# MACROINFAUNAL COMMUNITIES IN SEAGRASS BEDS IN ATLANTIC CANADA: REGIONAL VARIATION AND THE EFFECTS OF EUTROPHICATION AND FINFISH AQUACULTURE

by

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## Abstract

Seagrass beds are productive coastal ecosystems that harbour many different species of flora and fauna. The benthic macrofauna that live within the sediments perform important roles that contribute to the ecological functioning and productivity of seagrass habitats. This thesis examined variation in macroinfaunal communities associated with seagrass beds in Atlantic Canada, spatially, and locally along a gradient of human impact. Firstly, I examined the regional variation of seagrass beds and macroinfaunal communities across three provinces in eastern Canada and linked the observed infaunal variation with seagrass bed structure and environmental conditions. I found regional differences in infauna community structure, which were significantly influenced by benthic productivity (the microphytobenthos). While the microphytobenthos consistently came out as the best predictor of the infauna community, nutrient enrichment and eelgrass structure also played an underlying role. Secondly, I investigated changes in seagrass bed structure and macroinfaunal communities with respect to distance from a finfish farm. The infauna community was linked to changes in eelgrass structure, which in turn was significantly related to distance from the farm. In light of these results, I discuss the importance of large-scale spatial surveys, as well as local surveys across impact gradients, to inform the management and protection of seagrass ecosystems in Atlantic Canada.

# List of Abbreviations and Symbols Used

| | |
|---|---|
| δ | Delta- used for isotope notation |
| µ | Micro (unit of measurement: $1x10^{-6}$) |
| ABC | Abundance-biomass comparison |
| AG | Above-ground |
| BG | Below-ground |
| BI | Big Island Terra Nova |
| BT | Bouctouche |
| C | Carbon |
| CB | Carters Beach |
| CG | Cocagne |
| CH | Canopy height |
| Chl *a* | Chlorophyll *a* |
| CR | Croucher Island |
| DFO | Fisheries and Oceans Canada |
| FG | Franks George |
| FP | False Passage |
| GB | Goose Bay |
| JB | Jordan Bay |
| KB | Kouchibouguac |
| LM | Lamèque |
| MPB | Microphytobenthos |
| N | Nitrogen |
| NB | New Brunswick |
| NL | Newfoundland |
| NS | Nova Scotia |
| OW | Old Warf |
| PJ | Port Joli |
| SB | Sweet Bay |
| SC | St.Chads |
| SD | Shoot density |
| SI | Spectacle Island |
| SM | Inner Sambro |
| ST | Strawberry Island |
| TB | Tabusintac |
| TH | Taylor's Head |

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# Chapter 1- Introduction

Eelgrass, *Zostera marina*, is the dominant seagrass in Atlantic Canada that forms extensive meadows in shallow coastal waters with high above- and belowground biomass (Short & Short. 2003, Short et al. 2007, Schmidt et al. 2011, 2012). These eelgrass beds provide important three-dimensional structure that harbours diverse communities of flora and fauna (Orth et al. 1984, Heck et al. 2003, Moore.A. & Short. 2006, Schmidt et al. 2011). Eelgrass beds also provide key ecological services (Barbier et al. 2011), such as nutrient cycling and sediment stabilization, that are largely dependent on functions provided by macrobenthic communities (Snelgrove et al. 1997, Norling et al. 2007). With the accelerated loss of seagrass habitats worldwide, the importance of understanding the consequences of this loss to the provision of ecosystem functions and services is critical (Duarte 2002, Orth et al. 2006, Waycott et al. 2009).

One important component of the associated species community in seagrass beds is the macroinfauna which perform important ecological roles such as bioturbation, nutrient cycling, and sediment oxygenation (Snelgrove et al. 1997, Aller & Aller 1998, Norling et al. 2007). In addition to contributing to ecological functioning, changes in the diversity and composition of these communities can provide insight into pollution effects and overall health of marine ecosystems (Pearson & Rosenberg 1978, Henderson & Ross 1995, Smith et al. 2010). Despite the plethora of studies on the importance of macrobenthic communities to marine ecosystem functioning (e.g. Aller & Aller 1998, Desrosiers et al. 2000, Bolam et al. 2002, Bremner et al. 2006, Bremner 2008, Karlson et al. 2016) and their relationship to seagrass bed structure (e.g. Orth 1973, Edgar 1990, Webster et al. 1998, Frost et al. 1999, Bologna & Heck 2002, Gartner et al. 2013, Wong

& Dowd 2015), the linkage between macroinfauna and seagrass beds across spatial scales, particularily in Atlantic Canada, is limited.

Previous research has shown that the extent of the services provided by seagrass habitats depends on the physical structure of the beds and the composition of the associated species (Heck & Wetstone 1977, Orth et al. 1984, Heck et al. 1995, Boström et al. 2010, Schmidt et al. 2011, 2012, Gartner et al. 2013). Spatial differences in seagrass bed structure can be due to environmental conditions, such as temperature, depth and physical exposure (Thom et al. 2003, Frederiksen et al. 2004, Moore.A. & Short. 2006) or anthropogenic impacts, such as nutrient, organic or sediment loading or physical disturbance (Cancemi et al. 2003, Lee et al. 2004, Burkholder et al. 2007, Holmer et al. 2008, DFO 2011, Schmidt et al. 2012). Additionally, these differences have been shown to have strong effects on associated species communities as well as functions and services (Boström et al. 2002, Warren et al. 2010, Coll et al. 2011, Schmidt et al. 2012). To my knowledge, large-scale regional variation in the structure of seagrass beds and their associated macroinfauna has only been examined in the Baltic Sea (Boström & Bonsdorff 1997), however this was specifically comparing seagrass communities to bare sand habitats. In Atlantic Canada, one large-scale study has investigated the overall community composition of flora and fauna of eelgrass habitats in New Brunswick, Nova Scotia and Prince Edward Island (Namba 2015), however this expanded on Schmidt et al. (2012) by strictly looking at low impact sites from 2007 and did not extensively focus on infauna. In the present study I include both high and low impact sites from Schmidt et al. (2012), I hone in on the macroinfauna communities in great detail and extend the survey on a larger biogeographical scale to Newfoundland.

Additionally, there is limited scientific data on the local impacts of nutrient and organic enrichment from finfish aquaculture farms on surrounding eelgrass habitats and associated

macroinfauna communities in Atlantic Canada. This presents a novel opportunity to examine large-scale regional variation of eelgrass habitats in Atlantic Canada, as well as local-scale variation across an impact gradient, and will further provide baseline data for applications in management and conservation as well as future research.

## 1.1. Thesis Structure

This thesis is structured into two distinct data chapters which examine variation in eelgrass bed structure and associated macroinfaunal communities both regionally (Chapter 2) and locally (Chapter 3) in Atlantic Canada.

Chapter 2 uses large-scale field surveys to quantify the spatial variation of eelgrass habitats and macroinfauna communities across three biogeographic regions (New Brunswick, Nova Scotia and Newfoundland). Next, variation in the macroinfauna community structure is linked to variation in regional and local eelgrass and environmental conditions to determine if infauna variation can be explained by region or local study sites. I then discuss the importance of spatial surveys to inform conservation and management of coastal habitats and how these results can be applied in future research.

In Chapter 3, I examine local impacts of organic enrichment from finfish aquaculture on eelgrass beds and their associated macroinfauna communities. Again using field surveys along a local impact gradient, I then link changes in macroinfauna communities and indicator species to differences in eelgrass beds and environmental conditions. I discuss the importance of quantifying these impacts not only directly beneath fish pens, but also on adjacent eelgrass habitats within a bay. I conclude this

chapter by discussing the management implications and future possibilities for finfish aquaculture in Atlantic Canada.

In Chapter 4, I conclude the thesis with a discussion of the overall findings, as well as management implications.

# Chapter 2 – Spatial variation of macroinfaunal communities associated with *Zostera marina* beds in Atlantic Canada

## 2.1. Abstract

Seagrass beds and associated macrobenthic communities are important for ecological functioning in coastal ecosystems. The importance of the ecological functions provided by eelgrass and macroinfauna are well understood, however the spatial variation and linkage of the two have never been studied in Atlantic Canada. This study fills that knowledge gap by performing large-scale field surveys across three biogeographic regions (New Brunswick, Nova Scotia and Newfoundland). First, we examined variation in eelgrass bed structure (shoot density, canopy height, biomass) and environmental parameters (tissue nitrogen and carbon content, sediment organic content, microphytobenthos and annual algae) across the three regions. Next, we examined the regional variation in macroinfauna community composition and summary measures (species richness, diversity, total abundance and biomass). Lastly, we linked the eelgrass structure/environmental variables to the infauna community to determine what best explained patterns in the infauna. Our results indicate that eelgrass structure and most environmental parameters vary at the site level, however most variation in the infauna community was explained by region. Furthermore, the microphytobenthos was explained best by region and consistently came out as the best predictor of the infauna community. We suggest that in moving forward with protecting and managing eelgrass habitats, eelgrass structure should be assessed on a site-by-site basis, however benthic productivity (microphytobenthos) may be a useful tool in evaluating macroinfauna and ecosystem health on a region-scale.

## 2.2. Introduction

Seagrass beds are diverse and productive habitats in coastal ecosystems around the world (Moore & Short 2006, Kuo & Hartog 2007). They create important three dimensional structure and provide critical functions and services including nutrient cycling, carbon sequestration, sediment stabilization as well as food and habitat for various species of ecological and economical importance (Duarte 2002, Heck et al. 2003, Orth et al. 2006, Schmidt et al. 2011). Additionally, seagrass beds can have a strong influence on the spatial distribution of associated fauna by modifying the hydrodynamics of the marine environment (Fonseca & Fisher 1986), stabilizing sediments (Orth et al. 2006) and providing increased habitat complexity both above- and below-ground (Heck & Wetstone 1977, Orth et al. 1984, Gartner et al. 2013). Despite their ecological importance, proximity to human settlement and various anthropogenic activities has led to the decline of seagrass beds over past decades and centuries (Lotze et al. 2006, Waycott et al. 2009) leading them to become one of the most threatened ecosystems in the world (Duarte 2002, Orth et al. 2006, Halpern et al. 2008, Short et al. 2010).

Eelgrass, *Zostera marina*, is the most widely distributed seagrass species in the world and is the dominant seagrass in the Northwest Atlantic (Short & Short 2003, Short et al. 2007). Moreover, it has been designated as an ecologically significant species in eastern Canada due to its important role in sediment stabilization and ecological services (DFO 2009a, 2011). It can be found in estuaries and sheltered bays along coastlines in the Gulf of St. Lawrence, the Atlantic coast of Nova Scotia and in most parts of Newfoundland and Labrador (DFO 2011; Moore and Short 2006; Short and Short 2003).

While the eelgrass shoots and leaves provide important habitat for a variety of pelagic, epiphytic and epibenthic species (Orth et al. 1984, 2006, Heck et al. 2003, Schmidt et al. 2011), the extensive root-rhizome system provides sediment stability and below-ground habitat complexity which supports an abundant and diverse infaunal community (Orth 1977, Orth et al. 1984). Usually, infaunal abundance and diversity is much higher in these vegetated areas compared to bare sediments (Heck et al. 1995, Boström & Bonsdorff 1997, Wong & Dowd 2015). This below-ground ecosystem also provides a rich food source for both epifaunal and infaunal communities (Orth et al. 1984, Boström & Bonsdorff 1997) and can influence the spatial variation in benthic community structure, in addition to food supply in the water column (Grebmeier & McRoy 1989, Desrosiers et al. 2000) and deposition of organic matter (Pearson & Rosenberg 1978). Benthic infaunal species perform important roles in regulating ecological processes such as secondary production, pollution metabolism, bioturbation, nutrient cycling and oxygenation of the sediments (Snelgrove et al. 1997, Aller & Aller 1998, Norling et al. 2007). Not only are the functions provided by infauna communities fundamental to the maintenance of ecological processes, but changes in their community structure can be used as a way to identify pollution effects and eutrophication in the marine environment and therefore contribute to evaluating ecosystem health (Pearson & Rosenberg 1978, Henderson & Ross 1995, Smith et al. 2010). Therefore, the ramifications of seagrass decline or loss to the associated communities and their functions are of growing importance to science (Waycott et al. 2009) as well as the management and conservation of coastal ecosystems (DFO 2011).

In Atlantic Canada, most studies have examined variation in eelgrass bed structure and associated flora and fauna on either local scales (Laurel et al. 2003, Joseph et al. 2006, Warren et al. 2010, Schmidt et al. 2011, Wong et al. 2013), over a gradient of human activities such as eutrophication (Schmidt et al. 2012), or specifically at low impact sites (Namba 2015), but not across several biogeographic regions combining natural and anthropogenic variation. Nova Scotia (Scotian Shelf), New Brunswick (Gulf of St. Lawrence) and Newfoundland (Newfoundland-Labrador Shelf) are accepted as the three biogeographic regions in Atlantic Canada due to their distinct differences in bathymetry and oceanographic processes (DFO 2009b, 2015). While these oceanographic processes are most likely delineating the community dynamics of the marine taxa in each region, the need for species composition data, particularly benthic community data, has become increasingly important for understanding spatial variation in Atlantic Canada (DFO 2009b). To our knowledge, no large-scale spatial data on macroinfaunal communities exists in Atlantic Canada, especially those associated with seagrass habitats. Therefore, the first objective of this study was to quantify the regional variation in eelgrass bed structure, environmental parameters, and macroinfaunal communities across three provinces throughout Atlantic Canada. Our second objective was to link the observed variation in eelgrass bed structure and environmental parameters to the observed variation in the associated macroinfaunal community. More specifically, our aim was to determine which environmental and/or eelgrass bed variables were driving differences in infauna communities and whether some of the variation could be explained by province or biogeographic region. Understanding differences in macrobenthic assemblages across Atlantic Canada provides important information on regional-scale

conditions of eelgrass habitats and how these conditions are influencing infauna community structure. These results provide insight into how to best manage and conserve these important coastal ecosystems.

## 2.3. Methods

### *2.3.1. Study area*

Sampling sites were located in soft sediment eelgrass habitats across three provinces in Atlantic Canada (Figure 1, Table 1). In this study, each province was considered as its own region because in Atlantic Canada these three provinces not only represent different political boundaries, but also different biogeographic regions (DFO 2009b). The six New Brunswick (NB) sites and six of the nine Nova Scotia (NS) sites (FP, TH, FG, SM, CR, ST) were sampled in July-August of 2013. Newfoundland sites (NL) were sampled in July 2014 and the remaining three NS sites (CB, PJ, JB) sampled in July 2015. The six NB sites and three of the NS sites (FP, TH, FG) were previously selected based on different eutrophication levels and human impacts (Coll et al. 2011, Schmidt et al. 2012). The remaining sites were randomly selected within sheltered to moderately exposed embayments based on availability and size of a continuous eelgrass bed (>50 m). In order to complete the extensive field sampling, surveys had to be completed over a period of three years to ensure that the time of year (July) remained consistent between regions. Eelgrass and associated communities experience large seasonal fluctuation in Atlantic Canada (Cullain 2014) whereby consistency between time of year was of more importance than differences between years.

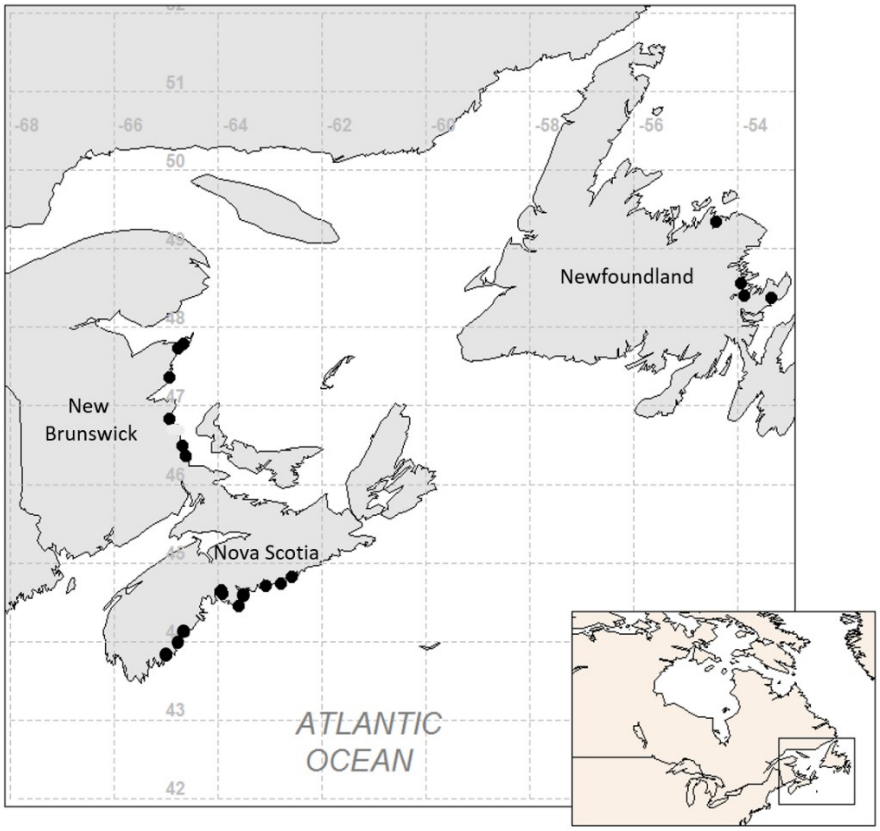


Figure 1. Map of study sites (black dots) in the provinces of New Brunswick, Nova Scotia and Newfoundland in Atlantic Canada (see Table 1 for site details).

Table 1. Site names and abbreviations (ID) for each sampling location with associated latitude (Lat.), longitude (Long.), bottom temperature (Temp.) and bottom depth. Regions include the eastern coast of New Brunswick (NB), Atlantic coast of Nova Scotia (NS) and the northeast coast of Newfoundland (NL).

| Site | ID | Lat. | Long. | Temp. (°C) | Depth (m) |
|---|---|---|---|---|---|
| **New Brunswick** | **NB** | | | | |
| Cocagne | CG | 46.37 | -64.62 | 23 | 1.0 |
| Bouctouche | BT | 46.50 | -64.68 | 23 | 0.75 |
| Kouchibouguac | KB | 46.84 | -64.94 | 23 | 0.75 |
| Tabusintac | TB | 47.37 | -64.94 | 22 | 0.8 |
| Baie St. Simon | SS | 47.73 | -64.77 | 20 | 1.0 |
| Lamèque | LM | 47.79 | -64.67 | 20 | 1.5 |
| **Nova Scotia** | **NS** | | | | |
| False Passage | FP | 44.44 | -62.47 | 12 | 4.6 |
| Taylor Head | TH | 44.49 | -62.34 | 10 | 4.9 |
| Inner Sambro | SM | 44.27 | -63.35 | 12 | 4.8 |
| Croucher Island | CR | 44.38 | -63.57 | 15 | 3.6 |
| Strawberry Island | ST | 44.39 | -63.56 | 14 | 4.4 |
| Franks George Island | FG | 44.35 | -63.53 | 15 | 4.3 |
| Carters Beach | CB | 43.91 | -64.82 | 12 | 2.5 |
| Port Joli | PJ | 43.84 | -64.88 | 15 | 2.9 |
| Jordan Bay | JB | 43.72 | -65.17 | 14 | 1.4 |
| **Newfoundland** | **NL** | | | | |
| Goose Bay | GB | 48.22 | -53.51 | 17 | 2.3 |
| Sweet Bay | SB | 48.26 | -53.39 | 16 | 2.5 |
| Big Island Terra Nova | BI | 48.33 | -53.57 | 16 | 2.2 |
| St.Chads | SC | 48.39 | -53.45 | 13 | 2.3 |

### *2.3.2. Sampling design and data collection*

Expanding upon the design by Schmidt et al. (2011, 2012), at each site we laid two 50 x 4 m transects parallel to shore inside the eelgrass bed ≥10 m from the vegetation-bare substrate interface. Three quadrats (0.5 x 0.5 m, with 0.25 m subsections) every 25 m along each transect (n = 6) were used to delineate the collection area of all samples. All data were collected using SCUBA during high tide. Bottom temperature and depth at each sampling location were recorded on SCUBA dive computers during the field survey.

Shoot density was examined using the 0.25 x 0.25 m subsection of the sampling quadrat and canopy height was determined by holding the zero end of the measuring tape against the substrate in the centre of the quadrat and extending it to the average height of the plants. The percent cover of each epiphytic and benthic macroalgae species was recorded in each quadrat. For epiphyte cover, I considered both sides of the blade as habitable space and estimated the cover of all the blades as a whole for each quadrat. The cover of benthic algae was estimated with respect to the bottom. Benthic and epiphytic algae species were then separated into perennial and annual algae groups whereby the sum for an individual quadrat could exceed 100%. To examine the eelgrass above- (AG) and below-ground (BG) biomass as well as infauna density and biomass, a sediment core (0.2 m diameter; 0.2 m deep) was pressed into the sediment within each of the quadrat subsections and brought to the surface where all above- and below-ground tissue was removed, rinsed in a 500 µm sieve to capture any fauna, bagged and kept on ice. On site, all infauna species were identified to the lowest possible taxon using identification keys and guidebooks. If organisms needed further identification they were brought back to the laboratory and examined under the microscope. Individuals of each species were counted (abundance $m^{-2}$) and weighed (g $m^{-2}$).

In the laboratory, the eelgrass blades, roots and rhizomes were rinsed again and all epiphytes were carefully scraped off the blades and then weighed for biomass (wet weight, g $m^{-2}$) prior to drying in an oven at 60˚C for 48 hours and weighed again for dry weight (g $m^{-2}$). After eelgrass biomass weights were recorded, a 50 mg dry weight subsample of each of the above- and below-ground tissue were taken and samples were sent to the University of California Davis Stable Isotope facility for analysis of % tissue

nitrogen (N) and carbon (C), and nitrogen ($\delta^{15}N$, $^{15}N$:$^{14}N$) and carbon ($\delta^{13}C$, $^{13}C$:$^{12}C$) stable isotopes.

To assess sediment organic content, a 60 mL syringe core (2.6 cm diameter) was used to collect two samples from the upper 5 cm of sediments (volume of sample ~ 8.83 mL) at the first 5 quadrat locations for the 2013 sites and at all 6 quadrat locations for the 2015 sites. No sediment samples were collected for the 2014 Newfoundland sites due to logistic reasons. Both samples were placed in a plastic bag and frozen until processed. The same protocol was followed for both the 2013 and 2015 samples, however the 2013 samples were sent to the Department of Fisheries and Oceans Canada (DFO) to be processed and the 2015 samples were processed in the laboratory at Dalhousie University. The samples were thawed and mixed and approximately 1 g of wet sediment was placed in a crucible which was previously ashed and weighed. Crucibles were placed in the drying oven at 60°C for 48 hours, removed and weighed for dry weight. Samples were then placed into a muffle furnace and combusted at 500°C for 6 h followed by 2 h in the drying oven (Luczak et al. 1997). We then weighed the crucible + ashed sample for ash weight. Percentages were calculated to determine overall percent organic content.

Also using a 60 mL syringe core, three microphytobenthos (MPB) samples were collected from the upper 2 cm of the sediments (volume of sample ~ 3.53 mL) at the six core sampling locations. Each set of three samples were combined together on site, placed in plastic cryovials and stored in liquid nitrogen while in the field and then a freezer (-20°C) until analysis in the laboratory. Samples were always kept in a darkened room throughout processing. First, frozen sediment samples were placed in labeled glass scintillation vials with 10 mL of 90% acetone, vortexed for 1 minute and then placed

back in the freezer to be digested for 24 hours. The following day samples were vortexed for one minute, placed in falcon tubes and centrifuged for 30 minutes at 3250 rpm (T. Whitsit, Dalhousie, pers. comm.). The supernatant was subsequently pipetted into clean scintillation vials and measured in a Turner Designs 10005R fluorometer to determine chlorophyll *a* concentrations.

Due to logistical reasons, at three sites in Nova Scotia (CR, SM, ST) only eelgrass structure and infauna data were collected. Therefore, these three sites were not included in any analyses where environmental data was used.

### *2.3.3 Data analysis*

The three questions we wanted to answer about regional patterns in eelgrass bed structure and associated macroinfaunal communities were: a) Does eelgrass bed structure vary between regions in Atlantic Canada, b) Does the macroinfaunal community also vary between these regions, and c) Is the variation in infauna communities linked to eelgrass bed structure and/or regional environmental parameters. All statistical analyses we performed in PRIMER (version 6) and R (version 3.3.1, vegan package).

#### *2.3.3i Eelgrass bed structure and environmental parameters*

Multivariate permutational analysis of variance (PERMANOVAs) were first applied to assess the effect of region (fixed factor) and site nested within region (random factor) on normalized variables that were not independent (i.e., shoot density and canopy height, AG and BG eelgrass biomass, % tissue nitrogen and carbon, AG and BG $\delta^{13}C$, and AG and BG $\delta^{15}N$), and these were only assessed individually if significant differences were found ($p \leq 0.05$). Using a Euclidean distance matrix, univariate PERMANOVAs were then used to assess whether there was a significant effect of region

or site within region on individual environmental and eelgrass parameters. Analogous to ANOVA, PERMANOVA can get unbiased estimates of each of the components of variation in the model using mean squares (Anderson et al. 2008). The estimates will be in terms of squared units of the dissimilarity measure chosen and can be put back into their original units using the square root (√V) (Anderson et al. 2008). Lastly, if significant effects of region were found, post-hoc pairwise tests were performed to determine which regions were significantly different from each other.

2.3.3ii *Macroinfauna*

To determine differences in community composition between sites, multivariate PERMANOVAs were applied on zero adjusted Bray-Curtis similarity matrices based on abundance (density) and biomass data separately. Abundance and biomass data were square-root transformed in order to down-weight the influence of highly abundant or large species (Clarke & Gorley 2006). If a significant effect of region was detected, we used post-hoc pairwise tests to determine which regions were significantly different from each other. We also calculated species richness, total abundance, total biomass and Shannon-Wiener Diversity (H') and used univariate PERMANOVAs to identify significant differences in individual summary measures between regions and sites nested within regions. Estimates of the components of variation (√V) were calculated for community assemblage and summary measures of macroinfauna to determine which factors in the model explained the most variation.

To visualize the data and support PERMANOVA results, centroids were computed for each site and group average cluster analysis performed on the centroids for both the infauna community abundance and biomass. To determine which species

contributed most consistently (>10%) to the differences between regions and sites, we used similarity percentages (SIMPER) analysis (Anderson et al. 2008) and then univariate PERMANOVAs on each SIMPER species to determine significant differences between regions and sites nested within region.

*2.3.3iii Linking the environment/eelgrass structure to the macroinfauna community*

First, I tested for correlations amongst all environmental/eelgrass variables (depth, temperature, sediment organic content, MPB, % cover annual algae, AG and BG % tissue nitrogen and carbon, AG and BG $\delta^{13}C$ and $\delta^{15}N$, AG and BG biomass, shoot density, canopy height) and selectively removed individual variables with a high correlation (>0.7) to one or more of the other eelgrass/environmental variables. Due to sediment organic content missing from NL, we ran all analyses with only NS and NB to determine if it was important in explaining infauna patterns. Because it never came out as a significant explanatory variable and because we were primarily interested in regional patterns with NL included, we chose to remove sediment organic content from all analyses that linked the environment to the biological community. While we ran analyses with different combinations of all uncorrelated variables, we chose to remove temperature and depth due to their high correlation to each other and MPB, and we also chose to remove % carbon and AG $\delta^{13}C$ due to the high correlation with BG $\delta^{13}C$ which was of more interest in this study. Consequently, the uncorrelated variables used in all multivariate analyses were MPB, % cover annual algae, eelgrass shoot density and canopy height, AG and BG eelgrass biomass, %N in AG and BG tissue, and $\delta^{15}N$ and $\delta^{13}C$ in BG tissue.

The BEST/BIOENV procedure was used to identify possible correlations between combinations of variables for the environment and/or eelgrass structure (Euclidean distance matrix) and Bray-Curtis similarity matrices of the infauna community based on abundance and biomass data separately. Because individual species did not fit the models using a parametric approach, we also used the BIOENV procedure to test correlations between environmental/eelgrass variables and individual SIMPER species. BIOENV provides a non-parametric index rho (ranging from 0 to 1) that indicates how closely the environmental variables explain the multivariate pattern of the species. We then used a permutation test to determine the significance level of the sample statistic (rho).

To link the overall response of the infauna community to different environmental and eelgrass variables, we used generalized linear models (GLMs) using R (R version 3.2.1). Models were fitted to total abundance, total biomass, species richness and Shannon diversity index (H') using various sets of uncorrelated environmental and eelgrass canopy variables as predictors. GLMs were fitted to the data using a normal Gaussian distribution (species richness, diversity and biomass) and a negative binomial distribution (total abundance). For each model, residuals were examined to check the assumptions of normality and homogeneous variance. All models fit the assumptions with the exception of biomass which experienced some heteroscedasticity. We applied different distribution families to the model, however the normal Gaussian distribution was the best fit. We also looked at individual linear models between infauna summary measures (dependent variable) and the environment/eelgrass structure (independent variable). Only the regressions with significant relationships ($p \leq 0.05$) and good/reasonable fits ($R^2 > 0.2$) were included.

## 2.4 Results

### *2.4.1 Eelgrass bed structure and environmental parameters*

Shoot density, canopy height and BG biomass did not significantly differ between regions, however they did differ between sites nested within region (Table 2, Appendix 2A, 2B). In all cases except canopy height, the residuals explained most of the variation, though usually only slightly more than site (Table 2). Region and site both had a significant effect on AG biomass with more variation in the model being explained by region. Although significant differences by region were only found in AG biomass, the same regional patterns were observed for all other eelgrass parameters with NS having higher shoot density, canopy height and AG and BG biomass than both NB and NL (Figure 2).

Table 2. Univariate PERMANOVA results of the effect of region (Re) and site nested within region (Si(Re)) on eelgrass bed structure and environmental variables in New Brunswick, Nova Scotia and Newfoundland. √V estimates the components of variation for each factor in the model. Res are the residuals. Significant effects ($p \leq 0.05$) are bolded.

| Variable | | Factor | DF | pseudo-F | p | (√V) |
|---|---|---|---|---|---|---|
| **Eelgrass bed Structure** | | | | | | |
| Shoot Density | | Re | 2 | 0.17 | 0.85 | -0.30 |
| | | Si(Re) | 13 | 5.37 | **0.001** | 0.68 |
| | | Res | 80 | | | 0.79 |
| Canopy Height | | Re | 2 | 1.97 | 0.17 | 0.34 |
| | | Si(Re) | 13 | 8.86 | **0.001** | 0.74 |
| | | Res | 80 | | | 0.64 |
| Biomass | | Re | 2 | 7.39 | **0.013** | 0.55 |
| | -Above | Si(Re) | 13 | 2.21 | **0.02** | 0.37 |
| | | Res | 80 | | | 0.82 |
| | | Re | 2 | 1.07 | 0.38 | 0.079 |
| | -Below | Si(Re) | 13 | 4.00 | **0.001** | 0.58 |
| | | Res | 80 | | | 0.82 |
| **Environmental variables** | | | | | | |
| % C | -Above | Re | 2 | 0.24 | 0.77 | -0.22 |
| | | Si(Re) | 13 | 2.04 | **0.021** | 0.40 |
| | | Res | 74 | | | 0.94 |
| | -Below | Re | 2 | 17.37 | **0.002** | 0.58 |
| | | Si(Re) | 13 | 0.72 | 0.71 | -0.20 |
| | | Res | 74 | | | 0.90 |
| % N | -Above | Re | 2 | 0.076 | 0.93 | -0.32 |
| | | Si(Re) | 13 | 5.52 | **0.001** | 0.70 |
| | | Res | 74 | | | 0.78 |
| | -Below | Re | 2 | 7.51 | **0.012** | 0.58 |
| | | Si(Re) | 13 | 2.58 | **0.006** | 0.41 |
| | | Res | 74 | | | 0.77 |
| $\delta^{13}C$ | -Above | Re | 2 | 5.79 | **0.02** | 0.71 |
| | | Si(Re) | 13 | 14.21 | **0.001** | 0.71 |
| | | Res | 74 | | | 0.46 |
| | -Below | Re | 2 | 4.57 | **0.038** | 0.59 |
| | | Si(Re) | 13 | 8.21 | **0.001** | 0.67 |
| | | Res | 74 | | | 0.59 |
| $\delta^{15}N$ | -Above | Re | 2 | 1.12 | 0.379 | 0.14 |
| | | Si(Re) | 13 | 45.75 | **0.001** | 0.96 |
| | | Res | 74 | | | 0.34 |
| | -Below | Re | 2 | 0.87 | 0.441 | -0.16 |
| | | Si(Re) | 13 | 40.14 | **0.001** | 0.97 |
| | | Res | 74 | | | 0.37 |
| MPB | | Re | 2 | 46.84 | **0.001** | 1.04 |
| | | Si(Re) | 13 | 3.82 | **0.001** | 0.30 |
| | | Res | 78 | | | 0.43 |
| Sediment | | Re | 1 | 0.40 | 0.542 | -0.31 |
| Organic | | Si(Re) | 10 | 22.85 | **0.001** | 0.95 |
| | | Res | 51 | | | 0.46 |
| Annual Algae | | Re | 2 | 1.24 | 0.34 | 0.19 |
| | | Si(Re) | 13 | 14.45 | **0.001** | 0.85 |
| | | Res | 69 | | | 0.53 |

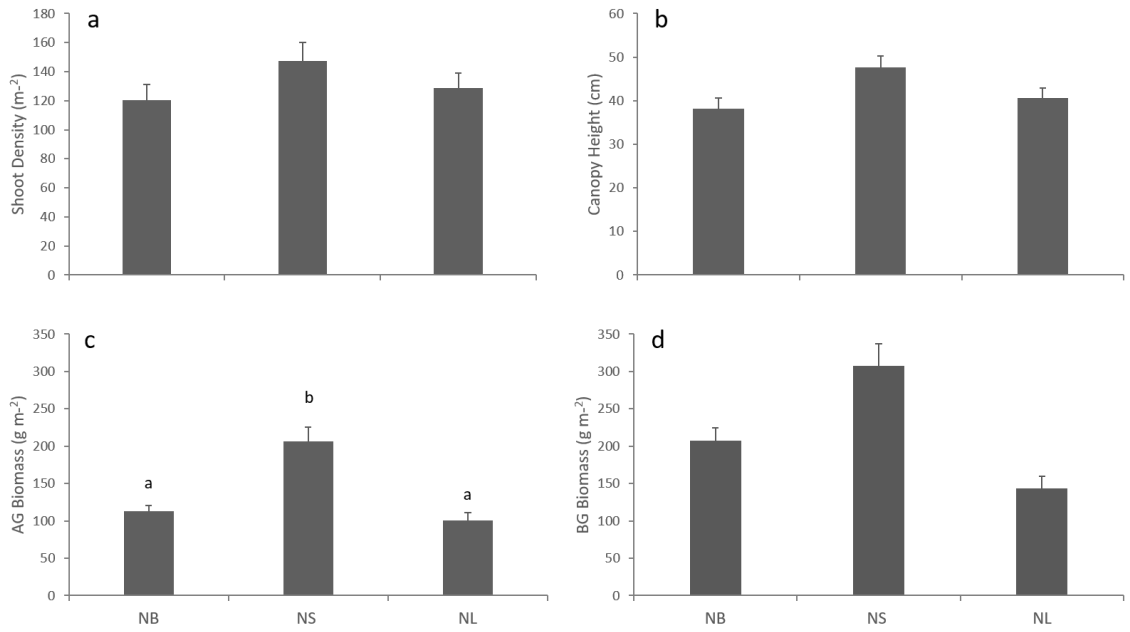


Figure 2. Average (+SE) eelgrass shoot density (a), canopy height (b), AG biomass (c) and BG biomass (d) across three provinces, New Brunswick (NB, n = 36), Nova Scotia (NS, n = 54) and Newfoundland (NL, n = 24) in Atlantic Canada. Lower cases letters indicate significant differences ($p \leq 0.05$) between regions.

No significant regional differences were found in AG % C and % N, AG and BG $\delta^{15}N$, or total annual algae, however site within region did have a significant effect on these parameters (Table 2, Appendix 2A, 2B). Further, BG % C and % N, AG and BG $\delta^{13}C$ and MPB all had significant regional effects and in all cases except for $\delta^{13}C$ more of the variation was explained by region than site (Table 2). Where regional differences were detected, post-hoc tests revealed that NB and NL had significantly higher % C in BG tissue than NS, and NB had significantly higher % N in BG tissue than NS and NL (Figure 3a-b). Additionally, NS and NB had significantly higher $\delta^{13}C$ in AG tissue than NL, and NS had higher $\delta^{13}C$ in BG tissue than both NB and NL (Figure 3c), but there were no regional differences in AG and BG $\delta^{15}N$ (Figure 3d).

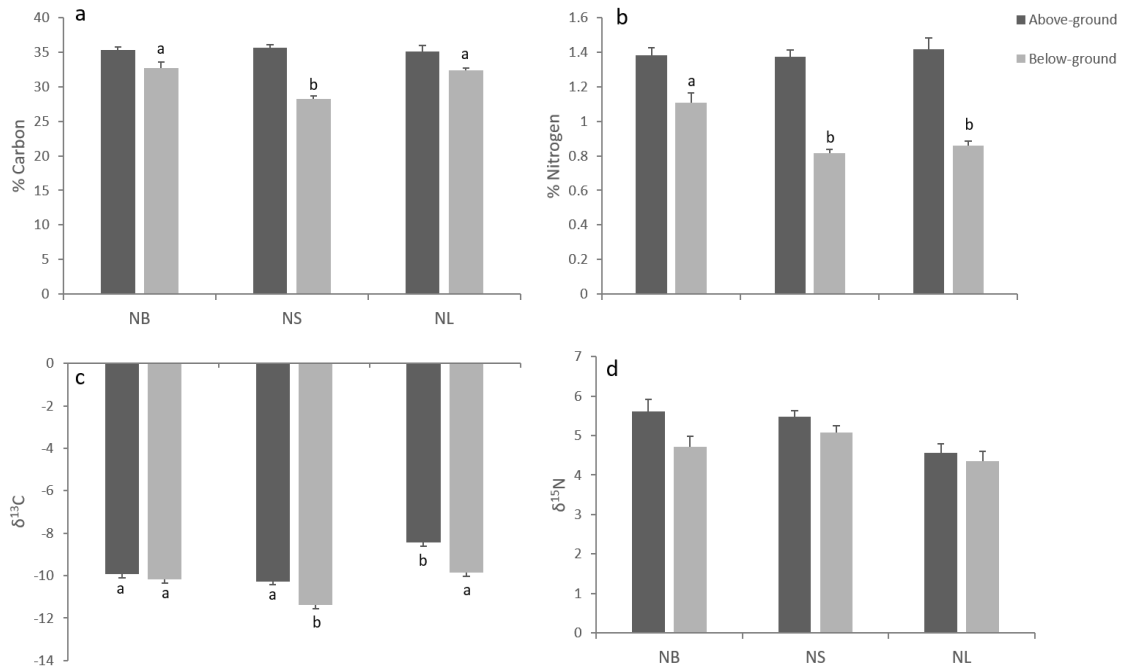


Figure 3. Percent tissue carbon (a) and nitrogen (b), and stable-isotope ratios $\delta^{13}C$ (c) and $\delta^{15}N$ (d) (average +SE) in above- and below-ground eelgrass tissue across New Brunswick (NB, n = 36), Nova Scotia (NS, n = 36) and Newfoundland (NL, n = 24) in Atlantic Canada. Lower cases letters indicate significant differences ($p \leq 0.05$) between regions.

Since sediment organic content was not collected at any of the NL sites, comparisons could only be made between NB and NS (Figure 4a). No regional differences were found, however there was a significant effect of site within region (Table 2, Appendix 2A, 2B). Similarly, percent cover of annual algae had no significant regional differences, but a site within region effect. NB did show higher percentages in both cases, particularly with annual algae (Figure 4b). Furthermore, significant regional differences were found in the MPB with NB being significantly higher than the two other regions (Figure 4c). The significant relationship between depth and MPB (Figure 4d) also shows the shallower NB sites having higher MPB concentrations.

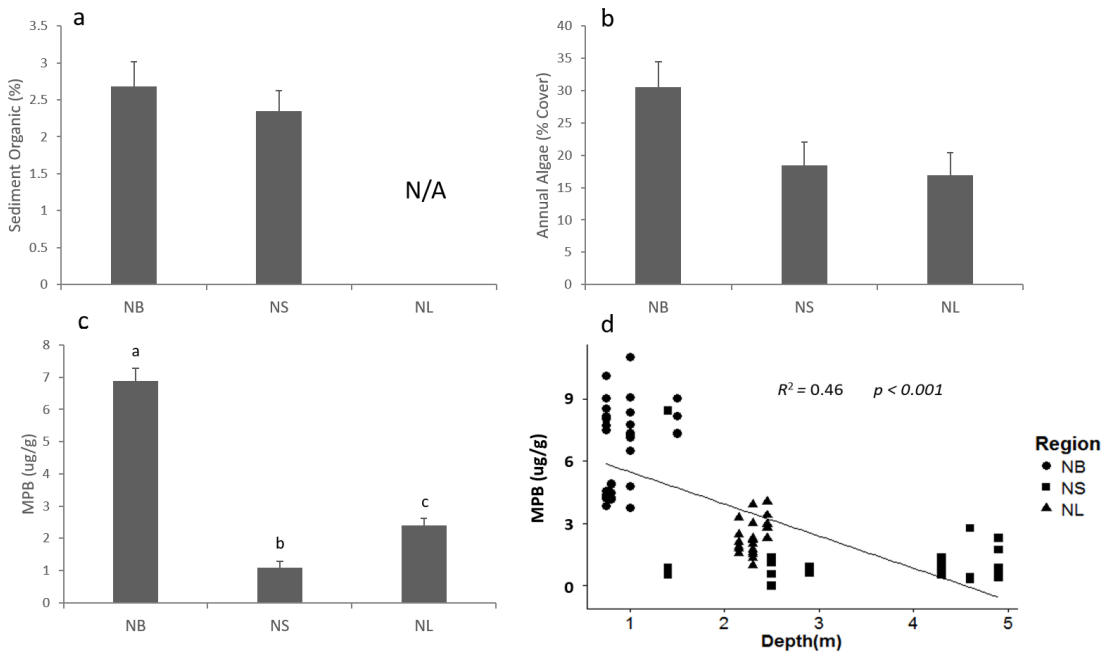


Figure 4. Average (+SE) percent sediment organic (a), percent cover of annual algae (b), MPB concentration (c) and a linear regression of MPB and depth (d) for New Brunswick (NB, n = 36) and Nova Scotia (NS, n = 36) and Newfoundland (NL, n = 24) in Atlantic Canada. Lower cases letters indicate significant differences ($p \leq 0.05$) between regions.

### *2.4.2 Macroinfauna*

In total 39 species and genera were identified (Appendix 2A: Table 1). Using both abundance and biomass of the infauna assemblage, significant differences were found regionally as well as at the site within region level (Table 3, Appendix 2B). For abundance more variation was explained by region, while site explained slightly more for biomass. When examining the community centroids of the infauna assemblage, clear regional clusters were identified (Figure 5). Further, NL appears to be clustering more closely with NS while most of the NB sites are clustering together separately.

Table 3. Multivariate PERMANOVA results of the effect of region (Re) and site nested within region (Si(Re)) on macroinfauna assemblage using abundance and biomass (top) and univariate PERMANOVA results on individual summary measures (bottom). √V estimates the components of variation for each factor in the model including residuals (Res). Significant effects ($p \leq 0.05$) are bolded.

| | Factor | DF | pseudo-F | p | √V |
|---|---|---|---|---|---|
| Community Assemblage (Abundance) | Re | 2 | 7.44 | **0.001** | 35.54 |
| | Si(Re) | 16 | 3.87 | **0.001** | 29.55 |
| | Res | 95 | | | 42.71 |
| Community Assemblage (Biomass) | Re | 2 | 5.24 | **0.001** | 29.31 |
| | Si(Re) | 16 | 3.96 | **0.001** | 30.14 |
| | Res | 95 | | | 42.89 |
| Species Richness | Re | 2 | 25.71 | **0.001** | 25.01 |
| | Si(Re) | 16 | 2.78 | **0.001** | 9.86 |
| | Res | 95 | | | 18.11 |
| Diversity (H') | Re | 2 | 19.44 | **0.001** | 14.76 |
| | Si(Re) | 16 | 3.05 | **0.001** | 6.9 |
| | Res | 95 | | | 11.82 |
| Total Abundance | Re | 2 | 23.16 | **0.001** | 32.15 |
| | Si(Re) | 16 | 2.89 | **0.001** | 13.53 |
| | Res | 95 | | | 24.09 |
| Total Biomass | Re | 2 | 9.14 | **0.001** | 25.59 |
| | Si(Re) | 16 | 3.88 | **0.001** | 18.94 |
| | Res | 95 | | | 27.32 |

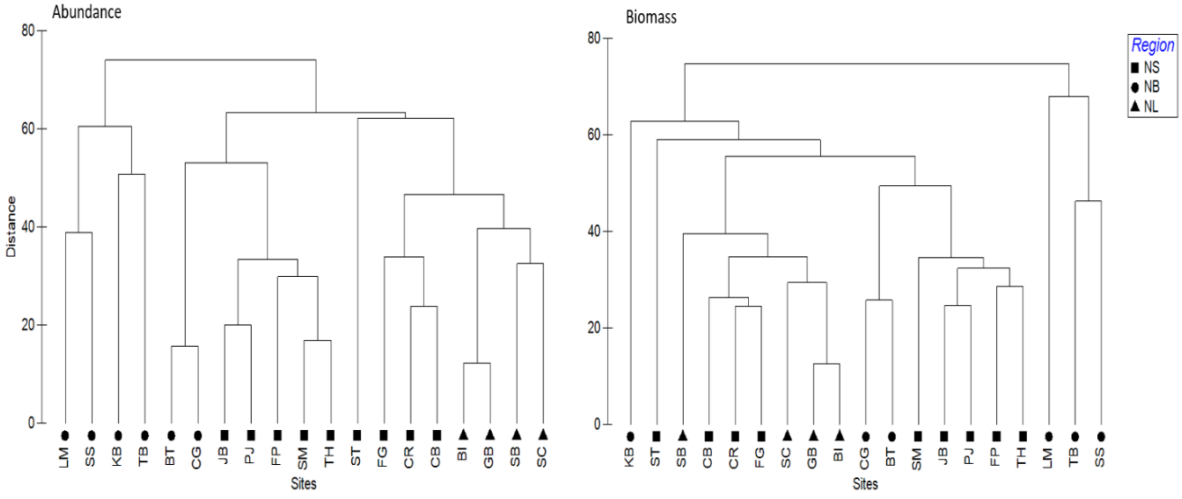


Figure 5. Cluster analysis for macroinfauna assemblage using abundance (left) and biomass (right) across three provinces, New Brunswick (NB), Nova Scotia (NS) and Newfoundland (NL) in Atlantic Canada. Refer to Table 1 for site details.

Individual summary measures (species richness, diversity, total abundance and total biomass) had significant effects of both region and site, however for all measures, most of the variation in the model was explained by region (Table 3). Post-hoc tests revealed that for all measures, each region was significantly different from each other with NB having the highest richness, diversity, abundance and biomass while NL had the lowest (Figure 6).

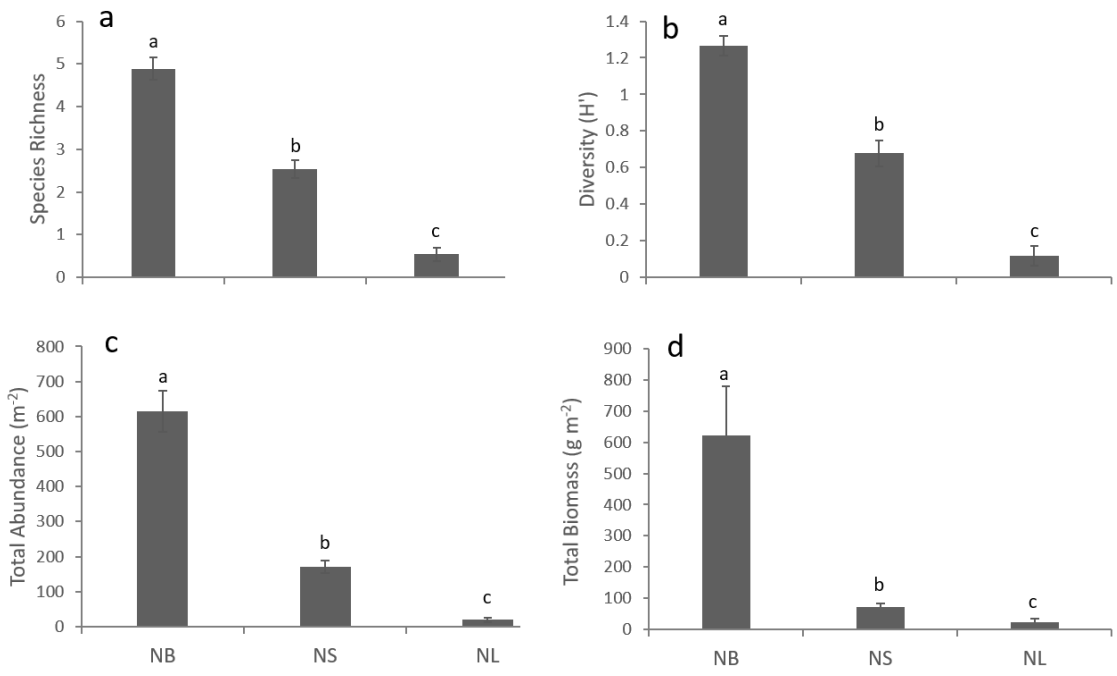


Figure 6. Average (+SE) species richness (a), Shannon's H diversity (b), total abundance (c) and total biomass (d) across three provinces, New Brunswick (NB, n = 36), Nova Scotia (NS, n = 54) and Newfoundland (NL, n = 24), in Atlantic Canada. Lowercase letters indicate significant differences ($p \leq 0.05$) between regions.

The SIMPER species contributing to >10% of the differences between regions consisted of five polychaetes: *Clymenella torquata, Glycera* sp., *Nereis* sp., *Nephtys* sp.

and *Pectinaria gouldii*; one bivalve: *Tellina agilis* and one gastropod: *Ilyanassa obsoleta.* While the presence of these SIMPER species, as well as the most abundant and large individuals were similar between regions, their contributions differed (Figure 7). All SIMPER species with the exception of *Glycera* sp. and *Nereis* sp. biomass were significantly different between regions (Table 4).

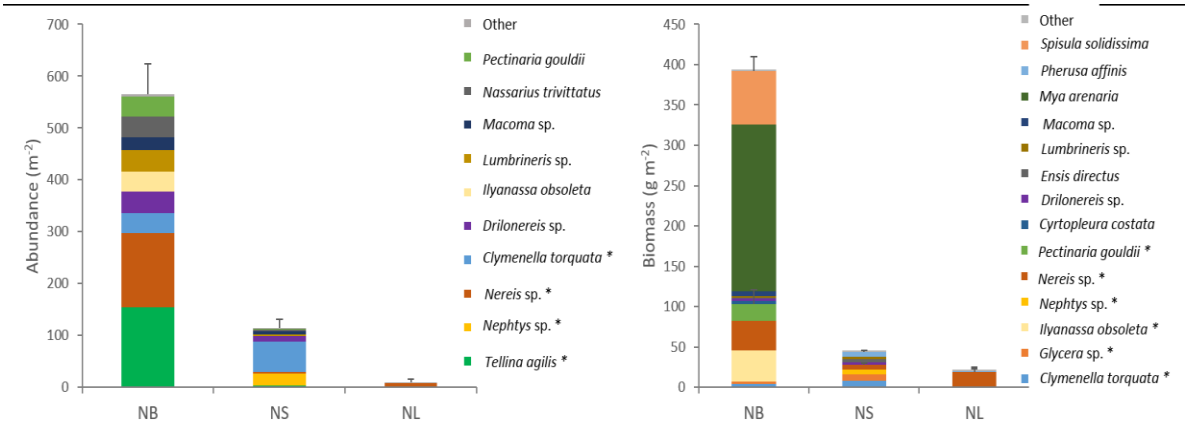


Figure 7. Abundance (left) and biomass (right) of macroinfauna species with the highest abundance and biomass (average +SE) across New Brunswick (NB, n = 36), Nova Scotia (NS, n = 54) and Newfoundland (NL, n = 24) in Atlantic Canada. All SIMPER species were included and indicated with an asterisk.

Table 4. Univariate PERMANOVAs of the effect of region (Re) and site nested within region (Si(Re)) on individual SIMPER species for abundance and biomass in Atlantic Canada.
Significant effects ($p \leq 0.05$) are bolded.

| | Factor | pseudo-F | p |
|---|---|---|---|
| **Abundance** | | | |
| *Clymenella torquata* | Re | 3.93 | **0.032** |
| | Si(Re) | 5.82 | **0.001** |
| *Nephtys* sp. | Re | 5.44 | **0.018** |
| | Si(Re) | 2.84 | **0.002** |
| *Nereis* sp. | Re | 3.71 | **0.046** |
| | Si(Re) | 18.08 | **0.001** |
| *Tellina agilis* | Re | 29.18 | **0.001** |
| | Si(Re) | 1.79 | **0.03** |
| **Biomass** | | | |
| *Clymenella torquata* | Re | 3.78 | **0.035** |
| | Si(Re) | 5.56 | **0.001** |
| *Glycera* sp. | Re | 2.00 | 0.162 |
| | Si(Re) | 2.03 | **0.021** |
| *Ilyanassa obsoleta* | Re | 7.30 | **0.004** |
| | Si(Re) | 6.26 | **0.001** |
| *Nephtys* sp. | Re | 4.65 | **0.03** |
| | Si(Re) | 2.87 | **0.001** |
| *Nereis* sp. | Re | 3.19 | 0.064 |
| | Si(Re) | 12.09 | **0.001** |
| *Pectinaria gouldii* | Re | 9.38 | **0.001** |
| | Si(Re) | 7.92 | **0.001** |

### *2.4.3 Linking the environment/eelgrass structure to the macroinfauna community*

Using the PRIMER BEST/BIOENV procedure we were able to determine any association between the Euclidean distance of environmental and eelgrass parameters (MPB, annual algae, BG $\delta^{15}N$, BG $\delta^{13}C$, AG % N, BG % N, eelgrass shoot density, canopy height, and AG and BG eelgrass biomass) and the Bray Curtis similarity of infauna community structure based on both abundance and biomass. For both abundance and biomass, MPB was identified as the best correlated variable for the infauna assemblage (Table 5).

When the SIMPER species were examined against the environmental/eelgrass variables, different combinations of variables were found for each species. Some species tended to be more correlated to the environment, while others correlated best with the environment in combination with eelgrass bed structure (Table 5). Similar to the community assemblage, MPB consistently showed up in many of these associations. All correlations were found to be significant with the exception of *Glycera* sp. biomass (Table 5).

Table 5. Results from the BEST/BIOENV procedure for the entire community (based on abundance above and biomass below) as well as SIMPER species using eelgrass and environmental (env.) data from New Brunswick, Nova Scotia and Newfoundland in Atlantic Canada. Significant ($p \leq 0.05$) correlations are bolded.

| Biological Variable | Best correlated env. variable(s) | Sample statistic (Rho) | Significance Rho |
|---|---|---|---|
| Community Assemblage (Abundance) | MPB | **0.298** | **0.01** |
| *Clymenella torquata* | MPB<br>AG eelgrass biomass<br>BG eelgrass biomass | **0.226** | **0.01** |
| *Nephtys* sp. | BG $\delta^{13}C$<br>Shoot density<br>Canopy height<br>AG eelgrass biomass | **0.314** | **0.01** |
| *Nereis* sp. | AG % N<br>MPB<br>BG $\delta^{15}N$ | **0.313** | **0.01** |
| *Tellina agilis* | BG % N<br>MPB | **0.384** | **0.01** |
| Community Assemblage (Biomass) | MPB | **0.389** | **0.01** |
| *Clymenella torquata* | MPB<br>BG eelgrass biomass | **0.194** | **0.01** |
| *Glycera* sp. | BG eelgrass biomass<br>Annual algae | 0.180 | 0.22 |
| *Ilyanassa obsoleta* | BG % N<br>MPB<br>BG $\delta^{15}N$ | **0.503** | **0.01** |
| *Nephtys* sp. | BG $\delta^{13}C$<br>Shoot density<br>Canopy height<br>AG eelgrass biomass | **0.317** | **0.01** |
| *Nereis* sp. | AG % N<br>MPB<br>BG $\delta^{15}N$<br>Shoot density | **0.309** | **0.01** |
| *Pectinaria gouldii* | BG % N<br>MPB | **0.418** | **0.01** |

Using GLMs we then examined which of these environmental/eelgrass variables were considered to be the best predictors of individual summary measures (species richness, diversity, total abundance and biomass) of the macroinfauna. Again, MPB consistently came out as the best predictor for all measures (Table 6). In addition to the MPB, the environment appeared to better explain infauna patterns than eelgrass structure. In particular BG $\delta^{15}N$ was a main predictor with a significant positive relationship across all measures except abundance, and BG $\delta^{13}C$ was a significant predictor of infauna biomass and AG % N for infauna diversity. Interestingly, species richness was the only measure to have an eelgrass structural variable (BG biomass) included as a predictor, however BG biomass was also marginal for total abundance (Table 6).

Table 6. Analysis of deviance table for macroinfauna total abundance and biomass, species richness and Shannon diversity (H'). For abundance a negative binomial GLM was applied and for biomass, species richness and diversity a normal GLM was used. Table contains test statistics (deviance for negative binomial and F-value for normal error distribution) and associated p-values. Significant results ($p \leq 0.05$) are bolded.

| Variable | Abundance | | Biomass | | Species Richness | | Diversity | |
|---|---|---|---|---|---|---|---|---|
| | Deviance | P-Value | F | P-Value | F | P-Value | F | P-Value |
| MPB | **15.35** | **< 0.001** | **23.56** | **< 0.001** | **39.37** | **< 0.001** | **11.42** | **0.001** |
| Annual algae | 0.38 | 0.54 | 1.06 | 0.31 | 1.12 | 0.29 | 1.13 | 0.29 |
| BG $\delta^{13}C$ | 2.69 | 0.10 | **4.97** | **0.03** | 0.53 | 0.47 | 0.29 | 0.59 |
| BG $\delta^{15}N$ | 0.43 | 0.51 | **5.54** | **0.02** | **6.61** | **0.01** | **4.80** | **0.03** |
| AG % N | 0.048 | 0.83 | 0.00 | 0.99 | 0.27 | 0.61 | **4.31** | **0.04** |
| BG % N | 0.50 | 0.48 | 1.41 | 0.24 | 0.04 | 0.84 | 0.64 | 0.43 |
| AG biomass | 0.36 | 0.55 | 0.13 | 0.72 | 0.01 | 0.91 | 2.52 | 0.12 |
| BG biomass | 3.55 | 0.06 | 1.89 | 0.17 | **4.53** | **0.04** | 0.18 | 0.68 |
| Shoot density | 0.21 | 0.65 | 0.73 | 0.40 | 0.001 | 0.98 | 0.21 | 0.65 |
| Canopy height | 2.29 | 0.13 | 0.64 | 0.43 | 1.28 | 0.26 | 1.60 | 0.21 |

To further examine the relationship of the individual infauna summary measures and the best explanatory variable (MPB) between regions, we used linear regressions (Figure 7). Species richness, diversity, total abundance and biomass all had highly significant positive relationships with the MPB, although $R^2$ values were not particularly high (0.22-0.30).

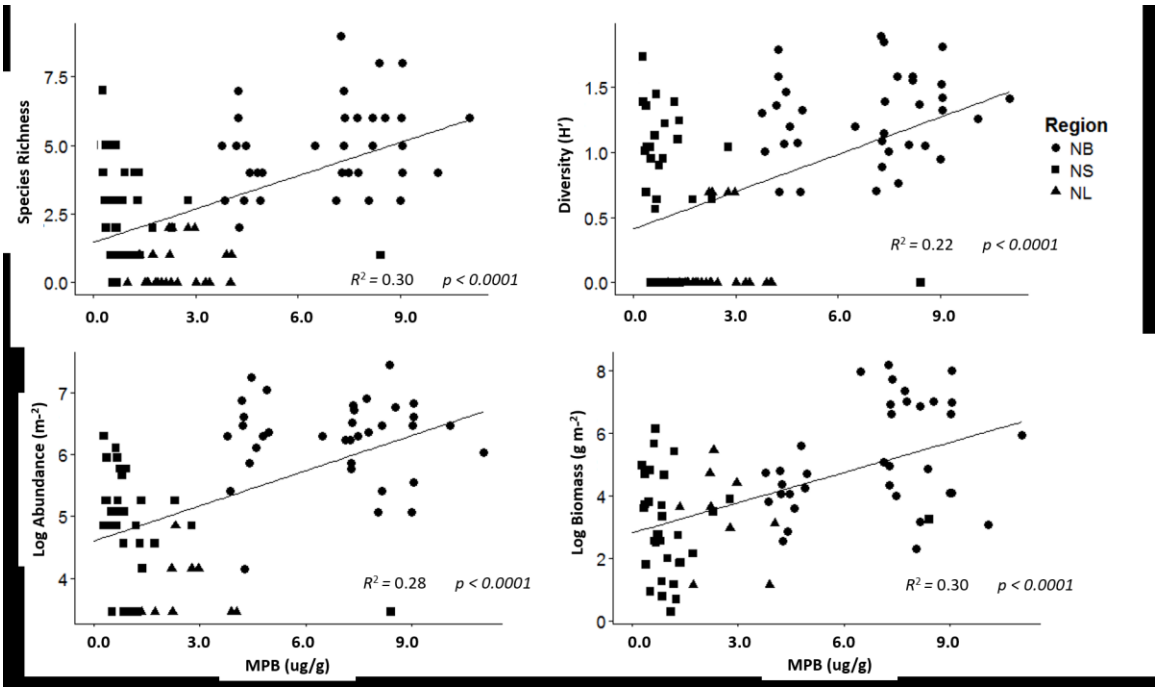


Figure 8. Linear relationships between MPB and individual infauna summary measures (species richness, diversity, log abundance and log biomass) across three provinces, New Brunswick (NB), Nova Scotia (NS) and Newfoundland (NL) in Atlantic Canada.

## 2.5 Discussion

Seagrass beds provide essential structure, functions and ecological services to coastal ecosystems and their associated macroinfaunal communities are important indicators of ecosystem health, yet how these differ across large spatial scales in Atlantic Canada has not been thoroughly studied. Our large-scale field surveys revealed clear regional patterns

in the macroinfaunal communities with distinct differences between Nova Scotia, New Brunswick and Newfoundland; however, eelgrass bed structure and environmental parameters tended to be more influenced by local conditions at the study site level. The abundance of microphytobenthos, a proxy of benthic primary production, was the clearest driver of regional patterns and consistently the best predictor for infauna species richness, diversity, abundance and biomass across all regions. In addition, structural eelgrass bed parameters as well as nitrogen tissue content and stable isotopes explained differences in individual species. Overall, our results provide insight into the regional variation of eelgrass beds and macroinfauna communities across Atlantic Canada as well as the potential drivers of these spatial differences.

### *2.5.1 Spatial variation in eelgrass bed structure and environmental variables*

Temperate seagrasses, such as eelgrass, are highly dependent on light availability (Dennison & Alberte 1985, Orth & Moore 1986, Lee et al. 2007) and reduced light penetration from depth and poor water quality can have a strong influence on their growth and production (Moore et al. 1996, Frederiksen et al. 2004). The three regions in this study had a clear depth gradient with eelgrass beds in Nova Scotia being located the deepest, followed by Newfoundland and New Brunswick. Our survey data indicated a pattern of higher shoot density, canopy height and above- and below-ground biomass in Nova Scotia, although this was only significant for above-ground biomass. A common response to increased water depth is longer eelgrass blades (canopy height) in order to obtain light for photosynthesis (Larkum et al. 2006), whereas areas of higher wave exposure tend to have increased below-ground biomass for stability (Fonseca & Bell 1998). Most Nova Scotia sites were deeper and more exposed than the other two regions, which could explain the

longer blades and higher biomass. In comparison, our New Brunswick sites were located in shallow, sheltered estuaries or bays that included areas of both high and low eutrophic conditions (Coll et al. 2011, Schmidt et al. 2012). Higher eutrophic conditions often lead to shorter blades and reduced shoot density and biomass due to light limitation from the increase in water column turbidity and overgrowth by benthic and epiphytic algae as well as the hostile chemical environment due to oxygen depletion (Short et al. 1995, Moore et al. 1996, Hauxwell et al. 2003). With the exception of one site (Goose Bay) in Newfoundland, which had observable eutrophic symptoms, all sites in Newfoundland and Nova Scotia were considered low impact with respect to nutrient loading. Interestingly, topography and exposure in Newfoundland were more similar to Nova Scotia, however the canopy structure was more similar to New Brunswick. Under these similar conditions, we would have expected eelgrass bed structure in Newfoundland to be more similar to Nova Scotia. The lower sample size in Newfoundland and higher variation explained by residuals, indicates that our survey did not capture one or more important driver(s) in determining the local patterns in eelgrass canopy structure.

Above-ground biomass was the only eelgrass component to have a significant regional effect while all other components were mostly explained by site. This is not surprising since hydrodynamics can have a strong influence on plant structure (Fonseca & Bell 1998) and each estuary or bay within a region is not exactly alike. It is interesting, however, that above-ground biomass is showing strong regional effects but canopy height and shoot density are not despite above-ground biomass being essentially a combination of these two metrics. A possible explanation is that the site variation in individual metrics is too large to establish regional effects, however when combining the two the site variation

dampens and a regional pattern emerges. This may indicate that while individual components such as canopy height, shoot density and below-ground biomass are strongly influenced by local conditions, overall above-ground biomass is varying on a larger scale.

The site effect in above-ground tissue carbon (%), and above- and below-ground tissue nitrogen (%), $\delta^{13}C$ and $\delta^{15}N$ is likely due to the integration of water column nutrients into eelgrass tissue which can also be used as a reflection of nutrient availability of the surrounding waters (Short 1987, Duarte 1990, Lee et al. 2007). Further, below-ground tissue plays an important role in the storage of nutrients (Duarte 2002, Schmidt et al. 2011, Greiner et al. 2013) and our regional effects of below-ground % tissue carbon and nitrogen suggest that this may be a reflection of longer-term conditions of carbon and nitrogen concentrations captured on a regional scale. Considering the location of each region, Nova Scotia and Newfoundland sites were located in the open Atlantic Ocean whereas New Brunswick sites were located in the southern Gulf of St. Lawrence, some of which were located in the more sheltered Northumberland Strait. We can expect that higher mixing is occurring in Nova Scotia and Newfoundland, which could be leading to similar above-ground % N as low- and high-impact sites in New Brunswick. However, the significantly higher below-ground % nitrogen in New Brunswick is likely indicating higher long-term nitrogen loading conditions (McIver et al. 2015). Nutrient input within a region can vary immensely due to different point and non-point sources within the area (Lepoint et al. 2004, McIver et al. 2015). Specifically, in New Brunswick we can see Lamèque driving the within region variability in $\delta^{15}N$ (Appendix 2A: Figure 3) due to the input from the seafood processing plant (McIver et al. 2015). We also see higher below-ground percent tissue nitrogen at the high impact sites (Lamèque, Cocagne and Bouctouche) compared to the low

impact sites (Kouchibouguac and Tabusintac) in New Brunswick (Appendix 2A: Figure 3). While the sources of nutrient loading have been identified for New Brunswick (McIver et al. 2015), sources have not yet been quantified for Nova Scotia and Newfoundland which may help to explain site variability.

Differences in the sediment organic content were more difficult to assess regionally due to the lack of Newfoundland data; however, the significant site effect suggests that organic content is varying locally within Nova Scotia and New Brunswick rather than on a larger regional scale. Sediment deposition is largely influenced by water movement as well as the reduction of water flow by the seagrass canopy (Fonseca & Fisher 1986, Cabaço et al. 2008) so it is likely that eelgrass structure and/or circulation patterns at each site are influencing organic deposition on the local level. In contrast, while both region and site had a significant effect on the microphytobenthos, most of the variation was explained by region. The highest amounts were found in New Brunswick followed by Newfoundland and then Nova Scotia, which also follows the depth gradient between these regions. The significant negative relationship between depth and microphytobenthos distinctly illustrates the shallower New Brunswick sites experiencing higher microphytobenthos concentrations. Increased light availability and a higher source of nutrients increases microphytobenthos productivity (MacIntyre et al. 1996) which can explain the higher amount of microphytobenthos in the shallower New Brunswick sites. Additionally, higher microphytobenthos concentrations are usually found in muddy, sheltered habitats as opposed to more exposed, sandy habitats (Cadée & Hegeman 1977, Delgado 1989). All New Brunswick sites were located in sheltered estuaries or bays with muddy sediment, whereas Nova Scotia and Newfoundland sites were most exposed and sediment ranged

from muddy-sand to cobble. Because our regions greatly differed in depth and exposure and these are known drivers of microphytobenthos biomass, we can more clearly understand the reasons behind the spatial variation of benthic microalgae in these areas.

### *2.5.2 Spatial variation in macroinfauna communities*

Contrary to the environmental and eelgrass parameters, the composition of the infauna community as well as its summary measures (species richness, diversity, total abundance and biomass) were strongly explained by region. This was particularly evident in the summary measures where New Brunswick had significantly higher species richness, diversity, total abundance and total biomass, and Newfoundland had the lowest. Regional clusters for Nova Scotia and Newfoundland were clearly illustrated for community composition based on both abundance and biomass, whereas some New Brunswick sites were more dissimilar from each other. In particular, Lamèque, Baie St. Simon, Tabusintac and Kouchibouguac are clustering away from Cocagne and Bouctouche. Based on the eutrophication levels categorized by Schmidt et al. (2012), Kouchibouguac, Tabusintac and Baie St. Simon were all considered to be low eutrophic sites, while Lamèque, Cocagne and Bouctouche were sites of high eutrophication. While we do see some indication of sites clustering with respect to high vs low impact in New Brunswick, it also appears that the physical environment and geographic location may also be influencing the similarities between these sites.

In terms of community composition based on infauna abundance, Lamèque and Baie St. Simon were found to be most similar to each other and may represent the similarity in physical structure of these sites such as the estuary shape and in/outflow, as well as the close geographic location in the most northern part of New Brunswick (Figure

1, Table 1). We also see Kouchibouguac being most similar to Tabusintac which may reflect them both being low impact sites; Kouchibouguac being surrounded by a National Park and Tabusintac a protected wetland area (Coll et al. 2011, Schmidt et al. 2012, McIver et al. 2015). Furthermore, Cocagne and Bouctouche are both high impact sites and located in the southernmost part of New Brunswick in the Northumberland Strait (Coll et al. 2011, Schmidt et al. 2012, McIver et al. 2015) which may be explaining the strong similarities in infauna community composition based on abundance at these two sites.

When looking at community composition based on infauna biomass we see similar patterns to abundance. Cocagne and Bouctouche are clustering together, however Tabusintac becomes more similar to Lamèque and Baie St.Simon, which are all located outside of the Northumberland Strait in northern New Brunswick. Further, Kouchibouguac is dissimilar from all the other New Brunswick sites and could be a result of being geographic located in the middle of these two groups of sites and/or being bordered by a National Park. These results suggest that community composition differences within New Brunswick, particularly based on biomass, may be more closely linked to geographic location rather than eutrophication impacts.

### *2.5.3 Links between the environment/eelgrass bed structure and macroinfauna*

Microphytobenthos consistently came out as the best predictor for the overall infauna community composition as well as for species richness, diversity, total abundance and biomass. Marine benthic microalgae are an important component of the coastal food web (Daehnick et al. 1992, Hillebrand et al. 2000) and our results demonstrate their significance in shaping macroinfaunal communities. The

microphytobenthos had a significant positive relationship with infauna richness, diversity, abundance and biomass with all of these measures being highest in New Brunswick where microphytobenthos was also highest. We also see the microphytobenthos correlating best with the individual SIMPER species. This is not surprising as the microphytobenthos are not only an important food source for deposit feeders, but the resuspension of particles into the water column also provides a rich food source for suspension feeders (Mayer et al. 1993, Miller et al. 1996). The microphytobenthos can be used as a proxy of benthic productivity (MacIntyre et al. 1996) and play an important role in the exchange of nutrients between the sediments and the water column (Rizzo et al. 1992, Sundback et al. 2000, Engelsen 2008). In the present study, the combination of shallower habitats and nutrient enrichment in New Brunswick appears to be leading to higher benthic productivity and in turn, more diverse, abundant macroinfaunal communities.

When examining other predictors of the macroinfaunal assemblage and summary measures, results from the BIOENV procedure as well as the GLMs indicate that there are complex associations between eelgrass bed structure and environmental parameters driving responses in the macroinfauna. Particularly below-ground $\delta^{15}N$ which came out as a significant predictor for all summary metrics except total abundance, indicating that the source of nitrogen in the roots is influencing marcoinfauna. Except one site in New Brunswick (Lamèque), which is significantly influenced by wastewater from a seafood processing plant (McIver et al. 2015), no other sites had elevated $\delta^{15}N$ suggesting that it may be the available nitrogen atmospheric deposition or fertilizer application (+2 to +6‰) rather than wastewater sourced nitrogen (Lepoint et al. 2004, Kendall et al. 2007).

However, the positive influence of $\delta^{15}N$ on the macroinfauna could be driven by the higher $\delta^{15}N$ in Lamèque since it also had a higher species richness, diversity and biomass than all other sites (Appendix 2A: Figure 4). It is possible that the $\delta^{15}N$ within the range of atmospheric deposition has a strong influence on macroinfauna, however it appears that the effects of higher $\delta^{15}N$ from wastewater sources on macroinfauna may be influencing these patterns.

Interestingly, each individual SIMPER species was correlated with various different combinations of eelgrass bed structure and environmental parameters. Overall, however, combinations always included an eelgrass structural variable along with an underlying environmental variable(s). Tissue % nitrogen, carbon and nitrogen stable isotopes, microphytobenthos and annual algae were all correlated to one or more infauna species, indicating that eelgrass bed structure as well as some indicator of primary production or nutrient enrichment is important in influencing individual infauna species abundance and biomass.

## 2.6 Conclusion

Overall, our results demonstrate strong regional patterns in overall macroinfaunal communities that could be largely linked to an indicator of benthic primary production (microphytobenthos). However, we also found site-by-site variation in summary community measures (richness, diversity, abundance, biomass) and individual infauna species which could be linked to differences in eelgrass bed structure as well as indicators of primary production (microphytobenthos, annual algae) and nutrient availability (% nitrogen, $\delta^{15}N$, $\delta^{13}C$). These results have important implications for conservation and management strategies for eelgrass habitats in Atlantic Canada; the status of and changes

in eelgrass bed structure and individual infauna species should be assessed at a site-by-site spatial scale, while the overall infauna community composition and structure can be assessed on a larger, regional spatial scale. Further, this study illustrates the importance of assessing the microphytobenthos on a larger regional scale, a knowledge gap where research is lacking (MacIntyre et al. 1996, Miller et al. 1996). Because the microphytobenthos biomass is strongly influencing the macroinfauna community, this may serve as a good monitoring tool to assess changes in primary production and macroinfauna communities over space and time. Finally, since the macroinfauna community can serve as an important indicator of eelgrass bed health, we can use changes in community composition on both local and regional scales to implement monitoring and management of these ecosystems.

# Chapter 3 – Impacts of organic enrichment from finfish aquaculture on *Zostera marina* and associated macroinfaunal communities in Atlantic Canada

## 3.1. Abstract

Seagrass beds are among the most productive and diverse marine ecosystems. In Atlantic Canada, eelgrass, *Zostera marina*, provides habitat and a rich food source for many epibenthic and infauna deposit feeders. Changes in these benthic communities have been linked to organic enrichment and eutrophication and are used as important tools for evaluating ecosystem health. In Nova Scotia, there has been growing concern about the impacts of the finfish aquaculture, and this research aimed to quantify the impacts of organic enrichment from finfish aquaculture on eelgrass beds and their associated macroinfaunal communities. We selected 3 study sites with eelgrass habitats at increasing distances from a finfish farm and a reference site in an adjacent unimpacted bay. Using extensive field surveys, we analyzed differences in environmental parameters, eelgrass bed structure and macroinfauna communities across sites and aimed to link observed differences in macroinfauna communities to the environment or eelgrass bed structure using multivariate distance matrices and generalized linear models. Our results show increased organic enrichment, decreased eelgrass biomass and shoot density, and decreased infauna biomass closer to the finfish farm. Although there were no significant differences in infauna richness and diversity across sites, community structure significantly differed and some sensitive species disappeared while tolerant species increased closer to the farm. Observed differences in macroinfauna communities could be linked to differences in eelgrass structure and underlying environmental parameters.

These results provide new insight into the impacts of finfish aquaculture on eelgrass habitats in Nova Scotia and prove useful in assessing and monitoring ecosystem changes.

## 3.2. Introduction

Seagrass beds are among the most productive ecosystems on the planet and are the most diverse of all the soft-bottom marine communities, however they continue to be threatened worldwide (Orth et al. 2006, Kuo & Hartog 2007, Waycott et al. 2009). Increased nutrient loading from anthropogenic inputs such as municipal and industrial effluent discharge (e.g. sewage, wastewater), land run-off, and more recently, marine aquaculture, have become some of the most influential causes of degradation to macrophyte habitats in coastal waters (Arzul et al. 1996, Hauxwell et al. 2003, Lotze et al. 2006, Waycott et al. 2009). Eelgrass (*Zostera marina*) is Atlantic Canada's local seagrass and has been designated as an ecologically significant species (ESS) due to its crucial role in sediment stabilization and essential habitat for numerous species (DFO 2009a, 2011). Eelgrass also provides key ecological services including nutrient cycling, carbon sequestration, reduction of wave action (Moore et al. 1996, Short & Wyllie-Echeverria 1996, Schmidt et al. 2011), and has an extensive below-ground root and rhizome system that stabilizes sediments and provides a rich food source for epibenthic and infauna deposit feeders (Orth 1973, Orth et al. 1984, Boström & Bonsdorff 1997). Changes in these benthic macrofaunal communities have been an important tool in determining the impacts of organic enrichment and eutrophication in the marine environment and contribute to evaluating ecosystem health (Pearson & Rosenberg 1978, Henderson & Ross 1995).

The impacts of marine fish farms on seagrass and macrofaunal communities have been documented in several regions worldwide (Delgado et al. 1999, Ruiz et al. 2001, Cancemi et al. 2003, Apostolaki et al. 2007). These studies repeatedly show that an increase in organic material in the form of faeces and food debris from fish farms leads to a decline in shoot density and biomass, and in some cases complete disappearance of seagrass habitats under and around the farm. Additionally, the ecological impacts of waste discharges from net pens have been shown to reduce water quality, simplify the community structure beneath the pen and reduce the abundance, diversity and species richness of the benthos (Henderson & Ross 1995, Milewski 2001). Some benthic species, such as the opportunistic polychaete *Capitella capitata*, are more successful under anaerobic conditions and therefore become dominant as sediment quality decreases due to organic loading in the marine environment (Pearson & Rosenberg 1978). While the impacts of organic enrichment from finfish aquaculture on seagrass beds have been extensively studied, most of these studies have been performed in the Mediterranean where seagrass can be found directly beneath fish pens at 16 to 39 meter depths (Apostolaki et al. 2007, Holmer et al. 2007, 2008). In Atlantic Canada, eelgrass is found at much shallower depths due to light limitation in temperate waters (Hemminga & Duarte 2000, DFO 2009a) and therefore often not directly under but adjacent to fish pens. This study was therefore unique in that aquaculture impacts were assessed on a bay-wide scale to assess how eelgrass beds differed based on their proximity to the finfish farm.

In 1994, a finfish (*Salmo salar* and *Oncorhynchus mykiss*) aquaculture farm opened in Port Mouton Bay, Nova Scotia and was active for 15 years before being fallowed from 2009-2012 and then reopening again in 2012 (Loucks et al. 2012, Friends

of Port Mouton Bay 2014). Using local ecological knowledge it was determined that eelgrass was not only present but lush and healthy before the farm opened but showed a gradual decline, and in some areas complete disappearance, while the farm was in operation (Lee 2014). However, after the fallowing period the reappearance of eelgrass was recorded (Friends of Port Mouton Bay 2014, Lee 2014). To date there is limited scientific data on the effects that these finfish aquaculture farms are having on the structure of eelgrass beds and their associated macroinfaunal communities in Nova Scotia. This study aimed to address this gap by assessing the changes in eelgrass bed structure and associated macroinfaunal communities based on their proximity to the finfish farm and in comparison to a reference site with no finfish aquaculture present. Our results will provide insight into the changes in eelgrass habitats due to organic enrichment and nutrient loading from finfish farms in Nova Scotia and could be considered as a way to assess and monitor the local impacts of organic enrichment in these ecosystems.

## 3.3. Methods

### *3.3.1 Study area*

Study sites were located along the Atlantic coast of southern Nova Scotia (Table 1, Figure 1). In Port Mouton Bay, three sites were selected at varying distances from the finfish farm. Initially, two additional sites (Jackie's Island and Port Mouton Island, Figure 1) were included based on a pilot survey in spring; however, when revisited during the summer sampling period, the eelgrass beds at both of these sites had nearly disappeared and therefore could not be sampled. A reference site was selected in an adjacent bay (Port Joli, Figure 1) and considered unimpacted due to being bordered by

Kejimkujik National Park and Thomas Raddall Provincial Park and located near little human development. It is important to note that approximately 4 months prior to sampling a super chill event occurred, killing almost all of the fish at the farm (CBC 2015). The farm was not restocked before the sampling period; however, the site was given a five-year lease renewal with plans to restock the following spring (2016). All four sites were located in shallow, soft-sediment areas with eelgrass as the dominant macrophyte (continuous beds >50m).

Table 1. Site characteristics and abbreviations for the four study sites sampled in July 2015. Three sites were located in Port Mouton Bay, Nova Scotia where a finfish farm is present and one site located in adjacent Port Joli Bay as a reference site.

| Site | Abbreviation | Distance from farm | Temperature (°C) | Depth (m) | Bottom Type |
|---|---|---|---|---|---|
| Spectacle Island | SI | 300 m | 15 | 2.0 | Mud |
| Carters Beach | CB | 700 m | 12 | 2.5 | Sandy-mud |
| Old Warf Port Joli | OW | 3000 m | 14 | 1.7 | Mud |
| | PJ | Reference (>10 km) | 15 | 2.9 | Sandy-mud |

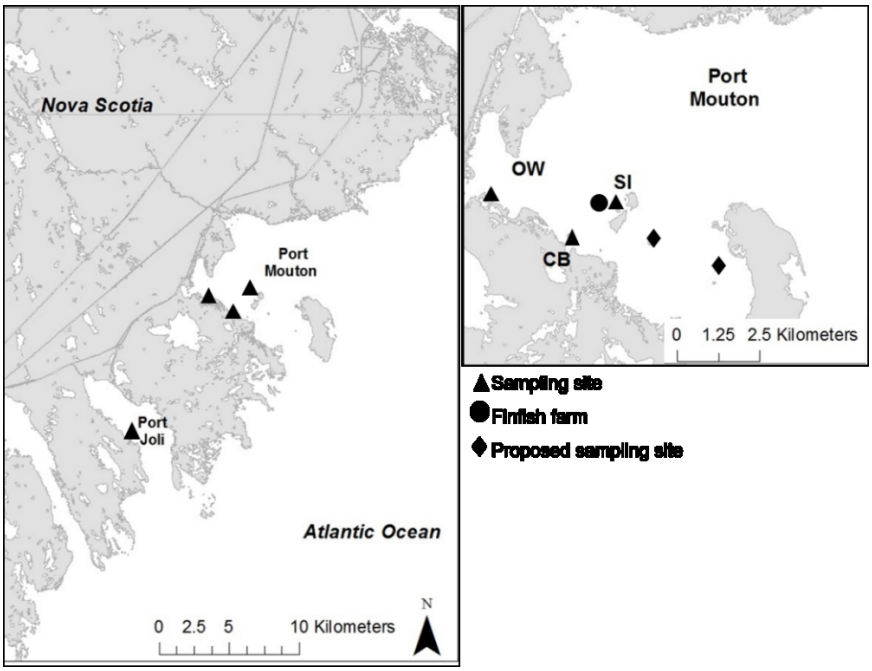


Figure 1. Map of the four sampling sites on the Atlantic coast (left) and detailed map with the location of the finfish farm and sampling sites in Port Mouton Bay (right). Proposed sampling sites indicate areas where eelgrass was no longer present. Refer to Table 1 for full site names and details.

### *3.3.2 Sampling design and data collection*

From July 14-21st 2015, we conducted extensive field surveys that followed and expanded upon the sampling design of Schmidt et al. (2011, 2012, in review). At each site, two 50 x 4 m transects were laid parallel to the shore inside the eelgrass bed ≥10 m from the vegetation-bare substrate interface. Using SCUBA, eelgrass canopy structure (shoot density and canopy height) was assessed using 6 quadrats (0.5 x 0.5 m, with 0.25 m subsections) at 0, 30, 50 m (first transect) and 5, 25, 45 m (second transect) during high tide. In the same six quadrats, percent cover of all epiphytic and benthic macroalgae was recorded. From these species, we then identified the annual macroalgae used as a common indicator of eutrophication (Worm & Lotze 2006, Schmidt et al. 2012). This included the green algae *Ulva intestinalis* and *Spongomorpha* sp., and the brown algae

*Ectocarpus siliculosus, Pilayella littoralis* and *Sphaerotrichia divaricata.* Seagrass biomass as well as the abundance of sediment infauna were collected using a sediment core (0.2 m diameter; 0.2 m deep) at the same 6 locations along each transect. In addition, microphytobenthos (MPB) and sediment organic content were collected using syringes (2.6 cm diameter; 2 cm and 5 cm depth, respectively) at the same 6 locations (see below for details). Sediment type of each core was recorded (e.g. sand, mud, sandy-mud) as well as the presence of any sulfur smell indicating hypoxia or anoxia. Sea surface temperature and sampling depth were recorded on SCUBA dive computers during the field survey.

To determine sediment organic content, two samples were taken from the upper 5 cm of sediments (volume of sample ~ 8.83 mL) with a 60 mL syringe core at the six core sampling locations as described above. Both samples were placed in a plastic bag and frozen until processed. In the laboratory, samples were thawed and mixed. Approximately 1 g of wet sediment was placed in a crucible which was previously ashed and weighed. Crucibles were placed in the drying oven at 60°C for 48 hours, removed and weighed for dry weight. Samples were then placed into a muffle furnace and combusted at 500°C for 6 h followed by 2 h in the drying oven. We then weighed the crucible + ashed sample for ash weight. Percentages were calculated to determine overall percent organic content.

Also using a 60 mL syringe core, three MPB samples were removed from the upper 2 cm of the sediments (volume of sample ~ 3.53 mL) at the six core sampling locations. Each set of three samples were combined together on site, placed in plastic cryovials and stored in liquid nitrogen while in the field and then a freezer (-20°C) until

analysis in the laboratory. Samples were always kept in a darkened room throughout processing. First, frozen sediment samples were placed in labeled glass scintillation vials with 10 mL of 90% acetone, vortexed for 1 minute and then placed back in the freezer to be digested for 24 hours. The following day samples were vortexed for one minute, placed in falcon tubes and centrifuged for 30 minutes at 3250 rpm (T. Whitsit, Dalhousie, pers. comm). The supernatant was subsequently pipetted into clean scintillation vials and measured in a Turner Designs 10005R fluorometer to determine chlorophyll *a* concentrations.

Canopy structure was examined using the 0.25 x 0.25 m inset of the sampling quadrat to count shoot density and measure canopy height. To determine canopy height, we held the zero end of the measuring tape against the substrate in the centre of the quadrat and extended it to the average height of the plants. To examine the eelgrass above- (AG) and below-ground (BG) biomass, the sediment core was pressed into the sediment at each sampling location and brought to the surface where all above- and below-ground tissue was removed, rinsed in a 500 µm sieve to capture any fauna, bagged and kept on ice. In the laboratory, the blades, roots and rhizomes were rinsed again and all epiphytes were carefully scraped off the blades and then weighed for biomass (wet weight, g $m^{-2}$) prior to drying in an oven at 60˚C for 48 hours and weighed again for dry weight (g $m^{-2}$).

Carbon and nitrogen content was determined using a 50 mg dry weight subsample of each of the above- and belowground tissue after biomass weights were recorded. Tissue samples were sent to the University of California Davis Stable Isotope facility for

analysis of % tissue nitrogen (N) and carbon (C), and nitrogen ($\delta^{15}N$, $^{15}N$:$^{14}N$) and carbon ($\delta^{13}C$, $^{13}C$:$^{12}C$) stable isotopes.

Macroinfauna abundance was collected using the same sediment core samples that were used for the above- and below-ground biomass collection. The core samples were sieved on site using a 500 µm sieve and all species identified to the lowest possible taxon. If organisms needed further identification they were brought back to the laboratory and examined under the microscope. Individuals of each species were counted (abundance $m^{-2}$) and weighed (g $m^{-2}$).

### *3.3.3 Data analysis*

The aim of this study was to: a) Test for differences in environmental parameters (sediment organic content, MPB, percent tissue carbon and nitrogen, $\delta^{13}C$ and $\delta^{15}N$, percent cover of annual algae) and eelgrass structure (shoot density, canopy height, AG and BG biomass) between sites based on distance from the finfish farm, b) Test for differences in macroinfauna abundance, biomass, richness, diversity and community composition based on distance to the finfish farm, and c) Link observed differences in macroinfaunal communities to the environment and/or eelgrass bed structure using multivariate distance matrices and generalized linear models.

#### *3.3.3i Eelgrass structure and environmental parameters*

First, multivariate permutational analysis of variance (PERMANOVA) were used to assess the effect of site (fixed factor) on variables that were not independent (i.e., shoot density and canopy height, AG and BG eelgrass biomass, % tissue nitrogen and carbon, AG and BG $\delta^{13}C$, and AG and BG $\delta^{15}N$), and these were only assessed individually if

significant differences were found ($p \leq 0.05$). Next, univariate PERMANOVAs were used to assess whether there was a significant effect of site (fixed factor) on individual environmental and eelgrass parameter. If significant effects were found, post-hoc pairwise tests were performed to determine which sites were significantly different from each other.

*3.3.3ii Macroinfauna*

Species richness, total abundance, total biomass and Shannon-Wiener Diversity (H') were first calculated and then averaged (+SE) across the 6 cores at each site. We then used univariate PERMANOVAs to identify significant differences in individual summary measures between sites. To determine differences in community composition between sites, multivariate PERMANOVAs were applied on zero adjusted Bray-Curtis similarity matrices based on abundance (density) and biomass data separately. Abundance and biomass data were square-root transformed in order to down-weight the influence of highly abundant or large species (Clarke & Gorley 2006). If a significant effect of site was detected, we used post-hoc pairwise tests to determine which sites were significantly different from each other. To visualize the data and support PERMANOVA results, centroids were computed for each site and group average cluster analysis performed on the centroids. To determine which species contributed most consistently (>10%) to the differences between sites, we used similarity percentages (SIMPER) analysis (Anderson et al. 2008).

We used the abundance-biomass comparison (ABC) method as a graphical way to detect pollution effects on the macrobenthic community (Warwick 1986) . This technique uses the log species rank (x-axis) and the cumulative percent dominance (y-axis) to create

the comparison of *k*-dominance curves for abundance and biomass at each site. In unpolluted sites, the biomass curve will lie above the abundance curve, in moderately polluted areas the two curves will closely coincide, and in grossly polluted sites the abundance curve will lie above the biomass curve (Warwick 1986, Warwick et al. 1987). This graphical demonstration expands on the theory by Pearson and Rosenburg (1978) where unpolluted sites will have less but larger individuals, but will shift to higher abundances of small opportunistic species as pollution level increases.

*3.3.3iii Linking the environment/eelgrass structure to the macroinfauna*

First, correlations among all variables (sediment organic content, MPB, % cover annual algae, AG and BG % tissue nitrogen and carbon, AG and BG $\delta^{13}C$ and $\delta^{15}N$, AG and BG biomass, shoot density, canopy height) were tested and any variables with high correlation (>0.7) were never included in the same analysis. Consequently, the uncorrelated variables used in all multivariate analyses were sediment organic content, MPB, % cover annual algae, eelgrass shoot density and canopy height, below-ground eelgrass biomass and $\delta^{15}N$ in below-ground tissue. If variables were equally correlated with others (e.g. AG and BG biomass), we chose to include those that were more relevant for infauna (e.g. BG biomass).

The BEST/BIOENV procedure was used to identify possible correlations between combinations of variables for the environment and/or eelgrass structure (Euclidean distance matrix) to Bray-Curtis similarity matrices of the infauna community based on abundance and biomass data separately. We further tested correlations between environmental/eelgrass variables and individual SIMPER species. BIOENV provides a non-parametric index rho (ranging from 0 to 1) that indicates how closely the

environmental variables explain the multivariate pattern of the species. We then used a permutation test to determine the significance level of the sample statistic (rho).

To link the overall response of the infauna community to different environmental and eelgrass variables, we used generalized linear models (GLMs). Models were fitted to total abundance, total biomass, species richness and Shannon diversity index (H') using various sets of uncorrelated environmental and eelgrass canopy variables as predictors. GLMs were fitted to the data using a normal Gaussian distribution (species richness and diversity) and a negative binomial distribution (total abundance and biomass). For each model, residuals were examined to check the assumptions of normality and homogenous variance.

All PERMANOVAs, cluster analyses, ABC method and the BEST/BIOENV procedure were carried out using PRIMER (version 6.1.11) with PERMANOVA+ (version 1.0.1, PRIMER-E, Plymouth) while regressions and generalized linear models were completed using R (R version 3.2.1).

## 3.4 Results

### *3.4.1 Environmental parameters*

Bottom temperature ranged from 12-15$^{o}$C between the four sites, while sampling depth ranged from 1.7-2.9 m (Table 1). Sediment organic content differed between sites (PERMANOVA: Pseudo-F = 7.63, $p < 0.001$) with SI and OW having significantly higher organic content than CB and PJ (Figure 2). Microphytobenthos (MPB) did not differ significantly between sites (Pseudo-F = 1.07, $p = 0.205$), although higher average

values were observed at the three Port Mouton sites (SI, CB and OW) compared to PJ (Figure 2).

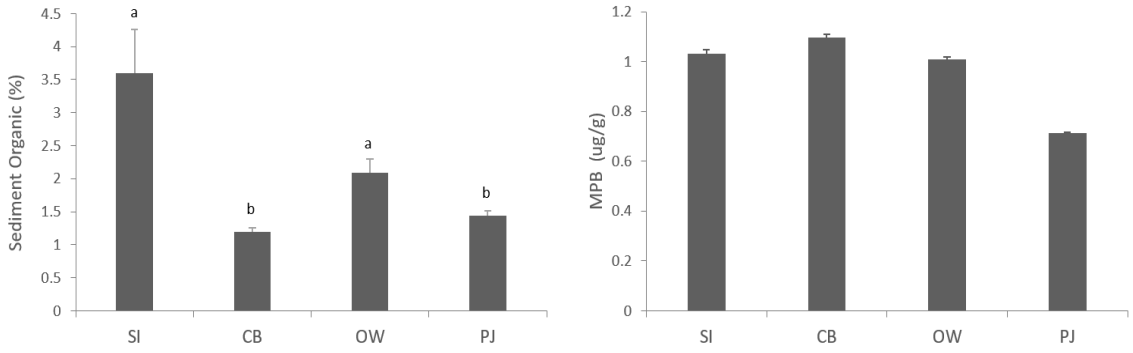


Figure 2. Sediment organic content and microphytobenthos (MPB) concentration (mean + SE, n = 6) at the four study sites (from left to right: increasing distance from farm and PJ reference site) in Nova Scotia, Canada. Lower case letters indicate significant differences ($p \leq 0.05$). Refer to Table 1 for site abbreviations and details.

No significant differences were found between tissue % nitrogen (N) and carbon (C) (Table 2) and no distinct patterns were observed across sites. Furthermore, tissue % N and C were correlated with N and C stable isotopes and were therefore no longer used in the analyses. Nitrogen stable-isotope ratios ($\delta^{15}N$) did show marginally non-significant differences across sites for AG and BG tissue (Table 2) with higher observed $\delta^{15}N$ at CB and PJ than SI and OW (Figure 3). Carbon stable-isotope ratios ($\delta^{13}C$) however, showed no significant differences across sites for AG or BG tissue (Table 2) and observed patterns varied (Figure 3).

Table 2. Results from multivariate and univariate PERMANOVAs on the effect of site on eelgrass structure as well as tissue nitrogen (%N) and carbon (%C) and stable isotopes ($\delta^{15}N$, $\delta^{13}C$). If no significant differences were found in the multivariate analysis, a univariate analysis was not performed. Significant differences ($p \leq 0.05$) are bolded.

| | | | | Multivariate | | Univariate | |
|---|---|---|---|---|---|---|---|
| | | DF | RDF | pseudo-F | p | pseudo-F | p |
| Shoot Density | | 3 | 20 | 1.24 | 0.32 | - | - |
| Canopy Height | | | | | | - | - |
| Biomass | - Above | 3 | 19 | 2.89 | **0.03** | 3.033 | **0.035** |
| | - Below | | | | | 2.767 | 0.074 |
| %C | - Above | 3 | 19 | 0.47 | 0.89 | - | - |
| | - Below | | | | | - | - |
| %N | - Above | | | | | - | - |
| | - Below | | | | | - | - |
| $\delta^{13}C$ | - Above | 3 | 19 | 0.45 | 0.84 | - | - |
| | - Below | | | | | - | - |
| $\delta^{15}N$ | - Above | 3 | 19 | 2.58 | 0.06 | - | - |
| | - Below | | | | | - | - |

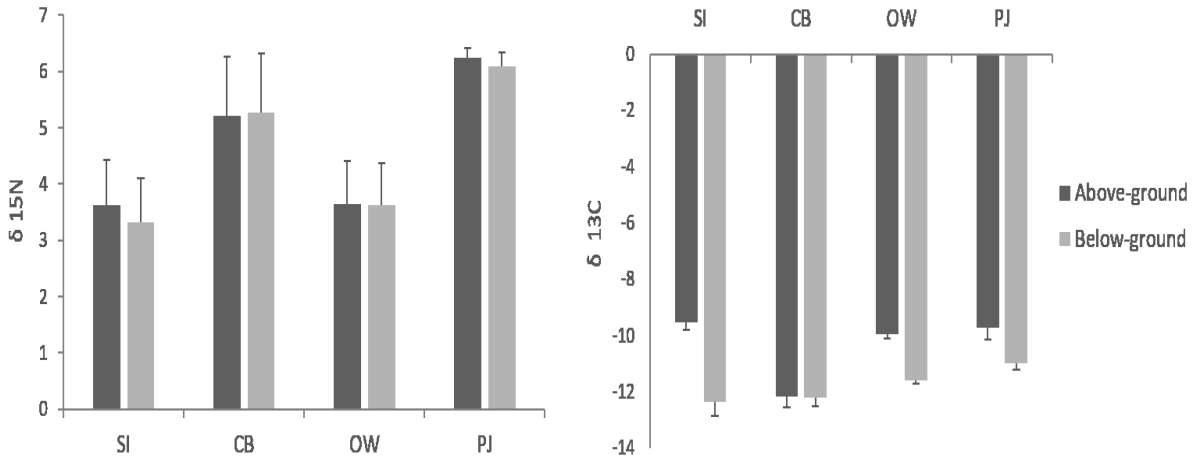


Figure 3. Stable-isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) from above and below-ground eelgrass tissue (mean + SE, n = 6) at the four study sites (from left to right: increasing distance from farm and PJ reference site) in Nova Scotia, Canada. Refer to Table 1 for site abbreviations and details.

### *3.4.2 Eelgrass bed structure*

Multivariate PERMANOVA detected no significant differences for shoot density and canopy height across sites (Table 2, Figure 4). However, a linear regression for shoot density with increasing distance from the farm was almost significant (Figure 5c). AG and BG biomass also decreased at sites closer to the fish farm (Figure 4, 5). Multivariate PERMANOVA found significant differences across sites, which were significant for AG biomass yet marginally non-significant for BG biomass (Table 2). Post-hoc tests revealed that SI and CB had significantly lower AG biomass than the reference site (Figure 4) and linear regression analyses confirmed that these trends were significant across distance (Figure 5). As it appeared that there may be a threshold, specifically in BG biomass and shoot density, we also tried non-linear regressions, however these results did not differ much from the linear regressions shown here.

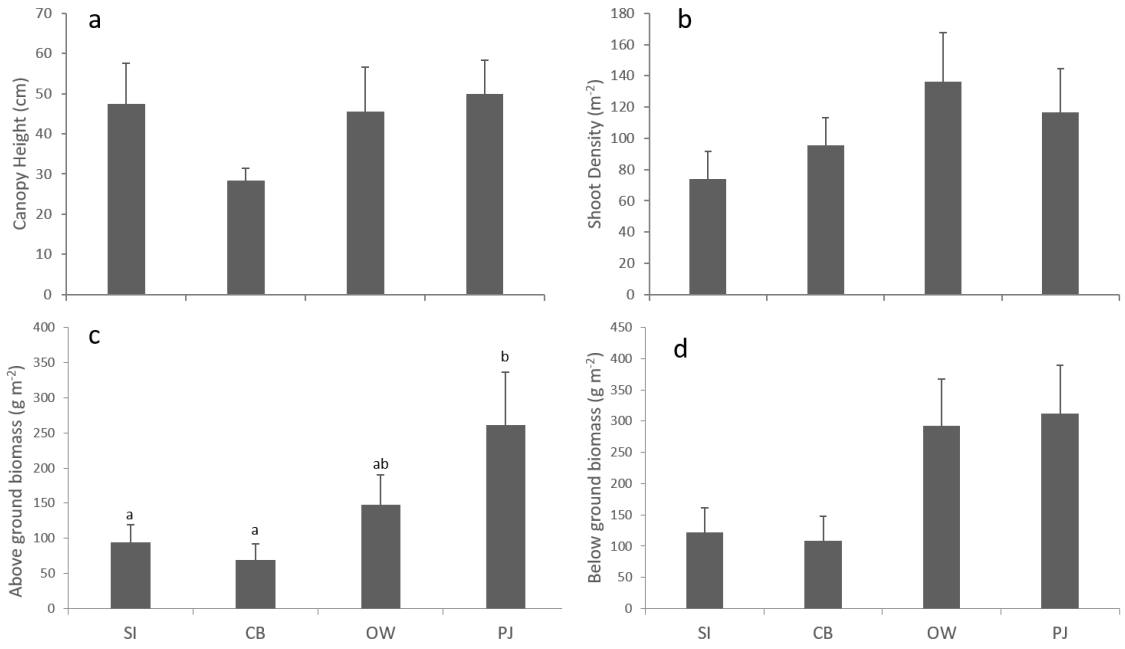


Figure 4. Eelgrass canopy height (a), shoot density (b), above-ground biomass (c) and below-ground biomass (d) (mean + SE, n = 6) at the four study sites (from left to right: increasing distance from farm and PJ reference site) in Nova Scotia, Canada. Lower case letters indicate significant ($p \leq 0.05$) differences. Refer to Table 1 for site abbreviations and details.

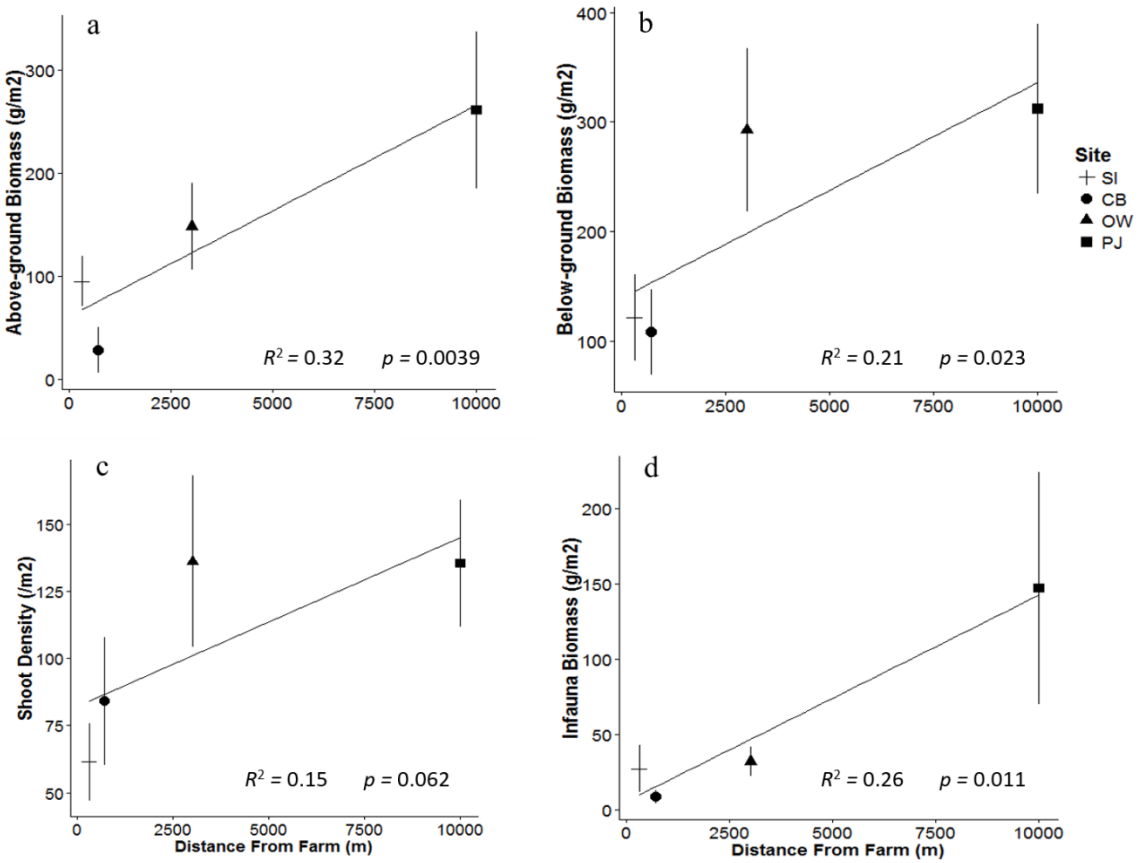


Figure 5. Linear regression of the mean (± SE) of AG eelgrass biomass (a), BG eelgrass biomass (b), shoot density (c) and infauna biomass (d) with distance from a finfish farm in Nova Scotia, Canada. See Table 1 for full site names and details.

### *3.4.3 Macroinfauna community*

A total of 20 macroinfauna genera were identified across all sites (Appendix 3A: Table 1), 10 of which were identified down to species level. Patterns of summary measures varied across sites (Figure 6), yet univariate PERMANOVAs did not detect any significant differences in species richness (pseudo-F = 0.48, p = 0.76), Shannon-Wiener Diversity (pseudo-F = 0.74, p = 0.53), total abundance (pseudo-F = 1.45, p = 0.2) or total biomass (pseudo-F = 1.67, p = 0.12) of infauna between sites. However, PJ did show higher infauna richness, diversity and biomass (Figure 6) and linear regression found a significant positive trend of infauna biomass with distance from the fish farm (Figure 5d).

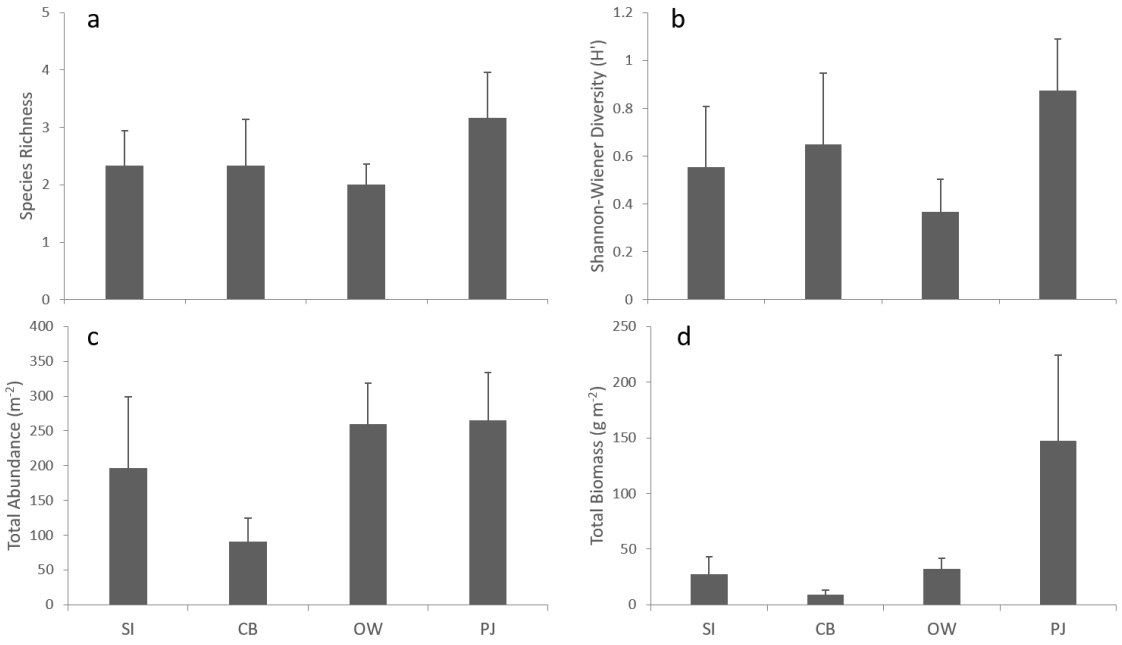


Figure 6. Average (+ SE, n = 6) infauna species richness (a), diversity (b), total abundance (c) and total biomass (d) at the four study sites (from left to right: increasing distance from farm and PJ reference site) in Nova Scotia, Canada. Refer to Table 1 for site abbreviations and details.

Despite no significant differences in summary measures, infauna community composition based on both abundance and biomass data significantly differed between sites (PERMANOVA: pseudo-F = 2.25, p = 0.005 and pseudo-F = 2.20, p = 0.003; respectively). A Hierarchical Cluster analysis of centroids showed SI and OW clustering for abundance and SI and CB clustering for biomass (Figure 7).

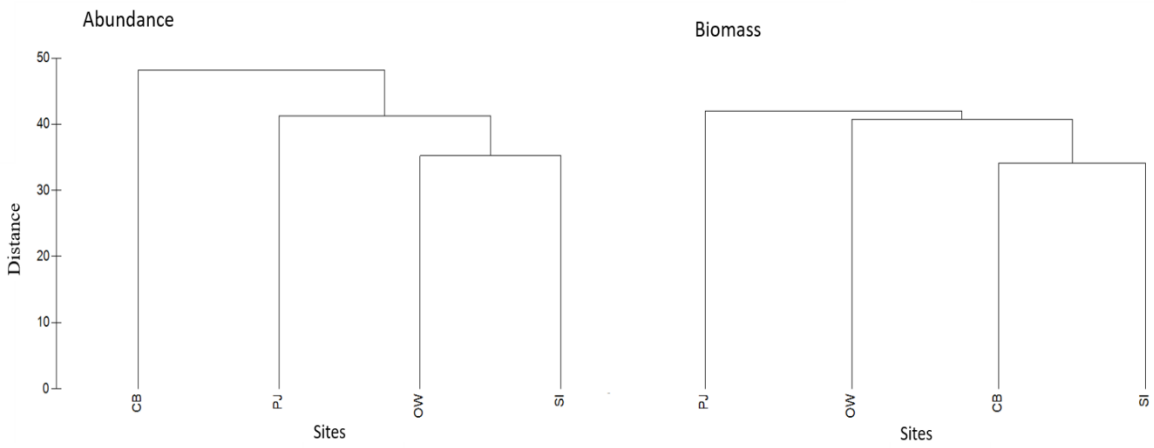


Figure 7. Cluster analysis using infauna community centroids based on abundance (left) and biomass (right) at the four study sites with differing distances from a finfish farm in Nova Scotia, Canada. See Table 1 for site abbreviations and details.

The main species identified by SIMPER contributing >10% of differences in abundance between sites included three polychaetes: *Clymenella torquata*, *Capitella capitata,* and *Nephtys* sp. The SIMPER species remained the same when looking at community structure based on biomass, with the addition of *Amphitrite* sp., however the contribution of each species to the community differed when considering abundance or biomass, respectively (Figure 8). When considering infauna abundance (density), *Clymenella torquata* clearly dominated the community across all sites except CB. This species also dominates at the three sites in Port Mouton Bay (SI, CB, OW) when considering their biomass, while *Amphitrite* sp. clearly dominates at the reference site in Port Joli (Figure 8). Interestingly, the opportunistic *Capitella capitata* only occurred at sites in Port Mouton, with highest abundance and biomass, respectively, at SI closest to the fish farm (Figure 8).

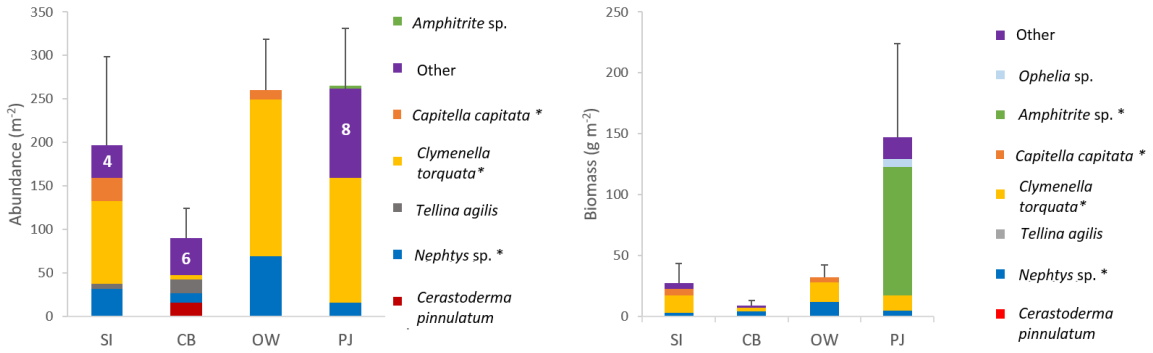


Figure 8. Species with the highest abundance (left) and biomass (right) at the four study sites (from left to right: increasing distance from farm and PJ reference site) in Nova Scotia, Canada. Refer to Table 1 for site names and details. SIMPER species are marked with an asterisk. The number of remaining species included in the "other" column.

Looking at the ABC curves for cumulative dominance (Figure 9) shows that the infauna biomass curve lies distinctly above the abundance curve for both CB and PJ, indicating unpolluted condition. The biomass and abundance curve for the SI site approach each other but do no overlap, suggesting that it is likely approaching moderately polluted condition. The OW site is the only site where the abundance curve lies above the biomass curve, indicating grossly polluted condition.

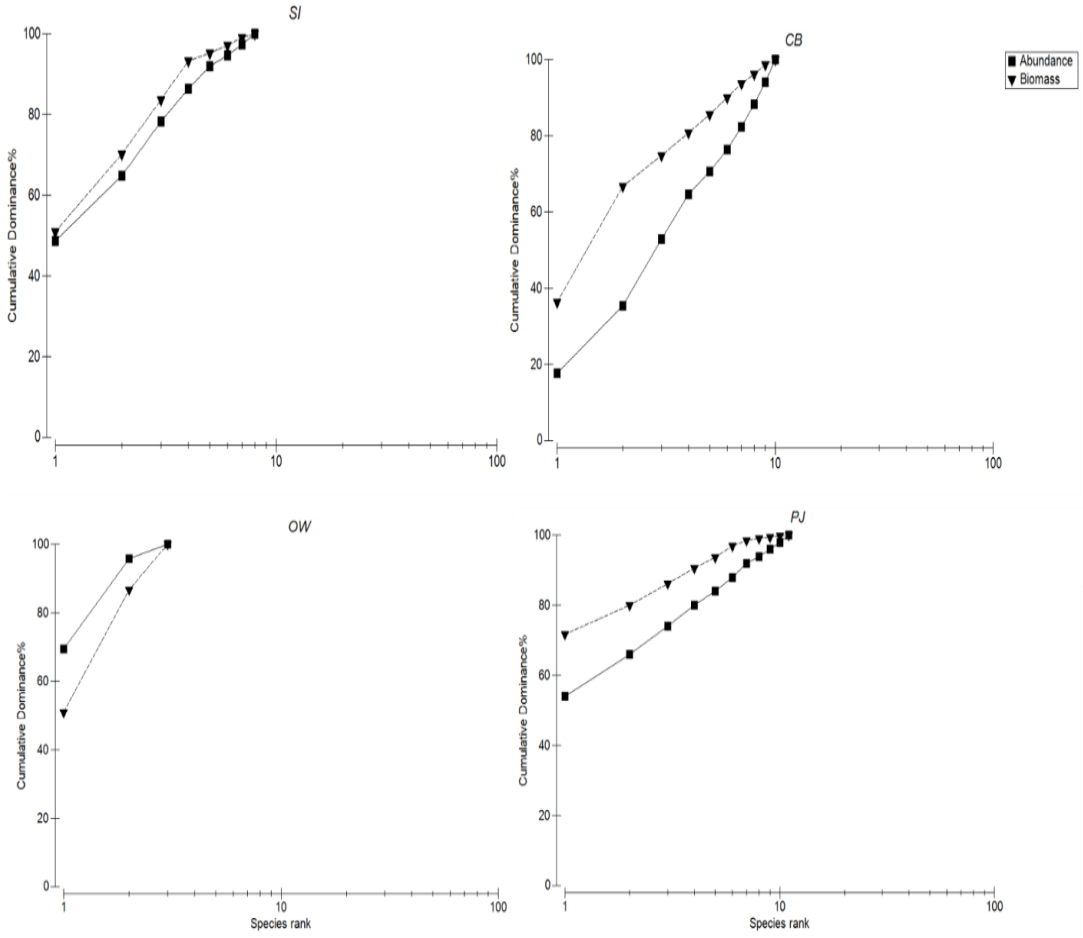


Figure 9 Abundance-biomass comparison (ABC) curves using cumulative dominance for infauna species for the four study sites in Nova Scotia, Canada. See Table 1 for site names and details.

### *3.4.4 Linking the environment/eelgrass structure to the infauna community*

The PRIMER BEST/BIOENV procedure was used to determine any association between the Euclidean distance of environmental and eelgrass parameters (sediment organic content, MPB, annual algae, $\delta^{15}N$, eelgrass shoot density, canopy height and BG eelgrass biomass) and the Bray Curtis similarity of infauna community structure (Table 3). The infauna community abundance assemblage was best correlated with annual algae, $\delta^{15}N$ and BG eelgrass biomass with a sample statistic rho ($\rho$) of 0.421 at a significance

level of 1.2% (or p = 0.012). Abundances of individual SIMPER species were then used in the analysis to see which environmental/eelgrass variables they were best correlated to. *Capitella capitata* was significantly correlated strictly to sediment organic content, while the other two species were more correlated with eelgrass structure (Table 3). When infauna biomass was used in the analysis, the best correlation of the community assemblage was with BG eelgrass biomass and annual algae, however this was not significant (Rho = 0.203, p = 0.142). The biomass of the SIMPER species correlated more or less to the same variables, with slightly different values (Table 3). The only species with significant correlation was *Clymenella torquata* to BG eelgrass biomass, however *Capitella capitata* was marginally (p = 0.065) correlated with sediment organic content.

Table 3. Results from the BEST/BIOENV procedure for the entire community (based on abundance above and biomass below) as well as SIMPER species using biological and environmental data from the four study sites in Nova Scotia, Canada. Significant ($p \leq 0.05$) correlations are bolded.

| Biological variable | Best correlated environmental variable(s) | Sample statistic (Rho) | Significance of sample statistic |
|---|---|---|---|
| **Community assemblage (abundance)** | **BG eelgrass biomass<br>BG $\delta^{15}N$<br>Annual algae (% cover)** | **0.421** | **0.012** |
| ***Capitella capitata*** | **Sediment organic** | **0.433** | **0.042** |
| ***Clymenella torquata*** | **BG eelgrass biomass** | **0.431** | **0.001** |
| *Nephtys* sp. | BG eelgrass biomass<br>Shoot density<br>Sediment Organic | 0.174 | 0.202 |
| Community assemblage (biomass) | BG eelgrass biomass<br>Annual algae (% cover) | 0.267 | 0.142 |
| *Amphitrite* sp. | BG eelgrass biomass,<br>Canopy height | 0.211 | 0.494 |
| *Capitella capitata* | Sediment organic | 0.413 | 0.065 |
| ***Clymenella torquata*** | **BG eelgrass biomass** | **0.378** | **0.007** |
| *Nephtys* sp. | BG eelgrass biomass,<br>Shoot density | 0.216 | 0.11 |

Finally, GLMs were used to identify which environmental or eelgrass structural variables best explained the observed patterns in infauna total abundance and biomass, species richness and diversity. Eelgrass structure (BG biomass, shoot density and canopy height) was always a better predictor for patterns in the infauna components than the environmental variables. More specifically, eelgrass BG biomass significantly explained

all infauna components better than any other explanatory variables (Table 4), whereby infauna species richness and total abundance increased with increasing BG biomass.

Table 4. Analysis of deviance table for infauna total abundance and biomass, species richness and Shannon diversity (H'). For abundance and biomass a negative binomial GLM was applied and for species richness and diversity a normal GLM was used. Table contains test statistics and associated p-values. Significant results ($p \leq 0.05$) are bolded.

| Variable | Abundance | | Biomass | | Species Richness | | Diversity | |
|---|---|---|---|---|---|---|---|---|
| | Deviance | P-Value | Deviance | P-Value | F | P-Value | F | P-Value |
| BG $\delta^{15}N$ | 0.0001 | 0.9907 | 0.0000 | 0.9960 | 0.3706 | 0.5518 | 1.508 | 0.2384 |
| Sediment organic | 1.836 | 0.1755 | 0.6074 | 0.4358 | 0.6610 | 0.4289 | 0.7565 | 0.3982 |
| MPB | 0.6234 | 0.4298 | 0.3625 | 0.5471 | 0.0153 | 0.9031 | 0.1325 | 0.7209 |
| Annual algae | 2.593 | 0.1073 | 2.801 | 0.0942 | 0.0254 | 0.8754 | 0.1135 | 0.8200 |
| BG biomass | **9.346** | **0.0022** | **21.66** | **3.25e-6** | **10.97** | **0.00474** | **6.522** | **0.0220** |
| Shoot density | 0.7090 | 0.3998 | **6.886** | **0.0087** | **5.502** | **0.03316** | 4.150 | 0.0597 |
| Canopy height | 0.2261 | 0.6344 | 0.4229 | 0.5155 | **5.539** | **0.03265** | **4.590** | **0.0489** |

## 3.5 Discussion

Our field surveys and multivariate statistics established differences in the macroinfauna community assemblage and eelgrass bed structure across study sites. In particular, eelgrass above- and below-ground biomass and shoot density decreased with proximity to a finfish farm. While Port Joli was located outside of Port Mouton Bay, its position as the closest unimpacted bay was used as a reference site in this study. Moreover, Port Joli was more similar to other unimpacted Nova Scotia sites in eelgrass bed structure, environmental parameters and infauna metrics (see Chapter 2, Appendix 2A: Figures 1-4) than other regions and could therefore be used for comparison among impacted sites in Nova Scotia. Our results also indicate that the observed differences in

the infauna community assemblage appear to be closely linked to differences in the eelgrass canopy structure as well as some underlying environmental parameters. Using individual general linear models, canopy structure (specifically BG biomass) consistently best explained patterns in summary measures of the infauna community, while the BEST/BIOENV procedure also captured some underlying environmental parameters (BG $\delta^{15}N$ and % cover of annual algae) in addition to BG biomass correlating with the composition of the infauna assemblage. This demonstrates the importance of assessing environmental and ecosystem changes as a whole rather than looking at just individual parameters. Such an approach should be considered in the management, monitoring and further development of finfish aquaculture farms in Nova Scotia.

### *3.5.1 Environmental parameters*

Due to its temperate latitude, eelgrass (*Zostera marina*) experiences large seasonal fluctuations in temperature and light availability in Atlantic Canada. During the sampling period, SST was quite similar at all five sites (12-15$^{o}$C), while depth ranged from 1.7-2.9 m. These are typical conditions for eelgrass habitat in Nova Scotia, which usually occurs at depths of 1-5 m (Schmidt et al. 2011, see also Chapter 2), with maximum depths observed at 12 m (DFO 2009a), and optimal growth temperatures of 10-25 $^{o}$C (Marsh et al. 1986, Touchette et al. 2003, DFO 2009a). Additionally, several other physical factors are known to influence eelgrass growth and survival, including hydrology, wave exposure, sediment type and water quality (Short 1987, Moore et al. 1996, Frederiksen et al. 2004). While all four sites were located in relatively sheltered areas with similar wave exposure, SI and OW were the most sheltered due to being nestled behind an island (SI) and positioned in the inner part of the bay (OW). Grain size

was not measured in this study; however, CB and PJ likely had larger grain sizes due to their more sandy bottom type, opposed to SI and OW which had muddier bottoms.

The closest site to the fish farm (SI) showed higher amounts of organic matter in the sediments than the other sites in Port Mouton, as well as the reference site in PJ, indicating organic enrichment. Grain size can also influence the amount of organic matter in the sediments (Luczak et al. 1997) and could explain why we are seeing higher percentages of organic matter in the muddier sites SI and OW; however, the observed organic content at OW is only slightly higher than CB and PJ and much lower than at SI. Other possible factors influencing the deposition of organic matter include hydrodynamic properties like current speed and flushing time. Port Mouton Bay has slow current speeds (mean of 2 cm/s) and a long 111.7 hour flushing time (Gregory et al. 1993) with weak recirculating currents (Friends of Port Mouton 2010), which may explain the high organic deposition from the fish farm at the SI site. While there was no RPD layer observed at any of the sites, it is likely that hypoxic or anoxic conditions were present at the SI and OW sites due to the strong sulfur smell and dark black sediments. This would also explain the higher abundance and biomass of the opportunistic polychaete *Capitella capitata*, which is hypoxia tolerant and can serve as an indicator species for organically enriched and oxygen-depleted sediments (Pearson and Rosenberg 1978). A community monitoring program has also indicated higher sulfide levels around the farm (Friends of Port Mouton 2011).

The microphytobenthos is a good indicator of benthic productivity and can contribute significantly to the primary productivity in shallow waters (MacIntyre et al. 1996). Biomass of the microphytobenthos is known to vary under different environmental

conditions; higher biomass being notably found in muddy, sheltered habitats and lower biomass in more exposed, sandy habitats (Cadée & Hegeman 1977, Delgado 1989). In the present study, although not significant, higher amounts of chlorophyll *a* in the sediments were observed at the three Port Mouton sites compared to the reference site in PJ. According to the generalization of sediment type on microphytobenthos biomass, we would have expected higher amounts at SI and OW and lower amounts at CB and PJ, however CB showed similar levels to that of SI and OW. Due to the high variability of the microphytobenthos, it is difficult to attribute the higher biomass in Port Mouton to any one specific environmental factor, however these results may indicate higher benthic productivity in Port Mouton opposed to Port Joli.

Increases in nutrient loads from anthropogenic sources such as sewage effluent and mariculture activities can have profound effects on coastal ecosystems, particularly seagrasses which are notably sensitive to changes in water quality (Short & Wyllie-Echeverria 1996, Waycott et al. 2009, Short et al. 2011, Schmidt et al. 2012). Tissue nitrogen content and stable-isotope ratios are therefore commonly used to trace the amount and source of nitrogen within seagrass ecosystems (McIver et al. 2015). Our results indicate no significant differences in tissue % nitrogen and % carbon and delta values of nitrogen and carbon stable isotopes within the range of natural variation in seagrass ecosystems (Hemminga & Mateo 1996, Lepoint et al. 2003, 2004). Although not significant, PJ and CB did have higher $\delta^{15}N$ compared SI and OW. These higher signatures do not suggest that the eelgrass tissues are incorporating the nitrogen from wastewater sourced nitrogen, but rather atmospheric deposition (+2 to +6‰) (Lepoint et al. 2004, Kendall et al. 2007). The SI site, which is located behind an island and closest to

the fish farm, was expected to show higher signatures considering animal waste typically showing $\delta^{15}N$ values ranging from +10 to +20‰ (Lepoint et al. 2004, Schubert et al. 2013). These results may suggest that $\delta^{15}N$ signatures are actually stronger at sites located closer to land run-off as opposed to more open-ocean sites located close to aquaculture activities which may have higher water circulation and/or flushing time. Another explanation could be the absence of stocked fish during the time of sampling, following the super chill event a few months earlier, allowing time for the $\delta^{15}N$ in the eelgrass tissue to be adequately used or cycled within the system. This would also explain why there were no enhanced tissue % nitrogen values at SI, which were expected due to nitrogen-rich animal and food wastes. For $\delta^{13}C$ a more negative isotopic signature represents the input of $^{13}C$-depleted carbon from the decomposition of organic material (Hemminga & Mateo 1996). While no significant differences in $\delta^{13}C$ were found between sites, a clear pattern of decreasing amounts of carbon were observed as we moved away from the finfish farm, specifically in the roots. This particular pattern of $\delta^{13}C$ values becoming less negative as distance from the source increases has been observed in seagrass beds which receive organic material from land run-off (Simenstad & Wissmar 1985, Hemminga et al. 1994, Hemminga & Mateo 1996, Peterson 1999). Thus, while the nitrogen signal may have disappeared due to the not-stocked fish farm at the time of sampling, the organic carbon signal was still visible. These $\delta^{15}N$ and $\delta^{13}C$ isotopes signatures could be used as an important tool in detecting and monitoring the sources of nitrogen and carbon in Port Mouton Bay and should be investigated further once the farm is restocked.

### *3.5.2 Eelgrass bed structure*

A common response of *Zostera marina* to increased eutrophication is a decrease of shoot density and biomass and increase in canopy height (Short et al. 2011, Schmidt et al. 2012). In our study, we see a decrease in shoot density and biomass with increasing proximity to the fish farm. These patterns and trends may become more significant with a larger sample size and more statistical power. However, it is clear that SI and CB, the two sites closest to the farm (< 1 km), showed different canopy structure than the other sites, particularly lower eelgrass biomass. Though estimated dispersion distances from fish farms are variable, the furthest distances of organic-enriched material has not exceeded 1000 m (Sarà et al. 2004, Holmer et al. 2007). Interestingly, many studies suggest that waste dispersion will only degrade the surrounding environment up to a maximum of 100 m from marine fish cages (Holmer 1992, Delgado et al. 1999, Pearson & Black 2000), yet our SI and CB sites were located past this distance with observed impacts from the farm. While the mechanisms behind the decline at SI and CB are likely complex, these patterns support the literature on the impacts on seagrasses from eutrophication and organic loading (Delgado et al. 1999, Ruiz et al. 2001, Perez et al. 2007, Short et al. 2011, Schmidt et al. 2012).

### *3.5.3 Macroinfauna*

Although summary measures of the infauna community (total abundance and biomass, species richness and diversity) did not significantly differ between sites, community composition based on abundance and biomass did. These multivariate community analyses have been used extensively to assess changes in macroinfaunal communities due to pollution disturbance (Warwick & Clarke 1993, Lee et al. 2006,

Apostolaki et al. 2007, Kutti et al. 2007, Lin & Bailey-Brock 2008). Our results indicate differences in infauna community structure across sites, particularly when using infauna biomass based on distance from the farm. The cluster analysis for infauna community biomass identified similarities between the two sites closest to the farm (SI and CB) with the reference site (PJ) being the least similar and OW falling between the SI/CB cluster and the PJ site.

The ABC method allowed us to examine the sites based on a pollution gradient (Warwick 1986). Our results indicate that OW is considered the most polluted site, followed by SI, while PJ can be considered unpolluted. The location of the OW site may explain why it is coming out as the most polluted site. This site is in an area of high boat traffic, residential homes and an old former fish processing plant. The ABC method appears to be capturing greater pollution from these multiple stressors as opposed to the single source of pollution from the fish farm. Since these sites were only sampled during one time period, it is also important and necessary to monitor the changes in these ABC curves over time.

The SIMPER analysis is commonly used to identify which species contribute most to the observed differences in community composition (Anderson et al. 2008). Interestingly, in our case the opportunistic polychaete, *Capitella capitata,* was a main contributor to differences in community composition based on both abundance and biomass at the SI site compared to all other sites. This may indicate that the sediments at this site are organically enriched and transitioning to (or recovering from) hypoxic conditions, as mentioned above. This is corroborated by our BEST/BIOENV analysis, that determined sediment organic content to be the best explanatory variable for observed

differences in *Capitella capitata* where the higher amounts of sediment organic at SI and OW are leading to higher abundances of this indicator species. In contrast, patterns observed in the other SIMPER species (*Clymenella torquata, Nephtys sp., Amphitrite* sp.) could be mostly linked to eelgrass canopy structure or biomass. Because the fish kill occurred approximately 4 months before the sampling period, it is difficult to tease apart the effects from the previous impacts of the farm or the recent fallow period. It is therefore important to continue to monitor these sites and the future impacts once the farm is restocked in 2016.

### *3.5.4 Linking the environment/eelgrass structure to infauna community composition*

Investigating the link between environmental parameters and/or eelgrass structure and the associated macroinfauna community using the BEST/BIOENV procedure as well as GLMs, we established that canopy structure was consistently the best predictor for species composition; however, annual algae and BG $\delta^{15}N$ were also correlated with the infauna assemblage. This supports the literature which illustrate that faunal assemblages are often proportional to seagrass biomass and structural complexity (Heck & Wetstone 1977, Bologna & Heck 2002, Gartner et al. 2013). In our study, the highest amount of annual macroalgae was found at CB (mean 39.5% ±7.27), followed by PJ (1.67% ±1.67). This may indicate higher eutrophication compared to the other sites although sources of the nutrient enrichment are likely complex. Interestingly, our results reveal that changes in the infauna communities are more closely linked to changes in eelgrass biomass, rather than direct organic or nutrient enrichment from the finfish farm. However, because we

did find lower eelgrass biomass at the sites closest to the farm, we can expect that this has an overall impact on the associated infauna community.

## 3.6 Conclusions

Determining the impacts of organic enrichment from finfish aquaculture on eelgrass habitats in Nova Scotia is challenging and complex, but should not be overlooked. While eelgrass beds in these temperate waters are not located directly under fish pens, our results reveal that a decrease in eelgrass biomass is occurring as far as 1 km from the farm. Our results also indicate that changes in infauna community structure are closely linked to changes in eelgrass biomass, rather than directly to environmental parameters. The only change clearly linked to organic enrichment was the increase in the opportunistic and hypoxia-tolerant indicator species *Capitella capitata*. However, environmental parameters still need to be considered, since they are likely contributing to the underlying differences in eelgrass structure. It is therefore necessary to not only study and monitor environmental impacts directly under the fish cages, but also the impacts on adjacent ecosystems on a bay-wide scale.

# Chapter 4- Discussion

Quantifying regional and local variation within eelgrass ecosystems is important for understanding and conserving these vulnerable habitats. Coastal ecosystems have been impacted by human activities for centuries (Lotze et al. 2006, Orth et al. 2006, Waycott et al. 2009), yet our knowledge on how best to monitor and manage these ecosystems, particularly in Atlantic Canada, is still limited (DFO 2009a). Obtaining baseline data so that longer term and larger scale monitoring can be implemented is crucial (DFO 2011). Additionally, the local impacts of finfish aquaculture on coastal habitats have important management implications on how to properly monitor and develop mariculture farms in Atlantic Canada (Fisheries and Oceans Canada 2003, Doelle & Lahey 2014).

In this thesis, I have examined large- and local-scale variation in macroinfaunal communities associated with eelgrass beds in Atlantic Canada. In Chapter 2, I examined the spatial variation in eelgrass structure, environmental parameters and macroinfauna communities across three distinct biogeographic regions, the Atlantic shore of Nova Scotia, the southern Gulf of St. Lawrence in New Brunswick, and the exposed northeastern shore of Newfoundland. My results illustrate that while spatial differences in eelgrass ecosystems are often complex, there are some measures that can be adequately assessed on a larger-spatial scale. While variation in eelgrass structure should be assessed and monitored on the site-by-site level, macroinfaunal communities can be evaluated across larger regional-scales in Atlantic Canada. Most underlying environmental conditions should also be evaluated on a site-by-site basis; however, variation in the microphytobenthos can be explained by region and may be an important tool in

monitoring regional-scale differences in benthic productivity and its influence on macroinfauna communities.

In Chapter 3, I examined the impacts of finfish aquaculture on eelgrass beds and associated macroinfaunal communities based on distance from a finfish farm in Port Mouton Bay, southern Nova Scotia. Here, I found changes in eelgrass bed structure as proximity to the farm increased, particularly with sites closest to the farm having lower shoot density and biomass than sites further away. Interestingly, infauna communities did not appear to be responding directly to organic enrichment, but rather indirectly to the changes in the eelgrass bed structure. These results are important for management implications for the finfish farm in Port Mouton Bay and may be extended to other existing and future farms in Atlantic Canada.

Both of these chapters linked changes in macroinfauna communities to changes in eelgrass structure and/or environmental parameters. In both chapters, it is evident that eelgrass bed structure and environmental variables vary within the site level, which is likely influenced by local hydrodynamic conditions (Thom et al. 2003, Schückel et al. 2013). Interestingly, on the larger scale, microphytobenthos have distinct regional patterns, however on a bay-wide scale these differences are not detected, even across a local impact gradient. Furthermore, links between the infauna and eelgrass bed structure were much more prominent on the local scale, whereas regionally stronger links were found with the environmental parameters and in combination with eelgrass bed structure. Therefore, regional and/or local scale assessments are dependent the parameters that are of interest and should be addressed accordingly. This illustrates the importance of

understanding the local and regional scale variation of eelgrass habitats in Atlantic Canada for conservation and management practices.

## 4.1 Management Implications

This thesis provides insight into potential monitoring approaches that could be used to assess ecosystem health within eelgrass habitats over large spatial scales and local impact gradients. For example, these results should be taken into consideration and applied to regional assessments such as those performed by the Department of Fisheries and Oceans (e.g. DFO 2009a, 2011) and/or regional monitoring programs such as the Northumberland Strait Environmental Monitoring Program (NorST-EMP). My results indicate that regardless of eutrophication impacts, seagrass bed structure varies between sites within a region in Atlantic Canada. This means that assessing the status of eelgrass habitat at one site is not representative of the entire biogeographic region. This has important management implications in the assessment and monitoring of seagrass beds whereby assessment must be made on a site-by-site level. On the contrary, assessing the microphytobenthos as a proxy for benthic productivity (MacIntyre et al. 1996), as well as macroinfauna community composition within seagrass beds, can be examined across biogeographic regions. Further, monitoring changes in these macroinfaunal communities can be used as an important tool in evaluating ecosystem health within each region (Pearson & Rosenberg 1978, Warwick et al. 1987).

On the local-scale, impact gradients can be used on the effects of finfish aquaculture (Ruiz et al. 2001, Sarà et al. 2004), or other human impacts such as nutrient loading, sewage effluent and seafood processing plants (Hauxwell et al. 2003, Cardoso et

al. 2004, Lee et al. 2004, McIver et al. 2015). While these studies show that these individual impacts can have profound effects on coastal ecosystems, each impact may result in different ecosystem effects and should be investigated at the local level. My study was the first to assess seagrass beds and associated infauna communities on a bay-wide scale in the presence of finfish aquaculture in Atlantic Canada. My results support the report by Doelle & Lahey (2014) for the need of more monitoring and regulations for finfish farms, especially where important habitats such as eelgrass beds are present. Furthermore, working with local community groups (e.g. Friends of Port Mouton Bay) can be a useful way to assess and monitor ecosystem changes on a local scale. Monitoring these changes over time is critical in determining the long-term effects of anthropogenic impacts such as eutrophication and organic enrichment from finfish aquaculture on the marine environment. Moreover, understanding these complex ecosystems will help to protect and manage them in the future.

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# Appendix 2A – Supplementary Materials for Chapter 2

Table 1. Species list of all species identified at each site in New Brunswick (NB), Nova Scotia (NS) and Newfoundland (NL). If a species was present within a site it is indicated with a plus (+), if it was absent it was left blank. See Chapter 2, Table 1 for full site names and details.

| | NB | | | | | | NS | | | | | | | | | NL | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CG | BT | KB | TB | SS | LM | FP | TH | SM | CR | ST | FG | CB | PJ | JB | GB | SB | BI | SC |
| *Amphipod* | | | | | | | + | | | | | | | | | | | | |
| *Amphitrite* sp. | | | | | | | | | | | + | | | + | | | | | |
| *Aphiopholis aculeata* | | | | | | | | | | + | | | | | | | | | |
| *Arabella iricolor* | | | | | | | | | | | | | | | + | | | | |
| *Asychis elongata* | | | | | | | | | + | | | | | | | | | | |
| *Capitella* sp. | | | | + | | | | | | | | | | | | | | | |
| *Cerastoderma pinnulatum* | | | | | | | | | | + | + | | + | | | | | | |
| *Cerebratulus* sp. | | | | | | | + | | | | | + | + | | | + | | | |
| *Clymenella torquata* | + | + | | + | + | + | + | + | + | + | + | + | + | + | + | | | | |
| *Corophium* sp. | + | | | | | | + | | | + | | | + | + | + | | | | |
| *Cyclocardia borealis* | | | | | | | | | + | | | | | | | | | | |
| *Cyrtopleura costata* | | | | + | | + | | | | | | | | | | | | | |
| *Drilonereis* sp. | | | | + | + | + | + | | | + | + | | + | + | | | | | |
| *Dysponetus pygmaeis* | | | | | | | | | | + | | | | | | | | | |
| *Ensis directus* | + | | | | | | | | | | + | | | | | | | | |
| *Glycera* sp. | + | + | | | | | + | + | + | + | + | + | | + | + | | | | |
| *Harmothae imbricata* | | | | | + | | | | | | | | + | + | | | | | + |
| *Hiatella arctica* | | | | | | | | | | | | | + | | | | | | |
| *Ilyanassa obsoleta* | + | + | + | | + | + | | | | | | | | | | | | | |
| *Lepidonotus squamatus* | | | + | | | | | | | | | | | | | | | | |
| *Lumbineris* sp. | | | + | + | | + | + | | | | | + | | | | | | | |
| *Macoma* sp. | + | + | + | | + | + | + | | | | | | + | | | | | | |
| *Melampus bidentatus* | | | | | | | | | + | | | | | | | | | | |
| *Mya arenaria* | | | | | | + | | | | | | | | | | | | | |
| *Nassarius trivittatus* | + | + | | + | | | + | | | + | + | + | | | | | | | |
| *Nephtys* sp. | | + | | | | | + | + | + | | | + | + | + | + | | | | + |
| *Nereis* sp. | | | | + | + | + | | | + | | | | | | + | | + | | + |
| *Ophelia* sp. | | | | | | | | | | | | | | + | | | | | |
| *Oysponetus pygmaeus* | | | | | | | | | | | + | | | | | | | | |
| *Panopeus herbstii* | | | | + | | | | | | | | | | | | | | | |
| *Pectinacria gouldi* | + | + | + | | + | + | | | | | | | | + | | | | | |
| *Petricola pholadiformis* | | | | | | | | | | + | | | | | | | | | |
| *Pherusa affinis* | | | | | | | + | + | + | | + | | | + | + | | | | + |
| *Platynereis* sp. | + | + | | | | | | | + | | | | | | | | + | | |
| *Saccoglossus kowalevskii* | | | | | | | | | | | | | | + | | | | | |
| *Solemya borealis* | | | | | | | + | | | | | | | | | | | | |
| *Spisula solidissima* | + | + | | | | | | | | | | | | | | | | | |
| *Tellina agilis* | + | + | + | + | + | + | + | | + | + | | | + | | | | | | |

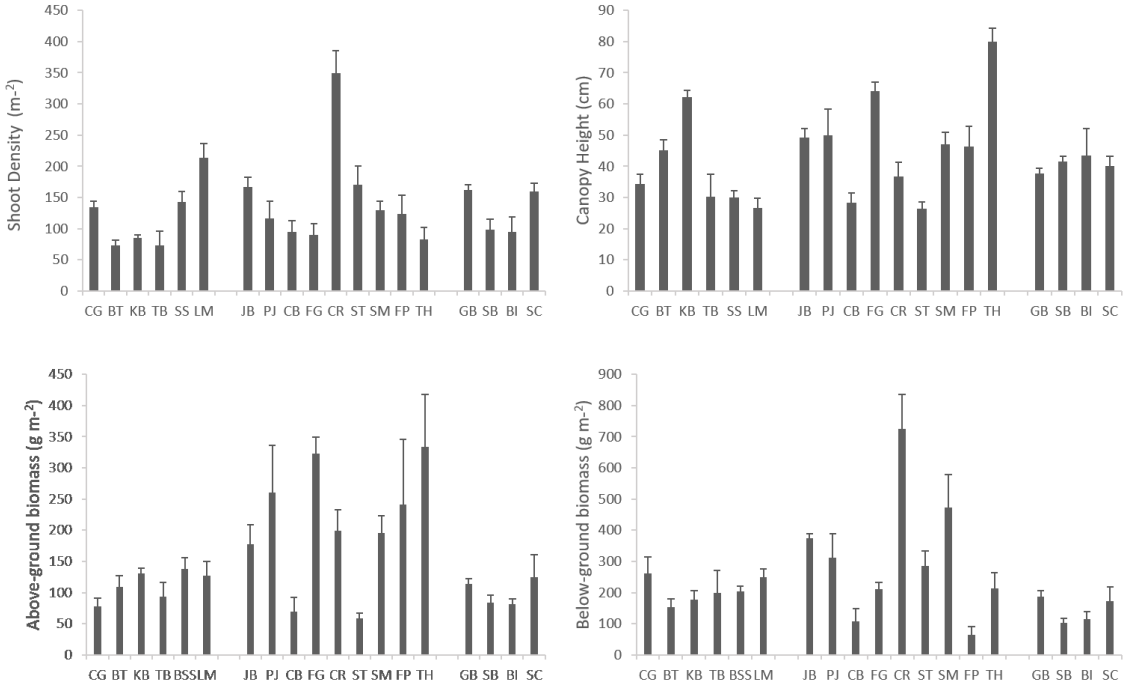


Figure 1. Average (+SE) of eelgrass structure (shoot density, canopy height, above- and below-ground biomass at each site within New Brunswick (NB), Nova Scotia (NS) and Newfoundland (NL). See Chapter 2, Table 1 for full site names and details.

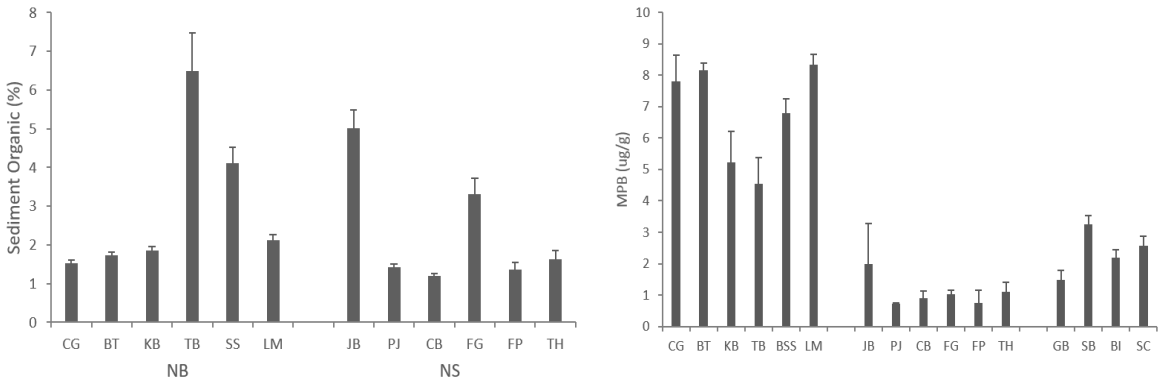


Figure 2. Average (+SE) sediment organic content and microphytobenthos (MPB) at each site within New Brunswick (NB), Nova Scotia (NS) and Newfoundland (NL). See Chapter 2, Table 1 for full site names and details.

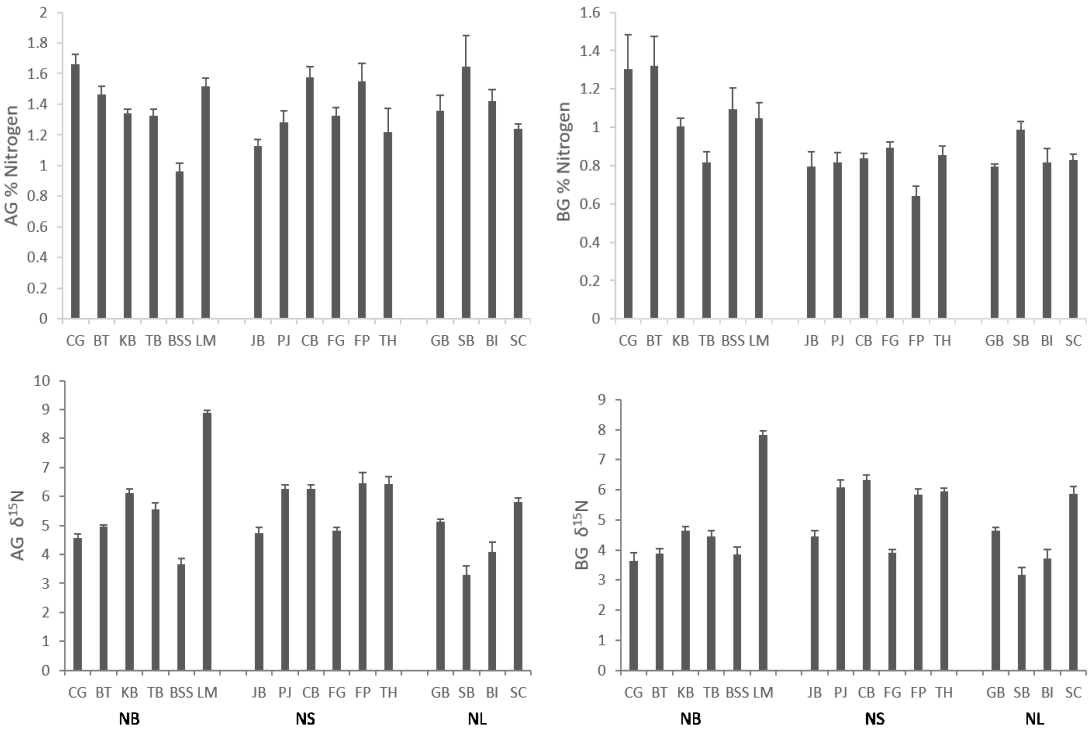


Figure 3. Average (+SE) AG and BG percent tissue nitrogen (top) and AG and BG $\delta^{15}N$ at each site within New Brunswick (NB), Nova Scotia (NS) and Newfoundland (NL). See Chapter 2, Table 1 for full site names and details.

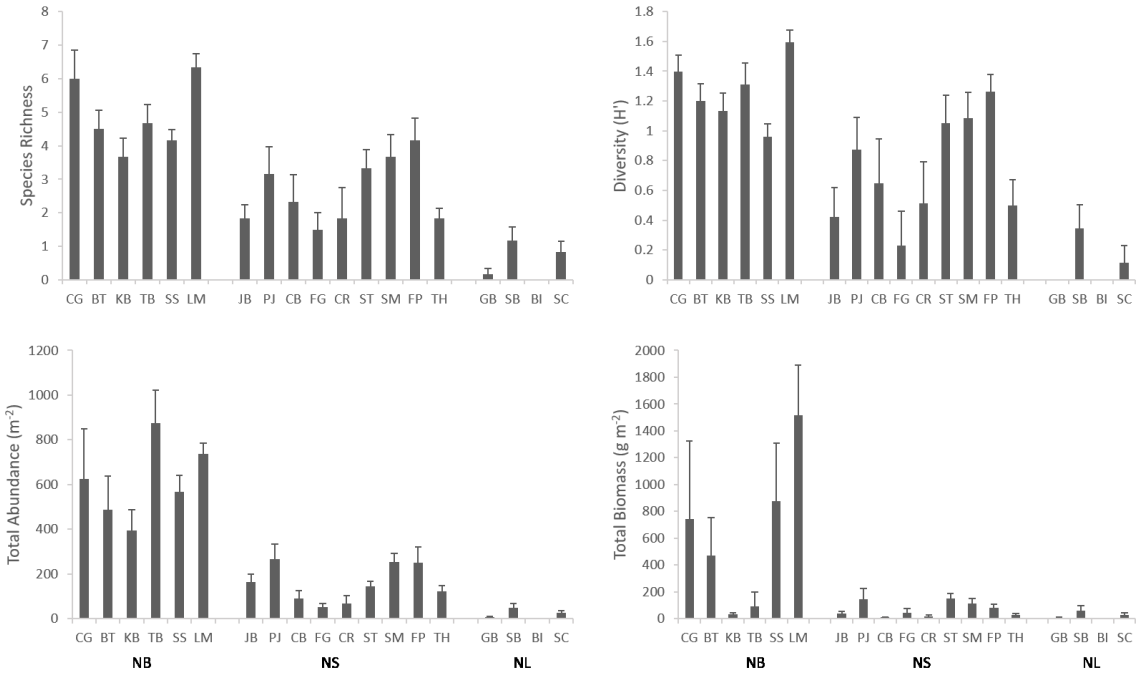


Figure 4. Average (+SE) species richness, Shannon diversity (H'), total abundance and total biomass at each site within New Brunswick (NB), Nova Scotia (NS) and Newfoundland (NL). See Chapter 2, Table 1 for full site names and details.

Appendix 2B- PERMANOVA output for pairwise comparisons of eelgrass bed structure, environmental variables and infauna community structure between sites within each region Si(Re) for New Brunswick (NB), Nova Scotia (NS) and Newfoundland (NL). T value and associated p-value for pairwise comparisons are reported. Significant p-values (≤0.05) are bolded.

**SHOOT DENSITY**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CG, BT** | **4.8434** | **0.005** | 42 |
| **CG, KB** | **4.2331** | **0.003** | 38 |
| **CG, TB** | **2.5043** | **0.039** | 54 |
| CG, SS | 0.41384 | 0.731 | 35 |
| **CG, LM** | **3.219** | **0.014** | 61 |
| BT, KB | 1.1061 | 0.299 | 22 |
| BT, TB | Negative | | |
| **BT, SS** | **3.677** | **0.01** | 52 |
| **BT, LM** | **5.7949** | **0.005** | 85 |
| KB, TB | 0.50839 | 0.658 | 43 |
| **KB, SS** | **3.1512** | **0.004** | 44 |
| **KB, LM** | **5.4144** | **0.002** | 81 |
| **TB, SS** | **2.459** | **0.047** | 63 |
| **TB, LM** | **4.3858** | **0.003** | 88 |
| **SS, LM** | **2.518** | **0.043** | 64 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CB, PJ | 0.64777 | 0.533 | 59 |
| **CB, JB** | **3.0997** | **0.024** | 55 |
| CB, FP | 0.80727 | 0.447 | 63 |
| CB, TH | 0.46078 | 0.627 | 44 |
| CB, FG | 0.21061 | 0.851 | 44 |
| PJ, JB | 1.6019 | 0.143 | 58 |
| PJ, FP | 0.16344 | 0.889 | 73 |
| PJ, TH | 0.98767 | 0.378 | 58 |
| PJ, FG | 0.80328 | 0.448 | 59 |
| JB, FP | 1.3155 | 0.25 | 60 |
| **JB, TH** | **3.4467** | **0.011** | 60 |
| **JB, FG** | **3.2768** | **0.018** | 58 |
| FP, TH | 1.128 | 0.268 | 67 |
| FP, FG | 0.95416 | 0.363 | 66 |
| TH, FG | 0.25276 | 0.828 | 47 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **SC, BI** | **2.3458** | **0.043** | 60 |
| **SC, SB** | **2.8901** | **0.019** | 50 |
| SC, GB | 0.16909 | 0.906 | 32 |
| BI, SB | 0.11585 | 0.9 | 45 |
| **BI, GB** | **2.6437** | **0.02** | 56 |
| **SB, GB** | **3.4749** | **0.019** | 50 |

**CANOPY HEIGHT**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CG, BT** | **2.3343** | **0.039** | 38 |
| **CG, KB** | **7.2336** | **0.001** | 62 |
| CG, TB | 0.52163 | 0.646 | 54 |
| CG, SS | 1.1298 | 0.319 | 31 |
| CG, LM | 1.7021 | 0.112 | 40 |
| **BT, KB** | **4.2515** | **0.005** | 48 |
| BT, TB | 1.8607 | 0.095 | 57 |
| **BT, SS** | **3.8042** | **0.003** | 44 |
| **BT, LM** | **3.9924** | **0.007** | 47 |
| **KB, TB** | **4.1926** | **0.003** | 78 |
| **KB, SS** | **10.628** | **0.001** | 66 |
| **KB, LM** | **9.2469** | **0.001** | 74 |
| TB, SS | 2.1854E-2 | 1 | 47 |
| TB, LM | 0.4384 | 0.705 | 53 |
| SS, LM | 0.87105 | 0.407 | 32 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CB, PJ | 2.4505 | 0.056 | 76 |
| **CB, JB** | **5.1405** | **0.004** | 56 |
| **CB, FP** | **2.4972** | **0.02** | 60 |
| **CB, TH** | **9.609** | **0.002** | 108 |
| **CB, FG** | **8.6725** | **0.001** | 76 |
| PJ, JB | 7.6618E-2 | 0.963 | 59 |
| PJ, FP | 0.34806 | 0.757 | 74 |
| **PJ, TH** | **3.1992** | **0.006** | 88 |
| PJ, FG | 1.624 | 0.15 | 60 |
| JB, FP | 0.42645 | 0.702 | 52 |
| **JB, TH** | **5.9635** | **0.002** | 75 |
| **JB, FG** | **3.8813** | **0.01** | 45 |
| **FP, TH** | **4.2857** | **0.005** | 91 |
| **FP, FG** | **2.5252** | **0.046** | 65 |
| **TH, FG** | **3.0569** | **0.01** | 50 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| SC, BI | 0.34159 | 0.836 | 55 |
| SC, SB | 0.39242 | 0.75 | 26 |
| SC, GB | 0.74026 | 0.466 | 25 |
| BI, SB | 0.20519 | 0.954 | 51 |
| BI, GB | 0.63479 | 0.671 | 55 |
| SB, GB | 1.666 | 0.13 | 19 |

**ABOVE-GROUND BIOMASS**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 1.3755 | 0.206 | 382 |
| **CG, KB** | **3.2665** | **0.017** | 413 |
| CG, TB | 0.57969 | 0.571 | 404 |
| **CG, SS** | **2.5623** | **0.025** | 402 |
| CG, LM | 1.8163 | 0.082 | 406 |
| BT, KB | 1.0914 | 0.303 | 414 |
| BT, TB | 0.51656 | 0.638 | 408 |
| BT, SS | 1.1036 | 0.305 | 406 |
| BT, LM | 0.62044 | 0.545 | 410 |
| KB, TB | 1.4865 | 0.18 | 398 |
| KB, SS | 0.32757 | 0.777 | 406 |
| KB, LM | 0.14406 | 0.907 | 407 |
| TB, SS | 1.465 | 0.167 | 412 |
| TB, LM | 1.0136 | 0.342 | 410 |
| SS, LM | 0.34912 | 0.722 | 407 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CB, PJ** | **2.4162** | **0.023** | 398 |
| **CB, JB** | **2.7982** | **0.023** | 395 |
| CB, FP | 1.6103 | 0.186 | 304 |
| **CB, TH** | **3.0333** | **0.002** | 406 |
| **CB, FG** | **7.3527** | **0.002** | 407 |
| PJ, JB | 1.019 | 0.394 | 417 |
| PJ, FP | 0.14824 | 0.866 | 408 |
| PJ, TH | 0.64584 | 0.501 | 409 |
| PJ, FG | 0.77232 | 0.46 | 414 |
| JB, FP | 0.59203 | 0.58 | 401 |
| JB, TH | 1.7454 | 0.121 | 424 |
| **JB, FG** | **3.5571** | **0.008** | 394 |
| FP, TH | 0.68849 | 0.509 | 415 |
| FP, FG | 0.7539 | 0.496 | 417 |
| TH, FG | 0.12666 | 0.9 | 406 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| SC, BI | 1.1892 | 0.282 | 395 |
| SC, SB | 1.0923 | 0.298 | 417 |
| SC, GB | 0.30814 | 0.757 | 371 |
| BI, SB | 0.14914 | 0.88 | 377 |
| BI, GB | 2.6996 | 0.033 | 278 |
| SB, GB | 1.9837 | 0.08 | 407 |

**BELOW-GROUND BIOMASS**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 1.8372 | 0.089 | 413 |
| CG, KB | 1.4067 | 0.189 | 406 |
| CG, TB | 0.70174 | 0.493 | 404 |
| CG, SS | 1.0463 | 0.301 | 410 |
| CG, LM | 0.19483 | 0.845 | 408 |
| BT, KB | 0.58687 | 0.545 | 404 |
| BT, TB | 0.60361 | 0.643 | 414 |
| BT, SS | 1.5535 | 0.134 | 397 |
| **BT, LM** | **2.6367** | **0.032** | 407 |
| KB, TB | 0.29217 | 0.843 | 404 |
| KB, SS | 0.76975 | 0.437 | 403 |
| KB, LM | 1.8884 | 0.089 | 403 |
| TB, SS | 5.6035E-2 | 0.963 | 407 |
| TB, LM | 0.67155 | 0.551 | 413 |
| SS, LM | 1.5085 | 0.17 | 406 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CB, PJ | 2.3477 | 0.053 | 402 |
| **CB, JB** | **6.3625** | **0.003** | 422 |
| CB, FP | 0.93695 | 0.37 | 309 |
| CB, TH | 1.6079 | 0.15 | 397 |
| **CB, FG** | **2.3169** | **0.048** | 403 |
| PJ, JB | 0.78125 | 0.507 | 373 |
| **PJ, FP** | **3.0206** | **0.007** | 406 |
| PJ, TH | 1.069 | 0.302 | 411 |
| PJ, FG | 1.2453 | 0.265 | 417 |
| **JB, FP** | **9.9744** | **0.004** | 410 |
| **JB, TH** | **2.9972** | **0.021** | 404 |
| **JB, FG** | **6.13** | **0.005** | 412 |
| **FP, TH** | **2.5428** | **0.021** | 406 |
| **FP, FG** | **4.2396** | **0.003** | 400 |
| TH, FG | 1.2035E-2 | 0.99 | 407 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| SC, BI | 1.0925 | 0.335 | 402 |
| SC, SB | 1.4226 | 0.169 | 402 |
| SC, GB | 0.30036 | 0.789 | 416 |
| BI, SB | 0.39763 | 0.677 | 412 |
| **BI, GB** | **2.2696** | **0.049** | 295 |
| **SB, GB** | **3.3592** | **0.014** | 401 |

**ABOVE-GROUND % NITROGEN**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CG, BT** | **2.3558** | **0.048** | 412 |
| **CG, KB** | **4.2013** | **0.005** | 400 |
| **CG, TB** | **4.0991** | **0.006** | 414 |
| **CG, SS** | **8.4204** | **0.002** | 407 |
| CG, LM | 1.6977 | 0.136 | 414 |
| BT, KB | 1.8872 | 0.098 | 411 |
| BT, TB | 1.9331 | 0.084 | 413 |
| **BT, SS** | **6.7201** | **0.003** | 416 |
| BT, LM | 0.68534 | 0.512 | 415 |
| KB, TB | 0.30599 | 0.743 | 126 |
| **KB, SS** | **6.0371** | **0.004** | 398 |
| **KB, LM** | **2.6183** | **0.03** | 399 |
| **TB, SS** | **5.206** | **0.004** | 404 |
| **TB, LM** | **2.6027** | **0.024** | 411 |
| **SS, LM** | **7.277** | **0.002** | 419 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CB, PJ** | **2.8986** | **0.013** | 411 |
| **CB, JB** | **5.6518** | **0.003** | 403 |
| CB, FP | 0.16518 | 0.834 | 394 |
| CB, TH | 1.6871 | 0.152 | 126 |
| **CB, FG** | **2.8511** | **0.016** | 407 |
| PJ, JB | 1.923 | 0.08 | 415 |
| **PJ, FP** | **1.9828** | **0.048** | 412 |
| PJ, TH | 0.30821 | 0.772 | 207 |
| PJ, FG | 0.47061 | 0.65 | 417 |
| **JB, FP** | **3.4713** | **0.005** | 418 |
| JB, TH | 1.6444 | 0.121 | 207 |
| **JB, FG** | **2.88** | **0.012** | 407 |
| FP, TH | 1.2329 | 0.273 | 208 |
| FP, FG | 1.7722 | 0.083 | 413 |
| TH, FG | 1.3121E-2 | 0.99 | 209 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| SC, BI | 2.3307 | 0.059 | 306 |
| **SC, SB** | **1.9185** | **0.037** | 399 |
| SC, GB | 1.2423 | 0.236 | 401 |
| BI, SB | 1.0224 | 0.401 | 311 |
| BI, GB | 0.51796 | 0.608 | 314 |
| SB, GB | 1.1595 | 0.319 | 410 |

**BELOW-GROUND % NITROGEN**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 6.5127E-2 | 0.95 | 411 |
| CG, KB | 1.4669 | 0.086 | 421 |
| **CG, TB** | **2.3691** | **0.013** | 412 |
| CG, SS | 0.98916 | 0.452 | 405 |
| CG, LM | 1.2929 | 0.234 | 405 |
| BT, KB | 1.7659 | 0.113 | 410 |
| **BT, TB** | **2.7858** | **0.015** | 412 |
| BT, SS | 1.1717 | 0.256 | 412 |
| BT, LM | 1.5373 | 0.178 | 410 |
| KB, TB | 2.645 | 0.054 | 126 |
| KB, SS | 0.68531 | 0.506 | 407 |
| KB, LM | 0.4336 | 0.645 | 401 |
| TB, SS | 2.0867 | 0.053 | 406 |
| TB, LM | 2.246 | 0.061 | 423 |
| SS, LM | 0.3345 | 0.724 | 410 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CB, PJ | 0.31889 | 0.766 | 404 |
| CB, JB | 0.47003 | 0.682 | 414 |
| **CB, FP** | **3.0963** | **0.01** | 414 |
| CB, TH | 0.36999 | 0.653 | 126 |
| CB, FG | 1.3084 | 0.244 | 410 |
| PJ, JB | 0.22375 | 0.835 | 395 |
| **PJ, FP** | **2.3789** | **0.036** | 415 |
| PJ, TH | 0.52471 | 0.561 | 208 |
| PJ, FG | 1.2498 | 0.228 | 412 |
| JB, FP | 1.7141 | 0.107 | 413 |
| JB, TH | 0.5975 | 0.594 | 208 |
| JB, FG | 1.1955 | 0.25 | 416 |
| **FP, TH** | **2.8211** | **0.016** | 209 |
| **FP, FG** | **4.1114** | **0.002** | 408 |
| TH, FG | 0.6527 | 0.519 | 209 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| SC, BI | 0.15111 | 0.902 | 411 |
| **SC, SB** | **3.0769** | **0.019** | 410 |
| SC, GB | 0.86823 | 0.431 | 415 |
| **BI, SB** | **2.2207** | **0.05** | 417 |
| BI, GB | 0.29033 | 0.72 | 310 |
| **SB, GB** | **4.2062** | **0.003** | 411 |

**ABOVE-GROUND $\delta^{13}C$**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 0.82089 | 0.428 | 410 |
| **CG, KB** | **4.8243** | **0.004** | 407 |
| **CG, TB** | **11.699** | **0.004** | 403 |
| **CG, SS** | **5.2914** | **0.004** | 412 |
| CG, LM | 1.8631 | 0.098 | 409 |
| **BT, KB** | **11.56** | **0.002** | 405 |
| **BT, TB** | **15.919** | **0.002** | 411 |
| **BT, SS** | **6.6666** | **0.004** | 409 |
| BT, LM | 1.6658 | 0.126 | 400 |
| **KB, TB** | **8.9331** | **0.014** | 126 |
| **KB, SS** | **2.2118** | **0.035** | 409 |
| **KB, LM** | **5.0698** | **0.006** | 401 |
| **TB, SS** | **4.3383** | **0.005** | 414 |
| **TB, LM** | **10.594** | **0.004** | 408 |
| **SS, LM** | **5.9449** | **0.006** | 412 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CB, PJ** | **4.3453** | **0.008** | 414 |
| **CB, JB** | **3.5219** | **0.009** | 410 |
| **CB, FP** | **3.8592** | **0.003** | 409 |
| **CB, TH** | **3.0178** | **0.046** | 126 |
| **CB, FG** | **4.6353** | **0.005** | 403 |
| PJ, JB | 1.625 | 0.138 | 412 |
| PJ, FP | 0.26715 | 0.801 | 408 |
| PJ, TH | 1.4693 | 0.192 | 206 |
| PJ, FG | 0.59002 | 0.566 | 416 |
| JB, FP | 1.2302 | 0.256 | 405 |
| JB, TH | 8.9736E-2 | 0.946 | 210 |
| JB, FG | 1.2836 | 0.225 | 403 |
| FP, TH | 1.116 | 0.347 | 209 |
| FP, FG | 0.25076 | 0.872 | 411 |
| TH, FG | 1.2449 | 0.233 | 209 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **SC, BI** | **6.3936** | **0.001** | 404 |
| **SC, SB** | **8.405** | **0.003** | 411 |
| **SC, GB** | **5.3879** | **0.002** | 397 |
| BI, SB | 0.42818 | 0.695 | 408 |
| BI, GB | 1.7991 | 0.097 | 406 |
| **SB, GB** | **2.8507** | **0.02** | 409 |

**BELOW-GROUND $\delta^{13}C$**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 0.23596 | 0.817 | 402 |
| **CG, KB** | **3.0739** | **0.011** | 402 |
| **CG, TB** | **4.9345** | **0.002** | 417 |
| **CG, SS** | **2.6183** | **0.026** | 412 |
| CG, LM | 5.8686E-2 | 0.94 | 403 |
| **BT, KB** | **6.5242** | **0.002** | 414 |
| **BT, TB** | **7.7868** | **0.001** | 405 |
| **BT, SS** | **4.0125** | **0.007** | 408 |
| BT, LM | 0.27 | 0.783 | 403 |
| **KB, TB** | **3.1342** | **0.018** | 126 |
| KB, SS | 0.5767 | 0.568 | 412 |
| **KB, LM** | **4.6117** | **0.003** | 393 |
| **TB, SS** | **3.2941** | **0.008** | 415 |
| **TB, LM** | **6.556** | **0.004** | 405 |
| **SS, LM** | **3.4618** | **0.013** | 407 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CB, PJ** | **3.363** | **0.015** | 404 |
| **CB, JB** | **2.322** | **0.024** | 406 |
| **CB, FP** | **2.724** | **0.036** | 403 |
| CB, TH | 1.7913 | 0.084 | 126 |
| CB, FG | 2.0977 | 0.063 | 418 |
| PJ, JB | 0.80166 | 0.448 | 418 |
| PJ, FP | 1.1249 | 0.292 | 401 |
| PJ, TH | 1.7384 | 0.137 | 207 |
| PJ, FG | 1.1681 | 0.246 | 422 |
| JB, FP | 1.5239 | 0.169 | 405 |
| JB, TH | 0.71616 | 0.628 | 209 |
| JB, FG | 0.30796 | 0.767 | 410 |
| FP, TH | 1.6681 | 0.147 | 207 |
| FP, FG | 1.717 | 0.13 | 410 |
| TH, FG | 0.42511 | 0.639 | 208 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **SC, BI** | **6.3316** | **0.006** | 411 |
| **SC, SB** | **6.7104** | **0.005** | 401 |
| **SC, GB** | **4.9424** | **0.005** | 407 |
| BI, SB | 1.1628 | 0.265 | 412 |
| **BI, GB** | **2.6346** | **0.037** | 400 |
| SB, GB | 2.2997 | 0.053 | 410 |

**ABOVE-GROUND $\delta^{15}N$**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CG, BT** | **2.4777** | **0.021** | 403 |
| **CG, KB** | **7.5251** | **0.007** | 416 |
| **CG, TB** | **3.9107** | **0.004** | 403 |
| **CG, SS** | **3.6956** | **0.008** | 406 |
| **CG, LM** | **26.441** | **0.005** | 398 |
| **BT, KB** | **7.4402** | **0.002** | 417 |
| **BT, TB** | **2.7904** | **0.004** | 408 |
| **BT, SS** | **6.0356** | **0.005** | 410 |
| **BT, LM** | **35.735** | **0.001** | 395 |
| KB, TB | 2.1159 | 0.063 | 125 |
| **KB, SS** | **9.3577** | **0.002** | 415 |
| **KB, LM** | **17.151** | **0.001** | 409 |
| **TB, SS** | **6.3261** | **0.003** | 415 |
| **TB, LM** | **15.358** | **0.003** | 409 |
| **SS, LM** | **23.726** | **0.002** | 407 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CB, PJ | 2.2267E-2 | 0.985 | 406 |
| **CB, JB** | **5.8538** | **0.003** | 401 |
| CB, FP | 0.47907 | 0.719 | 403 |
| CB, TH | 2.3756 | 0.062 | 126 |
| **CB, FG** | **7.6261** | **0.002** | 405 |
| **PJ, JB** | **5.8729** | **0.001** | 400 |
| PJ, FP | 0.52333 | 0.648 | 407 |
| PJ, TH | 2.0581 | 0.069 | 209 |
| **PJ, FG** | **7.1338** | **0.003** | 403 |
| **JB, FP** | **4.0726** | **0.002** | 416 |
| **JB, TH** | **7.5598** | **0.007** | 206 |
| JB, FG | 0.31636 | 0.782 | 413 |
| FP, TH | 0.46321 | 0.679 | 209 |
| **FP, FG** | **4.2104** | **0.004** | 408 |
| **TH, FG** | **11.606** | **0.004** | 208 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **SC, BI** | **4.8238** | **0.002** | 412 |
| **SC, SB** | **7.1835** | **0.003** | 403 |
| **SC, GB** | **3.5753** | **0.008** | 397 |
| BI, SB | 1.8264 | 0.096 | 406 |
| **BI, GB** | **2.8684** | **0.032** | 409 |
| **SB, GB** | **5.1799** | **0.007** | 405 |

**BELOW-GROUND $\delta^{15}N$**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 0.74644 | 0.485 | 412 |
| **CG, KB** | **3.079** | **0.027** | 409 |
| CG, TB | 2.3304 | 0.059 | 406 |
| CG, SS | 0.55369 | 0.582 | 409 |
| **CG, LM** | **13.402** | **0.003** | 415 |
| **BT, KB** | **3.4796** | **0.013** | 411 |
| **BT, TB** | **2.2847** | **0.05** | 414 |
| BT, SS | 0.10378 | 0.931 | 411 |
| **BT, LM** | **17.626** | **0.004** | 409 |
| KB, TB | 0.81179 | 0.492 | 126 |
| **KB, SS** | **2.6075** | **0.033** | 407 |
| **KB, LM** | **14.919** | **0.005** | 416 |
| TB, SS | 1.8373 | 0.096 | 412 |
| **TB, LM** | **13.817** | **0.004** | 411 |
| **SS, LM** | **13.468** | **0.006** | 412 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CB, PJ | 0.73195 | 0.489 | 398 |
| **CB, JB** | **6.9454** | **0.004** | 400 |
| CB, FP | 1.8533 | 0.095 | 418 |
| CB, TH | 1.7166 | 0.114 | 126 |
| **CB, FG** | **12.418** | **0.003** | 410 |
| **PJ, JB** | **5.1396** | **0.004** | 407 |
| PJ, FP | 0.79103 | 0.413 | 415 |
| PJ, TH | 0.45014 | 0.665 | 205 |
| **PJ, FG** | **8.074** | **0.004** | 395 |
| **JB, FP** | **5.1188** | **0.002** | 410 |
| **JB, TH** | **5.6404** | **0.008** | 207 |
| **JB, FG** | **2.4333** | **0.045** | 418 |
| FP, TH | 0.40018 | 0.743 | 209 |
| **FP, FG** | **9.0758** | **0.003** | 405 |
| **TH, FG** | **12.703** | **0.007** | 208 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **SC, BI** | **5.7115** | **0.002** | 405 |
| **SC, SB** | **7.8994** | **0.002** | 411 |
| **SC, GB** | **4.2397** | **0.001** | 401 |
| BI, SB | 1.4828 | 0.175 | 416 |
| **BI, GB** | **2.8458** | **0.027** | 412 |
| **SB, GB** | **5.3599** | **0.007** | 411 |

**SEDIMENT ORGANIC CONTENT**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 1.7151 | 0.114 | 126 |
| **CG, KB** | **2.6684** | **0.026** | 126 |
| **CG, TB** | **4.9235** | **0.009** | 126 |
| **CG, SS** | **5.8947** | **0.011** | 126 |
| **CG, LM** | **3.6579** | **0.01** | 126 |
| BT, KB | 1.0323 | 0.28 | 126 |
| **BT, TB** | **4.7192** | **0.004** | 126 |
| **BT, SS** | **5.4212** | **0.01** | 126 |
| **BT, LM** | **2.3821** | **0.044** | 126 |
| **KB, TB** | **4.585** | **0.012** | 126 |
| **KB, SS** | **5.0991** | **0.009** | 126 |
| KB, LM | 1.5324 | 0.193 | 125 |
| **TB, SS** | **2.1819** | **0.042** | 126 |
| **TB, LM** | **4.3067** | **0.013** | 126 |
| **SS, LM** | **4.3936** | **0.007** | 126 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CB, PJ | 2.4445 | 0.071 | 10 |
| **CB, JB** | **8.258** | **0.005** | 83 |
| CB, FP | 0.88232 | 0.381 | 176 |
| **CB, TH** | **2.1108** | **0.013** | 179 |
| **CB, FG** | **5.4798** | **0.003** | 178 |
| **PJ, JB** | **7.6854** | **0.007** | 80 |
| PJ, FP | 0.37945 | 0.725 | 222 |
| PJ, TH | 0.93785 | 0.432 | 226 |
| **PJ, FG** | **4.7975** | **0.005** | 223 |
| **JB, FP** | **6.8211** | **0.005** | 352 |
| **JB, TH** | **6.1924** | **0.003** | 345 |
| **JB, FG** | **2.7019** | **0.024** | 353 |
| FP, TH | 0.95346 | 0.35 | 126 |
| **FP, FG** | **4.21** | **0.007** | 125 |
| **TH, FG** | **3.5053** | **0.019** | 126 |

**MICROPHYTOBENTHOS**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 0.36645 | 0.742 | 418 |
| CG, KB | 1.8438 | 0.077 | 400 |
| **CG, TB** | **2.9558** | **0.022** | 400 |
| CG, SS | 0.92136 | 0.38 | 408 |
| CG, LM | 0.51474 | 0.685 | 399 |
| **BT, KB** | **2.9258** | **0.01** | 414 |
| **BT, TB** | **12.613** | **0.002** | 419 |
| **BT, SS** | **2.7811** | **0.006** | 408 |
| BT, LM | 0.40842 | 0.658 | 314 |
| KB, TB | 0.63746 | 0.945 | 414 |
| KB, SS | 1.464 | 0.158 | 405 |
| **KB, LM** | **2.997** | **0.01** | 415 |
| **TB, SS** | **4.4877** | **0.005** | 409 |
| **TB, LM** | **9.4367** | **0.003** | 402 |
| **SS, LM** | **2.7711** | **0.018** | 412 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CB, PJ | 0.90843 | 0.368 | 408 |
| CB, JB | 0.82883 | 0.794 | 413 |
| CB, FP | 0.3793 | 0.773 | 412 |
| CB, TH | 0.47847 | 0.64 | 414 |
| CB, FG | 0.47314 | 0.654 | 413 |
| PJ, JB | 0.99588 | 0.513 | 409 |
| PJ, FP | 6.3077E-2 | 1 | 413 |
| PJ, TH | 1.2165 | 0.357 | 404 |
| **PJ, FG** | **2.3758** | **0.042** | 412 |
| JB, FP | 0.93092 | 0.467 | 411 |
| JB, TH | 0.68003 | 0.811 | 408 |
| JB, FG | 0.74454 | 0.966 | 401 |
| FP, TH | 0.69534 | 0.461 | 409 |
| FP, FG | 0.68866 | 0.573 | 408 |
| TH, FG | 0.18605 | 0.873 | 401 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| SC, BI | 0.9208 | 0.401 | 410 |
| SC, SB | 1.623 | 0.129 | 404 |
| **SC, GB** | **2.8253** | **0.012** | 411 |
| **BI, SB** | **2.795** | **0.025** | 413 |
| **BI, GB** | **2.2289** | **0.034** | 412 |
| **SB, GB** | **5.0539** | **0.009** | 419 |

**INFAUNA ASSEMBLAGE (ABUNDANCE)**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| BT, CG | 0.70095 | 0.837 | 412 |
| **BT, KB** | **2.2094** | **0.004** | 411 |
| **BT, LM** | **2.8955** | **0.004** | 402 |
| **BT, SS** | **3.442** | **0.002** | 418 |
| **BT, TB** | **3.2486** | **0.005** | 420 |
| **CG, KB** | **2.6109** | **0.002** | 416 |
| **CG, LM** | **3.6984** | **0.004** | 408 |
| **CG, SS** | **4.3619** | **0.001** | 406 |
| **CG, TB** | **3.7153** | **0.001** | 415 |
| **KB, LM** | **3.3728** | **0.001** | 403 |
| **KB, SS** | **3.5281** | **0.006** | 414 |
| **KB, TB** | **2.4474** | **0.005** | 416 |
| **LM, SS** | **3.1613** | **0.002** | 413 |
| **LM, TB** | **4.0995** | **0.005** | 407 |
| **SS, TB** | **3.4235** | **0.002** | 409 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| BI, GB | 1 | 1 | 1 |
| BI, SB | 2.2448 | 0.074 | 6 |
| BI, SC | 1.8066 | 0.061 | 6 |
| GB, SB | 1.7549 | 0.11 | 9 |
| GB, SC | 1.4212 | 0.105 | 8 |
| SB, SC | 1.1128 | 0.254 | 42 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CR, FG | 0.95852 | 0.527 | 123 |
| **CR, FP** | **1.6961** | **0.018** | 306 |
| CR, ST | 1.4322 | 0.087 | 231 |
| **CR, SM** | **2.0014** | **0.004** | 310 |
| **CR, TH** | **1.948** | **0.012** | 236 |
| CR, CB | 0.69414 | 0.832 | 200 |
| **CR, JB** | **1.6672** | **0.017** | 313 |
| CR, PJ | 1.5086 | 0.07 | 201 |
| FG, FP | 1.2072 | 0.228 | 309 |
| **FG, ST** | **1.87** | **0.02** | 310 |
| FG, SM | 1.4554 | 0.054 | 313 |
| FG, TH | 1.1206 | 0.296 | 199 |
| FG, CB | 1.0746 | 0.312 | 239 |
| FG, JB | 1.3125 | 0.107 | 317 |
| FG, PJ | 1.2741 | 0.114 | 306 |
| **FP, ST** | **2.2964** | **0.007** | 405 |
| FP, SM | 1.1199 | 0.267 | 411 |
| FP, TH | 1.2222 | 0.207 | 416 |
| FP, CB | 1.4248 | 0.06 | 402 |
| **FP, JB** | **1.4259** | **0.038** | 401 |
| FP, PJ | 1.3499 | 0.055 | 415 |
| **ST, SM** | **2.8394** | **0.002** | 418 |
| **ST, TH** | **2.9972** | **0.005** | 415 |
| **ST, CB** | **1.5764** | **0.027** | 408 |
| **ST, JB** | **2.5841** | **0.005** | 401 |
| **ST, PJ** | **2.2189** | **0.008** | 409 |
| SM, TH | 0.74868 | 0.683 | 406 |
| **SM, CB** | **1.7693** | **0.008** | 399 |
| SM, JB | 1.163 | 0.319 | 409 |
| SM, PJ | 1.2092 | 0.134 | 406 |
| **TH, CB** | **1.8172** | **0.009** | 409 |
| TH, JB | 1.2165 | 0.268 | 415 |
| TH, PJ | 1.3339 | 0.117 | 412 |
| **CB, JB** | **1.7503** | **0.006** | 415 |
| CB, PJ | 1.4961 | 0.054 | 309 |
| JB, PJ | 0.73185 | 0.814 | 405 |

**INFAUNA ASSEMBLAGE (BIOMASS)**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 0.97761 | 0.488 | 407 |
| **CG, KB** | **2.7139** | **0.003** | 413 |
| **CG, TB** | **3.7714** | **0.003** | 411 |
| **CG, SS** | **3.8588** | **0.002** | 413 |
| **CG, LM** | **3.8216** | **0.003** | 412 |
| **BT, KB** | **1.9919** | **0.004** | 398 |
| **BT, TB** | **3.1927** | **0.004** | 417 |
| **BT, SS** | **2.9081** | **0.006** | 409 |
| **BT, LM** | **3.1372** | **0.003** | 414 |
| **KB, TB** | **3.2865** | **0.002** | 415 |
| **KB, SS** | **3.4765** | **0.001** | 402 |
| **KB, LM** | **4.2896** | **0.002** | 415 |
| **TB, SS** | **3.3311** | **0.004** | 418 |
| **TB, LM** | **6.2542** | **0.004** | 413 |
| **SS, LM** | **5.2537** | **0.006** | 413 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| GB, SB | 1.69 | 0.103 | 16 |
| GB, SC | 1.1764 | 0.247 | 9 |
| GB, BI | 1 | 1 | 1 |
| SB, SC | 1.0406 | 0.284 | 86 |
| SB, BI | 2.161 | 0.056 | 8 |
| SC, BI | 1.542 | 0.062 | 6 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CB, JB** | **1.4994** | **0.03** | 405 |
| CB, PJ | 1.3865 | 0.071 | 314 |
| **CB, SM** | **1.9446** | **0.003** | 397 |
| CB, CR | 0.88155 | 0.645 | 308 |
| **CB, ST** | **1.8706** | **0.003** | 403 |
| **CB, FP** | **1.5237** | **0.045** | 415 |
| **CB, TH** | **1.6648** | **0.048** | 400 |
| CB, FG | 0.88575 | 0.64 | 408 |
| JB, PJ | 0.84255 | 0.736 | 403 |
| JB, SM | 1.2803 | 0.189 | 400 |
| JB, CR | 1.258 | 0.182 | 407 |
| **JB, ST** | **2.0397** | **0.005** | 416 |
| JB, FP | 1.225 | 0.225 | 419 |
| JB, TH | 1.0387 | 0.419 | 419 |
| JB, FG | 1.1215 | 0.227 | 405 |
| **PJ, SM** | **1.342** | **0.05** | 409 |
| PJ, CR | 1.2383 | 0.219 | 315 |
| **PJ, ST** | **1.7306** | **0.022** | 415 |
| PJ, FP | 1.1881 | 0.204 | 406 |
| PJ, TH | 1.2264 | 0.214 | 411 |
| PJ, FG | 1.1579 | 0.2 | 414 |
| **SM, CR** | **1.8915** | **0.001** | 398 |
| **SM, ST** | **2.3378** | **0.001** | 411 |
| SM, FP | 1.1763 | 0.189 | 420 |
| SM, TH | 1.2989 | 0.189 | 415 |
| **SM, FG** | **1.8149** | **0.004** | 408 |
| CR, ST | 1.3031 | 0.092 | 396 |
| **CR, FP** | **1.5186** | **0.035** | 407 |
| **CR, TH** | **1.7618** | **0.014** | 402 |
| CR, FG | 0.79493 | 0.757 | 408 |
| **ST, FP** | **1.96** | **0.007** | 411 |
| **ST, TH** | **2.5723** | **0.008** | 408 |
| **ST, FG** | **1.8521** | **0.005** | 399 |
| FP, TH | 1.1006 | 0.313 | 414 |
| FP, FG | 1.3703 | 0.078 | 407 |
| TH, FG | 1.3745 | 0.073 | 414 |

## SPECIES RICHNESS

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| BT, CG | 1.3828 | 0.187 | 52 |
| BT, KB | 1.0625 | 0.385 | 27 |
| BT, LM | 2.5107 | 0.073 | 34 |
| BT, SS | 0.41638 | 0.822 | 20 |
| BT, TB | 0.22751 | 0.804 | 34 |
| CG, KB | 2.2852 | 0.056 | 65 |
| CG, LM | 0.63532 | 0.524 | 48 |
| CG, SS | 1.9985 | 0.137 | 30 |
| CG, TB | 1.2449 | 0.251 | 55 |
| **KB, LM** | **3.5807** | **0.011** | 63 |
| KB, SS | 0.98697 | 0.385 | 23 |
| KB, TB | 1.3212 | 0.232 | 41 |
| **LM, SS** | **4.1246** | **0.004** | 46 |
| **LM, TB** | **2.4209** | **0.025** | 53 |
| SS, TB | 0.68783 | 0.637 | 14 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| BI, GB | 1 | 1 | 1 |
| BI, SB | 3.0038 | 0.059 | 4 |
| BI, SC | 2.9328 | 0.068 | 4 |
| GB, SB | 2.1661 | 0.121 | 6 |
| GB, SC | 1.9047 | 0.174 | 5 |
| SB, SC | 0.50565 | 0.762 | 10 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CR, FG | 0.61989 | 0.686 | 17 |
| **CR, FP** | **2.3249** | **0.031** | 109 |
| CR, ST | 1.8451 | 0.083 | 48 |
| CR, SM | 1.9173 | 0.082 | 64 |
| CR, TH | 0.98086 | 0.391 | 23 |
| CR, CB | 0.52999 | 0.707 | 71 |
| CR, JB | 0.83527 | 0.431 | 30 |
| CR, PJ | 1.1621 | 0.267 | 71 |
| **FG, FP** | **3.7725** | **0.008** | 23 |
| **FG, ST** | **2.8316** | **0.027** | 26 |
| **FG, SM** | **2.9125** | **0.027** | 35 |
| FG, TH | 0.98704 | 0.502 | 8 |
| FG, CB | 0.83807 | 0.492 | 12 |
| FG, JB | 0.76337 | 0.546 | 6 |
| FG, PJ | 1.6513 | 0.099 | 35 |
| FP, ST | 0.99423 | 0.342 | 23 |
| FP, SM | 0.60432 | 0.595 | 47 |
| **FP, TH** | **3.4748** | **0.016** | 42 |
| FP, CB | 1.7712 | 0.134 | 47 |
| **FP, JB** | **3.1339** | **0.013** | 31 |
| FP, PJ | 0.97268 | 0.424 | 42 |
| ST, SM | 0.32557 | 0.843 | 26 |
| ST, TH | 2.3225 | 0.069 | 13 |
| ST, CB | 1.2541 | 0.245 | 53 |
| ST, JB | 2.1488 | 0.066 | 20 |
| ST, PJ | 0.5886 | 0.924 | 27 |
| SM, TH | 2.4612 | 0.062 | 34 |
| SM, CB | 1.3493 | 0.179 | 94 |
| SM, JB | 2.2886 | 0.056 | 45 |
| SM, PJ | 0.62069 | 0.831 | 52 |
| TH, CB | 0.6925 | 0.534 | 42 |
| TH, JB | 0.18953 | 1 | 9 |
| TH, PJ | 1.2997 | 0.208 | 47 |
| CB, JB | 0.59196 | 0.623 | 31 |
| CB, PJ | 0.67302 | 0.582 | 61 |
| JB, PJ | 1.2413 | 0.198 | 52 |

## SHANNON DIVERSITY

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| BT, CG | 1.2973 | 0.192 | 414 |
| BT, KB | 0.39601 | 0.704 | 422 |
| **BT, LM** | **2.8423** | **0.03** | 411 |
| BT, SS | 1.6554 | 0.144 | 412 |
| BT, TB | 0.55742 | 0.614 | 404 |
| CG, KB | 1.6369 | 0.138 | 409 |
| CG, LM | 1.549 | 0.162 | 409 |
| **CG, SS** | **3.2254** | **0.003** | 410 |
| CG, TB | 0.51874 | 0.666 | 405 |
| **KB, LM** | **3.0852** | **0.014** | 407 |
| KB, SS | 1.1283 | 0.271 | 415 |
| KB, TB | 0.87282 | 0.385 | 413 |
| **LM, SS** | **5.2909** | **0.002** | 400 |
| LM, TB | 1.6716 | 0.076 | 405 |
| SS, TB | 1.9752 | 0.081 | 410 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| BI, GB | Denominator is 0 | | |
| BI, SB | 2.2361 | 0.181 | 2 |
| BI, SC | 1 | 1 | 1 |
| GB, SB | 2.2361 | 0.175 | 2 |
| GB, SC | 1 | 1 | 1 |
| SB, SC | 1.1952 | 0.551 | 3 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CR, FG | 0.86417 | 0.411 | 8 |
| **CR, FP** | **2.6743** | **0.024** | 146 |
| CR, ST | 1.8906 | 0.104 | 152 |
| CR, SM | 1.9904 | 0.084 | 147 |
| CR, TH | 0.29929 | 0.974 | 22 |
| CR, CB | 0.32463 | 0.73 | 32 |
| CR, JB | 0.16904 | 0.964 | 23 |
| CR, PJ | 1.188 | 0.293 | 118 |
| **FG, FP** | **4.3183** | **0.015** | 32 |
| **FG, ST** | **3.2433** | **0.011** | 62 |
| **FG, SM** | **3.3644** | **0.014** | 61 |
| FG, TH | 1.2611 | 0.27 | 12 |
| FG, CB | 1.141 | 0.44 | 8 |
| FG, JB | 0.84554 | 0.542 | 6 |
| FG, PJ | 2.2678 | 0.069 | 32 |
| FP, ST | 1.0818 | 0.294 | 315 |
| FP, SM | 0.92097 | 0.387 | 199 |
| **FP, TH** | **3.2161** | **0.01** | 108 |
| FP, CB | 2.0251 | 0.121 | 149 |
| **FP, JB** | **3.3034** | **0.003** | 110 |
| FP, PJ | 1.5053 | 0.146 | 307 |
| ST, SM | 0.14567 | 0.929 | 313 |
| ST, TH | 2.0859 | 0.057 | 174 |
| ST, CB | 1.3751 | 0.182 | 110 |
| ST, JB | 2.2996 | 0.057 | 150 |
| ST, PJ | 0.65177 | 0.561 | 304 |
| **SM, TH** | **2.2193** | **0.049** | 126 |
| SM, CB | 1.4609 | 0.17 | 198 |
| SM, JB | 2.4199 | 0.052 | 144 |
| SM, PJ | 0.76458 | 0.475 | 413 |
| TH, CB | 0.35244 | 0.8 | 46 |
| TH, JB | 0.36898 | 0.759 | 34 |
| TH, PJ | 1.1846 | 0.25 | 149 |
| CB, JB | 0.48975 | 0.636 | 24 |
| CB, PJ | 0.7859 | 0.494 | 114 |
| JB, PJ | 1.4405 | 0.164 | 87 |

**TOTAL ABUNDANCE**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| BT, CG | 0.63809 | 0.578 | 226 |
| BT, KB | 0.27837 | 0.915 | 171 |
| BT, LM | 1.9699 | 0.118 | 232 |
| BT, SS | 1.1276 | 0.266 | 147 |
| BT, TB | 1.9725 | 0.075 | 314 |
| CG, KB | 0.92732 | 0.367 | 312 |
| CG, LM | 1.4479 | 0.176 | 313 |
| CG, SS | 0.46399 | 0.73 | 152 |
| CG, TB | 1.5047 | 0.146 | 410 |
| **KB, LM** | **2.323** | **0.016** | 116 |
| KB, SS | 1.4558 | 0.157 | 169 |
| **KB, TB** | **2.2937** | **0.021** | 234 |
| LM, SS | 2.1112 | 0.07 | 232 |
| LM, TB | 0.62361 | 0.581 | 231 |
| SS, TB | 1.8252 | 0.09 | 236 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| BI, GB | 1 | 1 | 1 |
| BI, SB | 3.0793 | 0.064 | 6 |
| BI, SC | 3.1276 | 0.055 | 4 |
| GB, SB | 1.929 | 0.119 | 9 |
| GB, SC | 1.8717 | 0.191 | 5 |
| SB, SC | 0.31667 | 0.743 | 16 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CR, FG | 1.3302 | 0.402 | 34 |
| **CR, FP** | **1.9479** | **0.019** | 145 |
| CR, ST | 1.7575 | 0.068 | 127 |
| **CR, SM** | **2.1417** | **0.006** | 149 |
| CR, TH | 1.5695 | 0.141 | 82 |
| CR, CB | 0.55588 | 0.82 | 91 |
| CR, JB | 1.6787 | 0.079 | 206 |
| CR, PJ | 1.2026 | 0.19 | 193 |
| **FG, FP** | **4.0308** | **0.008** | 32 |
| **FG, ST** | **3.422** | **0.017** | 60 |
| **FG, SM** | **5.4655** | **0.01** | 42 |
| FG, TH | 2.3281 | 0.056 | 26 |
| FG, CB | 0.93079 | 0.47 | 57 |
| **FG, JB** | **2.866** | **0.021** | 82 |
| **FG, PJ** | **2.0741** | **0.035** | 106 |
| FP, ST | 1.3534 | 0.199 | 87 |
| FP, SM | 0.5092 | 0.605 | 56 |
| FP, TH | 1.697 | 0.11 | 42 |
| FP, CB | 1.6087 | 0.068 | 99 |
| FP, JB | 0.89778 | 0.42 | 145 |
| FP, PJ | 0.87173 | 0.859 | 146 |
| **ST, SM** | **2.306** | **0.044** | 110 |
| ST, TH | 0.64985 | 0.601 | 71 |
| ST, CB | 1.2825 | 0.125 | 181 |
| ST, JB | 0.32152 | 0.864 | 289 |
| ST, PJ | 1.1899 | 0.125 | 372 |
| **SM, TH** | **2.5127** | **0.018** | 83 |
| **SM, CB** | **1.868** | **0.017** | 108 |
| SM, JB | 1.495 | 0.133 | 144 |
| SM, PJ | 0.92386 | 0.511 | 138 |
| TH, CB | 1.0384 | 0.274 | 80 |
| TH, JB | 0.70619 | 0.486 | 150 |
| TH, PJ | 1.2745 | 0.147 | 215 |
| CB, JB | 1.2204 | 0.131 | 104 |
| CB, PJ | 1.0344 | 0.461 | 170 |
| JB, PJ | 1.0081 | 0.481 | 289 |

**TOTAL BIOMASS**

Within level 'NB' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| CG, BT | 0.8051 | 0.497 | 404 |
| **CG, KB** | **2.8231** | **0.002** | 416 |
| CG, TB | 1.4614 | 0.122 | 406 |
| CG, SS | 0.8611 | 0.437 | 407 |
| **CG, LM** | **2.7904** | **0.019** | 408 |
| BT, KB | 1.5093 | 0.169 | 410 |
| BT, TB | 1.1577 | 0.411 | 411 |
| BT, SS | 1.3222 | 0.224 | 414 |
| BT, LM | 2.7237 | 0.053 | 403 |
| **KB, TB** | **4.2052** | **0.007** | 415 |
| **KB, SS** | **3.9024** | **0.007** | 407 |
| **KB, LM** | **9.3845** | **0.002** | 399 |
| **TB, SS** | **2.6988** | **0.014** | 403 |
| **TB, LM** | **8.9495** | **0.004** | 401 |
| SS, LM | 1.775 | 0.104 | 412 |

Within level 'NL' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| GB, SB | 1.7321 | 0.169 | 16 |
| GB, SC | 1.344 | 0.32 | 9 |
| GB, BI | 1 | 1 | 1 |
| SB, SC | 0.5771 | 0.725 | 87 |
| SB, BI | 2.8408 | 0.069 | 8 |
| SC, BI | 2.4786 | 0.057 | 6 |

Within level 'NS' of factor 'Region'

| Groups | t | P(perm) | Unique perms |
|---|---|---|---|
| **CB, JB** | **2.0447** | **0.036** | 403 |
| CB, PJ | 1.5674 | 0.128 | 308 |
| **CB, SM** | **3.6771** | **0.003** | 413 |
| CB, CR | 0.85738 | 0.508 | 311 |
| **CB, ST** | **3.2666** | **0.008** | 415 |
| **CB, FP** | **2.663** | **0.014** | 406 |
| CB, TH | 1.825 | 0.072 | 415 |
| CB, FG | 0.46567 | 0.928 | 414 |
| JB, PJ | 1.1371 | 0.26 | 399 |
| **JB, SM** | **2.706** | **0.016** | 402 |
| JB, CR | 0.95332 | 0.429 | 404 |
| **JB, ST** | **2.3586** | **0.035** | 408 |
| JB, FP | 1.4143 | 0.182 | 404 |
| JB, TH | 0.23096 | 0.962 | 413 |
| JB, FG | 1.6731 | 0.101 | 415 |
| PJ, SM | 1.1055 | 0.266 | 405 |
| PJ, CR | 1.0356 | 0.429 | 319 |
| PJ, ST | 0.94592 | 0.318 | 401 |
| PJ, FP | 0.77296 | 0.621 | 408 |
| PJ, TH | 1.114 | 0.297 | 420 |
| PJ, FG | 1.3337 | 0.147 | 410 |
| SM, CR | 2.4983 | 0.004 | 402 |
| SM, ST | 0.45116 | 0.715 | 401 |
| SM, FP | 0.81758 | 0.522 | 400 |
| **SM, TH** | **2.6772** | **0.01** | 400 |
| **SM, FG** | **3.2084** | **0.014** | 419 |
| **CR, ST** | **2.2958** | **0.02** | 412 |
| CR, FP | 1.6627 | 0.072 | 415 |
| CR, TH | 0.78235 | 0.534 | 318 |
| CR, FG | 0.69851 | 0.661 | 411 |
| ST, FP | 0.86215 | 0.432 | 401 |
| **ST, TH** | **2.3649** | **0.028** | 413 |
| **ST, FG** | **2.8514** | **0.029** | 410 |
| FP, TH | 1.4595 | 0.171 | 400 |
| FP, FG | 2.2795 | 0.054 | 411 |
| TH, FG | 1.4839 | 0.158 | 407 |

# Appendix 3A – Supplementary Materials for Chapter 3

Table 1. Species list of all species identified at Spectacle Island (SI), Carters Beach (CB), Old Warf (OW) and Port Joli (PJ). If a species was present within a site it is indicated with a plus (+), if it was absent it was left blank. See Chapter 3, Table 1 for site details.

| | SI | CB | OW | PJ |
|---|---|---|---|---|
| *Amphitrite* sp. | | | | + |
| *Arabella iricolor* | + | | | |
| *Capitella capitata* | + | | + | |
| *Cerastoderma pinnulatum* | | + | | |
| *Clymenella torquata* | + | + | + | + |
| *Corophium* sp. | | + | | + |
| *Drilonereis* sp. | | + | | + |
| *Glycera* sp. | | + | | + |
| *Harmothoe* sp. | | + | | + |
| *Hiatella arctica* | | + | | |
| *Lineus ruber* | + | | | |
| *Lumbrineris* sp. | + | | | |
| *Macoma* sp. | | + | | |
| *Nephtys* sp. | + | + | + | + |
| *Nereis* sp. | + | | | |
| *Ophelia* sp. | | | | + |
| *Pectinaria gouldii* | | | | + |
| *Pherusa affinis* | | | | + |
| *Saccoglossus kowalevskii* | | | | + |
| *Tellina agilis* | + | + | | |

Table 2. PERMANOVA pairwise comparison results for variables where a main effect was detected. T-value and associated p-values are reported. Significant p-values ($\leq 0.05$) are bolded.

| Site | Sediment organic | | Eelgrass AG biomass | | Infauna community (abundance) | | Infauna community (biomass) | |
|---|---|---|---|---|---|---|---|---|
| | t | p | t | p | t | p | t | p |
| CB, PJ | 2.44 | 0.06 | **2.42** | **0.02** | **1.50** | **0.05** | 1.39 | 0.09 |
| CB, SI | **3.25** | **0.003** | 1.47 | 0.21 | 1.17 | 0.16 | 1.16 | 0.19 |
| CB, OW | **4.05** | **0.003** | 1.64 | 0.15 | **2.07** | **0.001** | 1.94 | 0.23 |
| PJ, SI | **2.87** | **0.01** | **2.08** | **0.04** | 1.33 | 0.09 | 1.39 | 0.06 |
| PJ, OW | **2.87** | **0.01** | 1.30 | 0.24 | **1.70** | **0.03** | **1.68** | **0.01** |
| SI, OW | 1.78 | 0.097 | 0.69 | 0.52 | 1.31 | 0.13 | 1.44 | 0.06 |