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Impacts of organic enrichment from finfish aquaculture on seagrass beds and associated macroinfaunal communities in Atlantic Canada

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Eelgrass (Zostera marina) beds provide important habitat and food sources for a wide range of associated species both above- and belowground. Organic enrichment and nutrient loading from anthropogenic sources can change eelgrass canopy structure and macroinfauna community composition, making them important indicators of ecosystem health. In Atlantic Canada, there is growing concern about the impacts of finfish aquaculture on eelgrass habitats. To quantify these effects, we examined differences in environmental parameters, eelgrass bed structure and macroinfauna communities at increasing distances from a finfish farm in Port Mouton Bay, Nova Scotia and a reference site in an adjacent bay. We also compared the results to recently published large-scale survey results from the Atlantic coast. Results indicate increased organic enrichment and decreased eelgrass biomass, shoot density, and macroinfauna biomass closer to the farm. Moreover, community structure significantly differed between sites with some sensitive species disappearing while tolerant species increased closer to the farm. Changes in the macroinfauna community could be linked to observed differences in environmental and eelgrass bed variables. Our results provide new insights into the impacts of finfish aquaculture on eelgrass habitats in Atlantic Canada. We discuss possible assessment and monitoring metrics that would enable managers and regulators to evaluate the risk and potential changes to eelgrass habitat as a result of finfish aquaculture.
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ABSTRACT

Eelgrass (Zostera marina) beds provide important habitat and food sources for a wide range of associated species both above- and belowground. Organic enrichment and nutrient loading from anthropogenic sources can change eelgrass canopy structure and macroinfauna community composition, making them important indicators of ecosystem health. In Atlantic Canada, there is growing concern about the impacts of finfish aquaculture on eelgrass habitats. To quantify these effects, we examined differences in environmental parameters, eelgrass bed structure and macroinfauna communities at increasing distances from a finfish farm in Port Mouton Bay, Nova Scotia and a reference site in an adjacent bay. We also compared the results to recently published large-scale survey results from the Atlantic coast. Results indicate increased organic enrichment and decreased eelgrass biomass, shoot density, and macroinfauna biomass closer to the farm. Moreover, community structure significantly differed between sites with some sensitive species disappearing while tolerant species increased closer to the farm. Changes in the macroinfauna community could be linked to observed differences in environmental and eelgrass bed variables. Our results provide new insights into the impacts of finfish aquaculture on eelgrass habitats in Atlantic Canada. We discuss possible assessment and monitoring metrics that would enable managers and regulators to evaluate the risk and potential changes to eelgrass habitat as a result of finfish aquaculture.
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 by anthropogenic activities around the world (Orth et al., 2006; Waycott et al., 2009; Short et al., 2011). Discharge of nutrients and accumulation of organic matter from human sources, such as municipal and industrial wastewater and land run-off are some of the most influential causes of degradation to seagrass habitats in coastal waters (Hauxwell, Cebrian, Valiela 2003; Waycott et al., 2009; Schmidt et al., 2012). Over the past two decades, the development and rapid expansion of finfish aquaculture (such as open netpens) into sensitive coastal habitats has further increased the risk of degradation to seagrasses (Duarte, 2002; Vandermeulen, 2005; Holmer et al., 2008).

The impacts of marine fish farms on seagrass beds have been examined extensively, but not exclusively, on *Posidonia oceanica* meadows along the Mediterranean coast. These studies have shown that increased nutrient loading, organic matter and sedimentation rates resulting from fish farm activities can lead to changes in the size of seagrass meadows (e.g., decrease, disappearance, Pergent-Martini et al., 2006), its structure (e.g., shoot density, leaf morphometrics, rhizome growth, epiphyte loads, carbon sequestration, Pérez et al., 2008; Apostolaki et al., 2011, 2012; Rountos, Peterson & Karakassis, 2012), as well as changes in sediment composition and chemistry (e.g., % organic matter, grain size, sulphides, primary production, Holmer et al., 2008), and macroinfauna communities (e.g., abundance, composition, functional groups, Terlizzi et al., 2010; Martinez-Garcia et al., 2013; Leopardas et al., 2016). The distance at which effects from the fish farm can be measured vary with the environmental variable being measured and the intensity and duration of farming, husbandry practises and local oceanographic conditions.

*Zostera marina*, commonly known as eelgrass, is the dominant species of seagrass in Atlantic Canada. It has been designated an ecologically significant species (ESS) due to its crucial role in providing essential habitat for numerous species and other key ecological services, including nutrient cycling, carbon sequestration, and reduction of wave action (DFO, 2009a, 2011). With their extensive root and rhizome system, eelgrass beds also stabilize sediments and provide a rich food source and habitat for infauna communities (Orth et al. 1984; Boström & Bonsdorff 1997). Unlike seagrasses in the Mediterranean Sea that occur at depths down to 40-45 m (Telesca et al., 2015), eelgrass meadows along the Atlantic coast of Canada are found at shallower depths (1-12
m, DFO, 2009a) due to stronger light limitation in temperate waters (Hemminga & Duarte, 2000). Since open netpen fish farms in Atlantic Canada typically require a minimum depth of 12 m (net depth plus 3 m clearance from bottom; Hargrave, 2002), eelgrass meadows are typically not located directly under, but adjacent to, fish pens.

The potential impacts of finfish aquaculture on eelgrass beds in Canada have been acknowledged for more than a decade (Vandermeulen, 2005). To date, however, there have been no quantitative studies in Atlantic Canada to describe the potential impacts of finfish aquaculture on eelgrass habitat. This study aimed to address this gap by (i) assessing the changes in eelgrass bed structure and associated macroinfaunal communities at increasing distances to a finfish farm in Port Mouton Bay, Nova Scotia, and a reference site and (ii) link the observed changes in macroinfauna to changes in environmental and eelgrass bed variables. We also place our results in the context of recently published survey results from eelgrass beds across Atlantic Canada (Cullain et al., 2017). Overall, our study provides insights into changes in eelgrass habitats due to nutrient and organic enrichment from finfish farms in Atlantic Canada and we suggest possible metrics for assessing and monitoring local and broader-scale impacts of organic enrichment on these ecosystems.

**MATERIALS AND METHODS**

**Study area**

Our study sites were located along the Atlantic coast of southwestern Nova Scotia (Table 1, Fig. 1). Port Mouton Bay, site of the finfish farm, is a partially sheltered bay covering an area of 55.6 km² (Fig. 1). Tides, averaging 1.5 m, are semi-diurnal and water depth throughout the bay ranges from 8-18 m. Tidal currents tend to be low (2-3 cm s⁻¹, Gregory et al., 1993) and surface currents are strongly influenced by winds (DFO, 2007, 2009b). A finfish farm has been operating in the Bay since 1995. The current fish farm lease (43°54'54.11" N; -64°48'43.62" W) near Spectacle Island (Fig. 1) occupies an area of 8 hectares (ha) and the sea cages occupy ~0.58 ha of the lease area (McIver et al., 2018). The fish farm was initially stocked with rainbow trout (*Oncorhynchus mykiss*) until ~2000, followed by Atlantic salmon (*Salmo salar*) until 2009, fallowed from 2010-2012, stocked with rainbow trout in 2012-2014, and fallowed since 2015. The farm lease has been re-licensed for Atlantic salmon and rainbow trout for the period of March 2015 to March 2020 (NSDFA, 2017). Information on production at the farm site is deemed proprietary, however
it has been estimated at 760 mt annually for the last production period (2012-2014, McIver et al., 2018). In general, the Bay is ice-free in the winter months, but ice conditions do occur with the most recent event in 2015, five months prior to our sampling. This super chill event killed almost all of the fish and the farm was not restocked in 2015.

**Sampling design and data collection**

Three sites were selected at varying distances from the finfish farm (Fig. 1, Table 1): an eelgrass bed closest to the fish farm at Spectacle Island (SI), an eelgrass bed close to Carters Beach (CB), and an eelgrass bed near an Old Wharf (OW) inside Port Mouton Bay. Initially, two additional sites (Jackie’s Island and Port Mouton Island, Fig. 1) were included based on local ecological knowledge and a pilot survey in April 2015; however, when revisited during the summer sampling period, the eelgrass beds at both sites had nearly disappeared and were not sampled. A reference site was selected in adjacent Port Joli Bay (PJ) with similar physical and biogeographical conditions (Fig. 1). Port Joli Bay is bounded by protected land (Kejimkujik National Park and Thomas Raddall Provincial Park) which restricts human development and nutrient loading in the area (Nagel et al., 2018). All four study sites were in shallow, soft-sediment areas (Table 1) with eelgrass as the dominant macrophyte (continuous beds >50 m).

Field surveys were conducted from July 14-21, 2015. 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. All sampling was conducted during high tide at the same 6 locations; 0, 30, and 50 m along the shoreward transect and 5, 25, and 45 m along the seaward transect. Using SCUBA, eelgrass canopy structure (shoot density, canopy height, percent cover) and the percent cover of all epiphytic and benthic macroalgae and epiphytic fauna were assessed using quadrat sampling (0.5 x 0.5 m). A 0.25 x 0.25 m inset was used to count the number of shoots, whereas canopy height was measured in the centre of the quadrat by holding the zero end of a measuring tape against the substrate and extending it to the average height of the plants. The percent cover measures were estimated to the nearest 2% and we considered both sides of all eelgrass blades in the quadrat as habitable space for epiphytic cover. Therefore, if both sides of all the blades in the quadrat were covered with epiphytes, this would represent 100% cover. For the macroalgae, we separated all species used as common indicators of eutrophication for further analysis, including all annual green and brown algae (*Ulva intestinalis*, *Spongomorpha* sp., *Ectocarpus siliculosus*, *
Pilayella littoralis, Sphaerotrichia divaricata, Worm & Lotze 2006; Schmidt et al., 2012).

Similarly, from the epiphytic fauna, we separated those species (e.g. hydrozoans, bryozoans, poriferans, tunicates) identified as potential indicators of environmental disturbance such as increased turbidity and organic loading (Ben Brahim et al., 2014) which in our surveys were primarily hydroids (Campanularia sp.). Temperature and depth were recorded on SCUBA dive computers.

Seagrass biomass as well as the abundance of sediment macroinfauna were collected using sediment cores (0.2 m diameter; 0.2 m deep) at each of the 6 sampling locations. First, all above-ground (AG) and below-ground (BG) eelgrass tissue was removed, rinsed in a 500 µm sieve to capture any fauna, bagged and kept on ice. Sediment type in each core was recorded (e.g. sand, mud, sandy-mud), and the presence of any sulfur smell indicating hypoxia or anoxia. The remainder of the core contents were sieved (500 µm) on site and all macroinfauna species were identified to the lowest possible taxon. If organisms needed further identification, they were kept on ice and brought back to the laboratory and examined under a dissecting microscope.

Individuals of each species were counted (abundance m⁻²) and weighed (biomass g m⁻²). In the laboratory, the eelgrass blades (AG) and roots and rhizomes (BG) were rinsed again and all epiphytes were carefully scraped off the blades. AG and BG tissue was then weighed (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). Once dry, a 50 mg subsample of each of the AG and BG tissue were collected and sent to the University of California Davis Stable Isotope facility to analyze the tissue nitrogen (%N) content as well as the carbon (δ¹³C) and nitrogen (δ¹⁵N) stable isotopes.

Microphytobenthos (MPB) and sediment organic content (SOC) were collected using 60 ml syringe cores (2.6 cm diameter; 2 cm and 5 cm depth, respectively). At each of the 6 sampling locations, two samples were collected for SOC (volume of sample ~8.83 mL) and three for MPB (volume of sample ~3.53 mL). Both SOC samples were placed in a plastic bag and frozen until processed whereas each set of three MPB samples were combined on site, placed in plastic cryovials and stored in liquid nitrogen while in the field and in a freezer (-20°C) until analysis in the laboratory. In the laboratory, SOC 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. SOC 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, Janquin & Kupka, 1997). We then weighed the crucible + ashed sample for ash weight. Percentages were calculated to determine overall percent organic content. All MPB samples were processed in a darkened room. 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 University, pers. comm). The supernatant was subsequently pipetted into clean scintillation vials and measured in a Turner Designs 10005R fluorometer to determine chlorophyll \( a \) concentrations.

**Data analysis**

The aims of our study were to test for differences in 1) environmental parameters (SOC, MPB, tissue \( %N, \delta^{13}C \) and \( \delta^{15}N \), percent cover of annual algae and hydroids), 2) eelgrass structure (shoot density, canopy height, percent cover, AG and BG biomass), 3) macroinfauna abundance, biomass, richness, diversity and community composition between sites with increasing distance from the finfish farm, and 4) to link the observed differences in macroinfaunal communities to the environment and eelgrass bed structure using multivariate distance matrices. To place our results in a broader context, we also compared our results to a recently published survey of eelgrass beds and macroinfauna communities across 9 sites on the Atlantic coast of Nova Scotia (mean ± SE, \( n = 9 \), Cullain et al., 2017). The survey methodology was the same as our study, with two of the sites (CB and PJ, Table 1) being part of the present study.

We used multivariate permutational analysis of variance (PERMANOVA) to assess the effect of site (fixed factor) on the Euclidean distance matrix of each non-independent pair of canopy and environmental variables. If significant (\( p \leq 0.05 \)) differences were found in the paired variables, univariate PERMANOVA was used to assess the effect of site (fixed factor) on each of the individual environmental and eelgrass parameters followed by post-hoc pairwise tests between sites if there was a significant main effect. Similarly, univariate PERMANOVA and pairwise tests were used for the independent environmental variables (SOC, MPB) and zero-adjusted arcsine transformed Bray-Curtis similarity matrix for each of annual algae, hydroids and eelgrass % cover.
To determine differences in macroinfauna community composition between sites, multivariate PERMANOVA was used on the zero adjusted Bray-Curtis similarity matrix of abundance (density) and biomass 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 and group average cluster analysis using the centroids of each site to visualize the community data. To determine which species contributed most consistently (>10%) to the differences between sites, we used similarity percentages (SIMPER) analysis (Anderson et al., 2008). Univariate PERMANOVA on the Euclidean distance matrix of species richness and diversity (H’) as well as the square-root transformed Bray-Curtis similarity matrix of each of total macroinfauna abundance and biomass was used to identify differences in individual summary measures between sites.

We used abundance-biomass comparison (ABC) curves using the log species rank (x-axis) and the cumulative percent dominance (y-axis) to compare the k-dominance curves for macroinfauna abundance and biomass at each site (Warwick, 1986). In unpolluted sites, the biomass curve will be above the abundance curve, in moderately polluted areas the two curves will closely coincide, and in grossly polluted sites the abundance curve will be above the biomass curve (Warwick 1986, Warwick et al., 1987). This method expands on the theory by Pearson & Rosenberg (1978) where unpolluted sites will have less but larger individuals, but will shift to higher abundances of small opportunistic species as pollution level increases.

BIOENV was used to link the overall response of the macroinfauna community to different environmental and eelgrass variables. Prior to these analyses, we examined the correlations among all environmental (SOC, MPB, annual algae, hydroids, AG and BG tissue %N, AG and BG δ13C and δ15N) and canopy variables (shoot density, canopy height, percent cover, AG and BG biomass) and those with high correlation (≥0.7) were never included in the same analysis. If variables were equally correlated (e.g. AG and BG biomass), we chose to include the variable most relevant for infauna (e.g. BG biomass). Therefore, seven environmental (SOC, MPB, annual algae, hydroids, BG %N, BG δ13C, BG δ15N) and four eelgrass canopy (shoot density, canopy height, percent cover, BG eelgrass biomass) variables were used in the analyses. BIOENV provides a non-parametric index rho (ranging from 0 to 1) that indicates how closely different combinations of environment and eelgrass canopy variables explain the multivariate
pattern of the macroinfauna community based on the abundance and biomass data and individual SIMPER species. We then used a permutation test to determine the significance level of the sample statistic (rho).

All PERMANOVA, cluster, ABC and BIOENV analyses were carried out using PRIMER (version 6.1.11) with PERMANOVA+ (version 1.0.1, PRIMER-E, Plymouth).

RESULTS

Environmental parameters and eelgrass bed structure

Bottom temperature ranged from 12-15°C between the four sites and sampling depth ranged from 1.7-2.9 m (Table 1). Sediment organic content differed between sites (Table 2), with SI and OW having significantly higher organic content than CB and PJ, and SI also having higher values compared to average Nova Scotia (NS) sites (Fig. 2a). Microphytobenthos did not differ significantly between sites, although higher average values were observed at the three Port Mouton Bay sites compared to PJ (Table 2, Fig. 2b), but these were comparable to average NS sites. There were significant site effects in both the multi- and univariate analyses of the cover of annual algae and hydroids (Table 2) with CB and PJ having significantly more annual algae cover and SI having significantly more hydroids (Fig. 2c). Hydroid cover at SI and annual algal cover at CB were much higher compared to average NS sites. No significant differences were found in the multivariate analysis for above- (AG) and below-ground (BG) tissue nitrogen (%N, Table 2). For AG %N, however, the three Port Mouton sites had slightly enhanced values compared to PJ and average NS sites (Fig. 2d). Both AG and BG carbon (δ¹³C) and nitrogen (δ¹⁵N) stable-isotope ratios significantly differed between sites (Table 2, Fig. 2e, f), with CB having higher AG δ¹³C than the other sites and CB and SI having higher BG δ¹³C than PJ. For both AG and BG δ¹⁵N, SI and OW had lower values than CB and PJ, while average NS sites were in the middle.

Multivariate PERMANOVA detected no significant differences for shoot density and canopy height across sites (Table 2). Looking at the overall patterns, however, SI and CB had lower shoot density than the other sites including average NS sites (Fig. 3a), while canopy height was more similar across sites except for lower values in CB (Fig. 3b). Eelgrass cover (Fig. 3c) as well as both AG and BG biomass decreased at sites closer to the fish farm (Fig. 3d), but this was only
significant for AG biomass (Table 2). Post-hoc tests revealed that SI and CB had significantly
lower AG biomass than PJ, which were also lower compared to average NS sites.

**Macroinfauna community**

A total of 20 macroinfauna genera were identified across all sites, 10 of which were identified to
the species level. Due to the overall low number of species and high variability, univariate
PERMANOVA did not detect any significant differences in the summary measures of total
abundance (pseudo-$F_{3,20} = 1.45$, $p = 0.2$), total biomass (pseudo-$F_{3,20} = 1.67$, $p = 0.12$), species
richness (pseudo-$F_{3,20} = 0.56$, $p = 0.65$), or diversity (pseudo-$F_{3,20} = 0.83$, $p = 0.49$) of
macroinfauna between sites. However, the three sites in Port Mouton Bay tended to have lower
macroinfaunal biomass, richness and diversity compared to PJ and average NS sites (Fig. 4b-d),
while abundance was lowest at CB (Fig. 4a).

The ABC curves for cumulative dominance showed that the macroinfauna biomass were
distinctly above the abundance curve for both CB and PJ (Fig. 5b, d), indicating unpolluted
conditions. The biomass and abundance curves for SI approached each other but did no overlap
(Fig. 5a), suggesting that this site is approaching moderately polluted conditions. The OW site
was the only site where the abundance curve lied above the biomass curve (Fig. 5c), indicating
polluted conditions.

Macroinfauna community composition based on both abundance and biomass did differ
significantly between sites (multivariate PERMANOVA: pseudo-$F_{3,20} = 2.3$, $p = 0.005$ and
pseudo-$F_{3,20} = 2.2$, $p = 0.003$; respectively). Cluster analysis of centroids based on infauna
abundance data (Fig. 6a) showed a clustering of SI and OW, which were the more polluted sites
as identified by the ABC curves, while cluster analysis of infauna biomass data (Fig. 6b) showed
a clustering of SI and CB, the two sites closest to the fish farm. The main species identified by
SIMPER contributing $>10\%$ of differences in abundance and biomass between sites included
three polychaetes: *Clymenella torquata*, *Capitella capitata*, and *Nephtys* sp. with the addition of
a fourth species *Amphitrite* sp. for biomass. However, the contribution of each species to the
community differed when considering abundance or biomass, respectively (Table 3). *C. torquata*
dominated the community in abundance across all sites except CB, and in biomass except in PJ,
where *Amphitrite* sp. was the dominant species (Table 3). The opportunistic polychaete *C.
capitata* only occurred at the two more polluted sites in Port Mouton Bay (SI, OW), with the
highest abundance and biomass closest to the fish farm (Table 3). The polychaete *Nephthys* sp. also showed higher abundance at the two more polluted sites (SI, OW). In turn, *Amphitrite* sp. as well as *Ophelia* sp only occurred at the reference site PJ, whereas bivalves *Cerastoderma pinnulatum* and *Tellina agilis* were most abundant at the beach site CB (Table 3).

**Linking the environment to the macroinfauna community**

BIOENV was used to determine any associations between the Euclidean distance of environmental (SOC, MPB, annual algae, hydroids, BG %N, BG δ^{15}N, BG δ^{13}C) and eelgrass (shoot density, canopy height, percent cover, BG eelgrass biomass) parameters and the Bray-Curtis similarity matrix of macroinfauna community structure (Table 4). The community assemblage based on abundance data was most correlated to BG %N, BG δ^{13}C and the percent cover of annual algae (%A), and the assemblage based on biomass data to BG eelgrass biomass, BG %N and BG δ^{13}C (Table 4). Both the abundance and biomass of *C. capitata* were most correlated to the percent cover of hydroids (%H), while the next best correlations included SOC and MPB. In contrast, the other species were more correlated with eelgrass bed structure, with BG eelgrass biomass being the most important (Table 4).

**DISCUSSION**

Our study quantitatively assessed the potential impacts of finfish aquaculture on eelgrass bed structure and associated macroinfaunal communities in Atlantic Canada. Our results show that eelgrass biomass, shoot density and percent cover all decreased closer towards the finfish farm. Macroinfauna biomass and abundance were also reduced closer to the fish farm with significantly altered species composition. Importantly, the opportunistic polychaete *Capitella capitata*, a known indicator of polluted conditions, specifically organic enrichment (Pearson and Rosenberg 1978) reached its highest abundance close to the fish farm, and also occurred at an old wharf site with a history of pollution. This was strongly correlated with higher loads of epiphytic suspension feeders and higher sediment organic content pointing to organic enrichment at these sites. The combined assessment of multiple eelgrass structural and environmental variables was critical to evaluating ecological changes, particularly differences between impacted and unimpacted ecosystems. We discuss possible metrics for assessing and monitoring local and broader-scale impacts of organic enrichment on eelgrass ecosystems.
Environmental parameters

In Atlantic Canada, *Zostera marina* experiences large seasonal fluctuations in temperature and light availability due to its temperate latitude. During the summer sampling period, sea surface temperatures were quite similar at all five sites (12-15°C) and 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, Romanuk & Lotze, 2011; Cullain et al., 2017), and optimal growth temperatures of 10-25°C (DFO 2009a). Thus, conditions at our study sites were well comparable. In addition, several physical factors are known to influence eelgrass growth and survival, including wave exposure, sediment type and water quality (Frederiksen et al., 2004; Vandermeulen, 2005). In general, surficial sediments in Port Mouton Bay and Port Joli Bay are a mix of muddy, sandy and gravelly sand, but in sheltered areas muddy sands predominate (Piper et al, 1986). While all our four study sites were in relatively sheltered areas with similar wave exposure, Spectacle Island (SI) and Old Wharf (OW) sites were the most sheltered, with SI nestled behind an island and OW positioned in the inner most part of the bay. Current speeds in Port Mouton Bay are generally low (2-3 cm sec⁻¹, Gregory et al., 1993) and flushing times are long (114 hours, Nagel et al., 2018), which could lead to higher sedimentation rates compared to adjacent Port Joli Bay, which has faster flushing times (53 hours). However, we found the highest amount of organic matter in sediments closest to the fish farm, followed by the Old Wharf site, while Carters Beach inside Port Mouton Bay and the reference site in Port Joli Bay had much lower sediment organic content. Both the Spectacle Island and Old Wharf sites have been affected by long-term anthropogenic activities. The (abandoned) Old Wharf is in an area of higher coastal development with some direct discharge of domestic sewage and a former fish processing plant. The fish farm at the Spectacle Island site has been in operation, on and off, since 1995. Although no redox potential discontinuity (RPD) layer was observed at any of the sites during sampling, strong sulfur smell and dark black sediments were observed at the Spectacle Island and Old Wharf sites.

A potential early indicator of nutrient enrichment is the increased concentration of microphytobenthos, composed primarily of diatoms and cyanobacteria (Lever & Valiela, 2005). We found higher microphytobenthos chlorophyll-α concentrations at sites within Port Mouton Bay compared to the reference site (PJ), but not higher than at other sites in Nova Scotia. The
enhanced microphytobenthos productivity in Port Mouton Bay may be a result of the overall higher nutrient loading from greater human development in the watershed and the finfish farm in the bay (McIver et al., 2018) compared to the more protected and less developed watershed in Port Joli Bay resulting in much lower nutrient loading (Nagel et al., 2018). Under enhanced nutrient loading, increased microphytobenthos concentration can shift sediment conditions from being autotrophic to net heterotrophic, where sediments become increasingly hypoxic or anoxic, sulphides accumulate, and denitrification and mineralization are enhanced or stimulated (Meyer-Reil & Köster, 2000; Sundbäck et al., 2004; Hardison et al., 2013).

Eelgrass tissue nitrogen content and stable-isotope ratios are commonly used to trace the amount and source of nitrogen, respectively, within seagrass ecosystems (Hemminga & Duarte 2000; Dolonec et al., 2006; Ruiz, Marco-Méndez & Sánchez-Lizaso 2010). The higher tissue nitrogen content within Port Mouton Bay compared to the reference site, and compared to other Nova Scotia sites, also point to higher nutrient availability within Port Mouton Bay, possibly due to higher nutrient loading (Nagel et al., 2018). However, this seemed to be distributed throughout the bay, as there was no higher tissue content at the fish farm site (SI). We also did not find elevated stable isotope $\delta^{15}N$ values at the fish farm site. Generally, wastewater from human or animal waste has a higher $\delta^{15}N$ signature (ratio of $^{15}N/^{14}N$) of 8-10‰ up to 20‰ (Lepoint, Dauby & Gobert, 2004; Schubert et al., 2013), and fish farm waste (feces, mucus, pellets) has $\delta^{15}N$ values of 6.5-10.5‰ (Dolonec et al., 2006; Sarà et al., 2006; Ruiz, Marco-Méndez & Sánchez-Lizaso 2010). Several studies have found significantly elevated $\delta^{15}N$ at varying distances (250-900 m) up to >1000 m from fish farms (Holmer et al., 2007; Ruiz, Marco-Méndez & Sánchez-Lizaso 2010; García-Sanz et al., 2011). These values depend on hydrographical characteristics (e.g. depth, current speeds) at and around the farm site, the scale of farm production and feeding efficiencies (Sarà et al., 2006; Holmer et al., 2007). At our sites, $\delta^{15}N$ values of 4-6.5‰ were within the range of natural variation in seagrass ecosystems (Hemminga & Mateo, 1996; Lepoint, Dauby & Gobert, 2004) and comparable to values reported in other Atlantic Canadian bays (McIver, Milewski & Lotze, 2015). This may be explained by the fact that production at the fish farm ceased 5 months prior to our sampling in July due to a super chill event that killed all the fish, allowing time for the $\delta^{15}N$ in the eelgrass tissue to be adequately used or cycled within the system. This may also explain why we did not measure enhanced tissue nitrogen content directly at the Spectacle Island site, but rather throughout Port Mouton Bay.
For carbon $\delta^{13}C$, a more negative isotopic signature represents the input of $^{13}C$-depleted carbon from the decomposition of organic material (Hemminga & Mateo 1996). Particularly in the below-ground roots and rhizomes $\delta^{13}C$, we found a clear pattern of less negative $\delta^{13}C$ further away from the finfish farm. Similar patterns of less negative $\delta^{13}C$ further away from the source have been observed in seagrass beds which receive organic material from land run-off (Hemminga & Mateo 1996, Hemminga & Duarte 2000). Thus, while the nitrogen signal may have disappeared due to the fish farm not being stocked at the time of sampling, the organic carbon signal was still visible. Overall, the tissue nutrient content and $\delta^{15}N$ and $\delta^{13}C$ isotope signatures could be used as tools in detecting and monitoring the amount and sources of nitrogen and carbon in Port Mouton Bay.

**Eelgrass bed structure**

Frequently responses of seagrasses to increased nutrient and organic loading are decreases in shoot density, biomass and cover, and increases in canopy height (Short et al., 2011; Schmidt et al. 2012). Our results suggest a decrease in shoot density, cover, and biomass with increasing proximity to the fish farm; trends that may have been more significant with a larger sample size. The two sites closest to the farm (<1 km), Spectacle Island and Carters Beach, did show different eelgrass bed structure, particularly lower eelgrass biomass. Interestingly, two additional sites we planned to sample, Jackies Island and Port Mouton Island (Fig. 1), used to have healthy eelgrass beds (Lee 2014) but these degraded and largely disappeared between our pilot survey in April and our sampling in July 2015. Similar responses have been reported in other studies on the impacts of fish farm effluent on seagrasses (Pergent-Martini et al., 2006). A synthesis of the effects of Mediterranean fish farms reported that the most important process affecting *Posidonia oceanica* was the sedimentation of organic material (Holmer et al., 2008). Estimated dispersion distances from fish farms are variable, but the furthest distances of organic-enriched material has not exceeded 1000 m (Sarà et al. 2004, Holmer et al. 2007). For the Mediterranean, Díaz-Almela et al. (2008) proposed a rate of 1.5 g organic matter $m^{-2} d^{-1}$ as a threshold to protect *P. oceanica* from the impacts of fish farms. Although we did not measure sedimentation rates, the development of a comparable threshold value for eelgrass habitat in Atlantic Canada would be a valuable tool for protecting eelgrass from the impacts of finfish aquaculture as well as other anthropogenic activities.
Macroinfauna

The ABC curves allowed us to use the total macroinfauna abundance and biomass to examine the sites based on a pollution gradient (Warwick, 1986). Our results indicate that the Old Wharf site is considered the most polluted site, followed by Spectacle Island, while Port Joli can be considered unpolluted. The location of Old Wharf may explain its polluted status as this site has a decades-long, cumulative history of coastal development including direct sewage outflow and a former fish processing plant which is no longer in operation. The Spectacle Island fish farm, on the other hand, has been in operation on and off for 19 (1995-2015) years while the reference site (Port Joli Bay) has no industrial development. The Spectacle Island site, however, did show the two ABC curves approaching each other, indicating that the site may be transitioning to/from a polluted state (Warwick 1986). Since our study sites were only sampled during one-time period, it would be important and valuable from a management perspective to monitor changes in these ABC curves seasonally and over years.

Although summary measures of the macroinfauna community (total abundance and biomass, species richness and diversity) did not significantly differ between sites, our results indicated lower biomass and significantly different community composition. Based on biomass data, the two sites closest to the farm (Spectacle Island and Carters Beach) had similar communities, while the reference site (Port Joli) was the most different. The opportunistic polychaete *Capitella capitata* was the main contributor to the differences at the Spectacle Island. This is not surprising as *C. capitata* has long been associated with sediment organic enrichment from human activities (e.g. sewage, seafood, and wood-processing facilities) including finfish aquaculture (Holmer et al., 2005; Kutti et al. 2007; Hargrave, Holmer & Newcombe 2008). In their study on the effects of fish farming on soft-bottom polychaete assemblages, Martinez-Garcia et al. (2013) reported that tolerance to higher total dissolved sulphides, silt and clay fractions, and sediment stable isotope nitrogen signatures were the main sediment factors that distinguished the occurrence of polychaete families such as Capitellidae compared to families that are more sensitive to fish farm pollution such as Maldanidae and Nephtyidae. These latter families include two polychaete species, *Clymenella* sp. and *Nephtys* sp. respectively, found at all our study sites.
Linking the environment/eelgrass structure to infauna community composition

The correlation between macroinfauna composition and below-ground eelgrass biomass and detritus is well documented and has been linked to the role eelgrass roots and rhizomes have in accumulating and stabilizing sediments which in turn provide habitat and food to the associated detritivore-dominated infauna, releasing oxygen to the sediments and providing protection from predators (Orth, Heck & Montfrans, 1984; Lee, Bailey-Brock & McGurr, 2001, Boström, Jackson & Simestad, 2006). Consequently, sediment grain size and stability can covary with below ground-ground biomass and shoot density to influence macroinfaunal assemblages (Boström, Jackson & Simestad, 2006). Terlizzi et al. (2010) reported that 98% of the changes in benthic faunal assemblages between control and fish farm impacted sites were the result of changes in sediment features (sediment organic matter, grain size) and meadow structure (shoot density, rhizome matte compactness). We found that eelgrass bed structure, specifically below-ground biomass, was a good predictor of community and individual species abundance or biomass, as well as tissue nitrogen as an indicator of nutrient enrichment and δ¹³C as an indicator for enhanced decomposition of organic material for both community biomass and abundance. As an important indicator species, the abundance and biomass of C. capitata was most correlated to epiphytic suspension feeder cover and biogeochemical sediment conditions (sediment organic content, microphytobenthos). Increases in suspension feeders and microphytobenthos are associated with increased organic and nutrient loading, respectively (Lever & Valiela 2005; Ben Brahim et al., 2014).

Our assessment of the correlation between macroinfaunal composition and environmental factors may be confounded by the fact that the fish farm was not in operation at the time of our sampling (July 14-21, 2015). Finfish aquaculture operations are known to discharge significant quantities of nutrient and organic waste to the surrounding environment. When in operation, the Port Mouton Bay fish farm increases the annual total dissolved nitrogen load from human sources to the entire bay by 14% or 30,400 kg (McIver et al., 2018), with the fish farm being the single largest contributor of dissolved nitrogen to the bay after atmospheric deposition. The release of particulate organic waste from fish farms can be significantly larger (10s-100s mt) depending on the species raised, the scale of production and feed conversion efficiencies (Olsen, Holmer & Olsen, 2008). Impacts of fish farms on surrounding ecosystems can persist for several months to years after production ceases (Delgado et al. 1999; Karakassis et al. 1999; Brooks,
Continued monitoring at the Spectacle Island and Carters Beach sites is highly recommended to assess eelgrass habitat recovery and to provide managers with scientific information on the development of monitoring metrics and impact thresholds that would protect eelgrass from the adverse effects of finfish aquaculture.

**Conclusions**

Within the last five to ten years, there has been a growing acknowledgement of the potential significant spread and persistence of aquaculture waste over large spatial areas (Price et al. 2015). Our results reveal changes to eelgrass bed structure as far as 1 km from the farm site in Port Mouton Bay. Given the acknowledged status of eelgrass as an ecologically significant species in Atlantic Canada (DFO, 2009a) and its sensitivity to anthropogenic disturbances, including finfish aquaculture (Vandermeulen, 2005), resource managers need to include comprehensive eelgrass habitat assessments as part of the process for evaluating proposed finfish aquaculture operations. Effective indicators, with threshold values, need to be developed to monitor existing operations to ensure that fish farms are operating in a sustainable manner. Optimal indicators should integrate impacts over time and include multiple measures of eelgrass bed structure and environmental variables rather than single-event and individual variables. Suggested indicators based on this study and studies done in other regions should include sedimentation rates, sediment sulphides and organic content, microphytobenthos, prevalence of *C. capitata*, epiphyte loads and composition, as well as eelgrass bed structure (shoot density, % cover, above- and below-ground biomass) and tissue nitrogen content and isotope signatures.

**Acknowledgements**

We thank K. Wilson, A. Dixon and T. Harington for support during the field surveys, A. Chan for help in the lab, and M. Wong, M. Skinner, J. Grant, and S. Courtenay for helpful comments and discussions. We would also like to thank the Friends of Port Mouton Bay Society for providing their historical and contemporary knowledge of the Port Mouton area and marine ecosystem.
References


Canadian Department of Fisheries and Oceans (DFO). 2009a. Does eelgrass (Zostera marina) meet the criteria as an ecologically significant species? DFO Canadian Science Advisory Secretariat Science Advisory Report 2009/018.


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 was located in adjacent Port Joli Bay as a reference site.
<table>
<thead>
<tr>
<th>Site</th>
<th>Abbreviation</th>
<th>Distance from farm (m)</th>
<th>Temperature (°C)</th>
<th>Depth (m)</th>
<th>Bottom type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectacle Island</td>
<td>SI</td>
<td>300</td>
<td>15</td>
<td>2.0</td>
<td>Muddy sand</td>
</tr>
<tr>
<td>Carters Beach</td>
<td>CB</td>
<td>700</td>
<td>12</td>
<td>2.5</td>
<td>Sandy mud</td>
</tr>
<tr>
<td>Old Warf</td>
<td>OW</td>
<td>3000</td>
<td>14</td>
<td>1.7</td>
<td>Muddy sand</td>
</tr>
<tr>
<td>Port Joli Bay</td>
<td>PJ</td>
<td>&gt;10 km</td>
<td>15</td>
<td>2.9</td>
<td>Sandy mud</td>
</tr>
</tbody>
</table>
Table 2 (on next page)

Results from multivariate and univariate PERMANOVAs on the effect of site on eelgrass bed structure and environmental variables.

Results from multivariate and univariate PERMANOVAs on the effect of site on eelgrass bed structure and environmental variables. Multivariate analyses were only performed for interdependent variables, and only followed up with univariate analyses if significant differences were found. Significant differences (p ≤ 0.05) are bolded.
<table>
<thead>
<tr>
<th>Eelgrass bed structure:</th>
<th>DF</th>
<th>RDF</th>
<th>Multivariate</th>
<th>Univariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pseudo-F</td>
<td>p-value</td>
</tr>
<tr>
<td>Shoot Density</td>
<td>3</td>
<td>20</td>
<td>1.24</td>
<td>0.32</td>
</tr>
<tr>
<td>Canopy Height</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Biomass</td>
<td>3</td>
<td>19</td>
<td>2.89</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Percent Cover</td>
<td>3</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Environmental variables: | | | |
|--------------------------|----|-----|--------------|------------|
| Annual Algae Cover       | 3  | 20  | 29.6         | **0.001**  | 48.1       | **0.001** |
| Hydroid Cover            |    |     | -            | -          | 7.4        | **0.001** |
| Sediment Organic Content | 3  | 19  | -            | -          | 7.6        | **0.001** |
| Microphytobenthos        | 3  | 19  | -            | -          | 1.1        | 0.21      |
| Tissue %N                |    |     | -            | -          | 3.17       | **0.03**  |
|  | - Above | 3  | 17  | 1.81         | 0.13       | 3.17       | 0.67      |
|  | - Below  |    |     | -            | -          | 3.17       | 0.67      |
| Tissue δ\textsuperscript{13}C |  |    | -            | -          | 13.7       | **0.002** |
|  | - Above | 3  | 17  | 7.88         | **0.001**  | 4.45       | **0.022** |
|  | - Below |    |     | -            | -          | 13.7       | **0.002** |
| Tissue δ\textsuperscript{15}N |  |    | -            | -          | 15.4       | **0.001** |
|  | - Above | 3  | 17  | 14.5         | **0.001**  | 13.7       | **0.002** |
Table 3 (on next page)

Mean abundance and biomass of the most abundant macroinfauna species at the four study sites

Mean abundance (ABUN; m$^{-2}$) and biomass (BIOM; g m$^{-2}$) of the most abundant macroinfauna species (SE) 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 in bold.
<table>
<thead>
<tr>
<th>Species</th>
<th>SI</th>
<th>CB</th>
<th>OW</th>
<th>PJ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABUN</td>
<td>BIOM</td>
<td>ABUN</td>
<td>BIOM</td>
</tr>
<tr>
<td>Amphitrite sp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Capitella capitata</td>
<td>26.5</td>
<td>5.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerastoderma pinnulatum</td>
<td>0</td>
<td>0</td>
<td>15.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Clymenella torquata</td>
<td>95.5</td>
<td>14.0</td>
<td>5.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Nephtys sp.</td>
<td>31.9</td>
<td>2.6</td>
<td>10.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Ophelia sp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tellina agilis</td>
<td>5.3</td>
<td>0.2</td>
<td>15.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>

1
**Table 4** (on next page)

BIOENV results for the macroinfauna community and SIMPER species

BIOENV results for the macroinfauna community and SIMPER species using abundance and biomass (below the line) with environmental (belowground (BG) %N, δ^{15}N and δ^{13}C; percent cover of annual algae (%A); percent cover of hydroids (%H); sediment organic content (SOC)) and eelgrass canopy (BG biomass, shoot density, canopy height, eelgrass cover) variables in Nova Scotia, Canada. Significant (p ≤ 0.05) correlations are bolded.
<table>
<thead>
<tr>
<th></th>
<th>Best correlated variable(s)</th>
<th>$\rho$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community abundance</td>
<td>BG %N, BG $\delta^{13}$C, %A</td>
<td>0.45</td>
<td>0.02</td>
</tr>
<tr>
<td>Capitella capitata</td>
<td>%H</td>
<td>0.45</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>SOC, %H</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MPB, %H</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Clymenella torquata</td>
<td>BG biomass</td>
<td>0.47</td>
<td>0.01</td>
</tr>
<tr>
<td>Nephtys sp.</td>
<td>BG biomass, shoot density, SOC, eelgrass cover</td>
<td>0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>Community biomass</td>
<td>BG biomass, BG %N, BG $\delta^{13}$C</td>
<td>0.40</td>
<td>0.04</td>
</tr>
<tr>
<td>Amphitrite sp.</td>
<td>BG biomass</td>
<td>0.21</td>
<td>0.82</td>
</tr>
<tr>
<td>Capitella capitata</td>
<td>%H</td>
<td>0.50</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>SOC, %H</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Clymenella torquata</td>
<td>BG biomass</td>
<td>0.43</td>
<td>0.01</td>
</tr>
<tr>
<td>Nephtys sp.</td>
<td>BG biomass, shoot density</td>
<td>0.17</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Figure 1

Map of the four sampling sites on the Atlantic coast of Canada.

Overview of site locations are shown on the left and a detailed map with the location of the finfish farm and sampling sites in Port Mouton Bay are shown on the right. Disappeared sampling sites indicate areas where eelgrass was no longer present. Refer to Table 1 for full site names and details.
Figure 2

Environmental variables across the four study sites

Environmental variables across the four study sites (from left to right: increasing distance from farm and PJ reference site; mean ±SE, n = 6) and in sites across Nova Scotia (NS, n = 9, hatched bars) for comparison: (a) Sediment organic content; (b) microphytobenthos (MPB) concentration; (c) percent cover of annual algae (dark color) and hydroids (light color); as well as eelgrass tissue (d) nitrogen content (%N) and stable-isotope ratios of (e) carbon (δ^{13}C) and (f) nitrogen (δ^{15}N) in aboveground (dark color) and belowground (light color) tissue. Lower case letters indicate significant differences (p ≤ 0.05). Refer to Table 1 for site abbreviations and details.
Figure 3

Eelgrass bed structure across the four study sites

Eelgrass bed structure across the four study sites (mean ±SE, n = 6) and in sites across Nova Scotia (NS, n = 9, hatched bars) for comparison: (a) canopy height; (b) shoot density, (c) percent cover; (d) aboveground (dark color) and belowground (light color) biomass. Lower case letters indicate significant (p ≤ 0.05) differences. Refer to Table 1 for site abbreviations and details.
Figure 4

Summary measures of the macroinfauna community across the four study sites

Summary measures of the macroinfauna community across the four study sites (mean ±SE, n = 6) and in sites across Nova Scotia (NS, n = 9, hatched bars) for comparison: (a) total abundance; (b) total biomass; (c) species richness; (d) diversity. Refer to Table 1 for site abbreviations and details.
Figure 5

Abundance-biomass comparison (ABC) curves using cumulative dominance for infauna species for the four study sites:

Abundance-biomass comparison (ABC) curves using cumulative dominance for infauna species for the four study sites: (a) Spectacle Island (SI); (b) Carters Beach (CB); (c) Old Wharf (OW); and (d) the reference site Port Joli (PJ).
Figure 6

Cluster analysis using infauna community centroids

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.