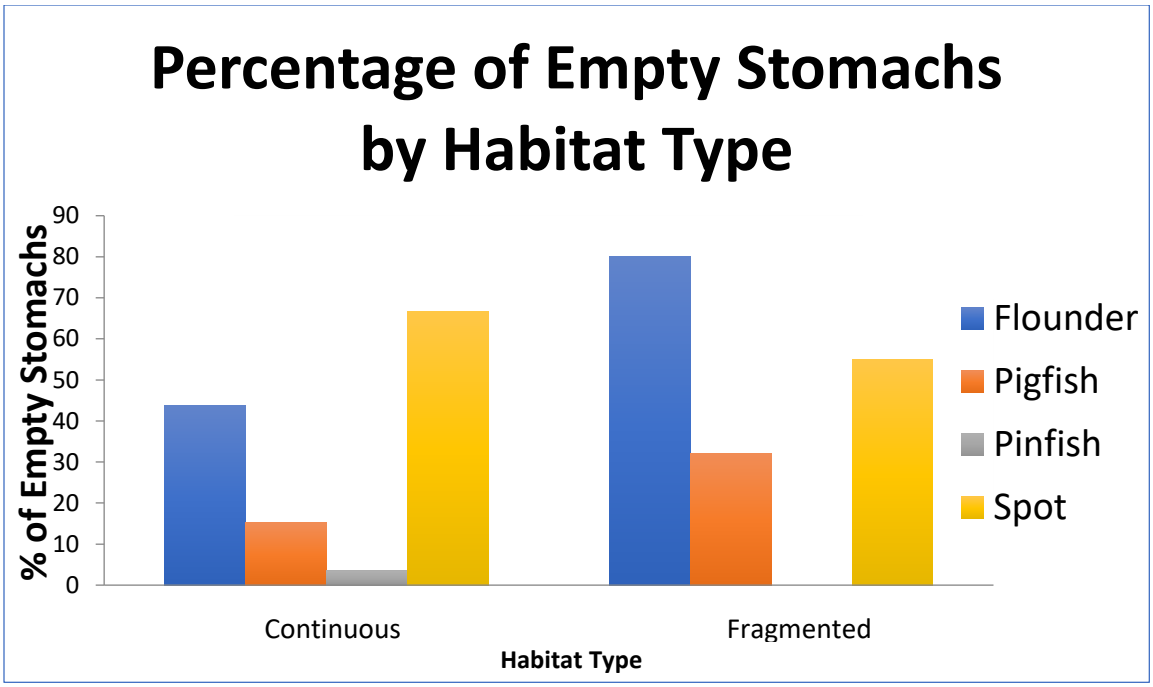

Figure 2: This graph shows the percentages of stomachs from each species in both continuous and fragmented seagrass beds that were empty and contained no prey species.

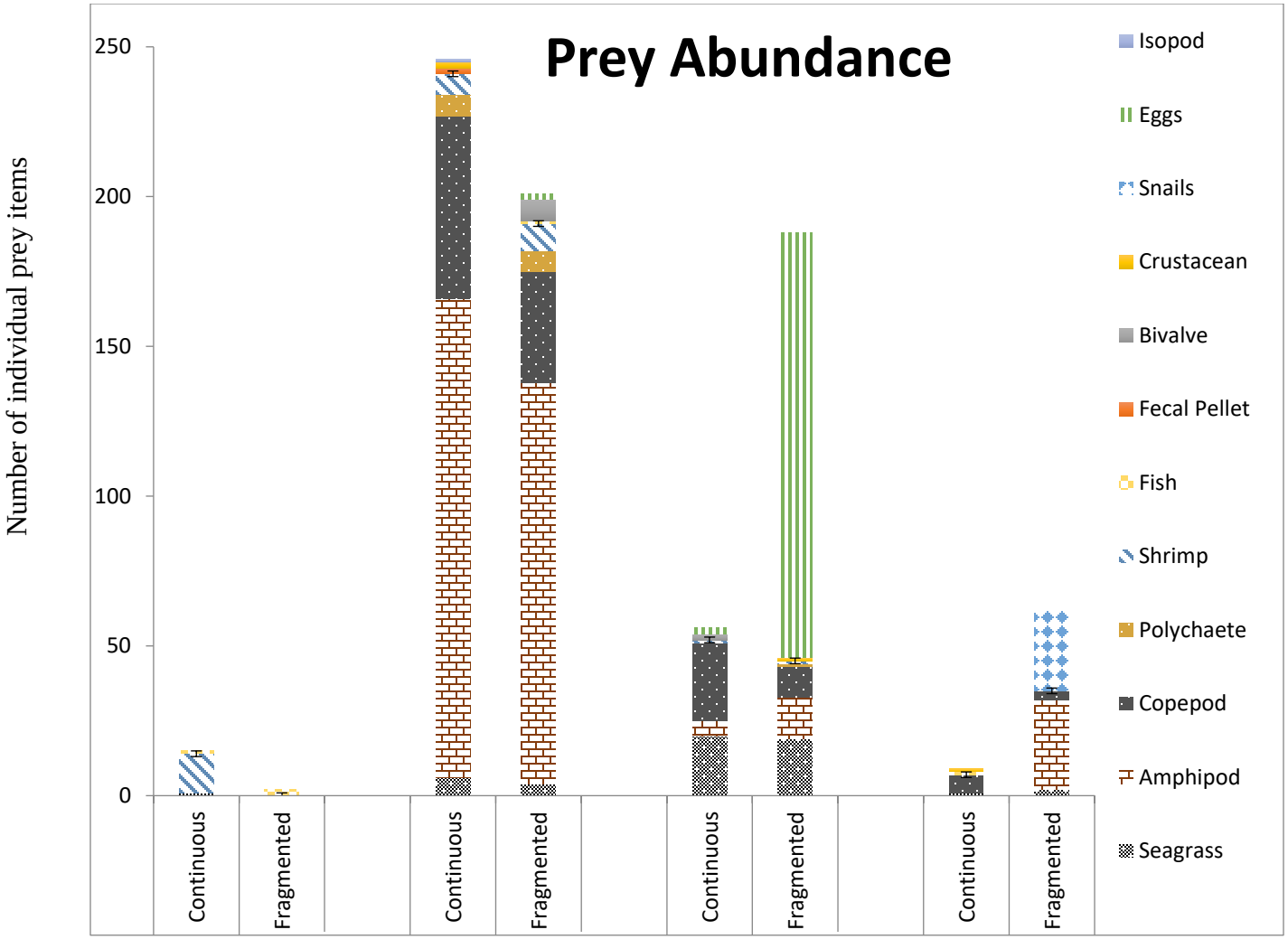

Figure 3: This graph shows the total number of prey individuals that were identified. Stacked columns were used to show the contributions of each prey species to the total species abundance.

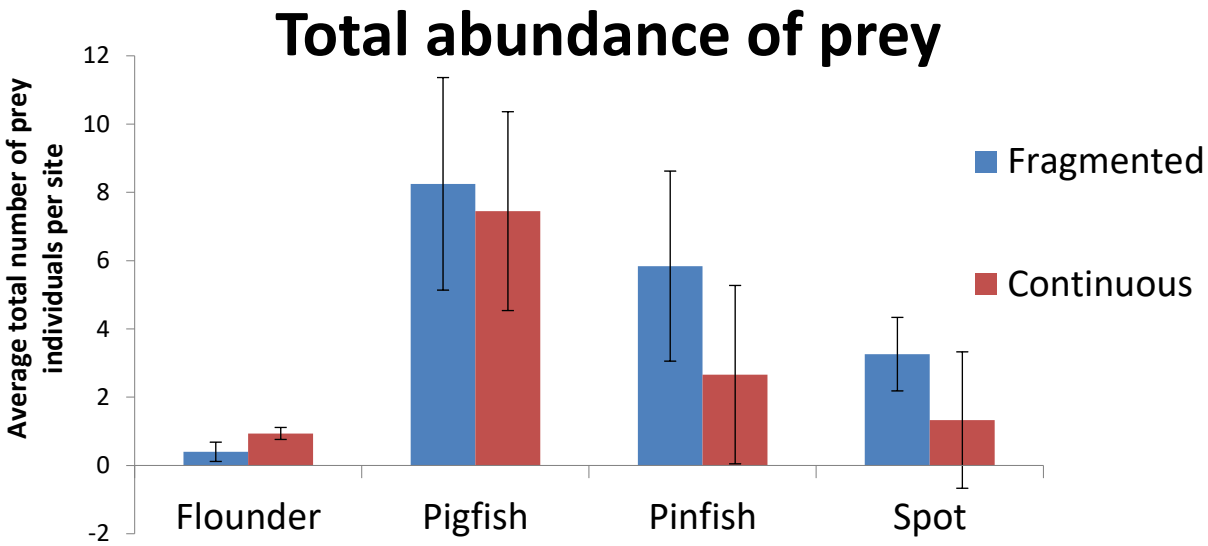

Figure 4. This graph shows the total prey abundances for each habitat type and species.

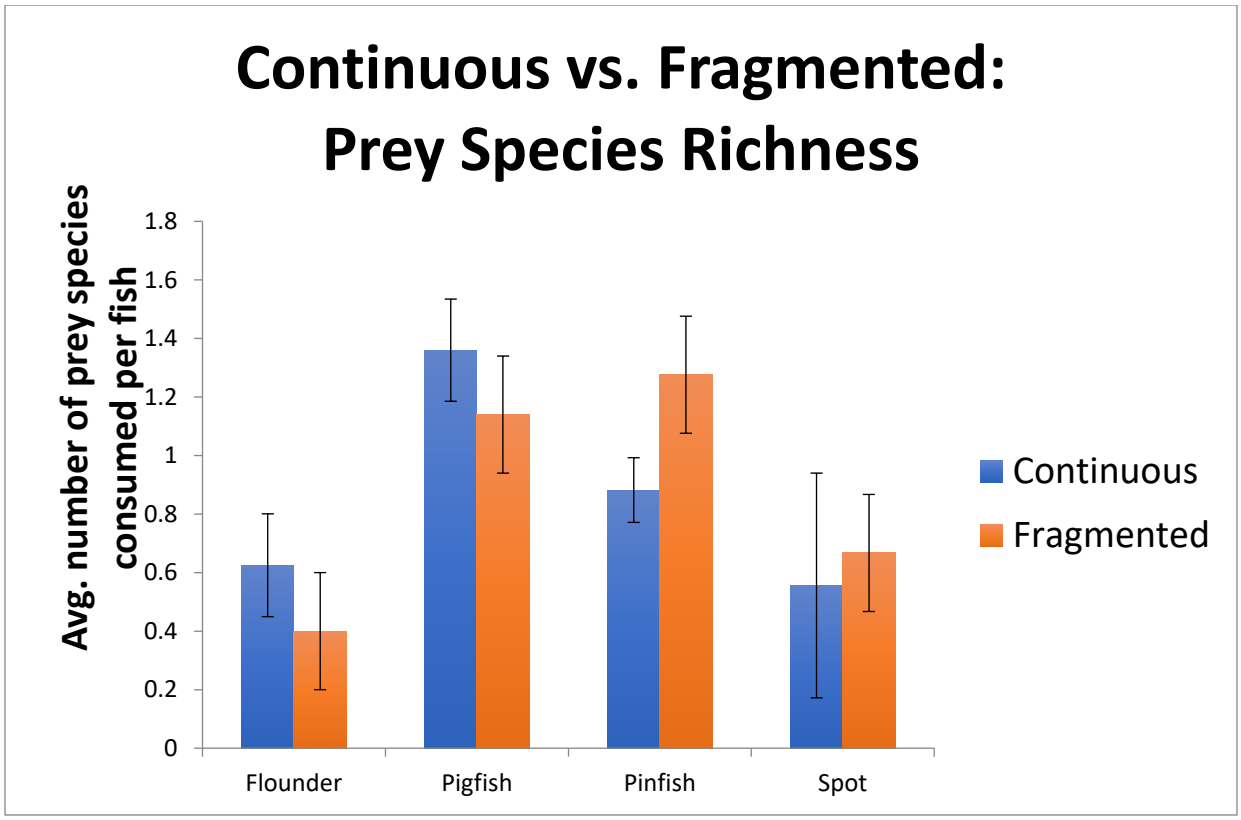

Figure 5. This graph shows the average number of different prey species consumed across species and habitat types.

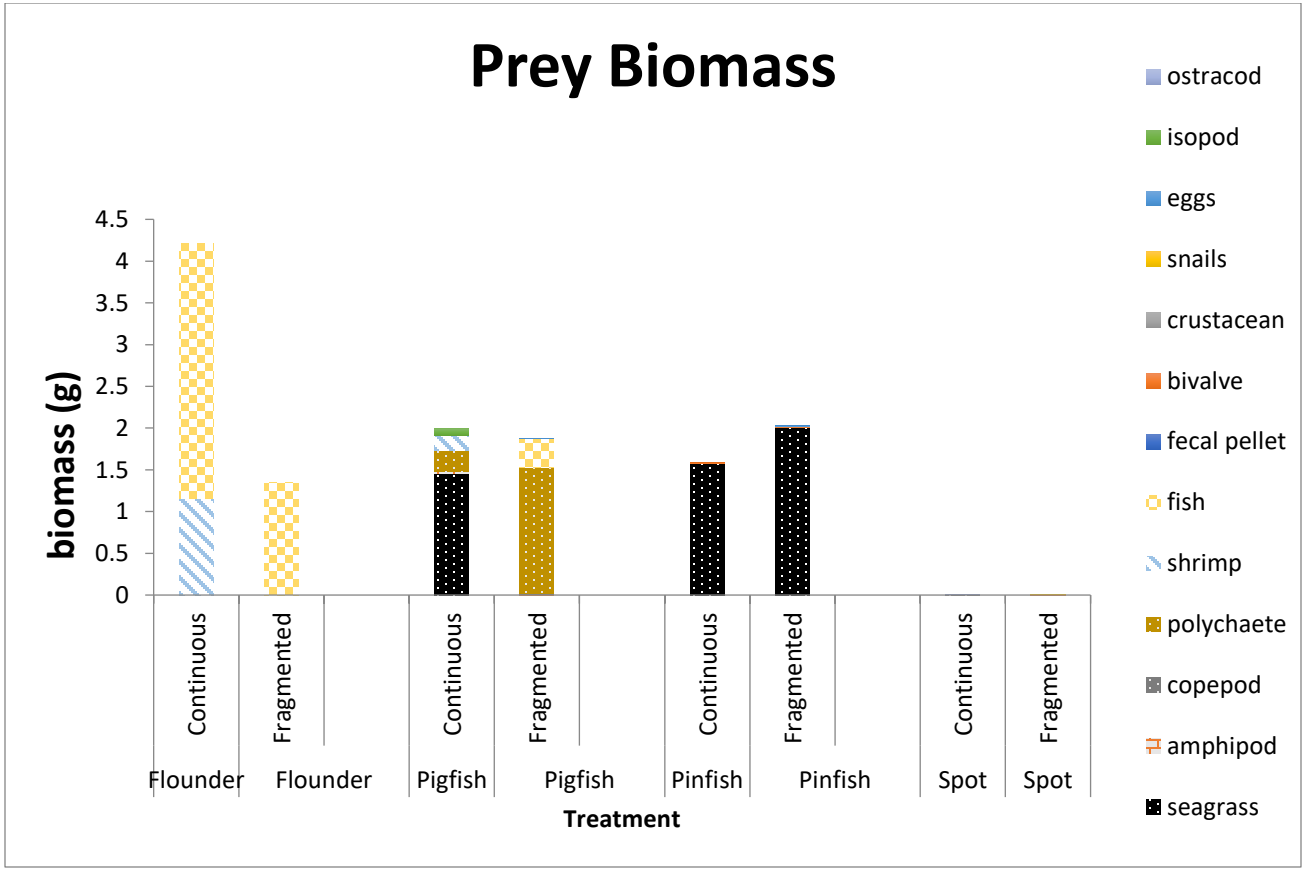

Figure 6. This graph shows the contribution of each prey item to the total biomass consumed by each species within each habitat treatment.

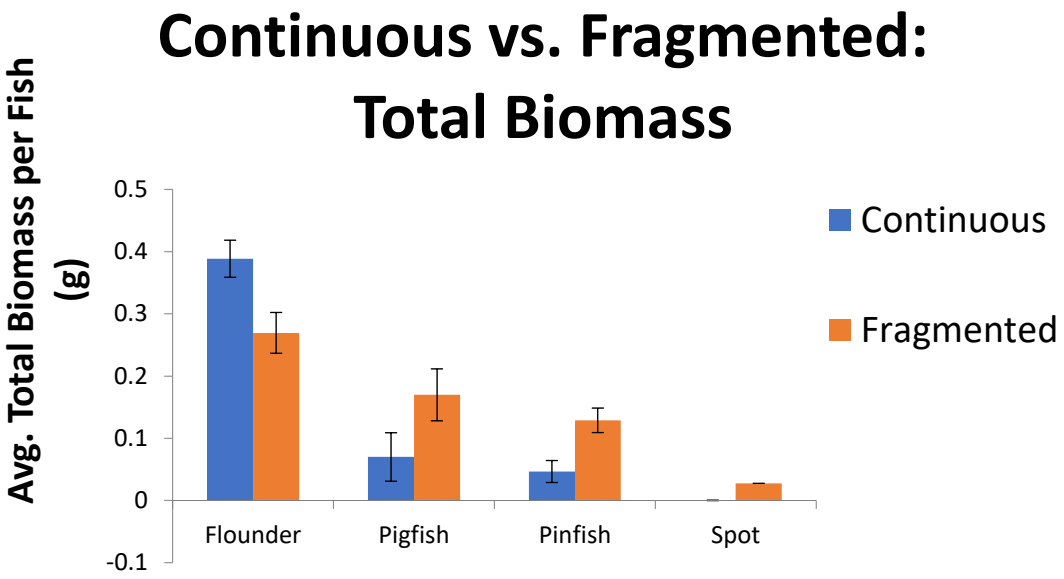

Figure 7. Total biomass consumed by each species in each habitat type.

The Effect of Salt-marsh Proximity upon Fish Diet in Seagrass Habitats: a Gut Content Analysis

Abstract

The proximity of one habitat type to another can have an impact on the foraging behavior of the organisms within either habitat. In this study, three different estuarine fishes were used to determine whether the presence of an adjacent saltmarsh affected the diet of fish in a seagrass habitat. Pinfish (*Lagodon rhomboides*), pigfish (*Bodianus unimaculatus*), and gulf flounder (*Paralichthys dentatus*) were collected from seagrass sites proximal to *Spartina alterniflora* saltmarsh and seagrass isolated from saltmarsh. Across all species, larger fish were found in isolated patches, whereas smaller fish were located in proximal areas. The effect of saltmarsh on diet may have been influenced by the trophic level of the fish; flounder showed no significant difference in prey diversity, richness, or percentage of fish with food in stomachs. Both pinfish and pigfish consumed a greater diversity of prey items, as well as number of prey consumed when in proximal areas compared to isolated treatments. Additionally, pinfish had some sort of prey item in their stomachs in all cases where samples were taken proximal to a saltmarsh, compared to approximately 70% of samples in isolated patches. For flounder, smaller flounder may be attracted to areas with smaller prey items, and both pinfish and pigfish were smaller in proximal areas. Regarding pinfish and pigfish, their greater prey diversity and richness could be caused by a lessening of predation, allowing a greater percentage of time for foraging. It could also be caused by a greater abundance of prey items in saltmarsh areas which may potentially lead to less intraspecific competition. Further research is necessary to distinguish the mechanism by which these differences arise. However, this study has future implications for seagrass conservation and estuary management. The restoration of seagrass proximal to saltmarsh supports a richer habitat for the growth of estuarine fish in the area, and this trophic transfer could promote a healthy ecosystem.

Introduction

In the last decades there has been a shift in ecology from studying the properties of a habitat as if it were an independent entity, towards analyzing the area around the habitat (Heck et al, 2008). It is now well known that both adjacent habitat and edge habitat can fulfill important ecological roles (Micheli & Peterson, 1999). For example, habitat adjacent to a rainforest can have a large impact on the species diversity of local tropical birds (Raman, 2006). In marine ecosystems, attention has been focused upon seagrass (Kayer et al. 1987, Kopp et al. 2010). Seagrass habitat is shrinking, and we still do not know the full importance of the habitat created by this subtidal plant. Seagrass habitat studies have included an evaluation of the habitat as a trophic transfer source, whether fragmented or continuous sections of seagrass are functionally different, and the differences in infaunal invertebrates between different seagrass species. This analysis focused on the interaction between salt marsh and seagrass; we attempted to determine the effect if any, the proximity of a salt marsh could have upon fish diet and behavior in that area. Specifically, we tested whether the proximity of a saltmarsh could affect prey diversity, richness, and percentage of fish with food in stomach. We expected fish near a saltmarsh to have a richer diet, based upon possible

trophic transfer between the saltmarsh and the seagrass habitats. This analysis spanned two trophic levels, that of the secondary consumer (pinfish, pigfish), and that of the tertiary consumer (flounder). A second aim of this study was to analyze the differences in diet contents between species.

Materials and Methods

Field Sampling of estuarine fishes

Three isolated seagrass sites were trawled for fish three times each; three sites proximal to saltmarsh were also trawled three times. Trawling was performed during July and August, a period when many estuarine fish are present due to the warm temperatures, and took place in Back Sound, North Carolina. No distinction between species of seagrass was made, typically *Zostera marina* and to a lesser extent *Halodule wrightii* were the species of seagrass at the locations. Only continuous patches of seagrass were used, to avoid the confounding variable of patchy habitat and associated edge effects. Prior to initial trawls, sites were studied in person and on Google Earth to ascertain that the habitat was suitable. (See figure 1). Trawls were taken during the day, with a trawl lasting two minutes at an average of 3.0 km/hr. Species, the number of fish, and the total weight of each species was recorded for each organism. Up to 10 flounder, pigfish, and pinfish from each trawl were placed into freezable bags for gut content analysis. After trawling, bags were placed into cold storage.

Gut-content analysis

Each bag of fish (representative of a single trawl) was individually defrosted by submerging it in cool water. Fish were subsequently thawed, blotted dry, weighed, and length from tip of snout to the end of the vertebral column (standard length, SL) was measured. A tissue sample was obtained by incision along the ventral line of the fish, or in larger fish, parallel incisions near the fish tail. After acquiring tissue samples of 0.4g when possible, the samples were labeled, wrapped in aluminum foil, and placed into cold storage for future tissue analysis. An incision was then made from the anus through the ventral cavity and into the pectoral muscles of the fish. Forceps were used to expose the abdominal cavity of the fish, and to pull out the stomach. Two small incisions severed the stomach from the rest of the gastrointestinal tract, and contents were squeezed out onto a petri dish. In situations where the stomach had pyloric caeca as much of the contents were removed as possible without addition of the projects themselves. Gut contents were then observed under a microscope, identified, and their quantity recorded. When possible, prey items were weighed and their volume measured. Any unidentifiable specimens were photographed for future identification. After all specimens were identified, enumerated, and weighed when appropriate, got contents were placed into a 50 ml vial. A 70% ethanol solution was subsequently added to the vial; the vial was labeled, and then stored.

To determine whether differences existed between the isolated and proximal seagrass sites, a spreadsheet was made containing all of the data previously collected. ANOVA tests were the preferred statistical test, given the variable sample size of each treatment. Each species was separately analyzed to determine the probability of differences between isolated and proximal sites. Prey richness (# of prey/fish), prey diversity, fish length, and the % of fish in each treatment with food in stomachs were all

compared between the two site types. Any significant differences (P<0.10) were further analyzed to determine whether outliers existed.

Results

Differences in diet were not similar between species, indicating that seagrass effects are species specific. A total of 177 fish and 1073 prey items were used for this analysis (Table 1).

Pinfish

Pinfish showed a decrease in their overall length and weight when proximal to a saltmarsh (figure 3); the average fish length in an isolated area was 61.6mm, whereas near fish near a saltmarsh averaged 53.4mm. This difference was non-significant (P=0.14), but the trend is also found in the other two species studied. The percentage of fish with food in stomachs increases dramatically to 100% near saltmarsh compared to 70% in isolated sites (P=0.0052), indicating that fish have a greater chance of finding prey at all in near a saltmarsh (see figure 4). Additionally, the number of prey items consumed does not drastically change (P=0.307); however; prey diversity strongly increases next to saltmarsh (P=0.0171)(see figure 5,6). Essentially, , and these fish are on average consuming a greater diversity of prey and spend less time without any prey in their stomachs.

Pigfish

Pigfish demonstrated a decrease in length (and weight) when near a saltmarsh (figure 3); the average fish length at isolated locations was 64.7mm, and 57.4mm when near saltmarsh (P=0.238). Again this difference is non-significant, but was found in other species studied. Pigfish showed a significant increase in prey diversity (P=0.0198), as well as prey richness (# of prey taxa appearing in stomachs per fish)(P=0.0198) in proximal sites compared to isolated sites using an ANOVA test. Additionally, marginally significant differences were found in percentage of fish with gut contents, Pigfish near saltmarsh exhibited a greater likelihood of having prey items in their stomachs (P=0.0718)., and these fish are consuming both a greater variety and number of prey items than those in isolated seagrass habitat.

Flounder

Flounder caught near a saltmarsh were smaller on average than those caught in isolated seagrass. In isolated areas, flounder averaged 109.7mm, whereas their standard length near a saltmarsh averaged 69.3mm (P=0.0269). No other differences were found between prey diversity (P=0.307), % of fish with gut contents (P=0.697), or prey richness (P=0.307) between habitat treatments. A primer multivariate analysis on diet dissimilarity yielded no significant differences in prey diet between the two site types.

Discussion

Pigfish and Pinfish

Pigfish and Pinfish occupy the same trophic level and exhibited similar results when comparing site types. Overall, fish were slightly smaller, though not significantly so, and they ingested a greater amount and variety of prey items near saltmarsh. There are a myriad of possible explanations for these differences. More abundant prey may be found near a saltmarsh, allowing trophic transfer through preying on marsh biota. It is also possible that the physical aspects of the environment allow for greater success when foraging for prey. More vegetation cover creates a safer environment to avoid predators. Smaller fish may be better able to avoid predation in these areas, and because of the high degree of shelter these fish could spend more time foraging for prey. These effects could be related to size, giving a greater incentive for smaller fish to occupy areas near a saltmarsh. Additionally, there may be less competition in areas near a saltmarsh, decreasing effort required to successfully forage. It is also possible that significant differences in diet may be related to fish size, and further analysis could be performed using fish size primarily over site type.

While there are many possible explanations for these differences in pigfish and pinfish diets between site types, this study could not distinguish the specific reason(s) for the change in diet. Further research would be necessary to determine which factors primarily drive these changes in diet and fish size. In the future, sampling the seagrass and saltmarsh for prey abundances could contribute to identifying a cause. If prey abundances were equal between the sites, it can be inferred that foraging success is not dependent on density, and may depend on other predation components such as encounter rates or capture success. To determine whether or not intraspecific competition is driving these differences, the number of fish found at each site could be compared to the prey abundances at that site. This comparison would address the strength of competition by providing an average ratio of # of prey per fish. Finally, predation on pinfish and pigfish may be lessened when they are near a saltmarsh. In order to verify this, a survey of other pigfish/pinfish predators in the area could be taken. An experiment could be devised to determine the predation success upon pinfish and pigfish in a saltmarsh vs. in isolated seagrass. Predators exert top-down pressure upon pigfish/pinfish and non-consumptive effects, such as the ecology of fear, can strongly impact the amount of time pinfish/pigfish spend foraging. A mesocosm lacking a flounder and a mesocosm with a flounder present could be used to compare foraging rates in the presence/absence of a predator. Different sized fish could be used as well, to analyze the possible effect of size on predation rate.

Flounder

The only significant difference in flounder was found to be their size; larger flounder were found in isolated areas and smaller flounder where found near a saltmarsh. Prey diversity, richness, and the percentage of flounder found with food in stomachs did not significantly differ between near and isolated locations. It is curious that such segregation by size does not have a corresponding change in diet. It may be that smaller flounder inhabit saltmarsh because their prey (pinfish, pigfish, shrimp) is smaller, whereas larger flounder can consume larger fish in isolated seagrass. Alternatively, flounder may be larger because of their increased success when preying on fish in isolated areas. Larger flounder could have difficulty concealing from, and preying upon prey with added vegetation from the saltmarsh. Conversely, smaller

flounder may be able to avoid predation by using the saltmarsh as shelter, where larger fish have difficulty reaching. To test this, an experiment could be conducted with flounder in seagrass areas vs. saltmarsh areas, with prey items restricted to the vegetation. Flounder success rate would be compared between the two trials.

Without further research, it will not be possible to distinguish which of these factors is primarily responsible for the difference in size between flounder in isolated vs. near sites. Similar experiments to those devised for pinfish/pigfish could be performed on flounder. Relative abundances of pigfish and pinfish in an area, along with a survey of shrimp abundances, would yield an estimation of the prey abundances in an area. A laboratory experiment could be performed to determine whether larger flounder prefer smaller or larger prey items, and subsequently whether prey items of the same size can better avoid flounder in a saltmarsh/seagrass mesocosm vs. an isolated seagrass mesocosm.

Conclusions

The effects of saltmarsh proximity are species-specific and many further experiments can test the ultimate causes of these differences. Experiments focusing on flounder interactions with pigfish and pinfish can potentially answer questions related to diet across all three species. Knowledge of how the physical habitat, the abundance of prey, and the number of intraspecific competitors in these habitats would contribute greatly to further understanding of these diet differences.

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Table 1: Fish and prey counts table. Approximately equal numbers of fish were sampled from proximal and isolated sites, however the number of prey items in proximal sites is over twice that of isolated areas.

	Total	**Near**	**Isolated**
Total Number of Fish	177	86	91
Flounder	44	28	16
Pigfish	60	27	33
Pinfish	62	29	33
Total Number of Prey Items	1073	747	326

Figure 1: Trawl locations: Ci's indicate isolated seagrass areas, Cn's represent areas near a saltmarsh.

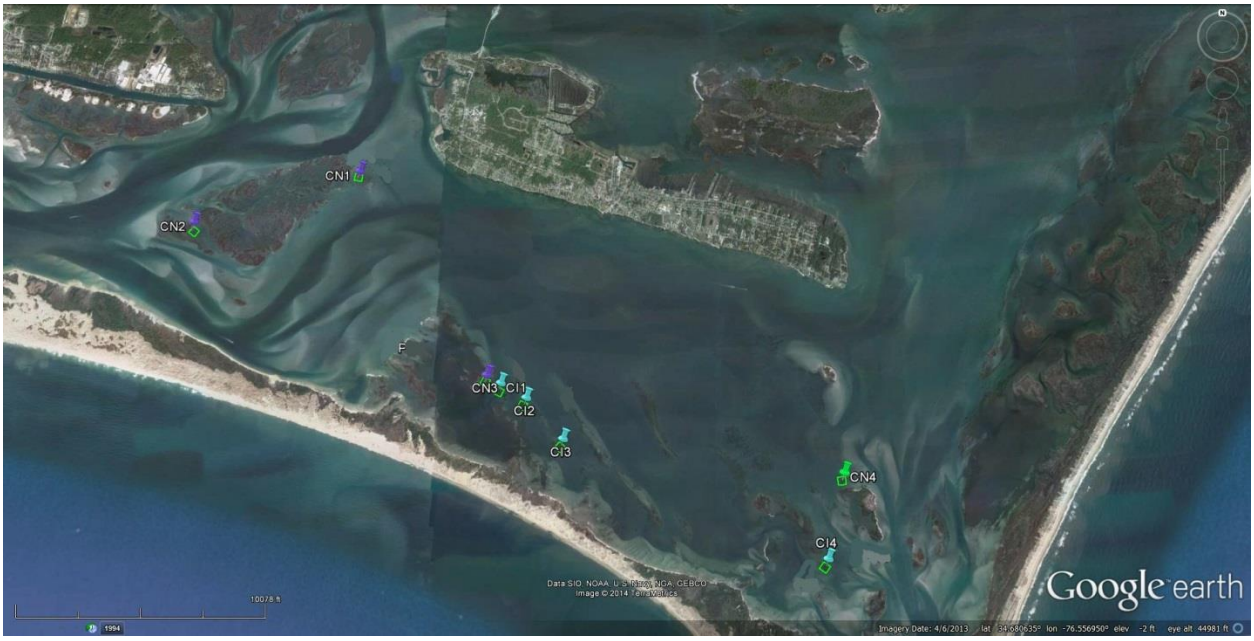

Figure 2: Fish length comparison across all treatments larger fish were found in isolated areas.

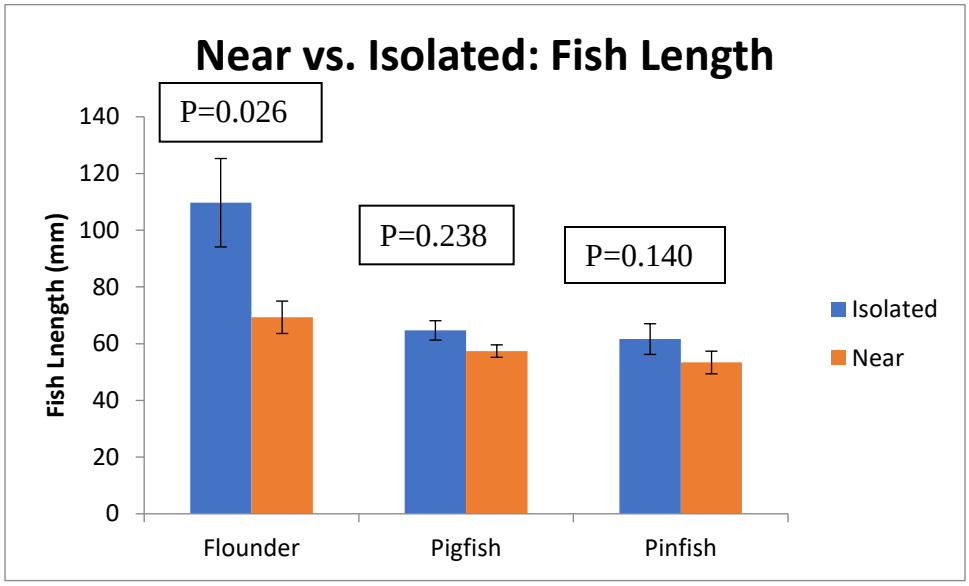

Figure 3: Prey richness comparison. Significant differences found between proximal and isolated sites in pigfish and pinfish.

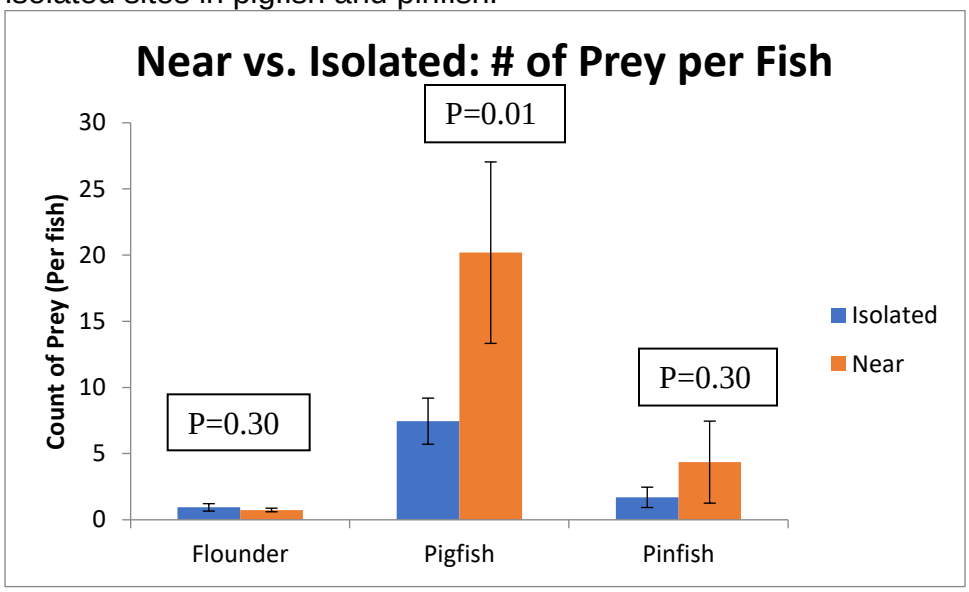

Figure 4: Prey Diversity comparison. Prey diversity was greater in proximal sites for pigfish and pinfish.

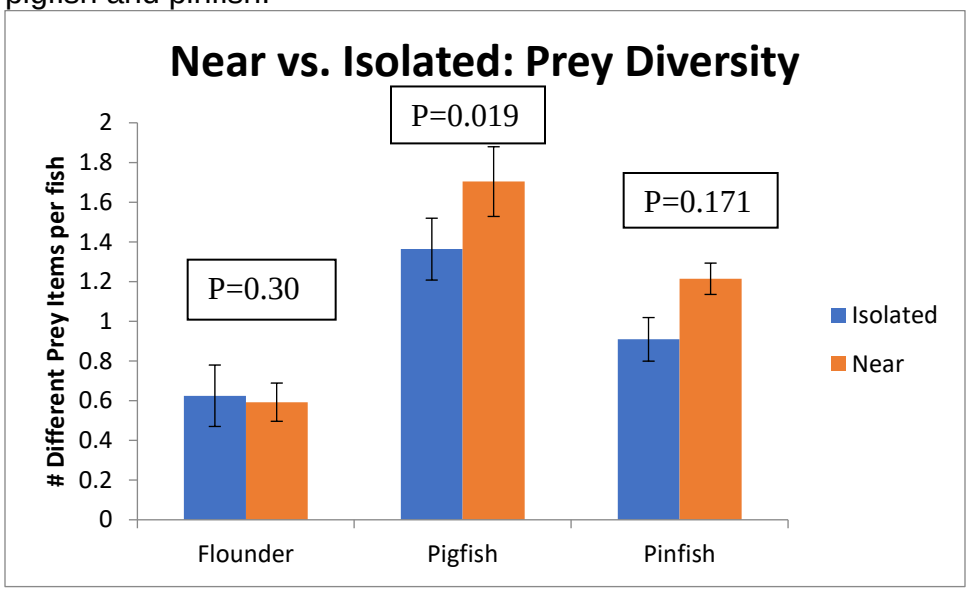
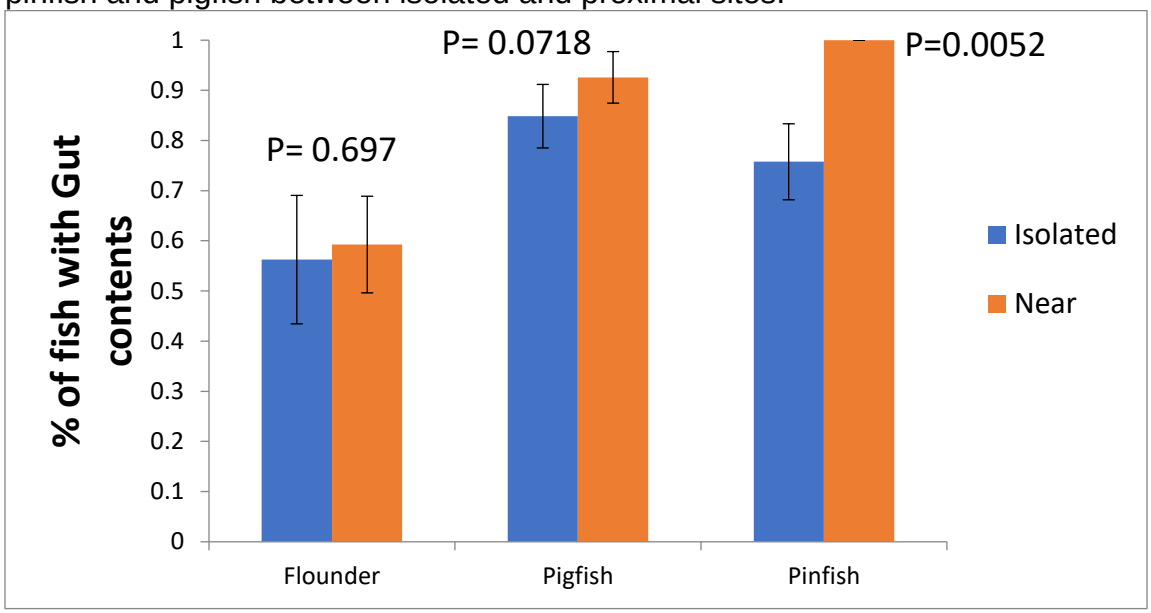
Figure 5: Percentage of fish with gut contents. Significant differences were found in both pinfish and pigfish between isolated and proximal sites.