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

Running head: Epifaunal diversity in seagrass

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ABSTRACT

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. 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. We sampled epifaunal invertebrate biodiversity in a systematic spatial grid within nine eelgrass meadows along a spatial gradient. We used univariate and multivariate diversity metrics to test for spatial and temporal diversity patterns. 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 or other biotic and abiotic conditions, though salinity may play a role in structuring these communities. 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: biodiversity; estuary; foundation species; grazer; invertebrate; metacommunity; seagrass; seascape ecology; *Zostera* *marina*.

INTRODUCTION

Understanding species’ distribution and abundance is essential to understanding ecological communities and to making informed decisions about seascape management and biodiversity conservation. In highly diverse coastal marine habitats, understanding can be 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 such highly productive animal assemblages, and the role of spatial arrangement of habitats, remains poorly understood (Bostrom et al. 2011). For example, rates of dispersal between seagrass patches likely vary between taxa and functional groups, potentially generating spatial patterns in community structure.

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. 2014, Furrer and Pasinelli 2016).

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 (kilometer-scale), evolutionary and colonization history produce a shared regional species pool (*gamma diversity*). At finer scales, meadow- or host plant-scale (meter- and centimeter-scales) 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 and Veech 2006, Sanders et al. 2007). Between local and regional scales, dispersal and colonization dynamics connect populations among habitat patches to produce metacommunities. The interactions between these local and regional processes generate variation in abundance and diversity across sites within the metacommunity (Leibold and Mikkelson 2002, Leibold et al. 2004, Crist and Veech 2006, Henriques-Silva et al. 2013). This variation, which sometimes produces spatial gradients in community structure (i.e.- turnover), is a form of beta diversity (Anderson et al. 2011).

Eelgrass-associated epifauna are the critical links in the food web that connect high eelgrass related algal primary production to the production of larger invertebrates, fish, and other vertebrates in the food chain, and the strength and stability of this link is likely mediated by epifaunal diversity (Duffy 2006, Hemminga and Duarte 2000). Biogeographic 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. 2003, 2015, Amundrud et al. 2015).

Epifaunal diversity can vary substantially within and among meadows (Hemminga and Duarte 2000, Boström et al. 2006, Yamada et al. 2007, Carr et al. 2011, Barnes and 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 and Ellwood 2012, Barnes 2013). In some regions total richness among meadows may remain relatively constant, while the proportion of rare and dominant species fluctuates suggesting that some meadows may be functioning at their carrying capacity and patterns of diversity are replacement-based, rather than additive (Boyé et. al, 2017). 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 (but see Boyé et. al, 2017). 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).

The purpose of this study is to explore potential metacommunity 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-dominated seascape to determine whether individual meadows, or a set of meadows, are representative spatial units for regional biodiversity (i.e. – patches of habitat that have similar species composition and diversity as found in the region). 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 the 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 (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 and Duarte 2000, Heck Jr. and 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 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. When a quadrat placed at a corner contained no seagrass, no sample was collected. For all meadows in all time periods, we removed a shoot from each corner quadrat to calculate leaf area, then dried and weighed eelgrass and its associated epiphytes, and standardized epiphyte dry mass to eelgrass dry mass. Additionally, we sampled epiphytes more thoroughly in the intertidal zone at three sites during low-tide periods (DC, WI, NB; < 0.5 m) in mid and late summer, by collecting 15 shoots per meadow from three tidal heights (Table S4). 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.

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 for a total of 2471 measurements across all nine sites (temperature = 1236, salinity = 1235). Means for each site and time period were calculated by integrating each variable measured at three stations per site across depths (at the surface, 2 m depth, and bottom; if the measurement location was shallower than 2 m, only surface and bottom measurements were taken). Measurements were pooled from approximately 15 days before and after biodiversity sampling to capture an approximate monthly mean. 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 (ESRI ArcGIS 10.3). A maximum distance of 50km was used for fetch lines, as this exceeds the distance between open ocean and the meadow furthest away from open ocean.

*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, seagrass was cut at the water-sediment interface with a diving knife and placed into a 250 µm drawstring mesh bag, collecting all eelgrass and epifauna. It is possible that some highly mobile organisms were able to escape during the collection process, particularly fishes, but the samples taken contained many relatively fast-moving invertebrates such as amphipods indicating that sample loss was minimal. 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 and 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 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 used four metrics to estimate alpha (quadrat-scale) diversity: rarefied species richness (R’), raw species richness values (R), Shannon diversity (H’), and Simpson evenness (S). We used the R package vegan (Oksanen et al. 2007, R Core Team 2016), and estimated R’ with the *rarefy* command in the vegan package and a minimum sample size of 5 individuals. 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, bRC,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), and post-hoc t-tests to identify meadows with distinct species richness compared to the overall mean. 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. We also used redundancy analysis (RDA), to model multivariate responses of the epifaunal community to environmental predictors including mean salinity, shoot density, epiphyte load, meadow area, and fetch. Community data was Hellinger-transformed and environmental variables were scaled and centered to Z-score values before analysis to avoid complications associated with using Euclidean distances and disparate units respectively (Legendre & Gallagher 2001). Permutations tests were used to determine the overall fit of the model as well as the significance of each axis, and forward selection of variables controlling for variance inflation factors (VIF) was used to identify explanatory variables.

To test our second hypothesis 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 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.

*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 and 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 and 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 S1). 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 S1). Distance from freshwater input captured correlations with other variables: temperature, salinity, shoot density (Table S2). Salinity increased and temperature declined with distance from freshwater inputs, though these changes were relatively small in magnitude (Table S3). Epiphyte load was highly variable between meadows, uncorrelated with position in the estuary and epiphyte abundances and the type of epiphytes present (periphyton vs. bladed algae) varied spatially and temporally (Table S4). Logistical constraints precluded a more thorough sampling of the epiphyte community, which likely increases the uncertainty around epiphyte loads in each meadow, and the standard errors given should be considered the minimum value. Intertidal epiphyte load standardized to seagrass dry weight (from more comprehensive surveys at DC, WI, and NB; Table S4) tended to be much higher than epiphytes sampled in the subtidal (Table S1). This was likely due to the larger size of subtidal plants compared to the shorter morphology of intertidal plants (pers. obs.). 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). Based on natural history knowledge and personal observation of many of these species, we excluded 17 from further analysis because either due to their life history stage or ecology, they do not spend much time on eelgrass blades (Light and Carlton, 2007; Table SX). 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). 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, S5). We found that alpha diversity did not vary systematically among meadows with any predictor we tested (Figure 2, Table 3). 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, n = 136, P < 0.001). We observed similar patterns in Shannon diversity (F = 4.6, df = 8, n = 136, P < 0.001) and Simpson diversity (F = 3.8, df = 8, n = 136, P < 0.001), though for these metrics BI is not different, and DC has lower within-quadrat evenness and ENS than other meadows (Figure 2B-D). The higher diversity of RP, lower evenness of DC, and differences in ENS were not explained by meadow-scale predictors such as distance from freshwater input, shoot density, meadow area or fetch (Table S5).

RDA RESULTS HERE.

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, Table S6). Comparison with the null model showed that both inter- and intra- meadow beta diversity (bRC) 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 S7). A permutation test of multivariate homogeneity of group dispersions showed that within-meadow beta diversity varied among meadows, but beta diversity did not vary predictably by distance from freshwater 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.

Meadow-scale patterns in species number and compositional variation were more similar among meadows than expected by chance, even though we observed different numbers and combinations of species in each meadow (Figure 4). 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 4).

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) 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.

Spatial patterns in epifauna suggest possible metacommunity structure, indicating 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 and 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. Absence of a signal of meadow area on diversity could indicate high connectivity (low isolation) among meadows. However, we only surveyed a small proportion of the larger meadows, so it is possible that higher diversity may be found in the larger meadows if sampled more extensively. Average quadrat scale diversity (7-20 species, Figure 3) and meadow-scale gamma diversity (Chao2 index estimate: 16 species per meadow ­+sd 3.39) was in all cases much lower than species diversity measured across the observed seascape (47 observed taxa). Together, these patterns suggest that some processes limit the presence of regional taxa in all meadows, and that within-meadow communities may be maintained by a balance between habitat filtering and biological connectivity that maintains population connections among nearby meadows. Such connectivity could maintain higher diversity in smaller meadows than expected based only on their size.

Contrary to some studies in other regions (Yamada et al. 2007, Barnes and Ellwood 2012), we did not observe a clear signal of continuous species turnover along the biotic and abiotic gradients associated with distance from freshwater 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 indicate that because the salinity regime of 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), these studies may have captured physiologically stressful, low-salinity conditions with a stronger influence on invertebrate assemblages that our study did not.

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 and 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. 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 seascape, and that the variation is not clearly predictable from an estuarine gradient and related biotic and abiotic predictors. Although *Z. marina* provides the appearance of a homogenous habitat, each *Z. marina* meadow appears to host a subset of epifaunal species observed in the larger seascape on multiple scales. 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.

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**Table SX: observed species identified as ‘not epifaunal’**

|  |  |  |  |
| --- | --- | --- | --- |
| **species** | **taxon** | **group** | **eelgrss epifauna** |
| **Amphipholis pugetana** | **echinoderm** | **echinoderm** | **no** |
| **Cockle** | **bivalve** | **gastropod** | **no** |
| **Dinophilus sp.** | **polychaete** | **polychaete** | **no** |
| **Lyonsia californica** | **bivalve** | **gastropod** | **no** |
| **Nephtys sp.** | **deposit feeder** | **polychaete** | **no** |
| **Nereis sp.** | **polychaete** | **polychaete** | **no** |
| **Phyllodoce sp.** | **polychaete** | **polychaete** | **no** |
| **Pisaster ochraceus** | **echinoderm** | **echinoderm** | **no** |
| **polycheate1** | **polychaete** | **polychaete** | **no** |
| **Solaster sp** | **echinoderm** | **echinoderm** | **no** |
| **Strongylocentrotus sp.** | **echinoderm** | **echinoderm** | **no** |
| **Telmessus cheiragonus** | **decapod** | **crustacean** | **no** |
| **Tetrastemmidae** | **Nemertean** | **nemertean** | **No** |
|  |  |  |  |

|  |
| --- |
| **Phyllodoce sp.** |
| **Amphipholis pugetana** |
| **Clinocardium nuttallii** |
| **Dinophilus sp.** |
| **Lyonsia californica** |
| **Nemertea spp.** |
| **Nephtys sp.** |
| **Nereis spp.** |
| **Pisaster ochraceus** |
| **Solaster sp.** |
| **Strongylocentrotus sp.** |
| **Telmessus cheiragonus** |
| **Anaitides sp.** |
| **Bittium sp.** |
| **Cypricercus sp.** |
| **Polychaeta spp.** |
| **Olivella sp.** |