**Within and among-meadow spatial structure in eelgrass-associated biodiversity**

R. Whippo1,2, N.S. Knight1,3, C. Prentice1,4 , M. Siegle1, M. 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, PO Box 37012, National Museum of Natural History, MRC 106, 20013-7012, Washington, USA

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

1B1, Montreal, Canada

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

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

Key words: seagrass, *Zostera marina*, foundation species, biodiversity, grazer, estuary, invertebrate, landscape ecology

ABSTRACT: Biodiversity varies in space and time, even in apparently stable, homogeneous environments such as biogenic habitat provided by foundation species. Quantifying spatial and temporal patterns in biodiversity is the first, critical step toward understanding the scale of processes that govern community structure and function, including resilience. We used a hierarchical biodiversity sampling scheme to answer the question: are the spatial distributions of taxonomic diversity and functional traits consistent among patches of a foundation species, eelgrass, distributed along a watershed gradient? To answer this question, we quantified subtidal faunal biodiversity at plot- and meadow-scales at three times throughout a summer season in Barkley Sound, B.C., Canada. We used a *an approach to estimate biodiversity that minimizes common confounding effects of spatial scale, and we applied a novel approach to estimating spatial turnover using a null model.* in diversity of all taxa as well as of the grazer taxa that play a key role in energy transfer in eelgrass-supported food webs. We found that eelgrass-associated faunal abundance, diversity and composition varied among meadows and in some cases, from month to month. The patterns of variation do not support a clear abiotic driver associated with position in the watershed, but instead suggest two different regions perhaps distinguished by ocean circulation patterns and bathymetry within the channel. We observed high species turnover within meadows, greater than expected based on a null model of random distribution among samples, suggesting aggregation of species within meadows. Overall, spatial and temporal variation in total and grazer biodiversity suggest metacommunity structure across the seascape. This finding directs future research and conservation efforts toward considering seagrass communities as spatially structured, connected communities in patchy seascapes.

**INTRODUCTION**

Foundation species promote biodiversity, providing habitat, food and other functions that support myriad species and ecological interactions (Altieri & Witman 2014, Bracken et al. 2007, Dayton 1972, Idjadi & Edmonds 2006, Nagelkerken et al. 2008). The ecological importance of foundation species for maintaining the integrity of biodiversity and ecosystem function is well known, and restoring and protecting foundation species are major conservation objectives (Byers et al. 2006, Gedan et al. 2014, van Katwijk et al. 2009). However, biodiversity can vary substantially among habitat patches of a single foundation species, even among adjacent patches, suggesting that full facilitation of biodiversity by foundation species occurs over landscapes of multiple habitat patches and through demographic connectivity among these patches (Robinson et al. 2011, Bishop et al. 2012, Irving & Bertness 2009, Boström et al. 2006). For example, biodiversity and associated ecosystem functions in salt marsh habitat on the foundation species *Spartina alterniflora* in eastern North America vary among patches depending on the presence and abundance of the mussel *Guekensia demissa,* which itself facilitates marsh biodiversity (Angelini et al. 2015). The patch-scale and regional community structure, ecosystem function, and ecosystem services associated with foundation species therefore are intimately linked with the processes that drive spatial and temporal patterns of biodiversity in these systems.

Foundation species can promote biodiversity within habitat patches (e.g., alpha diversity), but dispersal and connectivity among patches of foundation species can promote biodiversity at the landscape scale if patches host different species assemblages (beta diversity) that in total lead to a greater regional species pool (gamma diversity) than observed in any single foundation species habitat patch (Crist & Veech 2006). In many landscapes, foundation species form high quality habitat patches that are separated by less suitable habitat. Habitat patches defined by foundation species thus form spatial networks of populations residing in those patches. Variation among habitat patches can arise due to biotic and abiotic differences in patch properties that determine which species can persist there. If dispersal among patches is high, then variation among patches can reflect the interaction between local properties and colonization from other patches, or source/sink dynamics of some species (Leibold et al, 2003). Within each patch is therefore a community of species whose structure and function reflect local processes within habitat patches and ‘regional’ processes such as dispersal that connect communities among patches through source-sink dynamics. Even within patches, aggregation of individuals within species due to intra- or interspecific interactions can lead to distinct patterns of biodiversity that reflect the underlying biological processes. Understanding the scales of processes – within and among habitat patches – provides critical insight to the scale at which conservation or management efforts might best be targeted to preserve biodiversity and associated ecosystem function in marine systems characterized by patchy, foundation species habitats.

Seagrasses are foundation species that support diverse animal assemblages and high secondary productivity (Edgar & Shaw 1995, Heck & Wetstone 1977, Jackson et al. 2001). A major component of this biodiversity, and a major engine of ecosystem function, is the epifaunal grazer assemblage living on and in the seagrass blades (Barnes 2013, Duffy 2003, Duffy et al. 2015, Nelson 1979). Seagrass associated grazers include both mobile and sessile invertebrates (gastropods, arthropods, annelids, etc.); most of these animals feed on planktonic or seagrass-associated microalgae, though some feed on the seagrass itself (Douglass et al. 2011, Thom et al. 1995, Williams & Ruckelshaus 1993) or detritus (Douglass et al. 2011, Vizzini et al. 2002). They thus act as agents of energy transfer from primary producers into secondary productivity, and are then either consumed by fish and other invertebrates (Haegele 1997, Huang et al. 2015, Nelson 1979, Sutherland et al. 2013), or contribute directly to the detrital pool.

The diversity and composition of epifaunal grazers can influence ecosystem functions, including resilience to disturbance and control of algal epiphytes (Blake & Duffy 2012, Duffy et al. 2003, Duffy et al. 2015, Eklöf et al. 2012). Diversity of epifaunal grazers (algae-consumers) has been shown to stabilize trophic processes in seagrass meadows, with the additional effect of controlling epiphytic algal abundance and facilitating seagrass growth (Hughes et al. 2004). However, grazers are often analyzed as part of a larger pool of invertebrates that includes predators and detritivores, in large part because the natural history of many of these invertebrates is not well documented and consequently it is difficult to separate species into trophic groups. As a result, we have a limited understanding of how grazers vary in their distribution and abundance, and what proportion of invertebrates is actually contributing to algal consumption in seagrass meadows.

Seagrass habitat is considered an indicator of high biodiversity for assessments and prioritization schemes (Shokri & Gladstone 2013), yet seagrass-associated epifaunal diversity can vary substantially over relatively fine spatial and temporal scales (Boström et al. 2010, Carr et al. 2011, Robinson et al. 2011, Nelson 1979).  Seagrass meadows exist in discrete patches that range over several orders of magnitude in size (Mason et al. 2015), and are often separated by physical barriers such as deep water, fast currents, rocky substrates and human developments. Many factors can drive variation in diversity within meadows, including predation (Amundrud et al. 2015, Huang et al. 2015, Nelson 1979), productivity (De Troch et al. 2006), seagrass shoot density and phenotype (McCloskey & Unsworth 2015), and the regional species pool (Duffy et al. 2015, France & Duffy 2006). At the seascape scale, meadow size and abiotic factors including salinity (Yamada et al. 2007, Robinson et al. 2011), temperature (Barnes & Ellwood 2012), and connectivity (movement) among meadows (Boström et al. 2010, Tanner 2003) also influence biodiversity.

Here, we quantified eelgrass-associated epifaunal biodiversity to answer the following questions for a system of discontinuous eelgrass (*Zostera marina*; eelgrasses are seagrasses in the genus *Zostera*) meadows in British Columbia, Canada: 1a) How does epifaunal biodiversity vary within and among meadows? 1b) Do biotic attributes of the meadow (e.g., shoot density) or abiotic conditions such as temperature, salinity or position in the estuary explain variation in biodiversity? 2) Are spatial patterns of epifaunal biodiversity stable throughout the summer season? 3) Do we observe patterns in grazer diversity, and do some meadows have higher grazer diversity than others? To answer these questions, we surveyed nine meadows with a spatially structured sampling design to quantify eelgrass associated epifaunal diversity and to explore possible drivers of variation in biodiversity. To the best of our ability, we assigned trophic classifications to invertebrate taxa to estimate the characteristics of grazer assemblages in the seascape. We then considered the implications of differences among meadows for the scales of seagrass-associated biodiversity, ecosystem function and conservation in this region.

**MATERIALS AND METHODS**

**Study system**

We surveyed epifaunal biodiversity in meadows of the eelgrass *Zostera marina* in Trevor Channel, Barkley Sound, British Columbia, where *Zostera 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 with mean densities ranging from 14 - 53 shoots/m2 and a mean shoot length of 124 cm (Mason et al. 2015). Although in many parts of the world seagrass meadows have declined (Waycott et al. 2009), there has been no assessment of historical trends in seagrass abundance in British Columbia and the current trajectory of seagrass habitat is unknown. Shoreline development is minimal in Trevor Channel, and human impacts occur via sedimentation associated with clearcutting upstream, untreated sewage discharge from communities, and dredging or shoreline modification.

Trevor Channel is one of three channels in Barkley Sound that connect freshwater sources in Alberni Inlet with the open Pacific Ocean (Figure 1). Trevor Channel is approximately 200 m deep, with seagrass at coastal margins. Flow and circulation through the channel are restricted by a sill at the mouth of the Sound, and a rise to 30 m depth about 10 km west of Alberni Inlet. Temperature and salinity vary along a gradient due to the influence of warm freshwater input from Alberni Inlet in the northeast (~15 PPT), and cold, marine water from the Pacific Ocean in the southwest, about 30 km away. This situation is typical of west coast Vancouver Island, and the Pacific Northwest in general, in which upwelling of nutrient rich, cold water at the marine end of estuaries interacts with smaller volumes of freshwater inputs (McFarlane et al. 1997, Thomson 1981).  Evidence suggests that salinity gradients structure persistent spatial variation in eelgrass-associated fish assemblages in this region (Robinson et al. 2011, 2013). The region experiences high levels of precipitation; between 1981 and 2010 the average monthly rainfall ranged from 53 mm (in July) to 475 mm (in November; Canadian Climate Normals 2010).  During the same time period, average air temperatures ranged from 4.6 °C in December to 15.2 °C in August (Canadian Climate Normals 2010).  Data from a sampling station at Sarita Hole in Trevor Channel (near the middle of the estuary; data were recorded over nine years) show that sea surface temperatures at this one station ranged from approximately 5 °C in the winter to 20 °C in the summer, and that surface chlorophyll concentrations varied between lows of approximately 1 ug/L in the winter to highs of 10 ug/L in the summer (Pawlowicz 2013).

**Site characterization: abiotic and biotic properties**

We sampled eelgrass and associated fauna and abiotic features at nine meadows during the summer of 2012. To quantify site-scale abiotic conditions at each site, we monitored temperature and salinity using a hand held temperature/salinity probe (YSI Inc., OH USA). Measurements were taken at various times of day to characterize site conditions throughout the tidal cycle on biodiversity sampling days, and opportunistically on other days. Three stations were established at each site representing approximately the center and furthest edges of the largest contiguous meadow. At each station we recorded temperature and salinity at the surface, 2 m below the surface, and directly above the bottom. If the bottom was at 2 m, we only recorded surface and 2 m depth. If the bottom was shallower than 2 m, we recorded the bottom depth and took surface and bottom measurements. No measurements were taken below 5 m or taken in the intertidal zone.

To quantify biotic attributes of eelgrass meadows, we collected eelgrass from four 0.28 m2 quadrats outside each corner of a 4 x 4 m grid demarcated for community sampling (described below) in May and August at each of our main sites (i.e., the five sites that we sampled three times; Figure 1, Table 1). We estimated seagrass shoot density, epiphyte load, and leaf area index (LAI) concurrently with epifaunal sampling. To estimate shoot density, we counted all shoots within each quadrat. 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 seagrass 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). This resulted in a probable overestimation of total leaf area, however, this consistent measuring procedure allows for comparison of relative leaf area across sites and sampling times.

To estimate epiphyte load at the meadow-scale, we sampled epiphytes more thoroughly at three meadows (DC, WI and NB) once in mid and once in late summer during low tide events (< 0.5 m). We laid three 30 m transects parallel to shore (above, at, and below the low tide line) and from each transect we randomly picked five 1 m intervals from which we collected one eelgrass shoot, for a total of fifteen shoots per meadow at each time period. In the lab, we scraped all epiphytes from each shoot and separated bladed and diatom species. We dried and weighed epiphytes and each associated eelgrass shoot to estimate epiphyte weight per gram eelgrass.

We opportunistically sampled the fish community from six sites during the midsummer sampling period at the morning low tide (< 0.6 m) via beach seining (seine net dimensions: 4.5 m by 1.2 m; mesh size: 0.32 cm).

**Biodiversity sampling**

To estimate epifaunal biodiversity and characterize variation within meadows, we used a spatially structured set of 16 standard plots (0.28 m2) in each meadow (after Sanders 2007).  Plots were placed one meter apart and arranged in a 4 x 4 square grid. We placed sampling grids within contiguous meadows at least 2 m from any meadow edge to minimize the risk of capturing edge effects in diversity and composition of epifauna.  In each plot, we cut away seagrass within each sampled quadrat at the sediment-water interface and placed it into a 250 µm mesh bag, collecting all seagrass and epifauna. All samples were collected using SCUBA. We transported the seagrass back to the lab in seawater and removed all of the invertebrates; we then preserved the invertebrates in 70% EtOH within 24 hours of collection.  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. This sampling design allowed for comparison of diversity among plots and meadows while standardizing for total area sampled and the spatial arrangement of samples.

To estimate variation in diversity among meadows, we sampled nine subtidal seagrass meadows spanning the gradient of salinity beginning near the open coast and moving inland towards Alberni Inlet (Figure 1; Table 1).  We chose to sample these meadows because they are evenly distributed along Trevor Channel (Figure 1), they are large meadows (i.e., not fringing), and they capture 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, E), June/July (midsummer, M) and August (late summer, L) of 2012 (Table 1).

In the lab, we sorted invertebrate collections by size into the following fractions: 1-2 mm, 2-4 mm, 4-8 mm, and > 8 mm. It is likely that some organisms in the largest size class could swim and escape our field collection methods. We identified every invertebrate in each sample to the lowest taxonomic resolution possible using light microscopy.  The morphological appearance of some invertebrate groups can vary with life stage and sex; often organisms are damaged during preservation and handling.  This can be problematic while making identifications, as there are many epifaunal invertebrate species in this region about which very little is known. Though many of our identifications are to species level (gastropods, most amphipods and isopods), for many other taxa we have only identified them to family or even order. Each of these groups possibly includes numerous species that we have lumped; 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.  Vertebrates (in this case, fishes) were collected and analyzed separately.

We classified invertebrate species to broad trophic groups (grazer, predator, filter feeder, detritivore) based on published dietary data and personal experience. The grazer functional group includes organisms that consume micro- or macro-algae, including biofilms, growing on seagrass. Very few invertebrates directly consume seagrass in this region, and these animals also consume algae, and are therefore included as grazers.

**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 (i.e., no subsampling; Gotelli and Colwell 2010). We used this matrix for biodiversity analyses, allowing multiple metrics to be calculated to capture the roles of 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. Specifically, we estimated Shannon diversity, Simpson evenness, rarefied species richness, and the effective number of species (ENS), which is derived from the probability of an interspecific encounter (PIE), to characterize diversity at the plot scale (Dauby & 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. 2013) for biodiversity analyses.

To examine trends in grazers only, we assigned each taxon to a trophic functional group. Taxa were classified as grazers, (i.e., consumers of epiphytic algae, biofilm or seagrass) based on our own data, observations and published trait information (MacDonald et al. 2010). If not grazers, taxa were classified as predators, detritivores or filter feeders (Appendix 1).

To determine whether biodiversity varied more among meadows than within, and to compare within- and among-meadow variation, we estimated variation in species composition and turnover (beta diversity) with a Bray-Curtis dissimilarity matrix by measuring multivariate homogeneity of group dispersions (Anderson et al. 2006, 2010). Sites were grouped within sampling periods, and differences among meadows at each sampling period were detected with a permutational test for homogeneity of multivariate dispersions that generated pairwise comparisons based on 999 permutations. This method excludes joint absences and focuses on relative abundance among data sets, and is well suited to zero-rich community data.

We tested whether observed patterns of species turnover were likely a result of random distributions in space, or due to clumping or aggregation that might reflect biological processes such as species interactions or priority effects. To generate null models of beta diversity for each meadow, we created a statistical function in the program R (R Core Team 2013) that permutes observed community composition using the permat function from the vegan package (Oksanen 2013).  Beta diversity was calculated within each meadow with a Bray-Curtis dissimilarity matrix, and resampled 999 times to generate null expectations based on observed compositions. The resulting mean value of the median was used to represent the expected null value for multivariate dispersion, correcting for underestimation bias (Stier et al. 2013). We also used the Chao2 index to both estimate species richness at the meadow scale and to explore turnover within meadows. The Chao2 index emphasizes information gained from species represented in the data by only one individual, and thus is higher if samples within a meadow have several unique species in them. The Chao2 index is appropriate for estimating diversity with fewer than 50 samples (Chao & Bunge 2002, Colwell & Coddington 1994).

**Statistical analyses**

Our goals for analysis were to determine whether epifaunal biodiversity varied within and among meadows, and/or over time, and whether this variation could be attributed to biotic or abiotic factors and/or position within the watershed. Given the low number of meadows sampled (n = 9), robust tests of multiple possible predictors were impossible due to low statistical power. We therefore chose to explore the aggregated effects of biotic and abiotic factors by using position within the watershed as a fixed effect, which reflected the variation in temperature, salinity and seagrass biotic attributes (density, LAI) along this gradient. We assigned each meadow a ‘position’, estimated in kilometers from Alberni Inlet (the freshwater source). We tested the relationship between biotic and abiotic predictors (temperature, salinity, shoot density, leaf area index, and fish diversity) and position along the estuarine gradient using linear regression. Although this approach accounts for the non-independence of our predictor variables, it precludes us from attributing variation in invertebrate community characteristics to any particular environmental driver.

We used mixed effects models with meadow (site) as a random factor to test whether invertebrate assemblages varied predictably among meadows and sampling times using the nlme package in R (Pinheiro et al. 2014). Separate analyses were performed for a) all invertebrates in the assemblage, and b) the subset of grazers within the assemblage (as defined in Appendix 1). Abundance and ENS were log-transformed to meet the assumption of homoscedasticity. We tested our hypotheses about spatial and temporal variation by comparing models with and without terms for position, time (1, 2.5 or 4 to indicate May, June/July and August) and the interaction of position and time. 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 2002). 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 2002).  We did not observe any obvious 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).

We used simple linear regressions to test for the effects of position and time on estimated gamma diversity (Chao2 index) and beta diversity (distance to centroid) at the meadow scale.  Time, position, and their interaction were included as predictors in the model.  We observed no obvious deviations from the assumptions of linearity or homoscedasticity in plots of the residuals.

**RESULTS**

**Variation in meadow properties**

Abiotic and biotic attributes varied among meadows in Trevor Channel between Alberni Inlet and the open ocean. Temperature, salinity, shoot density and LAI were all correlated with each other and with position in the estuary (Table 1, Appendices 1, 2).  We thus used position in the watershed as the predictor of spatial variation for subsequent analyses. Eelgrass meadows toward the ocean end of the watershed were saltier (0.44 ppt/km) than meadows closer to the Alberni Inlet (Table 1, Appendices 1, 2). Seaward sites were cooler than fresher sites, although this effect was non-significant (0.07 °C/km, Appendices 1,2). Temperature increased at all sites from approximately 12 °C in May to at least 13 °C in July and 15 °C in August, and sites nearer Alberni inlet warmed to 22 °C by August (Tables 1, 2; Appendix 2; Figure S1). Biotic properties also varied along the watershed gradient. Moving from Alberni Inlet to the ocean, meadows increasing shoot densities (0.27 shoots/km) and associated LAI (240.73 cm2/km) (Tables 1, 2; Appendix 2). Shoot density and LAI increased between May and August at the fresher sites but remained relatively high all summer at the seaward meadows (Appendix 2; Tables 1, 2). Epiphyte load was highly variable between meadows, and did not change predictably with position in the estuary (Appendix 2; Tables 1, 2).

We systematically quantified functional types of epiphytes at three sites, and found that epiphyte abundances and the type of epiphytes present (periphyton vs. bladed algae) varied both spatially and temporally (Appendix 3). For example, the bladed brown epiphyte *Punctaria* *sp.* was much more abundant at two of the marine sites (Dodger Channel and Wizard Islet), and absent from the fresher Numukamis Bay. Furthermore, the timing of high abundances of the bladed epiphytes was not consistent between the two marine sites. Periphyton was present to some extent in all three meadows sampled (and observed across all sites); periphyton load varied among meadows. These results are consistent with the variation in epiphyte load that we observed between meadows (Appendix 2; Tables 1, 2).

Meadows also differed in the observed fish taxa, though position within the watershed was not a strong predictor of fish diversity (Appendix 2, Table 2). We identified 26 species of fish in 12 families living in six eelgrass meadows along Trevor Channel (Appendix 1).  Observed meadow-scale fish diversity ranged from 2 to 17 species (without correcting for sampling effort); mean observed richness was 6.75 species per meadow (+ 1.7 species).  However, the effective number of fish species (ENS) ranged only from 1.7 to 5.2 species, and mean ENS was 2.6 species per meadow (+ 0.5 species).

**Epifauna abundance and biodiversity**

In this study, we observed at least 47 epifaunal taxa in 42 families from a total pool of approximately 81,500 epifaunal invertebrates across the nine eelgrass meadows, five of which were sampled three times (N =304; Appendix 1). Of these, 24 taxa are herbivorous or omnivorous consumers of epiphytic algae, and therefore belong to the functional group “grazers” (Appendix 1). These include isopods, eelgrass-dwelling benthic copepods, amphipods, and several species of gastropod (Appendix 1). 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.

We observed between 0 and 1200 individual epifauna per 0.28 m2 plot of eelgrass. Mean total abundance of all taxa at the plot scale did not vary among meadows in May, but 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.

Grazer taxa comprised approximately 72% of all individuals; and 6.0 (± 0.15) grazer taxa were detected on average in each meadow; after accounting for sampling intensity with the Chao2 index we estimate that on average 7.0 (± 0.22) grazer taxa were present in each meadow. Spatial trends in grazer abundance differ somewhat from patterns in total invertebrates. In July, grazer densities were greatest toward Alberni inlet, while total invertebrates were most abundant nearest the ocean.

Univariate metrics suggested little variation in biodiversity along Trevor Channel despite variation in abundance and the biotic and abiotic meadow properties. Rarefied richness, which largely discounts the effects of species abundance on estimates of alpha diversity, did not vary spatially, though it did increase through time (Tables 2, 3; Figure 2A). Simpson diversity suggests periods of low diversity, likely reflecting dominance of one taxon, in two meadows near the ocean (Tables 2, 3; Figure 2A). Effective number of species (ENS) and Shannon diversity did not change predictably with time or position in the estuary (Appendix 4). Spatial results did not differ if we excluded the four additional sites sampled in July. All meadows show classic rank-abundance distributions (RADs), reflecting numerical dominance of several taxa (Appendix 7).

Grazer abundance and diversity varied among sites and sampling times, but no clear linear trends emerged (Figure 2G). Rarefied richness estimates suggested slightly higher grazer richness toward Alberni Inlet; however, neither time nor position in the estuary was a significant predictor of any grazer assemblage characteristics (Tables 5, 6).

Overall, we estimated that meadows in Trevor Channel supported between an estimated minimum of 13 and 53 epifaunal taxa, with an average of 28.1 (+ 6.4) epifaunal taxa per meadow.  Despite variation in meadow-scale diversity estimates (Chao2 index), differences were not explained by position in the watershed or by temporal progression through the summer season (Table 7). The best model of those we tested (Table 7) included an intercept term (estimate: 19.9, 95% CI: [6.4, 33.4]) and a time predictor (estimate: 3.3, 95% CI: [-1.7, 8.2]), but the coefficient for this term could not be distinguished from 0, indicating no convincing temporal trajectory in meadow-scale Chao2 richness.

**Beta diversity and aggregation: variation within and among meadows**

We detected variation in species composition, and clear evidence of spatial turnover in diversity within and among meadows. Within meadows, there is evidence of spatial aggregation of taxa consistent with clumping of species in space more than expected based on a random spatial distribution of species within the meadow (Figure 3). When the composition of each of the 16 plots is compared with the median composition, the average distance to the median serves as a metric of meadow-scale beta diversity. Average distance to median ranged from 0.20 to 0.49 across all sites and times (Appendix 5). In all cases, observed beta diversity exceeded the null expectation from <1% to >19%, with an average difference of 9.7% even with corrections for underestimation bias (Figure 3, Appendix 5).

We observed slightly higher beta-diversity among plots, suggesting greater spatial aggregation within meadows, at in meadows nearer Alberni Inlet (Figure 3). Further, comparing within-meadow estimates of beta diversity across time suggested that beta diversity declined slightly toward the end of the summer (May - 0.33, June/July - 0.34, August - 0.29). However, these trends were not statistically significant. Model selection showed that the best model predicting variation in beta diversity within meadows along the gradient had an intercept term (0.08, CI: [0.00, 0.15]) and a term for time (0.01, CI: [-0.02, 0.04]); neither term was significant (Table 7). Although beta diversity did not vary predictably along the watershed gradient, a permutation test of multivariate homogeneity of group dispersions showed that meadows did have significantly different values of beta diversity (Appendix 6).

Extending the comparisons to across meadows, we found that samples collected within meadows were typically more similar to samples from the same meadow than to samples from other meadows, suggesting meadow-scale aggregation and similarity in faunal assemblages (Figure 4). This species turnover among meadows was driven by both shifts in relative abundance, and by species turnover (Figure 4). Although many species were rare but present at most sites and times, no species was common everywhere at all times. 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. Conversely, four species were only detected at one time and place (*Alia carinata* at BI, *Strongylocentrotus* sp.at WI, *Solaster* sp.at CB, and *Dinophilus* sp. at DC; Appendix 1), and eight taxa were observed only twice. Whether a species was present at a site was fairly consistent through time. For example, *Photis brevipes*, *Pontogeneia* sp., *Platynereis bicanaliculata* and *Lacuna* sp. were not observed at Crow Cove at any of the three sampling times (48 samples in total).

**Community composition**

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, the 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.

We observed two community types in Trevor Channel, and these ‘types’ are characterized by species composition and the identities of dominant species. NMDS visualization reveals a community dominated by epiphyte grazers (*Caprella spp*., amphipods *A. columbiae, P*. *brevipes*, the isopod *Pentidotea resecata,* the sea hare *P. taylori*) in meadows toward the ocean end of Trevor Channel, and a community dominated by filter feeders (primarily mussels), *Pycnogonum* sp. and nematodes at meadows toward the Inlet (Figure 4). These differences are conserved through time, except at Wizard Islet (which resembled marine sites until a major recruitment of mussels in July; Figure 4).

The identity of dominant taxa varied through time. 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* let this species to outnumber *Caprella* spp*.* at Robbers Bank and Dodger Channel (at the marine end of the estuary).  However, these 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 Crickett Bay later during the same period.

The grazer species composition varied among meadows. *Pentidotea resecata*, amphipods *A. columbiae* and *Caprella* spp.*,* were observed in every meadow (Appendix 1), but tended to be less abundant in the meadows nearer Alberni Inlet. The eelgrass specialist *P. taylori* was not observed in every meadow (e.g., never at CC, and only 2 individuals observed at NB), but numerically dominated the fauna in some meadows (DC, RP and EI).

**DISCUSSION**

We found that invertebrate biodiversity and abundance observed associated with foundation species can vary substantially between habitat patches and over the course of a season. A watershed gradient of salinity and eelgrass shoot density did not explain variation in alpha (meadow-scale) diversity or species turnover among meadows. However, differences in species composition suggest that meadows nearer the ocean in Trevor Channel host grazer-dominated assemblages, while meadows nearer Alberni Inlet are dominated by filter feeders and other non-grazer taxa. This pattern suggests oceanographic circulation and dispersal among meadows might play a role in influencing species composition, even in the absence of clear trends in total diversity and a continuous effect of salinity, temperature or shoot density on total diversity.

Estimated species richness at the meadow scale varied by a factor of four from 13 to 53 species, and plot-level invertebrate abundance ranged over three orders of magnitude. However, we emphasize again that because we did not identify every individual to species, our estimates of diversity are minimums and the true diversity in these meadows is likely higher.

**Spatial variation in biodiversity**

Contrary to our expectations, we did not observe a clear signal of continuous variation in biodiversity along the biotic and abiotic gradients associated with the watershed. 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.

Despite the absence of a clear signal of an abiotic gradient or meadow area effect, we were able to distinguish between the composition of invertebrate assemblages at the marine and fresh ends of the estuary (Figure 4). Invertebrate assemblages in meadows including and to the southwest of Robber’s Passage (at the marine end of the estuary) tended to clump together in the NMDS similarity plot (Figure 4), and were dominated by grazers (amphipods, caprellids, the sea hare *Phyllaplysia taylori*). At the northeast (fresh) end of the estuary, invertebrate assemblages were dominated by juvenile mussels and nematodes. The one exception to this pattern was the invertebrate assemblage at WI, which showed a composition intermediate to those of the more marine and freshwater meadows despite its position at the marine end of the estuary (Figure 4). The emergence of two invertebrate assemblages associated with spatial areas in Trevor Channel could be explained by differences in connectivity, or meadow-to-meadow colonization and movement, within the two regions of Trevor Channel. The position of the break in species composition corresponds to a large sill in the bottom of Trevor Channel, at which the bottom depth rises to only 30 m from approximately 200 m. This sill could restrict mixing between the seaward and upstream ends of the Channel. Restricted exchange of water could be associated with distinct abiotic properties that influence species’ ability to persist or dominate in these environments. An alternate explanation is that population connectivity could be reduced between these two regions, such that meadows within one of the regions are more demographically connected and therefore more similar in composition than meadows in the other region.

Like alpha diversity metrics, beta diversity did not vary predictably along the watershed gradient. However, our results show that non-directional beta diversity 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.

To date, there are only a few other beta diversity estimates for seagrass-associated epifauna with which to compare our findings. These studies have typically focused on directional beta diversity, i.e., beta diversity along some gradient.  Previous research has shown 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; Barnes and Ellwood 2012, De Troch et al. 2001, De Troch et al. 2003, 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).

Eelgrass meadows are known for their ability to support a high diversity of fish species (Robinson et al. 2011, Robinson & Yakimishyn 2008). In British Columbia, over 80 fish species occur in eelgrass meadows (Robinson & Yakimishyn 2008). However, not all eelgrass meadows support the same fish assemblages (Robinson et al. 2011), and salinity is one abiotic driver of differences in fish assemblage composition. Though we did observe differences in species assemblages of fish across the meadows, there was no clear correlation between fish composition or diversity and invertebrate abundance or diversity. One limitation to this test in our study is the limited sampling effort for fish diversity and thus low sample sizes. Based on other reports from the region, there likely were many more fish species in these meadows that we missed, and we consequently limit our inferences on fish as a driver of invertebrate composition at this time.

**Temporal trends**

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. Despite the observed variation, there was no clear overall directional trend through time (Table 3). Dominant species did shift through time, within meadows. 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).

The temporal patterns we observed are different from patterns reported epifauna in eelgrass meadows in the east coast of North America. In those systems, grazer and invertebrate abundance and diversity peaks in winter, and from May declines substantially until grazers are virtually absent in late summer (Nelson 1979). This trend has been attributed to seasonal fish predation that intensifies in June each year. 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. These patterns are not clearly consistent with a major seasonal change in predation pressure in this system, despite fish assemblages that include seasonal juveniles (Robinson et al 2011).

**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, it is more important than ever that we develop a baseline understanding of how biodiversity in seagrass meadows is maintained across ecologically relevant scales. This study represents one of the first comprehensive assessments of eelgrass-associated biodiversity in the Pacific Northwest, and is perhaps the most comprehensive assessment and analysis of eelgrass-associated biodiversity on Vancouver Island.

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, *Z. marina* meadows host distinct communities, and each appears to host a subset of epifaunal species observed in the larger region. These patterns are indicative of a metacommunity system, and suggest that the 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.

**Acknowledgements**

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, J. Cristiani, F. Ratcliffe, K. Anderson, D. de Jonge, and S. Anthony for their assistance in the field and laboratory. We thank Alice Liou and the Bamfield Marine Sciences Center for their administrative support. Financial support for this project was provided by Bamfield Marine Sciences Center 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.

LITERATURE CITED

Altieri AH, Witman JD (2014) Modular mobile foundation species as reservoirs of

biodiversity.  Ecosphere 5:124

Amundrud SL, Srivastava DS, O’Connor MI (2015) Indirect effects of predators control herbivore

richness and abundance in a benthic eelgrass (*Zostera marina*) mesograzer community.  J Anim Ecol 84:1092-1102

Anderson MJ, Ellingsen KE, McArdle BH (2006) Distance-based test for homogeneity of

multivariate dispersions. Biometrics 62:245-253

Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL, Sanders NJ, Cornell HV,

Comita LS, Davies KF, Harrison SP, Kraft NJB, Stegen JC, Swenson NG (2010) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol Lett 14:19-28

Angelini C, van der Heide T, Griffin JN, Morton JP, Derksen-Hooijberg M, Lamers LPM, Smolders AJP, and Silliman BR (2015) Foundation species’ overlap enhances biodiversity and multifunctionality from the patch to landscape scale in southeastern United States salt marshes. Proc Roy Soc B 282: 20150421

Backman TWH (1991) Genotypic and phenotypic variability of *Zostera marina* on the west coast of

North America.  Can J Bot 69:1361-1371

Barnes RSK (2013) Distribution patterns of macrobenthic biodiversity in the intertidal seagrass beds

of an estuarine system, and their conservation significance.  Biodivers Conserv22:357-372

Barnes RSK, Barnes MKS (2011) Hierarchical scales of spatial variation in the smaller surface and

near-surface macrobenthos of a subtropical intertidal seagrass system in Moreton Bay,

Queensland.  Hydrobiologia673:169-178  
Barnes RSK, Ellwood MDF (2012) Spatial variation in the macrobenthic assemblages of intertidal

seagrass along the long axis of an estuary.  Estuar Coast Shelf S112:173-182

Best RJ, Stachowicz JJ (2014) Phenotypic and phylogenetic evidence for the role of food and

habitat in the assembly of communities of marine amphipods. Ecology 95: 775-786

Bishop MJ, Byers JE, Marcek BJ, Gribben PE (2012) Density-dependent facilitation cascades

determine epifaunal community structure in temperate Australian mangroves. Ecology 93:1388-1401

Blake RE, Duffy JE (2012) Changes in biodiversity and environmental stressors influence

community structure of an experimental eelgrass (*Zostera marina*) system. Mar Ecol Prog Ser 470:41-54

Borg JA, Rowden AA, Attrill MJ, Schembri PJ, Jones MB (2010) Spatial variation in the composition

of motile macroinvertebrate assemblages associated with two bed types of the seagrass *Posidonia oceanica.*  Mar Ecol Prog Ser 406:91-104

Boström C, Tomroos A, Bonsdorff E (2010) Invertebrate dispersal and habitat heterogeneity:

Expression of biological traits in a seagrass landscape.  J Exp Mar Biol Ecol 390:106-117

Bracken MES, Bracken BE, Rogers-Bennett L (2007) Species diversity and foundation

species: Potential indicators of fisheries yields and marine ecosystem functioning. California Cooperative Oceanic Fisheries Investigations Reports 48:82-91

Burnham KP, Anderson DR (2002) Model selection and multimodel inference. Springer-Verlag New York, NY

Byers JE, Cuddington K, Jones CG, Talley TS, Hastings A, Lambrinos JG, Wilson WG (2006) Using

ecosystem engineers to restore ecological systems. Trends Ecol Evol 21:493-500

Carr LA, Boyer KE, Brooks AJ (2011) Spatial patterns of epifaunal communities in San Francisco

Bay eelgrass (*Zostera marina*) beds. Mar Ecol-Evol Persp 32:88-103

Chao A, Bunge J (2002) Estimating the number of species in a stochastic abundance model.

Biometrics 58:531-539

Chase JM, Myers JA (2011) Disentangling the importance of ecological niches from stochastic

processes across scales.  Philos T Roy Soc B 366:2351-2363

Colwell RK, Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation.  Philos

T Roy Soc B345:101-118

Crist TO, Veech JA (2006) Additive partitioning of rarefaction curves and species-area relationships:

unifying α-, β-, and γ-diversity with sample size and habitat area. Ecol Lett 9:923-932

Dauby G, Hardy OJ (2012) Sampled-based estimation of diversity *sensu stricto* by transforming

Hurlbert diversities into effective number of species.  Ecography35:661-672

Dayton PK (1972) Toward an understanding of community resilience and the potential effects of

enrichments to the benthos at McMurdo Sound, Antarctica. Proceedings of the Colloquium on Conservation Problems 81–96. Allen Press, Lawrence, Kansas

De Troch M, Van Gansbeke D, Vincx M (2006) Resource availability and meiofauna in sediment of

tropical seagrass beds: Local versus global trends. Mar Environ Res 61:59-73

De Troch M, Fiers F, Vincx M (2003) Niche segregation and habitat specialisation of harpacticoid

copepods in a tropical seagrass bed.  Mar Biol 142:345-355

De Troch 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. Mar Ecol Prog Ser 215: 225-236

Douglass JG, France KE, Richardson JP, Duffy JE (2010) Seasonal and interannual change in a

Chesapeake Bay eelgrass community: Insights into biotic and abiotic control of community structure. Limnol Oceanogr 55:1499-1520

Douglass JG, Duffy JE, Canuel EA (2011) Food web structure in a Chesapeake Bay eelgrass bed as

determined through gut contents and C-13 and N-15 isotope analysis. Estuar Coast 34:701-711

Duarte CM (2002) The future of seagrass meadows. Environ Conserv 29:192-206

Duffy JE, Canuel EA, Richardson JP (2003) Grazer diversity and ecosystem functioning in seagrass

beds. Ecol Lett 6:637-645

Duffy JE, Reynolds PL, Boström C, Coyer JA, Cusson M, Donadi S, Douglass G, Eklöf JS, Engelen

AH, Eriksson BK, Fredriksen S, Gamfeldt L, Gustafsson C, Hoarau G, Hori M, Hovel K, Iken K, Lefcheck JS, Moksnes P, Nakaoka M, O’Connor MI, Olsen JL, Richardson JP, Ruesink JL, Sotka EE, Thormar J, Whalen MA, Stachowicz JJ (2015) Biodiversity mediates top–down control in eelgrass ecosystems: a global comparative-experimental approach.  Ecol Lett 18:696-705

Edgar GJ, Shaw C (1995) The production and trophic ecology of shallow-water fish assemblages

in southern Australia .3. General relationships between sediments, seagrasses, invertebrates and fishes.  J Exp Mar Biol Ecol 194:107-131

Eklöf JS, Alsterberg C, Havenhand JN, Sundbäck K, Wood HL, Gamfeldt L (2012)

Experimental climate change weakens the insurance effect of biodiversity. Ecol Lett, 15:864–872

France KE, Duffy JE (2006) Diversity and dispersal interactively affect predictability of ecosystem

function. Nature 441:1139-1143

Gedan KB, Kellogg L, Breitburg DL (2014) Accounting for multiple species in oyster reef

restoration benefits. Restor Ecol 22:517-524

Gotelli NJ, Colwell RK (2010) Estimating species richness. In: Magurran AE, McGill BJ (eds)

Biological Diversity: Frontiers in Measurement and Assessment. Oxford University Press, Oxford 39-54

Government of Canada (2010) Canadian Climate Normals: Bamfield East.

<http://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?stnID=231&lang=e&amp;StationName=Bamfield&amp;SearchType=Contains&amp;stnNameSubmit=go&dCode=5&dispBack=1>  (accessed 7/20/2015)

Haegele CW (1997) The occurrence, abundance, and food of juvenile herring and salmon in the

Strait of Georgia, British Columbia, in 1990 to 1994. Retrieved from the Department of Fisheries and Oceans Canada website: http://www.dfo-mpo.gc.ca/library/224393.pdf

Hansen JCR, Reidenbach MA (2013) Seasonal growth and senescence of a *Zostera marina* seagrass

meadow alters wave-dominated flow and sediment suspension within a coastal bay.  Estuar Coast 36:1099-1114

Heck KL, Wetstone GS (1977) Habitat complexity and invertebrate species richness and

abundance in tropical seagrass meadows. J Biogeogr 4:135-142

Huang AC, Essak M, O’Connor MI (2015) Top-down control by great blue herons *Ardea heroditas*

regulates seagrass-associated epifauna. OikosOnline (accessed 7/27/2015)

Hughes AR, Bando KJ, Rodriguez LF, Williams SL (2004) Relative effects of grazers and nutrients

on seagrasses: a meta-analysis approach. Mar Ecol Prog Ser 282:87-99

Idjadi JA, Edmunds PJ (2006) Scleractinian corals as facilitators for other invertebrates on a

Caribbean reef. Mar Ecol Prog Ser 319:117-127

Irving AD, Bertness MD (2009) Trait-dependent modification of facilitation on cobble beaches.

Ecology 90:3042-3050

Jackson EL, Rowden AA, Attrill MJ, Bossey SJ, Jones MB (2001) The importance of seagrass

beds as a habitat for fishery species.  Oceano Mar Biol 39:269-303

Jost L (2006) Entropy and diversity.  Oikos 113:363-375

Knight NS, Prentice C, Tseng M, O’Connor MI (2015) A comparison of epifaunal invertebrate

communities in native eelgrass *Zostera marina* and non-native *Zostera japonica* at Tsawwassen, BC.  Mar Biol Res 11:564-571

Kraft NJB, Comita LS, Chase JM, Sanders NJ, Sweson NG, Crist TO, Stegen JC, Vellend M,

Boyle B, Anderson MJ, Cornell HV, Davies KF, Freestone AL, Inouye BD, Harrison SP, Myers JA (2011) Disentangling the drivers of beta diversity along latitudinal and elevational gradients.  Science 333:1755-1758

Lannin R, Hovel K (2011) Variable prey density modifies the effects of seagrass habitat structure on

predator-prey interactions. Mar Ecol Prog Ser 442:59-70

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB,

Law R, Tilman D, Loreau M, Gonzalez A (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecol Lett 7:601-613

Macdonald TA, Burd BJ, Macdonald VI, 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) Community mapping network.

<http://cmnmaps.ca/EELGRASS/> (accessed 7/15/2015)

McCloskey RM, Unsworth RKF (2015) Decreasing seagrass density negatively influences associated

fauna.  PeerJ 3:e1053

McFarlane GA, Ware DM, Thomson RE, Mackas DL, Robinson CLK (1997) Physical, biological

and fisheries oceanography of a large ecosystem (west coast of Vancouver Island) and implications for management. Oceanol Acta 20:191-200

Nagelkerken I, Blaber SJM, Bouillon S, Green P, Haywood M, Kirton LG, Meynecke JO, Pawlik J,

Penrose HM, Sasekumar A, Somerfield PJ (2008) The habitat function of mangroves for terrestrial and marine fauna: A review.  Aquat Bot 89:155-185

Nelson WG (1979) An analysis of structural pattern in an eelgrass (*Zostera marina* L.) amphipod community. J Exp Mar Biol Ecol 39:231-264.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O’Hare RB, Simpson GL, Solymos P,

Stevens MHH, Wagner H (2013) vegan: Community Ecology Package. R package version

2.0-10

Pawlowicz R (2013) Barkley Sound Time Series.

<http://www.eos.ubc.ca/~rich/BSTS/bark_home.html> (accessed 7/20/2015)

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2014) nlme: Linear and Nonlinear Mixed

Effects Models. R package version 3.1-118

Powell KI, Chase JM, Knight TM (2013) Invasive plants have scale-dependent effects on diversity

by altering species-area relationships. Science 339:316-318

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for

Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org/>

Robinson CLK, Yakimishyn J (2008) Monitoring for the ecological integrity of eelgrass beds *(Zostera*

*marina*)in Canada’s coastal national parks of British Columbia. Western and Northern Service Centre Technical Report

Robinson CLK, Yakimishyn J, Dearden P (2011) Habitat heterogeneity in eelgrass fish assemblage

diversity and turnover. Aquat Conserv 21:625-635

Robinson CLK, Yakimishyn J (2013) The persistence and stability of fish assemblages with eelgrass

meadows (*Zostera marina*) on the Pacific coast of Canada. Can J Fish Aquat Sci 70:775-784

Sanders NJ, Lessard JP, Fitzpatrick MC, Dunn RR (2007) Temperature, but not productivity or

geometry, predicts elevational diversity gradients in ants across spatial grains. Global Ecol Biogeogr 16:640-649

Searle SR (1988) Parallel lines in residual plots.  Am Stat42:211-211

Shokri MR, Gladstone W (2013) Limitations of habitats as biodiversity surrogates for conservation

planning in estuaries.  Environ Monit Assess 184:3477-3492

Stier AC, Geange SW, Hanson KM,  Bolker BM (2013) Predator density and timing of arrival affect

reef fish community assembly. Ecology 94, 1057–1068

Sturaro N, Lepoint G, Perez-Perera A, Vermeulen S, Panzalis P, Navone A, Gobert S (2014)

Seagrass amphipod assemblages in a Mediterranean marine protected area: a multiscale approach.  Mar Ecol Prog Ser 506:175-192

Sutherland TF, Elner RW, O’Neill JD (2013) Roberts Bank: Ecological crucible of the Fraser

River estuary.  Prog Oceanogr 115:171-80

Tanner JE (2003) Patch shape and orientation influences on seagrass epifauna are mediated by

dispersal abilities. Oikos 100:517-524

Thom R, Miller B, Kennedy M (1995) Temporal patterns of grazers and vegetation in a temperate

seagrass system. Aquat Bot 50:201-205

Thomson RE (1981) Oceanography of the British Columbia Coast. Canadian Special Publications of Fisheries and Aquatic Sciences 56, 291pp.

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

van Katwijk MM, Bos AR, de Jonge VN, Hanssen LSAM, Hermus DCR, de Jong DJ (2009)

Guidelines for seagrass restoration: Importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. Mar Pollut Bull 58:179-188

Veech JA, Crist TO (2010) Toward a unified view of diversity partitioning. Ecology91:1988-1992

Vizzini S, Sara G, Michener RH, Mazzola A (2002). The role and contribution of the seagrass

*Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. Acta Oecol 23:277-285

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladne A,

Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens ecosystems. P Natl Acad Sci USA 106:12377-12361

Williams SL (2007) Introduced species in seagrass ecosystems: Status and concerns. J Exp Mar Biol

Ecol 350:89–110

Williams SL, Ruckelshaus MH (1993) Effects of nitrogen availability and herbivory on eelgrass

(*Zostera marina*) and epiphytes. Ecology 74:904-918

Wonham M, Carlton J (2005) Trends in marine biological invasions at local and regional scales: The

Northeast Pacific Ocean as a model system. Biol Invasions 7:369–92

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

**APPENDICES**

**Appendix 1:**  Identified taxa, their trophic classification, and the sites at which they were observed at least once. Trophic classifications for taxonomic groups not identified to the level of species are given as the most common feeding habits observed in that group. Whenever possible, references are provided below. Site abbreviations (from marine to fresh): DC = Dodger Channel, WI = Wizard Islet, BE = Bald Eagle Cove, EI = Ellis Island, RP = Robber’s Passage, NB = Numukamis Bay, CB = Crickett Bay, BI = Boyson Islands, CC = Crow Cove.

|  |  |  |  |
| --- | --- | --- | --- |
| **Taxonomic group** | **Highest resolution identification** | **Trophic group** | **Observed at** |
| **Polychaeta** |  |  |  |
| Nereidae | *Platynereis bicanaliculata* | Omnivore1-2 | DC, WI, BE, EI, RP, NB, CB, BI |
|  | *Nereis* sp. | Omnivore1-2 | DC, WI |
| Serpulidae | *Janua pagenstecheri* | Unknown | WI, RP, CB, BE |
| Nephtyidae | *Nephtys* sp. | Predator1 | DC, RP |
| Dinophilidae | *Dinophilus* sp. | Unknown | DC |
| Phyllodocidae | *Anaitides* sp. | Predator1 | DC, NB, CB |
| Syllidae | *Odontosyllis* sp. | Predator1 | DC, WI, NB, CB |
| **Nematoda** |  |  |  |
|  | Nematode sp. 1 | Unknown | DC, WI, EI, RP, NB, BI, CC |
|  | Nematode sp. 2 | Unknown | DC, RP |
| **Nemertea** |  |  |  |
| Tetrastemmatidae | *Tetrastemma* sp. | Predator1 | DC, NB, CB |
| **Amphipoda** |  |  |  |
| Aoridae | *Aoroides columbiae* | Grazer3 | DC, WI, BE, EI, RP, NB, CB, BI, CC |
| Hyalidae | *Photis brevipes* | Omnivore1 | DC, WI, BE, EI, RP, NB, CB, BI |
| Anisogammaridae | *Eogammarus confervicolus* | Predator1 | DC, WI, BE, EI, RP, NB, CB, BI, CC |
| Eusiroidae | *Pontogeneia* sp. | Predator1 | DC, WI, BE, EI, RP, NB, CB, BI |
| Corophiidae | *Monocorophium achersicum* | Omnivore1 | DC, WI, RP, NB, CB, BI, CC |
| Ampithoidae | *Amphithoe* sp. | Grazer1, 3 | DC, WI, EI, RP, NB, CB, BI |
| Isaeidae | Isaeidae sp. | Omnivore1 | DC, WI, BE, EI, RP, NB, CB, BI, CC |
| Caprellidae | *Caprella spp.* (*a. C. californica*  and b. *C. laeviuscula*) | a. Grazer1  b. Grazer1, 3 | DC, WI, BE, EI, RP, NB, CB, BI, CC |
| **Isopoda** |  |  |  |
| Idoteidae | *Pentidotea resecata* | Grazer1 | DC, WI, BE, EI, RP, NB, CB, BI, CC |
| Cirolanidae | *Cirolana harfordi* | Predator | DC, WI, BE, NB, CB |
| **Tanaidacea** |  |  |  |
| Leptocheliidae | *Leptochelia dubia* | Omnivore1 | DC, WI, BE, RP, NB, CB, BI, CC |
| **Leptostraca** |  |  |  |
| Nebaliidae | *Nebalia* sp. | Predator1 | DC, WI, NB |
| **Ostracoda** |  |  |  |
| Cyprididae | Cyprididae sp. | Omnivore1 | DC, WI, BE, EI, RP, NB, CB, BI |
| **Gastropoda** |  |  |  |
| Aplysiidae | *Phyllaplysia taylori* | Grazer\* | DC, WI, BE, EI, RP, NB, CB |
| Columbellidae | *Alia carinata* | Grazer\* | NB, BI |
| Littorinidae | *Lacuna* spp. | Grazer1, 3 | DC, WI, BE, EI, RP, NB, CB, BI |
| Haminoeidae | *Haminoea vesicular* | Grazer1 | DC |
| Cerithiidae | *Bittium* sp. | Omnivore1 | DC, WI, EI |
| Lottiidae | *Lottia pelta* | Grazer1 | DC, WI, RP, NB, CB, BI |
| Olivellidae | *Olivella* sp. | Omnivore1 | DC, CB |
| Turbinidae | *Margarites helicinus* | Grazer1 | NB, CB |
| **Bivalvia** |  |  |  |
| Mytilidae | *Mytilus trossulus* | Filter feeder1 | DC, WI, BE, EI, RP, NB, CB, BI, CC |
| Lyonsiidae | *Lyonsia californica* | Filter feeder1 | DC, WI, EI, RP, NB, CB, BI, CC |
| Cardiidae | *Clinocardium nuttalli* | Filter feeder1 | DC, CB |
| **Sessilia** |  |  |  |
| Balanidae | *Balanus* sp. | Filter feeder1 | DC, WI, EI, NB, CB |
| **Decapoda** |  |  |  |
| Epialtidae | *Pugettia richii* | Grazer1 | DC, WI, NB, CB |
| Paguridae | *Pagurus quaylei* | Omnivore1 | DC, WI, RP, NB, CB, BI |
| Atelecyclidae | *Telmessus cheiragonus* | Predator1 | RP, NB |
| Pandalidae | Pandalidae sp. | Detritivore\* | DC, NB, CB |
| **Asteroidea** |  |  |  |
| Amphiuridae | *Amphipholis pugetana* | Omnivore1 | DC, NB |
| Asteriidae | *Pisaster ochraceus* | Predator1 | CB |
| Solasteridae | *Solaster* sp. | Predator1 | DC, CB |
| **Echinoidea** |  |  |  |
| Strongylocentrotidae | *Strongylocentrotus* sp. | Grazer1 | WI |
| **Pycnogonida** |  |  |  |
| Pycnogonidae | *Pycnogonum* sp. | Predator1 | DC, WI, BE, EI, RP, NB, CB, BI, CC |
| **Arachnida** |  |  |  |
| Halacaridae | Halacaridae spp. | Predator1 | DC, WI, BE, EI, RP, NB, CB, BI, CC |
| **Copepoda** |  |  |  |
|  | Harpacticoida spp. | Omnivore1 | DC, WI, EI, RP, NB, CB |
| **Chordata** |  |  |  |
| Cottidae | [*Enophrys*](http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatget.asp?genid=1606) *bison* |  | RP, NB, CB |
|  | *Artedius fenestralis* |  | RP, NB, CB |
|  | *Artedius lateralis* |  | RP, EI, CB |
|  | *Oligocottus snyderi* |  | RP |
|  | *Leptocottus armatus* |  | RP |
|  | Cottidae sp. |  | RP |
| Hemitripteridae | *Blepsias cirrhosis* |  | WI |
| Pholidae | *Pholis laeta* |  | WI, EI, RP, NB, CB |
|  | *Pholis ornate* |  | NB |
|  | *Apodichthys fucorum* |  | EI |
|  | *Apodichthys flavidus* |  | NB |
| Embiotocidae | *Cymatogaster aggregate* |  | DC, WI, EI, RP, NB, CB |
|  | *Brachyistius frenatus* |  | EI, NB, CB |
|  | *Rhacochilus vacca* |  | EI, RP, CB |
|  | *Embiotoca lateralis* |  | RP |
| Hexagrammidae | *Hexagrammos decagrammus* |  | DC, WI, EI, RP |
| Paralichthyidae | *Citharichthys stigmaeus* |  | EI, RP |
| Sebastidae | *Sebastes caurinus* |  | RP, CB |
| Syngnathidae | *Syngnathus leptorhynchus* |  | RP |
| Pleuronectidae | *Parophrys vetulus* |  | RP |
|  | *Lepidopsetta bilineata* |  | WI |
|  | *Platichthys stellatus* |  | NB |
| Batrachoididae | *Porichthys notatus* |  | RP |
| Gasterosteidae | *Gasterosteus aculeatus* |  | RP |
| Salmonidae | *Oncorhynchus kisutch* |  | NB |
|  | *Oncorhynchus nerka* |  | WI |

\*Based on personal observations.

1. Macdonald TA, Burd BJ, Macdonald VI, 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

2. Woodin SA (1977) Algal “gardening” behaviour by nereid polychaetes: Effects on soft-

bottom community structure. Mar Biol 44:39-42

3. Norton TA, Benson MR (1983) Ecological interactions between the brown seaweed

*Sargassum muticum* and its associated fauna. Mar Biol 75:169-177

**Appendix 2:** Spatial and temporal variation in seagrass meadow temperature and salinity.Model comparisons for mixed effects models with meadow as a random effect (t = time, P = position).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Model** |  | **AICc** | ***w*** | **df** | **logLik** | **d** | **P** |
| **Temperature** | | | | | | | |
| **A3** | **T = t + P + t\*P** | **55.4** | **0.963** | **5** | **-20.410** | **0** | **--** |
| A2 | T = t + P | 62.0 | 0.037 | 4 | -25.554 | 6.53 | < 0.01 |
| A1 | T = t | 72.6 | 0.000 | 3 | -32.502 | 17.17 | < 10-4 |
| **Salinity** | | | | | | | |
| **B2** | **S = t + P** | **82.0** | **0.73** | **4** | **-35.591** | **0** | **--** |
| B3 | S = t + P +t\*P | 84.0 | 0.27 | 5 | -34.708 | 1.99 | 0.24 |
| B1 | S = t | 109.8 | 0.00 | 3 | -51.090 | 27.74 | < 10-6 |
| **Shoot density** | | | | | | | |
| **C2** | **Sh = t + P** | **51.8** | **0.836** | **4** | **-17.920** | **0** | **--** |
| C1 | Sh = t | 55.5 | 0.136 | 3 | -22.735 | 3.63 | 0.01 |
| C3 | Sh = t + P + t\*P | 58.6 | 0.028 | 5 | -16.810 | 6.78 | 0.27 |
| **Leaf area index (LAI)** | | | | | | | |
| **D2** | **LAI = t + P** | **185.4** | **0.906** | **4** | **-84.698** | **0** | **--** |
| D1 | LAI = t | 190.4 | 0.075 | 3 | -90.184 | 4.97 | 0.01 |
| D3 | LAI = t + P + t\*P | 193.1 | 0.019 | 5 | -84.059 | 7.72 | 0.40 |
| **Epiphyte load** | | | | | | | |
| E1 | Epi = t | -11.7 | 0.760 | 3 | 9.666 | 0.00 | -- |
| E2 | Epi = t + P | -9.1 | 0.207 | 4 | 9.996 | 2.60 | 0.46 |
| E3 | Epi = t + P +t\*P | -5.4 | 0.032 | 5 | 10.018 | 6.31 | 0.76 |

**Appendix 3:** Epiphyte loads at the three sites in the mid (M) and late (L) summer of 2012. Values are average epiphytes loads ± standard error (number of shoots). Site abbreviations: DC = Dodger Channel, WI = Wizard Islet, NB = Numukamis Bay. Epiphyte load is standardized to seagrass dry weight in grams.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Site** | **Bladed epiphyte load** | | **Periphyton load** | |
| **M** | **L** | **M** | **L** |
| DC | 0.45 ± 0.05 (14) | 0.15  (1) | 0.56 ± 0.06 (15) | 0.56 ± 0.05 (15) |
| WI | 0.04 ± 0.03 (7) | 0.43 ± 0.12 (13) | 061 ± 0.07 (15) | 0.57 ± 0.04 (15) |
| NB | − | 0.12  (1) | 0.87 ± 0.08 (15) | 1.05 ± 0.12 (15) |

**Appendix 4:** Model comparisons (Table A2.1) and coefficients (Table A2.3) for plot-scale diversity estimates for mixed effects with meadow as a random effect. Time = t, position = P. Results from models that only included the five main sites were nearly identical, and therefore results for all sites are shown here. Random effects not estimated because models are averaged.

**Table A4.1**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Model** |  | **AICc** | ***w*** | **df** | **logLik** | δAIC | **P** |
| Effective number of species (ENS) | | | | | | | |
| **D1** | **ln(ENS) = t** | **-102.8** | **0.639** | **6** | **57.566** | **0** | **--** |
| D2 | ln(ENS) = t + P | -101.0 | 0.264 | 7 | 57.746 | 1.77 | 0.55 |
| D3 | ln(ENS) = t + P + t\*P | -99.0 | 0.096 | 8 | 57.806 | 3.79 | 0.79 |
| Shannon Diversity (SH) | | | | | | | |
| **E2** | **SH = t + P** | **301.6** | **0.391** | **7** | **-143.538** | **0** | **--** |
| E1 | SH = t | 301.8 | 0.351 | 6 | -144.708 | 0.22 | 0.12 |
| E3 | SH = t + P + t\*P | 302.4 | 0.258 | 8 | -142.885 | 0.84 | 0.25 |

**Table A4.2**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Response** | **mod** | **Intercept** | **Time** | **Position** | **Time\*Pos.** |
| ln(ENS) | D1-3 | **0.39**  [0.21, 0.57] | 0.00  [-0.07, 0.06] | 0.00  [-0.01, 0.02] | 0.00  [-0.01, 0.01] |
| Shannon Index | E1-3 | **1.27**  [0.91, 1.63] | -0.02  [-0.14, 0.11] | 0.01  [-0.01, 0.03] | 0.01  [-0.01, 0.03] |

**Appendix 5**: Homogeneity of multivariate dispersions average distance to median for all sites and times. Values calculated from Bray-Curtis dissimilarity within each time period, larger values indicate greater variation within communities (beta diversity). See Figure 1 for site abbreviations.

|  |  |  |  |
| --- | --- | --- | --- |
| **Time Period** | **Site** | **Average distance**  **to median** | **Null distance**  **to median** |
| MAY | DC | 0.2686 | 0.1900 |
| WI | 0.4926 | 0.2958 |
| RP | 0.2483 | 0.2362 |
| NB | 0.3649 | 0.2313 |
| CB | 0.2570 | 0.2567 |
| JUNE/JULY | DC | 0.2797 | 0.2332 |
| WI | 0.4174 | 0.2191 |
| BE | 0.2318 | 0.1860 |
| EI | 0.2817 | 0.2448 |
| RP | 0.3439 | 0.2392 |
| NB | 0.3746 | 0.3092 |
| CB | 0.3282 | 0.1808 |
| BI | 0.3866 | 0.2180 |
| CC | 0.3834 | 0.2756 |
| AUGUST | DC | 0.2019 | 0.1292 |
| WI | 0.2772 | 0.1384 |
| RP | 0.2265 | 0.1305 |
| NB | 0.3271 | 0.2415 |
| CB | 0.4142 | 0.2303 |

**Appendix 6:** Observed p-value below diagonal, permuted p-value above diagonal. Test of homogeneity of multivariate dispersion: p-values below 0.05 suggest that two groups show different amounts of variation.

**MAY**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | DC | WI | RP | NB | CB |
| DC |  | **1.00E-03** | 6.20E-01 | **5.90E-02** | 0.7610 |
| WI | **4.22E-05** |  | **1.00E-03** | **1.60E-02** | **0.0010** |
| RP | 6.24E-01 | **1.98E-06** |  | **1.70E-02** | 0.8030 |
| NB | **6.94E-02** | **1.89E-02** | **1.70E-02** |  | **0.0290** |
| CB | 7.87E-01 | **7.43E-06** | 8.06E-01 | **3.23E-02** |  |

**JUNE/JULY**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | DC | WI | BE | EI | RP | NB | CB | BI | CC |
| DC |  | **0.0240** | 0.3930 | 0.9650 | 0.2930 | 0.1000 | 0.4190 | 0.0630 | 0.0550 |
| WI | **0.0205** |  | **0.0040** | **0.0190** | 0.2530 | 0.4390 | 0.1660 | 0.5540 | 0.5050 |
| BE | 0.3745 | **0.0015** |  | 0.2950 | 0.0670 | **0.0120** | 0.1170 | **0.0050** | **0.0040** |
| EI | 0.9668 | **0.0108** | 0.2887 |  | 0.3000 | 0.0630 | 0.4080 | **0.0310** | **0.0330** |
| RP | 0.3046 | 0.2449 | 0.0674 | 0.2773 |  | 0.5830 | 0.8220 | 0.4760 | 0.4780 |
| NB | 0.0918 | 0.4372 | **0.0091** | 0.0645 | 0.6133 |  | 0.4340 | 0.7930 | 0.8560 |
| CB | 0.4407 | 0.1641 | 0.1169 | 0.4202 | 0.8156 | 0.4497 |  | 0.3280 | 0.3580 |
| BI | 0.0508 | 0.5607 | **0.0036** | **0.0305** | 0.4696 | 0.8141 | 0.3287 |  | 0.9510 |
| CC | **0.0478** | 0.5011 | **0.0027** | **0.0265** | 0.4876 | 0.8558 | 0.3386 | 0.9458 |  |

**AUGUST**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | DC | WI | RP | NB | CB |
| DC |  | 1.70E-01 | 5.48E-01 | **1.50E-02** | **0.0010** |
| WI | 1.52E-01 |  | 3.36E-01 | 3.96E-01 | **0.0150** |
| RP | 5.20E-01 | 3.15E-01 |  | **3.40E-02** | **0.0010** |
| NB | **9.94E-03** | 3.77E-01 | **2.82E-02** |  | 0.0830 |
| CB | **1.68E-05** | **1.50E-02** | **4.34E-05** | 7.42E-02 |  |

**Appendix 7:** Log-transformed rank abundance curves of invertebrate assemblages at every meadow for every sampling period. The first two letters of each heading are site, the third letter is sampling period (A = May, C = June/July, E = August).