**Invertebrate beta diversity varies among eelgrass meadows**

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**Running head: Variation in epifaunal eelgrass diversity**

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ABSTRACT

In landscapes, spatial structure of habitat forming species can play an important role in supporting animal diversity and secondary productivity. In coastal ecosystems, spatial structure of eelgrass meadows is an overlooked dimension of their ecological and conservation value. We tested whether patterns of eelgrass-associated invertebrate biodiversity are consistent with spatially structured biodiversity processes across meadows. For 9 meadows in Barkley Sound, British Columbia, we quantified epifaunal biodiversity on eelgrass *Zostera marina.* We tested three hypotheses: taxonomic diversity and composition i) vary randomly within meadows but ii) vary systematically among meadows reflecting abiotic factors and metacommunity dynamics, and iii) biodiversity patterns are consistent through time. Though we identified two high-diversity meadows, faunal diversity varied as much within a few meters as among meadows separated by kilometers, of different sizes and exposures. Biotic or abiotic factors did not explain non-random patterns of community composition among meadows. Community similarity within and across meadows increased from May to August. These findings leave open the possibility that landscape scale metacommunity dynamics, and abiotic factors, influence eelgrass-associated faunal biodiversity.

INTRODUCTION

Understanding the distribution and abundance of species in spatially structured habitats is not only essential to understanding ecological communities but also to making informed decisions about landscape management and biodiversity conservation. In coastal marine habitats, rich in biodiversity, understanding is limited by a lack of basic information about where and when species occur and the spatial scales over which dominant diversity-influencing processes operate (Gaston 2000, Boström et al. 2006). While a spatially discrete habitat patch such as a seagrass meadow or a kelp bed may appear to host a complete or representative local faunal community, habitat provided by foundation species is just one of several processes that influence biodiversity of a local habitat patch. In addition, population dynamics and species interactions structure species assemblages at finer (within meadow) and broader (landscape) spatial scales (Leibold et al. 2004, Boström et al. 2006, Pillai et al. 2011).

The purpose of this study is to quantify faunal biodiversity across spatial scales in a seagrass meadow seascape to determine whether individual meadows, or a set of meadows, represent meaningful spatial units for biodiversity. Spatial variation in biodiversity is related to the spatial scales of ecological processes that influence species’ relative abundance and presence or absence (Leibold et al. 2004, Ricklefs 2004, Boström et al. 2006). Within a region, evolutionary and colonization history produce a shared regional species pool (gamma diversity). At finer scales, meadow- or plant-scale biotic interactions (predation, competition, facilitation, resource provision) and abiotic conditions (temperature, salinity, etc) filter species from the regional pool to a subset of regional diversity, called alpha diversity. Between local and regional scales, dispersal and colonization dynamics connect populations among habitat patches to produce metacommunities. In metacommunities, abundance and diversity vary among local sites, producing patterns of spatial turnover or variation (beta diversity) are jointly influenced by local and regional process (Leibold and Mikkelson 2002, Leibold et al. 2004, Crist and Veech 2006).

Despite the apparent similarity of seagrass meadows from patch to patch, faunal diversity can vary substantially (Hemminga and Duarte 2000). Within meadows, alpha diversity at fine scales (0.5 – 1 m2) is typically relatively consistent in patches within the same meadow, and much lower than meadow-scale species diversity (Barnes and Ellwood 2012, Barnes 2013). This pattern suggests that beta diversity should be high and a relatively important component of seagrass associated biodiversity within meadows, yet there are few reports of beta diversity from seagrass systems (but see Barnes and Ellwood 2012). Among meadows, variation in species composition and diversity is not readily explained by abiotic attributes (Carr et al. 2011), though a few studies report effects of fetch or some measure of energy (Boström et al. 2006, Robinson et al. 2011), or salinity (Yamada et al. 2007, Boström et al. 2014).

Here, we test the hypotheses that 1) alpha diversity does not vary systematically among meadows, but within-meadow beta diversity does vary systematically among eelgrass meadows, and 2) spatial variation in species composition is consistent with i) local filtering of species based on abiotic conditions, or ii) metacommunity-scale processes that involve dispersal among meadows. To test these hypotheses, we quantified spatial structure in seagrass-associated epifaunal biodiversity across nine meadows in British Columbia, Canada.

MATERIALS AND METHODS

*Study system*

We sampled epifaunal biodiversity in eelgrass *Zostera marina* meadows in Trevor Channel, Barkley Sound, British Columbia, where *Z. marina* is the only meadow-forming seagrass species (Figure 1).  In this region, *Z. marina* forms primarily subtidal, perennial meadows that range in size from < 10 m2 to > 25,000 m2 (Mason et al. 2015). *Z. marina*, like other seagrasses, hosts a rich faunal assemblage of gastropods, crustaceans and other taxa that live on and among the eelgrass blades. These epifauna consume epiphytic algae growing on seagrass, detritus and each other, forming the base of a highly productive food web (Hemminga and Duarte 2000, Heck Jr. and Valentine 2006).

We quantified several biotic attributes of eelgrass meadows that could explain variation in eelgrass associated invertebrate biodiversity. We estimated shoot density, leaf area index (LAI), and meadow area. Shoot density and LAI were estimated from four 0.28 m2 quadrats collected outside each corner of a 4 x 4 m grid demarcated for community sampling (described below) in May and August at each of our primary sites (i.e., the five sites that we sampled three times; Figure 1, Table 1) and in June/July at primary and secondary sites (Figure 1, Table 1) concurrently with epifaunal sampling. We removed, dried and weighed eelgrass and its associated epiphytes, and standardized epiphyte mass to eelgrass mass. To estimate LAI, we counted the number of blades per eelgrass shoot and measured the longest blade for length (from top of sheath to tip of blade) and width (at the midpoint). We then multiplied the width and length of the longest blade by the number of blades for each shoot (after Borg et al. 2010). We did not estimate plot-level density or LAI for the same plots as we sampled biodiversity.

To quantify meadow-scale abiotic conditions, we monitored temperature and salinity using a hand held temperature/salinity sensor (YSI Inc., OH USA). Measurements were taken throughout the tidal cycle on biodiversity sampling days, and opportunistically on other days. We used position in the watershed to represent the potential estuarine gradient of salinity and temperature. Position was estimated as linear distance in kilometers from the nearest freshwater source (Sarita or Alberni inlet). We estimated fetch by calculating the distance to nearest land from the eelgrass meadow in 10 degree increments and summed the distances ().

*Biodiversity sampling*

To estimate epifaunal biodiversity and characterize variation within meadows, we used a 4 x 4 m grid of 16 standard plots (0.28 m2), each separated by 1 m, in each meadow (after Sanders et al. 2007, Duffy et al. 2015). This sampling design allowed for comparison of diversity among plots and meadows while standardizing for total area sampled and the spatial arrangement of samples. We placed sampling grids within contiguous meadows at least 2 m from any meadow edge.  In each plot, we cut away eelgrass at the sediment-water interface and placed it into a 250 µm mesh bag, collecting all eelgrass and epifauna. All sampled areas were at least 1 m below lower low water large tide (LLWLT), and did not vary in depth by more than a meter at any given site. We collected samples using SCUBA, then transported to the lab in seawater, where all invertebrates were removed and preserved in 70% EtOH within 24 hours of collection.

To estimate variation in diversity among meadows, we sampled nine meadows between the open coast and Alberni Inlet in Trevor Channel (Figure 1; Table 1).  We chose these meadows among the subset of known meadows in Trevor channel (n ~ 20) because they are evenly distributed along the channel (Figure 1), they are large meadows (i.e., min 0.25 ha, not fringing), and they represent many of the shallow areas where eelgrass might occur. To determine whether spatial biodiversity patterns varied through time, we sampled five of these meadows three times, in May (early summer, time A), June/July (midsummer, time B) and August (late summer, time C) of 2012 (Table 1).

We identified every invertebrate > 1mm in each sample to the lowest taxonomic resolution possible using light microscopy. Though many of our identifications are to species level, many other taxa are identified to higher levels (Family or Order). Each of these groups possibly includes numerous species; consequently, our reference to taxa below reflects varying resolution, and our taxonomic diversity estimates should be considered minimum estimates. We did not include egg masses or colony-forming species (e.g., bryozoans) in our analyses. We classified invertebrate species to broad trophic groups (grazer, predator, filter feeder, detritivore) based on our observations and published information (Light and Carlton 2007, Macdonald et al. 2010). The grazer functional group includes organisms that consume micro- or macro-algae, including biofilms, growing on eelgrass.

*Biodiversity estimation*

To estimate diversity within and among meadows, we created species-plot and species-site matrices with abundance data for each taxon, using information on every individual collected from each plot (Gotelli and Colwell 2011). We estimated multiple biodiversity metrics: species richness (number of species observed), their relative abundance, and the likelihood that additional taxa were present but unobserved in estimating and comparing biodiversity among samples and meadows. We estimated alpha diversity by rarifying (ENS) and using raw richness values (R), Shannon diversity (H’), and Simpson evenness (S). The effective number of species (ENS) is derived from the probability of an interspecific encounter (PIE), to characterize diversity at the plot scale (Dauby and Hardy 2012). ENS can be interpreted as the number of equally-abundant species that would exist in a sample of a given diversity value (Jost 2006). We used the R package vegan (Oksanen et al. 2007) for biodiversity analyses.

Patterns in community similarity within and among meadows (beta diversity) can be used to infer the possible role of underlying ecological processes that could structure communities in space, or alternatively, can indicate random distributions of species not clearly explained by a particular ecological model (Leibold and Mikkelson 2002). We quantified beta diversity in two ways. First, we estimated beta diversity (βBC) within each meadow using the Bray-Curtis dissimilarity metric, which accounts for relative abundance of species and estimates differences among samples as their average distance from a group (meadow-level) centroid (Tuomisto 2010, Anderson et al. 2011). Second, we used a null model approach developed by Chase et al. (2011) to evaluate possible mechanisms that influence community similarity among meadows and to distinguish differences in beta diversity that reflect underlying patterns of community structure rather than random differences in alpha diversity among sites. The model uses a third metric of beta-diversity, the Raup-Crick metric, βRC,to compare pairwise dissimilarities between samples with a null expectation. This metric uses presence/absence of species and their relative occurrence across samples to assign probabilities that community samples are less similar (>-1), more similar (<1) or no different (=0) than expected by chance. We analyzed patterns in community composition across all nine sites sampled across all time periods for both intra-meadow and inter-meadow variation.

*Statistical analyses*

To test our first hypothesis that faunal beta diversity (turnover among samples) varies among meadows but alpha diversity (sample-level species diversity) does not, we used one-way anova on univariate diversity metrics among nine meadows sampled in July (midsummer, time B). To explore possible explanations for variation in diversity within and among meadows, we tested whether spatial patterns in grazer diversity varied with season, distance, biotic and abiotic meadow attributes. We conducted linear regression analyses using sampling date, distance among plots, meadow area, fetch and position in the watershed as predictors. We tested for correlations among these variables. Abundance and ENS were log-transformed to meet the assumption of homoscedasticity.

Beta and alpha diversity patterns can differ if species are aggregated within meadows (as opposed to randomly distributed throughout the meadow), or if aggregation varies among species and dominance varies among meadows. We estimated aggregation within meadows using standardized Morisita’s index (I), and then compared aggregation scores for species in each meadow with their rank. We specifically tested for aggregation within meadows, whether it is driven by the most abundant species, and whether the most abundant species the same across meadows.

*EMS methods*

To determine whether spatial patterns of biodiversity across meadows are consistent with metacommunity processes operating at the landscape scale, we applied the elements of metacommunity (EMS) framework (Leibold and Mikkelson 2002, Presley et al. 2010). This analysis first tests for coherence in species distributions across meadows by comparing observed species distributions with expected distributions based on a null model. If positive coherence is identified, spatial patterns can be further tested to identify patterns consistent with individualistic species distributions across the landscape (a Gleasonian model of species distributions), or clumped species distributions (Clementsian structure), or random, providing insight to possible types of community structure (Leibold and Mikkelson 2002, Presley et al. 2010, Henriques-Silva et al. 2013). We analyzed metacommunity structure for the 9 meadows sampled in July 2012 using the R package Metacom (Dallas 2014). We used a null model that fixed species richness within sites (‘fixed row) but allowed composition to vary based on their marginal probabilities (method = “R1”).

We ranked models using AICc, and compared them using likelihood ratio tests, δaic and Akaike weights (w). Models with a δaic < 2 can be considered equivalent to the best model (Burnham and Anderson 2003). In the case of multiple highly ranked models, we selected the set of models that produced a cumulative w > 0.95, representing our confidence (95%) that the set includes the best model, and we averaged these models to produce coefficients of effects (Burnham and Anderson 2003).  We examined residual plots for deviations from the assumptions of linearity or homoscedasticity, (with the exception of parallel lines in a plot of the models’ fitted values vs. residuals).  This phenomenon resulted from including repeated values of position in the estuary in the model (Searle 1988). We tested hypotheses on a subset of our full dataset - the meadows sampled at all three time points - to conservatively test for an effect of temporal variation. We then repeated the analysis with the full dataset (including the four sites sampled only once).

RESULTS

*Meadow attributes*

We collected 71 samples for seagrass structural metrics over the course of the summer 2012. Meadows increased in shoot densities (+0.27 shoots/km) and associated LAI (+240.73 cm2/km) with increasing proximity to the Pacific Ocean (Tables 1, 2; Appendix 2). Shoot density and LAI increased between May and August at meadows near freshwater but remained relatively high all summer at the seaward meadows (Appendix 2; Tables 1, 2). Position in watershed captured correlations with other variables: temperature, salinity, shoot density (Table S2). Mean salinity increased and temperature declined from the freshwater to marine end of the gradient, though these changes were relatively small in magnitude (Table S3). Epiphyte load was highly variable between meadows, and did not change predictably with position in the estuary (Appendix 2; Tables 1, 2). Epiphyte abundances and the type of epiphytes present (periphyton vs. bladed algae) varied both spatially and temporally (Appendix 3). The bladed brown epiphyte *Punctaria sp*. was abundant at two of the marine sites (Dodger Channel and Wizard Islet), and absent from the fresher Numukamis Bay.

*Epifauna summary*

We collected 304 seagrass samples with associated epifaunal across all sites and sample periods and identified approximately 81,500 epifaunal invertebrates, representing at least 47 taxa in 42 families. Of these, 30 taxa are considered epifaunal, meaning they regularly or always occur on the blades of eelgrass (rather than in or on the sediment) (Table 2). We observed between 0 and 1200 individuals per 0.28 m2 of seagrass meadow. Mean total abundance of all taxa at the plot scale did not vary among meadows in May, but did increase over the summer such that by August abundance was 2-3 times higher in seaward meadows (DC, WI, RP) compared to meadows nearer Alberni Inlet in which abundance was stable over time (CB, NB; Tables 2, 3; Figure 2). The seasonal increase in seaward meadows is clear in both the small (1-2 mm) and large (>2 mm) size fractions.

Of the 30 epifaunal taxa analyzed, 17 taxa are herbivorous or omnivorous consumers of epiphytic algae, and therefore belong to the functional group “grazers” (Appendix 1, Table 2). These comprised approximately 72% of all individuals. On average, in each meadow 6.0 (± 0.15) grazer taxa (isopods, harpacticoid copepods, amphipods, and gastropods) were detected in each meadow. Other functional groups include predators (polychaetes, crabs, free-living mites, two species of amphipod), filter feeders (bivalves), and deposit feeders/detritivores (shrimp). Across all samples, epifaunal assemblages were dominated by small (1-2 mm) invertebrates, which made up ~83% of individuals, whereas large invertebrates (> 8 mm) such as crabs, sea stars and urchins made up less than 3% of individuals.

*Spatial biodiversity patterns*

At the meadow scale, we estimate significantly different numbers of species among some meadows: Crow Cove likely has fewer species at the meadow scale than BI and RP (Table 1). Other meadows, despite differences in observed gamma, likely do not differ in actual meadow-scale richness (Table 1). Of the regional ‘species’ pool (30 epifaunal taxa) used in our analyses, in each meadow across the nine sampled plots, we observed between 10 and 22 taxa. Thus, there is substantial variation in observed diversity among meadows (Table 1).

Consistent with our first hypothesis, we found that alpha diversity did not vary systematically among meadows (Figure 2). We found that raw plot-level alpha diversity [R] differed as much within meadows as among meadows (Figure 2A), with the exception of high alpha diversity at Robbers Passage and Boyson Islands (F = 8.9, df = 8, 136, P < 0.001). We observed similar patterns in Shannon diversity (F = 4.6, df = 8, 136, P < 0.001) and Simpson diversity (F = 3.8, df = 8, 136, P < 0.001), though for these metrics Boyson Islands is not different, and Dodger Channel has lower within-plot evenness than other meadows (Figure 2). Rarified diversity estimates are more variable among meadows (Figure 2C; F = 3.62, df = 8, 120, P = 0.002). The higher diversity of Robbers Passage, lower S of Dodger Channel, and differences in ENS were not explained by meadow-scale predictors such as position in the watershed, shoot density, meadow area or fetch (Appendix Table 1).

Beta bray Curtis results here for *spatial* comparisons (Figure 3). Comparison with the null model showed that both inter- and intra- meadow beta diversity (βRC) was always greater than expected by chance (Figure 4, Figure S1). A permutation test of multivariate homogeneity of group dispersions showed that within-meadow varied among meadows (Appendix 6), but beta diversity did not vary predictably along the watershed gradient,

Spatial variation in species composition in July was consistent with metacommunity-scale processes structuring biodiversity. Across all nine meadows, elements of metacommunity structure (EMS) analysis based on presence/absence data suggested a checkerboard pattern of species distribution among meadows. For all epifaunal taxa (30 taxa), EMS suggested negative spatial coherence (86 embedded absences relative to 56.17 + 6.53 expected by the null model, z = - 4.57, P <0.01). For grazers (17 taxa), we observed a similar pattern of negative coherence (29 embedded absences relative to 16.42 + 3.76 expected by the null model, z = - 3.35, P <0.01).

*Temporal biodiversity patterns*

Over time, raw plot-scale alpha increased over time, and generally increased between July and August, except for at RP (Table 3, Figure X). Shannon diversity and Simpson’s evenness did not vary systematically over time, while ENS declined slightly overall between May and June (Table 3, Figure X). By August, there is a trend toward lower plot-scale H’, ENS and S in the marine meadows compared to the meadows nearer freshwater (Figure X).

Bray-Curtis dissimilarity metrics suggest that beta diversity increased in mid-summer in most sites except RP (Fig 4, Table 3). In contrast, we observed a general increase in dissimilarity within sites over time, while dissimilarity initially increased then decreased among sites. The model detected no difference between expected beta within and among sites but did support observed changes in expected beta over the course of the summer (two-way ANOVA, F = 45.222, df = 2, 2316, P < 2e-16). In addition, variance of observed dissimilarity generally increased among sites over the summer (0.31-0.76), and decreased within sites (0.53-0.44, Levene’s Test, F = 9.44, df = 5, 2316, P < 5e-09).

*Patterns in composition and dominance*

Differences in diversity (Figure 2) and composition (Figures 3, 4) among meadows can be explained by variation in the relative abundance of species across sites and times, rather than by changes in their presence in meadows. Many species were rare but present at most sites and times, yet no species was common everywhere at all times (Table 2). Five taxa were detected at every site and sampling time (the grazers *Pentidotea resecata*, *Caprella spp*., and *Aoroides columbiae*; the predator *Eogammarus confervicolus*; and the filter-feeding *Mytilus trossulus*), and eight additional taxa were detected at all sites but one (Table 2). Conversely, four species were only detected at one time and place (*Alia carinata* at BI, *Mesocentrotus* sp. at WI, *Solaster sp*. at CB, and *Dinophilus sp*. at DC; Appendix 1), and eight taxa were observed only twice. Species presence at a site was fairly consistent through time (Table 2).

Across all meadows, five taxa accounted for > 80% of invertebrates collected: the caprellid amphipods *Caprella spp*. (*C. laeviuscula* and *C. californica*), the sea hare *Phyllaplysia taylori* (primarily juveniles), the mussel *Mytilus trossulus* (primarily juveniles), the polychaete worm *P. bicanaliculata*, and the sea spider *Pycnogonum sp*.  However, abundances of these species were not consistently high across all meadows and all sampling periods.  For example, in the midsummer sampling period the number of caprellids ranged from 488 individuals/plot in Numukamis Bay to less than 0.1 individual/plot at Ellis Island.

The identity of dominant taxa varied through time (Table 2). We observed large temporal changes in the abundance of many common species: for example, *Caprella spp*. abundance increased more than ten-fold (from an average of 16 individuals/plot to 237 individuals/plot) from early to mid-summer, but by late summer a recruitment event of *P. taylori* led this species to outnumber *Caprella spp*. at Robbers Bank and Dodger Channel.  However, changes in abundance were not consistently observed across sites. For example, the *P. taylori* recruitment event occurred at two sites, while a major mussel recruitment event occurred at other sites including Numukamis Bay and Crickitt Bay later during the same period.

DISCUSSION

Seagrass meadows host vast biodiversity worldwide, and this biodiversity is part of the immense ecological and economic value associated with seagrass ecosystems (Heck, Duffy et al 2015, etc). Spatial processes have rarely been considered in the seagrass diversity literature (Bostrom et al 2006), yet the spatial patchiness and likely high dispersal of some eelgrass associated organisms suggest that spatially structured community dynamics may be important in maintaining local and regional seagrass faunal diversity. In an unprecedented description of the biodiversity of British Columbia seagrass epifauna within and among meadows over a summer season, we found that alpha diversity was relatively consistent across the landscape, despite variation in meadow size and abiotic conditions. Communities were more similar than expected by chance, and among meadows, species exhibited patterns consistent with a checkerboard distribution. Between May and August, communities became more similar across space, as abundance and observed species richness increased over time (?). The patterns we observe suggest spatially structured community dynamics are likely important to the ecology and conservation of these important coastal ecosystems.

Although we observed different numbers and combinations of species across different meadows, meadow-scale biodiversity is more similar across the landscape than expected. The null expectation considered the same regional species pool and relative abundance of species but species are distributed across the meadow landscape randomly. Instead, both within and across meadows, we observed patterns consistent with spatial aggregation within species and community similarity among locations. The metacommunity analysis also suggested spatial structure in species distributions that differs from a null expectation based on species presence across meadows (not patches within meadows), and suggests a negative coherence, or checkerboard, pattern of species distributions among meadows. Checkerboard patterns have been interpreted to reflect either negative species interactions such that pairs of species do not occur together; this pattern could also reflect shared environmental affinities among groups of species. The metacommunity analysis found no evidence for spatially nested or clumped community patterns. *Say more on this?*

Samples collected within meadows mid-summer were typically more similar to samples from the same meadow than to samples from other meadows with a general sorting by position along the watershed, suggesting meadow-scale aggregation and similarity in faunal assemblages (Figure S2). However, while dissimilarity was greater among sites than within sites at the beginning of the summer compared to null expectations, the reverse was true by the end of the summer (Figure 3). This species turnover among meadows was driven by both shifts in relative abundance, and by species turnover.

We did not observe a clear signal of continuous variation in biodiversity along the biotic and abiotic gradients associated with the watershed (Figure 2, Figure 4, Figure S1). The lack of a clear signal of salinity in the epifaunal community contrasts with previous findings in other seagrass systems. Barnes (2013) found that invertebrate assemblages in an extensive seagrass meadow in Knysna Bay (South Africa) respond strongly to estuarine gradients, with significant reductions in species diversity and richness at the fresh end of the estuary.  Yamada et al. (2007) also found a positive relationship between salinity and invertebrate diversity. In contrast, we observed only weak, temporally inconsistent trends in invertebrate abundance and diversity with estuarine position (correlated with salinity and eelgrass structure).  However, the sites sampled by Barnes (2013) and Yamada et al. (2007) spanned a greater range in salinity (< 5 - 35 ppt and 6.2 – 32.2 ppt, respectively) than did ours (~14 - 28 ppt), and may have captured physiologically stressful, low-salinity conditions with a stronger influence on invertebrate assemblages.

Variation in species diversity at the plot- or meadow-scale could also be explained by meadow area. Larger meadows would be expected to host more species per area than smaller meadows. We only have area estimates for five of the meadows we sampled, and the low sample size (n = 5) and low variation in meadow area prevent robust statistical analysis of area as a predictor. Numukamis Bay (NB) was by far the largest meadow (72 ha), while the others were on the order of <1 to several hectares (Table 1). However, species diversity patterns do not suggest vastly greater species richness in NB than other sites, nor are there fewer species at the smallest (< 1 ha) meadows (Figure 2). Further, average plot scale diversity (7-20 species, Figure 3) and meadow-scale alpha diversity (Chao2 index estimate: 28 species per meadow) was in all cases much lower than regional species diversity (gamma diversity = 47 observed taxa), suggesting that some processes limit the presence of all regionally-present taxa in all meadows.

Like alpha diversity metrics, beta diversity did not vary predictably along the watershed gradient. However, our results show that beta diversity as non-directional variation is significantly greater than expected by chance (Figure 3), suggesting aggregation of species within meadows is greater than expected at random. Spatial aggregation within meadows can indicate micro-habitat variation, species interactions such as competition, predation or facilitation, or historical effects such as recovery from disturbance. Despite this signal of aggregation, suggesting species turnover within meadows over fine scale, we observed greater turnover in composition among meadows than within meadows. Greater turnover among meadows could, again, reflect either spatial distance among samples, variation in meadow-scale properties (properties not measured in this study), connectivity, asynchronous community trajectories, or historical events such as disturbance and associated recovery.

Our study contributes to only a few other beta diversity estimates for seagrass-associated epifauna. These studies have typically focused on directional beta diversity, i.e., beta diversity along some gradient, finding that beta diversity in seagrass beds can be driven by variation in salinity, seagrass species, tidal height, and sub-habitat type (e.g., seagrass shoots or sediment; De Troch et al. 2001, 2003, Barnes and Ellwood 2012, Knight et al. 2015). However, we know very little about non-directional beta diversity within seagrass meadows, and (to our knowledge) no study has attempted to distinguish observed patterns in beta diversity from null expectations (Kraft et al. 2011).  The comparison of null expectations to empirical datasets allows us to infer whether random processes such as chance colonization and extinction, or deterministic processes like environmental filtering or competition are generating observed patterns in community composition (Chase and Myers 2011).

Temporal variation in abundance and diversity within meadows balanced in magnitude the variation we observed over 30 km and nine distinct meadows. Rather than a directional seasonal in abundance or diversity within meadows, these metrics tended to peak in mid-summer (July) and decline again in August. Dominant species did shift through time, within meadows (Table 2). These shifts through time reflected recruitment events of several species, notably *Phyllaplysia taylori* and *Mytilus trossulus*. Local reproductive events by the non-dispersing offspring of *P. taylori* dominated the marine sites RP and DC in August. At other sites, planktonic dispersing larvae of mussels colonized and dominated eelgrass assemblages. These trends suggest that reproductive events, regardless of dispersal type, characterize seasonal trends in epifauna diversity and abundance between May and August in this system. Other taxa shifted in abundance substantially, including an increase in nematode abundance at NB between May and July. Grazer taxa such as amphipods and isopods with continuous population dynamics and overlapping generations increased between May and July at all sites except NB. This latter pattern suggests some site-level factors such as food availability, seasonal warming, or reduction in predation between May and July that was then reversed in DC, CB and NB by August. Despite this variation in univariate metrics and the identities of dominant species, multivariate metrics suggested composition overall was relatively stable through time, suggesting that the differences in abundance and richness are reflecting changes in dominance (Figure 4).

In Chesapeake Bay eelgrass systems, epifaunal grazers remain relatively stable in abundance between May and August, though their relative abundance shifts from *Caprella* and *Gammarus* dominated assemblages to the amphipod *Erichsonella attenuata* (Douglass et al. 2010). Our observations are consistent with other reports of seasonal variation in epifaunal assemblages from Puget Sound, WA, showing increasing abundance of grazers (*P. resecata*, *Lacuna sp*., and *Caprella sp*.) between May and late summer (Thom et al. 1995). Best and Stachowicz (2014) also report peak abundance of epifaunal taxa in August, and that peak abundance can vary among habitat patches.

*Conclusions*

Understanding spatial and temporal variation is essential to linking the abiotic and biotic features of a marine seascape to the ecosystem functions that promote biodiversity as well as secondary productivity that emerges at higher trophic levels including fish, wading birds, and marine mammals.  Although biodiversity patterns in seagrass meadows have been to date quantified primarily within individual, discrete meadows, many of the processes that influence these patterns, including potentially damaging processes such as eutrophication, the introduction of non-native species, and severe weather, operate across much larger spatial scales (Duarte 2002).  This disconnect between sampling scale and the spatial extent of disturbance has been shown to lead to conflicting and misleading conclusions about the effects of disturbance on ecosystems, particularly with respect to changes in local population persistence and species richness (Powell et al. 2013).  As anthropogenic pressure on valuable coastal ecosystems continues to accelerate at local and regional scales, it is more important than ever that we develop a baseline understanding of how biodiversity in seagrass meadows is maintained across ecologically relevant scales.

In one of the first comprehensive assessments of eelgrass-associated biodiversity in western Canada, we have shown here that species diversity and abundance vary among seagrass meadows within a region, and that the variation is not clearly predictable based on an estuarine gradient and related predictors. Although Z. marina provides relatively homogenous habitat, each *Z. marina* meadow appears to host a subset of epifaunal species observed in the larger region. These patterns are indicative of a metacommunity system, and suggest that processes that maintain diversity in eelgrass communities may reflect a seascape of many meadows connected through dispersal. If true, then conservation of eelgrass systems will require a network approach that preserves numerous meadows within the region. Examining biodiversity patterns across a seascape has expanded our view of seagrass biodiversity, and additional research is required to meet the challenges of understanding how spatial turnover in diversity is related to the function and resilience of these ecosystems.

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