**Epifaunal diversity patterns among eelgrass meadows suggest metacommunity structure**

Running head: Epifaunal diversity in seagrass

Ross Whippo1,2, Nicole S. Knight1,3,4, Carolyn Prentice1,5, John Cristiani1, Matthew R. Siegle1, Mary I. O’Connor1\*

\*Corresponding author: [oconnor@zoology.ubc.ca](mailto:oconnor@zoology.ubc.ca)

1. Department of Zoology and Biodiversity Research Centre, University of British Columbia, 2212 Main Mall, V6T 1Z4, Vancouver, Canada

2. Smithsonian Institution, Tennenbaum Marine Observatories Network, 647 Contees Wharf Rd, Edgewater, MD, 21037, USA

3. Department of Biology, McGill University, 1205 Avenue du Docteur-Penfield, H3A

1B1, Montreal, Canada

4. Smithsonian Tropical Research Institute, Apartado Balboa 0843-03092, Ancon, Republic of Panama

5. School of Resource and Environmental Management, Simon Fraser University, 8888 University Drive, V5A 1S6, Burnaby, Canada

ACKNOWLEGEMENTS

We are very grateful to C. Harley, G. Crutsinger, N. Sanders, J. Bernhardt, N. Caulk and A. Gonzalez for their feedback on the manuscript. We also sincerely thank A. MacDonald, B. Harrower, K. Demes and M. Barbour for their help with statistical analyses, and S. Gray, W. Cheung, R. DeGraff, S. James, F. Ratcliffe, K. Anderson, D. de Jonge, S. Anthony, and the Robles lab for their assistance in the field and laboratory. Financial support for this project was provided by Bamfield Marine Sciences Centre to R.W. and M.I.O., an undergraduate research award to N.S.K. from the University of British Columbia, and an NSERC Discovery Grant and a Sloan Fellowship to M.I.O. The authors do not have any conflicts of interest with regard to this work.

ABSTRACT

AIM: In coastal seascapes, spatial habitat structure can support high animal diversity and secondary productivity. Still, seascape-scale connectivity among eelgrass meadows is an often overlooked dimension of their ecological and conservation value. We tested whether eelgrass-associated biodiversity patterns are consistent with spatial processes such as abiotic habitat filtering or metacommunity dynamics.

LOCATION: In Barkley Sound, British Columbia, we quantified epifaunal biodiversity on eelgrass (*Zostera marina*) among nine meadows to test three hypotheses: taxonomic diversity and composition i) vary randomly within meadows but ii) vary systematically among meadows reflecting seascape position and metacommunity dynamics, and iii) patterns are stable over time.

METHODS: We sampled epifaunal invertebrate biodiversity in a systematic spatial grid within 9 eelgrass meadows along a spatial gradient. We used univariate and multivariate diversity metrics to test for spatial and temporal diversity patterns.

RESULTS: Though we identified two high-diversity meadows, epifaunal diversity varied as much over a few meters within the same meadow as among meadows separated by kilometers and of different sizes and exposures. This variation was not clearly explained by seascape position. Community similarity increased from May to August.

MAIN CONCLUSIONS: These findings leave open the possibility that seascape scale metacommunity dynamics influence eelgrass-associated epifaunal biodiversity and its persistence in spatially patchy seascapes.

Key words: seagrass, *Zostera* *marina*, foundation species, biodiversity, grazer, estuary, invertebrate, seascape ecology, elements of metacommunity structure

INTRODUCTION

Understanding species’ distribution and abundance is essential to understanding ecological communities and to making informed decisions about seascape management and biodiversity conservation. In Canada’s highly diverse coastal marine habitats, 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). Coastal foundation species such as seagrass and kelp are often targeted for conservation for the biodiversity they harbor and the ecosystem services they provide. Yet, how exactly these habitats support the high and productive animal assemblages, and the role of spatial arrangement of habitats, remains poorly understood.

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 provision by foundation species is just one of several processes that influence biodiversity of a patch: population dynamics and species interactions structure species assemblages at finer (within meadow) and broader (seascape) spatial scales in spatially structured ‘metacommunities’ (Leibold et al., 2004; Boström et al., 2006; Pillai et al., 2011). Rates of colonization and rescue effects from source-sink dynamics can play a major role in maintaining the species composition and functional integrity of an ecosystem, and understanding these dynamics is critical for successful management (gerber et al ecosphere 2014… but there are many, just esarch connectivity and managmeent).

The purpose of this study is to explore spatial structure among eelgrass-associated epifaunal species as a first step to assess the most informative spatial scale for understanding how eelgrass meadows support biodiversity and its associated ecosystem functions. Specifically, our aim was to target epifaunal biodiversity among spatial scales in a seagrass meadow seascape to determine whether individual meadows, or a set of meadows, are representative spatial units for biodiversity (i.e. – groupings of habitat that reflect regional patterns of diversity). Spatial variation in biodiversity is influenced by the spatial scales of ecological processes (e.g.- local predation pressure vs. regional dispersal patterns) that affect species’ relative abundance and presence or absence (Leibold et al., 2004; Ricklefs, 2004; Boström et al., 2006). Across a region, evolutionary and colonization history produce a shared regional species pool (gamma diversity). At finer scales, meadow- or host 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* (DeTroch et al., 2001; Crist & Veech, 2006; Sanders et al., 2007). 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, forms of *beta diversity* (Anderson et al 2011), that are jointly influenced by local and regional process (Leibold & Mikkelson, 2002; Leibold et al., 2004; Crist & Veech, 2006; Henriques-Silva et al., 2013).

Eelgrass-associated epifauna are the critical links in the food web that connect high eelgrass related algal primary production to fish production in the food chain (Simonstad ref, others). Biogeograpic variation in eelgrass-associated epifaunal diversity influences the strength of top down control in eelgrass meadows, and is central to many of the ecosystem services provided by these systems (Duffy et al 2015, Amundrud et al 2015, Duffy et al 2003 eco letts).

Epifaunal diversity can vary substantially within and among meadows (Hemminga & Duarte, 2000; Boström et al., 2006; Yamada et al., 2007; Carr et al., 2011; Barnes & Ellwood, 2012; Barnes, 2013). Within meadows, alpha diversity at fine scales (0.5 – 1 m2) is typically relatively consistent among patches within the same meadow and much lower than meadow-scale species diversity (Carr et al., 2011; Barnes & Ellwood, 2012; Barnes, 2013). This pattern suggests that compositional differences, one form of beta diversity, should be high and a relatively important component of seagrass associated biodiversity within meadows. Though studies of variation in species distribution have reported high turnover at moderate spatial scales (< 150 m, Barnes and Ellwood 2012), few studies specifically assess beta diversity at the same scale. Among meadows, variation in species composition and diversity is in some cases explained partially by wave energy or exposure (e.g., fetch; Boström et al. 2006, Robinson et al. 2011) or salinity (Yamada et al. 2007, Boström et al. 2014, Barnes and Ellwood 2013). Still, in some cases diversity patterns are not readily explained by abiotic attributes (e.g., Carr et al. 2011, Barnes and Elwood 2012).

Here, we test the hypothesis that non-random spatial patterns in species composition and turnover within and among meadows distinguish meadows within a seascape and provide insight as to potential importance of abiotic factors and community dynamics in driving regional-scale epifaunal diversity patterns. Specifically, we hypothesize that 1) alpha (quadrat-scale) epifaunal diversity is the same among meadows, but within-meadow species composition varies in space to produce patterns of beta diversity that also vary 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. We also tested 3) whether patterns of diversity vary over time, and whether variation over time could provide insight to possible drivers of diversity patterns. To test these hypotheses, we used a nested sampling design to quantify spatial structure in seagrass-associated epifaunal biodiversity among nine meadows in British Columbia, Canada.

METHODS

*Study system*

We sampled epifaunal biodiversity in eelgrass *Zostera marina* meadows in summer of 2012 in Trevor Channel, Barkley Sound, where *Z. marina* is the only meadow-forming seagrass species (Table 1, Figure 1a).  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). A rich epifaunal assemblage of gastropods, crustaceans and other taxa live on and among eelgrass blades. Epifauna consume epiphytic algae growing on seagrass, detritus and each other, forming the base of a highly productive food web (Hemminga & Duarte, 2000; Heck Jr. & Valentine, 2006).

*Structure and abiotic conditions*

We quantified several biotic attributes of eelgrass meadows that could explain variation in eelgrass associated invertebrate biodiversity: shoot density, leaf area, epiphyte load, and meadow area. Shoot density and leaf area were estimated from three or four 0.28 m2 quadrats collected outside each corner of a 4 x 4 m grid demarcated for community sampling (described below) at each site concurrent with epifaunal sampling. For all meadows in all time periods, we removed a shoot from each corner quadrat, dried and weighed eelgrass and its associated epiphytes, and standardized epiphyte dry mass to eelgrass dry mass. Additionally, we sampled epiphytes more thoroughly at three sites during low-tide periods (< 0.5 m) in mid and late summer, by collecting 15 shoots per meadow from three tidal heights. We separated bladed epiphytes from periphyton and again standardized epiphyte mass to eelgrass mass. We estimated leaf area using methods described by Borg et al., 2010. We did not estimate quadrat-level density or leaf area for the same quadrats from which we sampled epifaunal biodiversity.

To quantify meadow-scale abiotic conditions, we measured 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 represented the estuarine abiotic gradient of salinity and temperature by quantifying the geographic position from the nearest freshwater input of each meadow. Position was estimated as linear distance in kilometers from the nearest freshwater source (Sarita River or Alberni inlet, Figure 1a). We estimated exposure to wave energy (using fetch as a proxy) by calculating the distance to nearest land from the eelgrass meadow in 10 degree increments around a centroid point and summed the distance.

*Biodiversity sampling*

To estimate epifaunal biodiversity and characterize variation within meadows, we used a 4 x 4 m grid of 16 standard quadrats (0.28 m2), each separated by 1 m, in each meadow (Figure 1b; after Sanders et al. 2007, Duffy et al. 2015). Repeated sampling within meadows was rotated 90° around a central reference point at a distance of 1-2 m so that seagrass removed at one time period would not impact the density of seagrass and associated epifaunal at subsequent samplings. This sampling design allowed for comparison of diversity among quadrats 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 quadrat, 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) but not deeper than 3 m below LLWLT. Samples were then transported back 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, they are large meadows (i.e., 0.12 – 11.33 ha), 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.

We identified every invertebrate > 1 mm 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). Consequently, our taxonomic diversity estimates should be considered minimum estimates. We classified invertebrate species to broad trophic groups (grazer, predator, filter feeder, detritivore) based on our observations and published information (Light & Carlton, 2007; Macdonald et al., 2010).

*Biodiversity estimation*

To estimate diversity within and among meadows, we created species-quadrat and species-site matrices with abundance data using information on every individual collected from each quadrat (Gotelli & 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 (quadrat-scale) diversity by rarifying (ENS) and using species 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; Dauby and Hardy 2012). ENS is a standard metric that can help distinguish changes in diversity from changes in abundance and 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; R Core Team, 2016).

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). This allows comparison of quadrats to all other quadrats within the meadow. 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 the Raup-Crick beta diversity metric, βRC,to compare pairwise dissimilarities between samples with a null expectation. This metric uses presence/absence of species and their relative occurrence among samples to assign probabilities that community samples are less similar (>0), more similar (<0) or no different (=0) than expected by chance. We analyzed patterns in community composition among all nine sites sampled at all time periods for both intra-meadow and inter-meadow variation.

*Statistical diversity analyses*

To test our first hypothesis that alpha diversity (quadrat-level species diversity) does not vary among quadrats 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 from each other, and meadow attributes. We conducted linear regression analyses using sampling date, distance among quadrats, meadow area, fetch and distance from freshwater inputs as predictors. We tested for correlations among these variables. Abundance and ENS were log-transformed to meet the assumption of homoscedasticity.

To test our second hypotheses that beta diversity within meadows varies among meadows, we compared beta diversity estimates with null expectations as described above. 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 are the same among meadows.

For all model comparisons, 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 & 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 & 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.

*Elements of metacommunity methods*

To test whether biodiversity patterns among meadows are consistent with metacommunity processes operating at the seascape scale, we applied the elements of metacommunity (EMS) framework (Leibold & Mikkelson, 2002; Presley et al., 2010). This analysis first tests for coherence in species distributions among 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 seascape (a Gleasonian model of species distributions), or clumped species distributions (Clementsian structure), or random, providing insight to possible types of community structure (Leibold & Mikkelson, 2002; Presley et al., 2010; Henriques-Silva et al., 2013). We analyzed metacommunity structure for the nine 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”).

RESULTS

*Meadow attributes*

We collected 71 samples for seagrass structural metrics. Meadows increased in shoot densities (+0.27 shoots/km) and associated leaf area (+240.73 cm2/km) with increasing proximity to the Pacific Ocean (Table S2). Shoot density and leaf area increased between May and August at meadows near freshwater but remained relatively high all summer at the seaward meadows (Table S2). Distance from fresh water input captured correlations with other variables: temperature, salinity, shoot density (Table S3). Salinity increased and temperature declined with distance from freshwater inputs, though these changes were relatively small in magnitude (Table S5). Epiphyte load was highly variable between meadows, uncorrelated with position in the estuary (Table S3). Epiphyte abundances and the type of epiphytes present (periphyton vs. bladed algae) varied spatially and temporally (Table S4). The bladed brown epiphyte *Punctaria* sp. was abundant at two of the marine sites (DC and WI), and absent from the fresher NB.

*Epifauna summary*

We collected 304 seagrass samples with associated epifauna and identified approximately 81,500 invertebrates (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 associated with 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 quadrat 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 relatively stable over time (CB, NB; Tables S7). The seasonal abundance increase in seaward meadows is clear in small (1-2 mm) and large (>2 mm) size fractions.

Of the 30 epifaunal taxa observed, 17 taxa are herbivorous or omnivorous consumers of epiphytic algae, classified as “grazers”. 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. Among 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*

Between 10 and 22 epifaunal taxa (of the 30 observed) occurred in each meadow. Thus, there was substantial variation in observed diversity among meadows (Tables 1, 2, S1). We found that alpha diversity did not vary systematically among meadows with any predictor we tested (Figure 2, Table S1). Quadrat-level species richness [R] varied as much within meadows as among meadows (Figure 2a), with the exception of high species richness (alpha diversity) at RP and BI (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 BI is not different, and DC has lower within-quadrat evenness than other meadows (Figure 2d). Rarified diversity estimates were more variable among meadows (Figure 2b; F = 3.62, df = 8, 120, P = 0.002). The higher diversity of RP, lower S of DC, and differences in ENS were not explained by meadow-scale predictors such as distance from fresh water input, shoot density, meadow area or fetch (Table S1).

Beta diversity within meadows, reflecting similarity in relative abundance and composition of species from each of the 16 sampled quadrats, varied substantially among meadows (Figure 3e-f). Comparison with the null model showed that both inter- and intra- meadow beta diversity (βRC) was always less than expected by chance indicating that meadow epifauna were more similar than would be expected if species were distributed among meadows randomly (Figure 4a, Figure S1). A permutation test of multivariate homogeneity of group dispersions showed that within-meadow beta diversity varied among meadows (Table S6), but beta diversity did not vary predictably by distance from fresh water input or with other abiotic and biotic factors. Finally, analyses of aggregation indicated that individuals within species were spatially aggregated within meadows for most species (>80% in 7 of 9 meadows, Table 1).

Spatial variation in species composition was consistent with metacommunity-scale processes structuring biodiversity patterns. Among all nine meadows in July, elements of metacommunity structure (EMS) analysis based on presence/absence data suggested a checkerboard pattern of species distribution among meadows, indicating differences among meadows that were non-random. 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, quadrat-scale (alpha) richness increased, primarily between July and August, except at RP where richness spiked mid-summer (Figure 3a). Shannon diversity and Simpson’s evenness did not vary systematically over time (Figure 3c-d), while ENS declined slightly overall between May and June (Figure 3b). By August, there was a trend toward lower quadrat-scale observed richness and lower ENS, which increased from marine to fresher meadows (Figure 3b).

Beta diversity as gamma/alpha and mean Bray-Curtis dissimilarity generally decreased over the summer, meaning quadrats within the same meadow became more similar. However, Bray-Curtis beta spiked at RP and CB in midsummer, likely due to a numerical increase in *Caprella* spp. and *Phyllaplysia taylori* at RP, and *Mytilus trossulus* at CB. These decreases in beta diversity over the course of the summer are reflected in comparisons to null predictions (Figure 4), which found that among- and within-meadow beta diversity exhibited less dissimilarity than predicted values, a trend that strengthened on both scales over the course of the summer. This indicates that meadows were becoming more similar across small (<1 m) and large (>10 km) scales simultaneously. This trend can be attributed to the observed recruitment events by increasingly common mesograzers that occurred as the summer progressed.

*Patterns in composition and dominance*

Increasing similarity within and among meadows over time co-occurred with increases in abundance in common taxa, rather than by changes in their presence in meadows (Table 2, Figure 3). For example, *Caprella* spp. abundance increased more than ten-fold (from an average of 16 individuals/quadrat to 237 individuals/quadrat) from early to mid-summer, but by late summer a recruitment event of *Phyllaplysia taylori* led this species to outnumber *Caprella* spp. at RP and DC.  However, changes in abundance were not consistently observed among sites. For example, the *P. taylori* recruitment event occurred at two sites, while a major mussel recruitment event occurred at other sites including NB and CB later during the same period, causing the species composition of these two sites to diverge from the others. Many species were rare but present at most sites and times, yet no species was common everywhere at all times (Table 2). Nearly half of epifaunal taxa analyzed (12) were common – five taxa were detected at every site and sampling time.

Among 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 *Platynereis bicanaliculata*, and the sea spider *Pycnogonum* sp.  However, abundances of these species were not consistently high among all meadows and all sampling periods.

DISCUSSION

Seagrass meadows host vast biodiversity, and this biodiversity is part of the immense ecological and economic value associated with seagrass ecosystems (Hemminga and Duarte 2000, Duffy et al. 2015, Heck and Williams 2000). Generalities and drivers of spatial patterns in eelgrass associated fauna are not well documented or understood (Boström et al., 2006). In an unprecedented spatially explicit description of the epifaunal biodiversity of British Columbia seagrass meadows over a summer season, we found that non-random spatial patterns in species composition and turnover distinguished meadows within the Trevor Channel seascape. In contrast, alpha diversity (species richness and ENS) was varied little over space and time. Differences in meadow area, fetch and distance from freshwater input did not explain variation in diversity. These patterns suggest that species interactions and population dynamics are playing out across meadows in this region, and seagrass meadow communities may form larger metacommunities linked by dispersal.

Meadow-scale patterns in species number and compositional turnover were more similar among meadows than expected by chance, even though we observed different numbers and combinations of species in each meadow. For example, samples collected within meadows mid-summer were typically more similar to samples from the same meadow than to samples from other meadows. These distinctive meadow species compositions reflected differences in numerically dominant species, rather than less striking differences in species presence or absence. 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 4A).

Spatial patterns in epifauna suggest possible metacommunity structure, indicateing that biodiversity may be maintained at the seascape scale rather than the meadow scale. Among meadows, species exhibited patterns consistent with a checkerboard distribution. Checkerboard patterns have been interpreted to reflect negative species interactions such that pairs of species do not occur together. Although this pattern could also reflect shared environmental affinities among groups of species (Leibold & Mikkelson, 2002; Presley et al., 2010; Henriques-Silva et al., 2013), the metacommunity analysis found no evidence for spatially nested or clumped community patterns that would have been more consistent with groups of species sharing environmental affiliations. This suggestion is also supported by the lack of an observed effect of meadow area or other abiotic predictors in this study.

Contrary to some studies in other regions (Yamada et al., 2007; Barnes & Ellwood, 2012), we did not observe a clear signal of continuous species turnover along the biotic and abiotic gradients associated with distance from fresh water input (Figure 2, Figure 3a-c), until, possibly, late summer (Figure 3d). We observed only weak, trends in invertebrate abundance and diversity with estuarine position (correlated with salinity and eelgrass structure).  This may be indicative of the salinity regime of the sites sampled by Barnes (2013) and Yamada et al. (2007), which 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.

Our findings are consistent with other studies that suggest that species compositional turnover in space is the main component of biodiversity in these systems. Quadrat-scale diversity seems to be quite constrained to a small number of species, regardless of meadow biotic or abiotic attributes. Thus, the processes that underlie spatial turnover are the ones that facilitate the high diversity of this system. Our study reveals spatial aggregation of most species within meadows. Aggregation can indicate micro-habitat variation, species interactions such as competition, predation or facilitation, or historical effects such as recovery from disturbance or stochastic events. We also observed turnover in composition among meadows that suggests a role for seascape-scale dispersal (>10 km), asynchronous community trajectories, or historical events such as disturbance and associated recovery.

Our study contributes to a limited number of existing beta diversity estimates for seagrass-associated epifauna. These studies have typically focused on turnover, i.e., directional change in 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 variation in 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 allowed us to infer whether random processes such as chance colonization and extinction, or deterministic processes like environmental filtering or competition are likely causes of observed patterns in community composition (Chase & Myers, 2011).

Over the summer season, species turnover within and among meadows declined, reflecting demographic changes that increased the abundance and prevalence of several numerically dominant species. These demographic changes were driven by very different specific processes: annual recruitment pulses by the weakly-dispersing seagrass specialist *Phyllaplysia taylori* in RP and DC in August, recruitment by the pelagic dispersing and cosmopolitan mussel *Mytilus trossulus* at other sites, and population increases by benthic eelgrass-dwelling caprellid amphipods. These increases were similar among quadrats, but each reflects a temporary increase that is expected to change over time with juvenile mortality and population dynamics. 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, consistent with observations in other systems (Douglass et al. 2010, Thom et al. 1995, Best and Stachowicz 2014). Still, the potential role of species interactions such as competition or predation in the temporal dynamics of these communities is not well understood.

*Conclusions*

Seagrass communities are known for their high epifaunal diversity and productivity, and fine-scale variation in species composition appears to be a critical component of this diversity. 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. 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 this comprehensive assessment of eelgrass-associated biodiversity, we have shown that species diversity and abundance vary among seagrass meadows within a region, and that the variation is not clearly predictable from an estuarine gradient and related biotic and abiotic 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, the 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 address the challenges of understanding how spatial turnover in diversity is related to the function and resilience of these ecosystems.

REFERENCES

Anderson, M.J., Crist, T.O., Chase, J.M., Vellend, M., Inouye, B.D., Freestone, A.L., Sanders, N.J., Cornell, H. V, Comita, L.S., Davies, K.F., Harrison, S.P., Kraft, N.J.B., Stegen, J.C., & Swenson, N.G. (2011) Navigating the multiple meanings of beta diversity: a roadmap for the practicing ecologist. *Ecology Letters*, **14**, 19–28.

Barnes, R.S.K. (2013) Distribution patterns of macrobenthic biodiversity in the intertidal seagrass beds of an estuarine system, and their conservation significance. *Biodiversity and Conservation*, **22**, 357–372.

Barnes, R.S.K. & Ellwood, M.D.F. (2012) Spatial variation in the macrobenthic assemblages of intertidal seagrass along the long axis of an estuary. *Estuarine Coastal and Shelf Science*, **112**, 173–182.

Borg, J.A., Rowden, A.A., Attrill, M.J., Schembri, P.J., & Jones, M.B. (2010) Spatial variation in the composition of motile macroinvertebrate assemblages associated with two bed types of the seagrass Posidonia oceanica. *Marine Ecology Progress Series*, **406**, 91–104.

Boström, C., Baden, S., Bockelmann, A.C., Dromph, K., Fredriksen, S., Gustafsson, C., Krause-Jensen, D., Moller, T., Nielsen, S.L., Olesen, B., Olsen, J., Pihl, L., & Rinde, E. (2014) Distribution, structure and function of Nordic eelgrass (Zostera marina) ecosystems: implications for coastal management and conservation. *Aquatic Conservation-Marine and Freshwater Ecosystems*, **24**, 410–434.

Boström, C., O’Brien, K., Roos, C., & Ekebom, J. (2006) Environmental variables explaining structural and functional diversity of seagrass macrofauna in an archipelago landscape. *Journal of Experimental Marine Biology and Ecology*, **335**, 52–73.

Burnham, K.P. & Anderson, D.R. (2003) *Model selection and multimodel inference: a practical information-theoretic approach.* Springer Science & Business Media,

Carr, L.A., Boyer, K.E., & Brooks, A.J. (2011) Spatial patterns of epifaunal communities in San Francisco Bay eelgrass (Zostera marina) beds. *Marine Ecology-an Evolutionary Perspective*, **32**, 88–103.

Chase, J.M., Kraft, N.J.B., Smith, K.G., Vellend, M., & Inouye, B.D. (2011) Using null models to disentangle variation in community dissimilarity from variation in α-diversity. *Ecosphere*, **2**, art24.

Chase, J.M. & Myers, J.A. (2011) Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **366**, 2351–2363.

Crist, T.O. & Veech, J.A. (2006) Additive partitioning of rarefaction curves and species-area relationships: unifying alpha-, beta- and gamma-diversity with sample size and habitat area. *Ecology Letters*, **9**, 923–932.

Dallas, T. (2014) metacom: an R package for the analysis of metacommunity structure. *Ecography*, **37**, 402–405.

Dauby, G. & Hardy, O.J. (2012) Sampled-based estimation of diversity sensu stricto by transforming Hurlbert diversities into effective number of species. *Ecography*, **35**, 661–672.

DeTroch, M., Fiers, F., & Vincx, M. (2001) Alpha and beta diversity of harpacticoid copepods in a tropical seagrass bed: the relation between diversity and species’ range size distribution. *Marine Ecology Progress Series*, **215**, 225–236.

DeTroch, M., Fiers, F., & Vincx, M. (2003) Niche segregation and habitat specialisation of harpacticoid copepods in a tropical seagrass bed. *Marine Biology*, **142**, 345–355.

Douglass, J.G., France, K.E., Richardson, J.P., & Duffy, J.E. (2010) Seasonal and interannual change in a Chesapeake Bay eelgrass community: Insights into biotic and abiotic control of community structure. *Limnology and Oceanography*, **55**, 1499–1520.

Duffy, J.E., Reynolds, P.L., Boström, C., et al. (2015) Biodiversity mediates top-down control in eelgrass ecosystems: a global comparative-experimental approach. *Ecology Letters*, **18**, 696–705.

Gaston, K.J. (2000) Global patterns in biodiversity. *Nature*, **405**, 220–227.

Gotelli, N.J. & Colwell, R.K. (2011) Estimating species richness. *Biological diversity: frontiers in measurement and assessment*, **12**, 39–54.

Heck Jr., K.L. & Valentine, J.F. (2006) Plant–herbivore interactions in seagrass meadows. *Journal of Experimental Marine Biology and Ecology*, **330**, 420–436.

Hemminga, M.A. & Duarte, C.M. (2000) *Seagrass ecology.* Cambridge University Press,

Henriques-Silva, R., Lindo, Z., & Peres-Neto, P.R. (2013) A community of metacommunities: exploring patterns in species distributions across large geographical areas. *Ecology*, **94**, 627–639.

Jost, L. (2006) Entropy and diversity. *Oikos*, **113**, 363–375.

Knight, N.S., Prentice, C., Tseng, M., & O’Connor, M.I. (2015) A comparison of epifaunal invertebrate communities in native eelgrass Zostera marina and non-native Zostera japonica at Tsawwassen, BC. *Marine Biology Research*, **11**, 564–571.

Kraft, N.J.B., Comita, L.S., Chase, J.M., Sanders, N.J., Swenson, N.G., Crist, T.O., Stegen, J.C., Vellend, M., Boyle, B., Anderson, M.J., Cornell, H. V, Davies, K.F., Freestone, A.L., Inouye, B.D., Harrison, S.P., & Myers, J.A. (2011) Disentangling the Drivers of beta Diversity Along Latitudinal and Elevational Gradients. *Science*, **333**, 1755–1758.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M., & Gonzalez, A. (2004) The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters*, **7**, 601–613.

Leibold, M.A. & Mikkelson, G.M. (2002) Coherence, species turnover, and boundary clumping: elements of meta-community structure. *Oikos*, **97**, 237–250.

Light, S.F. & Carlton, J.T. (2007) *The Light and Smith manual: intertidal invertebrates from central California to Oregon.* Univ of California Press,

Macdonald, T.A., Burd, B.J., Macdonald, V.I., & Van Roodselaar, A. (2010) Taxonomic and feeding guild classification for the marine benthic macroinvertebrates of the Strait of Georgia, British Columbia. *Canadian Technical Report of Fisheries and Aquatic Sciences*, **2874**, .

Mason, B., Knight, R., & Boyer, L. (2015) Available at: http://cmnmaps.ca/EELGRASS/.

Oksanen, J., Kindt, R., Legendre, P., O’Hara, B., Stevens, M.H.H., Oksanen, M.J., & Suggests, M. (2007) The vegan package. *Community ecology package*, **10**, 631–637.

Pillai, P., Gonzalez, A., & Loreau, M. (2011) Metacommunity theory explains the emergence of food web complexity. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19293–19298.

Presley, S.J., Higgins, C.L., & Willig, M.R. (2010) A comprehensive framework for the evaluation of metacommunity structure. *Oikos*, **119**, 908–917.

R Core Team (2016) R: A language and environment for statistical computing. .

Ricklefs, R.E. (2004) A comprehensive framework for global patterns in biodiversity. *Ecology Letters*, **7**, 1–15.

Robinson, C.L.K., Yakimishyn, J., & Dearden, P. (2011) Habitat heterogeneity in eelgrass fish assemblage diversity and turnover. *Aquatic Conservation-Marine and Freshwater Ecosystems*, **21**, 625–635.

Sanders, N.J., Lessard, J.P., Fitzpatrick, M.C., & Dunn, R.R. (2007) Temperature, but not productivity or geometry, predicts elevational diversity gradients in ants across spatial grains. *Global Ecology and Biogeography*, **16**, 640–649.

Searle, S.R. (1988) Parallel lines in residual plots. *American Statistician*, **42**, 211.

Tuomisto, H. (2010) A diversity of beta diversities: straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity. *Ecography*, **33**, 2–22.

Yamada, K., Hori, M., Tanaka, Y., Hasegawa, N., & Nakaoka, M. (2007) Temporal and spatial macrofaunal community changes along a salinity gradient in seagrass meadows of Akkeshi-ko estuary and Akkeshi Bay, northern Japan. *Hydrobiologia*, **592**, 345–358.

DATA ACCESSIBILITY STATEMENT

All species composition and abundance data is available as a spreadsheet file from Dryad at: <http://doi>.

BIOSKETCH

The O’Connor lab at University of British Columbia strives to understand what drives variation in ecosystem structure and function to better understand the ecological impacts of climate change and habitat modification, and to explore how conservation efforts can be most effective given natural environmental changes. More about our research can be found at <http://oconnorlab.weebly.com/>.