Impacts of hypoxia on estuarine macroinvertebrate assemblages across a regional nutrient gradient

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Abstract

In this study, we examined the effects of dissolved oxygen, via metrics based on hourly measurements, and other environmental variables on invertebrate assemblages in estuaries spanning a gradient of nutrient loading and geography in the southern Gulf of St. Lawrence, Canada. Upper areas (15–25 practical salinity units (PSU)) of 13 estuaries that were dominated by either seagrass (*Zostera marina* Linnaeus, 1753) or macroalgae (*Ulva* spp. Linnaeus, 1753) were sampled from June to September 2013. Macroinvertebrate assemblages from *Z. marina* were found to be distinct from *Ulva* assemblages for both epifauna and infauna. Small snails dominated each vegetation type, specifically cerithids in *Z. marina* and hydrobids in *Ulva*. Although *Z. marina* had higher species richness, approximately 70% of species were common to both habitats. Faunal communities differed among estuaries with large, within-estuary, temporal variance only observed at *Ulva* sites impacted by hypoxia and particularly at sites with long water residence time. Indeed, abundances varied by several orders of magnitude in *Ulva* ranging from zero to thousands of macroinvertebrates. There was a strong negative correlation between hypoxic or anoxic water, 48 h prior to sampling, with relative abundances of amphipods, and a positive correlation with the relative abundances of snails. As one of the first studies to use high-frequency oxygen monitoring, this study revealed probable impacts and the transient nature of hypoxia in eutrophication.

Key words: hypoxia, eutrophication, macroinvertebrate, estuary, *Ulva lactuca*, *Zostera marina*, dissolved oxygen

Introduction

Nutrient loading from anthropogenic sources such as wastewater and agriculture has significantly impacted estuaries and coastal systems around the world (Smith 2003; Lotze et al. 2006; Burkholder et al. 2007). The increase in productivity brought on by nutrient enrichment leads to the displacement of seagrasses by macroalgae and eventually the development of hypoxia (Valiela et al. 1997; Larkum et al. 2006; Burkholder et al. 2007). Algal proliferation exaggerates the normal diurnal cycle of photosynthesis-driven elevated dissolved oxygen during the day and respiration-driven decreased dissolved oxygen at night, resulting in supersaturation and hypoxia, respectively (Lovato et al. 2013; Coffin et al. 2018). Ultimately, macroalgae become space limited and begin to shade themselves and

decompose, depressing dissolved oxygen concentration further (Valiela et al. 1997; Larkum et al. 2006; Burkholder et al. 2007). Although faunal diversity is often lower in macroalgae than in seagrass habitat (Hauxwell et al. 2003; Cebrian et al. 2014), some animals are able to thrive there and can reach very high abundances (Norkko et al. 2000; Cardoso et al. 2004; Andersson et al. 2009; Schein et al. 2012).

Hypoxia and dissolved oxygen supersaturation clearly impact animal behaviour and vital rates (Fidler 1988; Deegan 2002; Miller et al. 2002; Vaquer-Sunyer and Duarte 2008; Riedel et al. 2014; Hrycik et al. 2017), yet they are not always fully integrated into studies examining the consequences of eutrophication. Given that dissolved oxygen is highly variable, infrequent point measurements are not likely representative of the conditions to which biota are exposed, potentially masking its influence. In systems where hypoxia is suspected, establishing exactly when hypoxia occurs and its impact on structuring invertebrate communities are therefore critical. Supporting this, preliminary research at a limited number of sites in the southern Gulf of St. Lawrence recently found that hypoxia (dissolved oxygen <2 mg/L) sustained for more than 30 h resulted in decreased crustacean abundance and an altered community (Coffin et al. 2017).

This study examined invertebrate assemblages in the upper estuaries of eelgrass- and algae-dominated systems in the southern Gulf of St. Lawrence, Canada, and how they related to environmental variables: metrics of dissolved oxygen, sediment grain size, water residence time, organic content of the sediment, temperature, salinity, chlorophyll, nitrate-N loading, and pH. The overarching research objective was to assess the effect of nutrient-induced hypoxia on macroinvertebrate assemblages in relation to other environmental factors in eelgrass (*Zostera marina* Linnaeus, 1753) and sea lettuce (*Ulva* Linnaeus, 1753) habitats. Additionally, it was expected that intermittent hypoxia/anoxia in eutrophic, *Ulva*-dominated habitat may be correlated with decreased faunal abundances and the dominance of fewer species compared with *Z. marina* habitat. Hypoxia is less likely to occur and persist in *Z. marina* habitat, and fauna are expected to be more diverse there. To our knowledge, this is the first study to investigate the relationship between hourly measures of dissolved oxygen and macroinvertebrates in multiple estuaries within a regional scale. Given the prevalence of nutrient-impacted estuaries and coastal seas around the world (Lotze et al. 2006), the methodology and findings presented herein should be of interest to those studying eutrophication worldwide.

Methods

Study area

Because of its relatively low salinity, the Gulf of St. Lawrence has been referred to as a very large estuary (Therriault 1991), but this study focuses on the many small estuaries within it, specifically those situated in the southern portion (henceforth referred to as the southern Gulf of St. Lawrence, SGSL), with watershed areas ranging from 37 to 386 km^2 in this study (**Fig. 1**). Low freshwater input results in estuaries that are well mixed throughout the water column (Bugden et al. 2014) and that remain saline throughout. Tides vary in both amplitude and nature and can be semi-diurnal or diurnal (Pingree and Griffithis 1980; Godin 1987; Koutitonsky et al. 2004). Because of local geography and low freshwater input, estuaries are generally shallow and small. On the north shore of Prince Edward Island most estuaries are lagoon-type, with barrier islands, and drain into the southern Gulf, whereas those on the south shore empty into the Northumberland Strait and are most often coastal embayments (Glibert et al. 2010). Eelgrass (*Z. marina*), the local seagrass, is in decline in the region (DFO 2009), particularly in the upper reaches of Prince Edward Island (PEI) estuaries (Hitchcock et al. 2017). Many PEI estuaries have agriculturally driven high nitrate–N loads (Danielescu et al. 2007; Danielescu and MacQuarrie 2011), are dominated by sea lettuce (*Ulva* spp.), and experience seasonal anoxia as a result (Bugden et al. 2014). The remaining provinces in the southern Gulf region
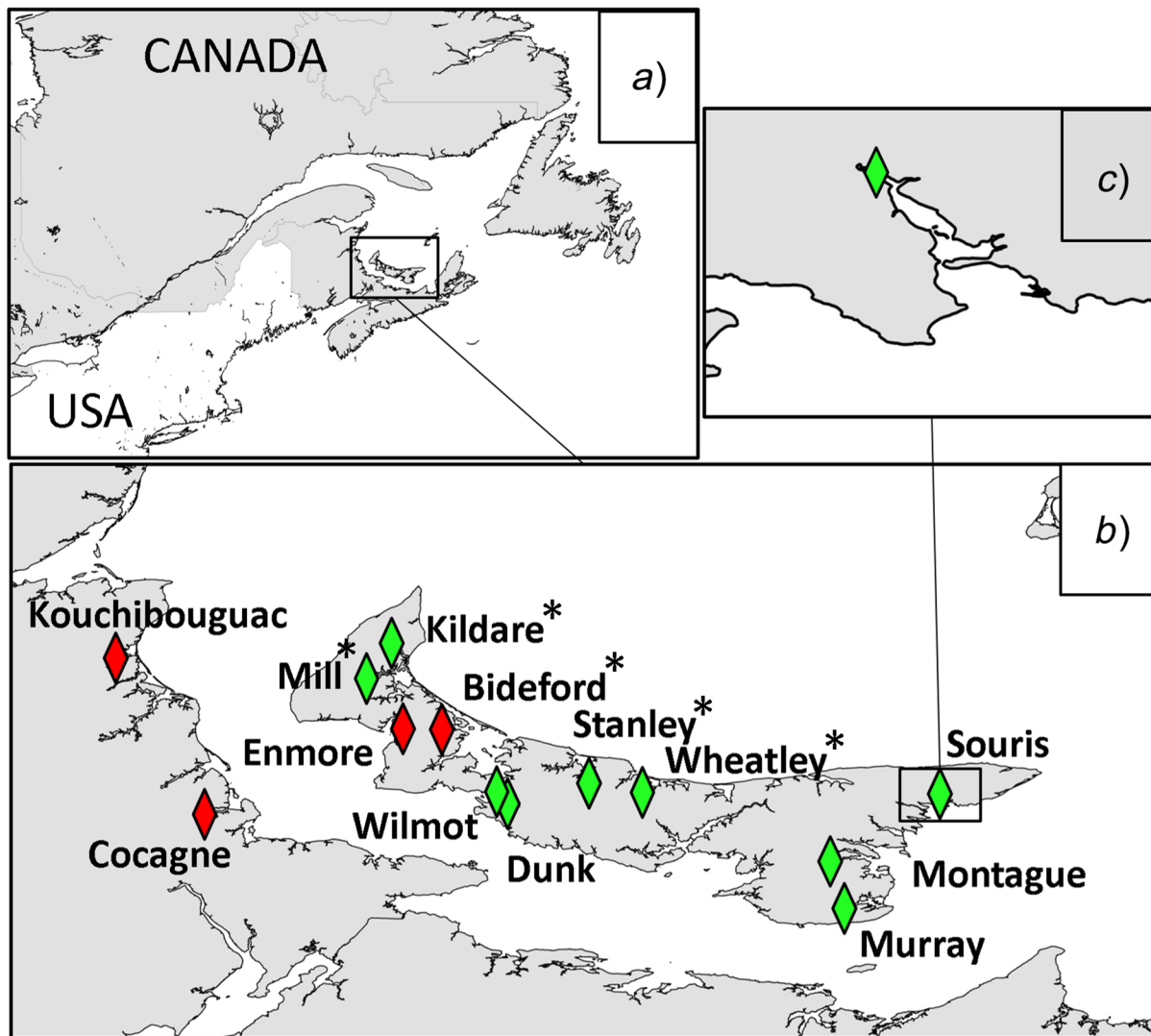
Fig. 1. Location of the study area in eastern North America (*a*). All estuaries sampled (*b*); those with an asterisk (*) drain directly into the southern Gulf of St. Lawrence, and those without an asterisk drain into the Northumberland Strait. Sites dominated by *Zostera marina* are indicated by red diamonds and those dominated by *Ulva* spp. are indicated by green diamonds. The relative location of sampling in each estuary (*c*). Maps were created using ArcGIS 10.3.

have ≤5% of their land use in annual crops (Natural Resources Canada 2009; Grizard 2013). Thus, the estuaries of the region constitute a gradient of nutrient enrichment through which to examine the interactions of factors structuring invertebrate communities (**Table 1**). The transition zone from 0 to 15 PSU salinity is short and creates a highly susceptible area to nutrient impact immediately downstream, and is also the area of interest in this study. This area is algae-dominated at nutrient-impacted sites (Bugden et al. 2014) and eelgrass-dominated at oligotrophic sites. At sites that were neither eutrophic nor oligotrophic (i.e., along the nutrient continuum), vegetation tended to be dominated by one or other type but was patchier. In total, 13 estuaries spanning a gradient of nutrient loading throughout the SGSL and that were dominated by either *Ulva* or *Z. marina*, were sampled monthly for epifauna and infauna and more frequently for standard physicochemical variables, e.g., salinity, temperature, and pH. Dissolved oxygen was the focus of this study and was continually measured using optical logger technology.

Table 1. Site characteristics, sediment characteristics, and average water chemistry measurements for each site over the course of sampling.

Site	Substrate grain size (D50) (μm)	Substrate organic content % (±0.02)	Salinity (PSU)	pH (±0.1)	Average depth (m)	Tidal amplitude (m)	Temperature (°C)	Nitrate-N (kg/d)	Residence time	Chlorophyll a (μg/L)	Dissolved oxygen (mg/L)	Hours below 6 mg/L oxygen	Hours above 10 mg/L oxygen
Kouchibouguac	42.7 ± 6.2	12.2	17.7 ± 3.7	7.6	1.22	0.85	17.4 ± 0.4	31	1.72	1.4 ± 0.2	9.3	0	0.28
Cocagne	36.3 ± 4.8	11.8	17.9 ± 3.3	7.5	1.13	1.10	21.1 ± 1.7	2	1.19	7.6 ± 1.8	7.6	0.07	0.02
Kildare	30.5 ± 3.1	15.4	23.4 ± 0.6	7.5	1.67	0.68	21.8 ± 1.4	38	3.72	13.2 ± 1.1	7.8	0.33	0.51
Mill	36.2 ± 1.4	14.6	23.6 ± 0.5	7.5	1.16	0.97	20.8 ± 1.5	189	2.74	19.0 ± 5.4	8.7	0.38	0.45
Enmore	89.2 ± 18.1	8.6	18.2 ± 1.9	7.5	1.57	1.10	22.6 ± 1.1	2	0.78	6.9 ± 2.0	7.8	0.15	0.09
Bideford	69.3 ± 6.2	17.0	26.8 ± 0.4	7.5	1.05	1.13	21.1 ± 1.0	1	2.13	9.8 ± 2.3	10.6	0.01	0.57
Wilmot	154.4 ± 5.0	3.0	21.1 ± 1.1	7.6	1.18	1.85	21.3 ± 1.4	421	0.83	10.2 ± 3.9	10.0	0.04	0.40
Dunk	239.9 ± 3.1	3.4	21.8 ± 2.2	7.6	1.19	1.93	20.9 ± 1.5	721	0.78	23.3 ± 1.5	8.6	0.21	0.30
Stanley	24.7 ± 2.3	17.8	25.6 ± 0.7	7.6	1.43	0.91	21.8 ± 0.8	34	3.93	21.5 ± 4.2	6.0	0.60	0.35
Wheatley	22.0 ± 1.9	20.7	25.4 ± 0.6	7.6	1.62	1.07	21.7 ± 0.6	119	3.80	14.6 ± 5.5	9.9	0.26	0.56
Murray	33.2 ± 1.9	16.1	26.7 ± 0.2	7.5	2.05	1.80	19.2 ± 1.1	53	2.40	22.3 ± 2.3	N/A	N/A	N/A
Montague	189.2 ± 73.5	8.1	25.0 ± 1.6	7.5	1.34	1.81	17.3 ± 0.8	374	2.85	7.4 ± 2.5	6.6	0.41	0.08
Souris	66.3 ± 3.3	9.8	23.8 ± 1.6	7.6	0.63	1.64	19.3 ± 1.8	57	2.30	12.3 ± 6.5	14.0	0	0.71

Note: N-loading is courtesy of the Prince Edward Island provincial government, based on 2010 land-use layers, and work conducted by Grizard (2013) and Jiang et al. (2015). Pressure loggers were deployed in summer 2015 at the same locations as the dissolved oxygen loggers. Residence time is the proportion of water remaining after the minimum low tide relative to mean tide (the overall average is presented here). Dissolved oxygen threshold values represent the average percentage of hours meeting the criteria across all sampling times. Variability is presented as ±1 SE, for $n = 3$ in all cases.

Water chemistry and dissolved oxygen

Water chemistry variables were measured biweekly, before noon and within 2 h of slack tide, 0.5 m from the substrate using a YSI V2 6600 multiparameter sonde (Yellow Springs Instruments, Yellow Springs, Ohio, USA). The sonde was equipped with optical probes for dissolved oxygen, pH, conductivity, and temperature. Environmental variables were taken biweekly from June to September 2013 and the overall averages are presented in Table 1. For the monthly invertebrate sampling period, however, these variables were averaged over the two biweekly samplings preceding it to represent typical conditions prior to sampling. One litre grab samples of water were taken for chlorophyll sampling concurrently with environmental variable measurements, about 0.5 m below the water surface, and stored in the dark until processing later that day. Water samples were filtered through glass filter fiber paper type F and then submerged in 5 mL of acetone to extract chlorophyll. Samples were stored in the dark at −80 °C and later analyzed using high-performance liquid chromatography (Schein et al. 2012).

An Onset HOBO (Bourne, Massachusetts, USA) dissolved oxygen logger, which recorded dissolved oxygen (mg/L) and temperature (°C) hourly, was used to capture fine-scale variability in the oxygen regime. Criteria for the area of deployment for the dissolved oxygen logger, and later for invertebrate sampling, were as follows: salinity (average PSU of 15–25), depth (∼1.5 m at slack tide), and vegetation presence (*Z. marina* or *Ulva*). Cumulative dissolved oxygen metrics for the 48 h preceding sampling were selected to capture symptoms of nutrient impact, i.e., hypoxia and dissolved oxygen supersaturation. Metrics were based on work by Coffin et al. (2018), which found that hypoxia (proportion of time below 4 mg/L), dissolved oxygen supersaturation (proportion of time above 10 mg/L), and the coefficient of variation of dissolved oxygen were all predicted by water residence time and nitrate loading. Additionally, metrics for the proportion of time <2 mg/L, <6 mg/L, and >15 mg/L were incorporated for greater resolution of sites that were minimally and maximally impacted by nutrients (Miller et al. 2002; Landman et al. 2005; Riedel et al. 2014; Coffin et al. 2018). The duration of 48 h was selected because effects from hypoxia prior to invertebrate sampling would likely still be evident (Coffin et al. 2017), and although not investigated directly in this manuscript, the impacts on behaviour and survivorship can occur after extended exposure to hypoxia (Miller et al. 2002; Riedel et al. 2014). There were two data quality issues over the course of the sampling in the Murray and Cocagne estuaries. Data from the Murray logger were unreliable as it was periodically buried in anoxic substrate and were, therefore, excluded from analyses or ordinations involving environmental variables. The logger from Cocagne, a *Z. marina* site, became fouled with vegetation immediately prior to the July sampling and so the first 48-h period without fouling was used instead (approximately 5 d earlier).

Water residence time and nutrient loading

A tidal prism model employing estuarine volume, mean tidal amplitude, and freshwater input was used to create a proxy for water residence time. Bathymetric data were collected in a related project and used here to calculate estuarine volume using ArcGIS 10.3. Tidal data were collected every 10 min for at least 30 d at the dissolved oxygen logger location in each estuary using Onset level loggers (one barometric and one submerged). Harmonic tidal models were created from those data using the t_tides program in Matlab (Pawlowicz et al. 2002). These models were used to simulate tides over the period of dissolved oxygen logger deployment (15 May to 30 November 2013), and mean tidal amplitude was derived by averaging the tidal amplitudes that occurred over that period. Higher residence time values are indicative of sites with low flushing rates. These values represent the ratio of overall estuarine volume relative to freshwater input and tidal exchange.

Nitrogen loading (nitrate-N) data were collected from two sources: primarily from Grizard (2013) and supplemented with data from Bugden et al. (2014). In short, daily water flow (m³/s) was

multiplied by mean nitrate-N concentration (kg/ha/year), which is the most bioavailable form of nitrogen and best correlated with plant productivity (Hemminga and Duarte 2000). A strong relationship was found between daily flow and watershed area for rivers in this region ($n = 8$, $R^2 = 0.97$). As our area of interest was farther downriver than the most downstream measurements, the calculated nutrient loads, and flow, were prorated for the larger watershed area and assumed to be proportional to the measured watershed area.

Sediment organic content and particle size analysis

A Birt–Flannagan-modified Ekman dredge (15 cm × 15 cm) was used to collect sediment samples at three locations, but only on one occasion, within the sampling region for particle size and organic content analysis. Sediment was homogenized and then dried at 60 °C for 48 h. A 5 g sediment subsample from each replicate was analyzed for organic content by loss on ignition at 550 °C for 24 h. The organic-free sediment sample was analyzed for grain size using laser diffraction (Horiba laser particle sizer model LA-960). During the analysis, ultrasonic dispersion was used to ensure homogeneity of the sample. For our analyses, the 10th, 50th, and 90th percentiles were highly correlated within estuaries, and only the 50th percentile (D50) was retained to characterize grain size.

Invertebrate sampling

Invertebrate sampling occurred before noon and very close to slack tide, monthly between 10 June and 25 September 2013. Sampling was boat-based, in 1–2 m of water, and was within a 200 m radius of the dissolved oxygen logger (location as described above). To ensure the independence of the samples, ephemeral algal mats were sampled by traveling in a random direction, mooring, and then attempting collection. Several sampling methodologies were employed to suit the assemblage or habitat being sampled (*Z. marina*, *Ulva*, or infauna). Epibenthic invertebrates, henceforth referred to as epifauna, in *Z. marina* habitat were sampled using a Birt–Flannagan-modified Ekman dredge (15 cm × 15 cm) dropped into a *Z. marina* bed. This method was ineffective for *Ulva* habitat as the dredge was incapable of cutting through the thick algal mats. Thus, an alternative method was devised using two modified bow-head garden rakes (40.6 cm long with 2.5 cm between tines). The sampler would place the rakes, one in each hand and shoulder width apart, on the substrate and bring them together underwater, sampling an approximate area of 0.25 m^2 (Coffin et al. 2017). Vegetation samples for either habitat were brought into the boat and into a bucket of invertebrate-free water. Vegetation was visually inspected for degradation (only healthy algae were retained), separated from sediment and manually cleared of invertebrates, and then placed into a plastic bag to be processed in the lab. *Ulva* samples were large and compact when brought into the boat, and a total of 135 *Ulva* samples were taken with an average dry mass of 20.9 ± 1.5 g (SE); although some invertebrates on exposed edges may have escaped during sampling, the majority were contained within the algae ball. The remaining water–sediment–macroinvertebrate slurry was immediately sieved through 500 μm mesh and stored in 95% ethanol to be processed later. Infauna were collected using a 7.6 cm diameter core, about 15 cm deep within 5 m of the vegetation samples, also sieved through 500 μm mesh and stored in 95% ethanol. For each sampling method, five replicate samples were taken each month (June, July, and August/September). All samples were further processed using a dissecting microscope (40× magnification), and macroinvertebrates were identified using appropriate taxonomic guides (Bousfield 1973; Appy et al. 1980; Pollock 1998; Merritt et al. 2008; Thorp and Covich 2010). As sampling methodologies differed for invertebrate collection, all analyses were performed using relative abundances. A selection bias for different fauna was likely given the variety of sampling equipment used, and thus infauna and epifauna were

not compared statistically. Vegetation was not measured explicitly in this study, but *Z. marina* coverage estimates were taken in a related study (Hitchcock et al. 2017). Within the sampling area, only Montague and Wilmot had sparse *Z. marina* near the sampling area at *Ulva*-dominated sites and none of the *Z. marina* sites had significant *Ulva* incursion (Hitchcock et al. 2017). Generally, the area sampled had continuous vegetation; sites where bare substrates existed were not sampled.

Data analysis

All data were analyzed using Plymouth Routines in Multivariate Ecological Research package version 6.1.18 (Clarke and Gorley 2006) with PERMANOVA+ (Anderson et al. 2008) and STATISTICA version 12. Environmental variables from the two weeks prior to each sampling time at each site were averaged and then analyzed using principal component analysis (PCA) to examine relative differences between sites. To meet assumptions of linearity and homoscedasticity for parametric analyses, environmental data were tested visually and with Cochran's C test, respectively, and, where necessary, log transformed and then normalized for subsequent analyses (Clarke and Gorley 2006). For invertebrate assemblage data, Bray–Curtis similarity resemblance matrices were created with the inclusion of a dummy variable to account for samples with no species (Clarke and Gorley 2006; Anderson et al. 2008). Principal coordinate analysis (PCoA) ordinations were created, using the Bray–Curtis dissimilarity matrices, for epifaunal and infaunal communities to visualize data by habitat type. Mixed-model PERMANVOAs were used to test between the fixed factor habitat type (*Z. marina* or *Ulva*) and "Month" (the convention for repeated measures designs (Anderson et al. 2008)), and the random factor "Site" nested within "Habitat Type". Additionally, species richness was calculated for each site and sampling time and then analyzed for each sampling methodology by habitat type using two one-way ANOVAs. Next, habitat types were analyzed independently using mixed-model PERMANOVAs to examine the effects of the fixed factor "Month", the random factor "Site", and the potential interaction between those factors. Sites were designated as a random factor because they were chosen from a larger group of potential estuaries on which inferences were to be made. In *Z. marina* habitat for both sampling methodologies, initial analyses resulted in a negative estimate for the component of variation and large p values ($p = 0.542$ and $p = 0.479$ for epifauna and infauna, respectively) for the temporal factor "Month". As suggested by Underwood (1997) and Anderson et al. (2008), this term was pooled with the interaction term, "Month × Site" in this case. Similarity percentages were also calculated across all sites and sampling times for *Z. marina* and *Ulva* habitats to determine which species contributed most to the dissimilarity.

Relationships between invertebrate assemblages and environmental predictor variables were examined using distance-based linear models (DISTLM) and visualized using distance-based redundancy analysis (dbRDA). Correlations between independent variables were restricted to $r < 0.7$ (Pearson's correlation), as strong correlations have undue influence over results and can lead to spurious interpretation of the dependent variables (Anderson et al. 2008). When independent variables were highly correlated, the variable that was most correlated with other independent variables was eliminated. DISTLM is used to analyze the relationship between the Bray–Curtis resemblance matrix and potential explanatory variables, in this case invertebrate assemblages and associated environmental variables. Because some of the environmental data were at the monthly scale, invertebrate data were pooled (averaged) by sampling time for each estuary to enable analyses. As with the mixed-model PERMANOVAs, analyses were conducted on each habitat type and for epifauna and infauna independently. Model selection for the DISTLM was performed using step-wise selection based on adjusted R^2 criteria.

Results

Site and plant habitat differences

Environmental variables from the 13 estuaries studied, except for Murray because of logger burial in anoxic substrate (Table 1), were visualized using a PCA (Fig. 2). The first three axes of the PCA explained 33.9%, 21.1%, and 19.8% of the total variation, respectively (Table 2). There was separation of sites that drain into the southern Gulf and those that drain into the Northumberland Strait. This can be explained by SGSL-draining sites tending to be more correlated with longer water residence time and higher values for variables associated with eutrophication, specifically chlorophyll, dissolved oxygen variability (DO CV), and hypoxic hours (dissolved oxygen <6) (Fig. 2). The primary axis was driven by variables related to nutrient impact (e.g., coefficient of DO CV, the percentage of hours of dissolved oxygen under 6 mg/L, organic content of the sediment sample, water residence time, and chlorophyll) (Table 2). Salinity was also generally higher at *Ulva* sites, most likely due to their dominance in SGSL-draining estuaries that have lower tidal variability compared with Northumberland Strait draining sites (Table 1). Conversely, the secondary axis was driven by nitrate-N loading, average substrate grain size (D50), and organic content (Fig. 2, Table 2). The tertiary axis was nearly as important as the secondary axis and, like the primary axis, was driven by eutrophic variables, specifically dissolved oxygen metrics related to high and low oxygen and also pH; temperature was not important for any of the first three axes (Table 2).

The PCoA ordinations examining relative abundance of epifauna and infauna between *Z. marina* and *Ulva* habitat showed similar patterns and in both cases the first two axes explained more than 50% of the total variation (Fig. 3). Samples were generally grouped according to vegetation type within the ordinations for epifauna and infauna. It is notable, however, that Kouchibouguac, a *Z. marina*-dominated site that had a high proportion of hydrobid snails, was more associated with *Ulva* sites. For both epifauna and infauna sampling methodologies, hydrobid snails dominated *Ulva* habitat but were also found regularly at low densities in *Z. marina*. Conversely, cerithid snails were uncommon in *Ulva* habitat but dominated in *Z. marina* (Table 3). These two taxa contributed most to the
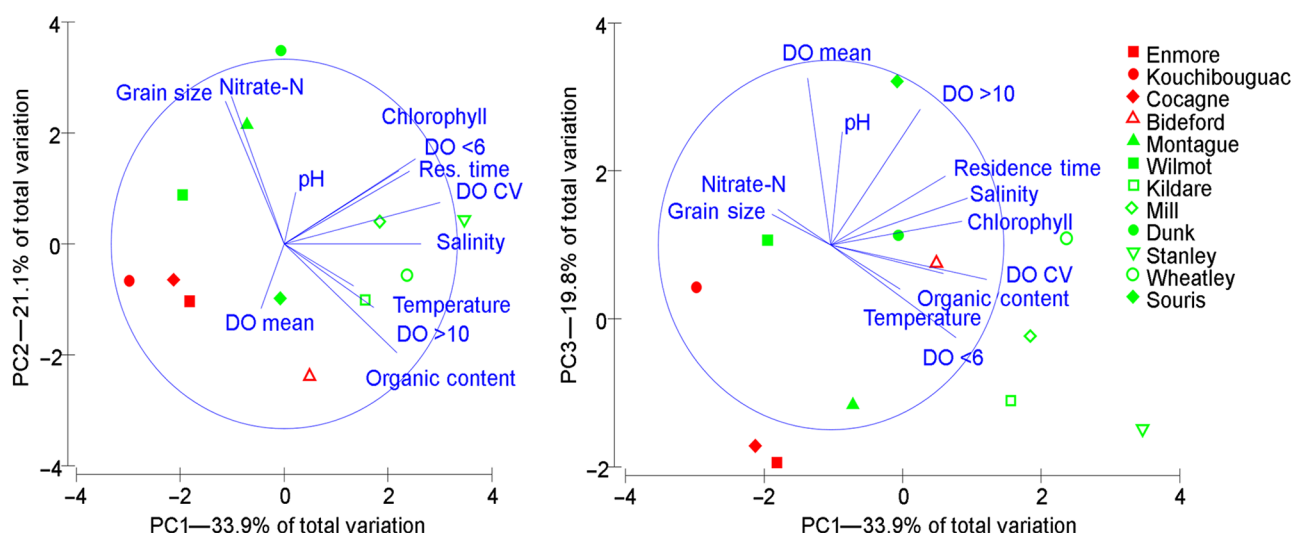

Fig. 2. Principal component analysis for each site using the average for each environmental variable. Vector length represents the contribution of that particular variable to the axes. *Zostera marina*-dominated habitats are in red and *Ulva* spp. dominated habitats are in green. Furthermore, estuaries draining into the Gulf of St. Lawrence are open and those draining into the Northumberland Strait are filled. DO, dissolved oxygen; CV, coefficient of variation.

Table 2. Coefficients in the linear combination of variables making up principal components (PC) 1–3 for the environmental principal component analysis (Fig. 2).

Variable	PC1—33.9%	PC2—21.1%	PC3—19.8%
<6 mg/L DO	**−0.359**	−0.246	**0.325**
>10 mg/L DO	−0.257	0.216	**−0.475**
DO mean	0.067	0.220	**−0.586**
DO CV	**−0.447**	−0.141	0.122
Grain size (D50)	0.169	**−0.486**	−0.170
Organic content	**−0.323**	**0.371**	0.100
Salinity	**−0.393**	0.000	−0.165
pH	−0.033	−0.176	**−0.396**
Temperature	−0.199	0.144	0.156
Nitrate-N	0.152	**−0.503**	−0.125
Residence time	**−0.328**	−0.249	−0.241
Chlorophyll	**−0.377**	−0.290	−0.084

Note: Values >0.300 or <−0.300 are bolded to highlight their relative contribution to the model. DO, dissolved oxygen; CV, coefficient of variation.
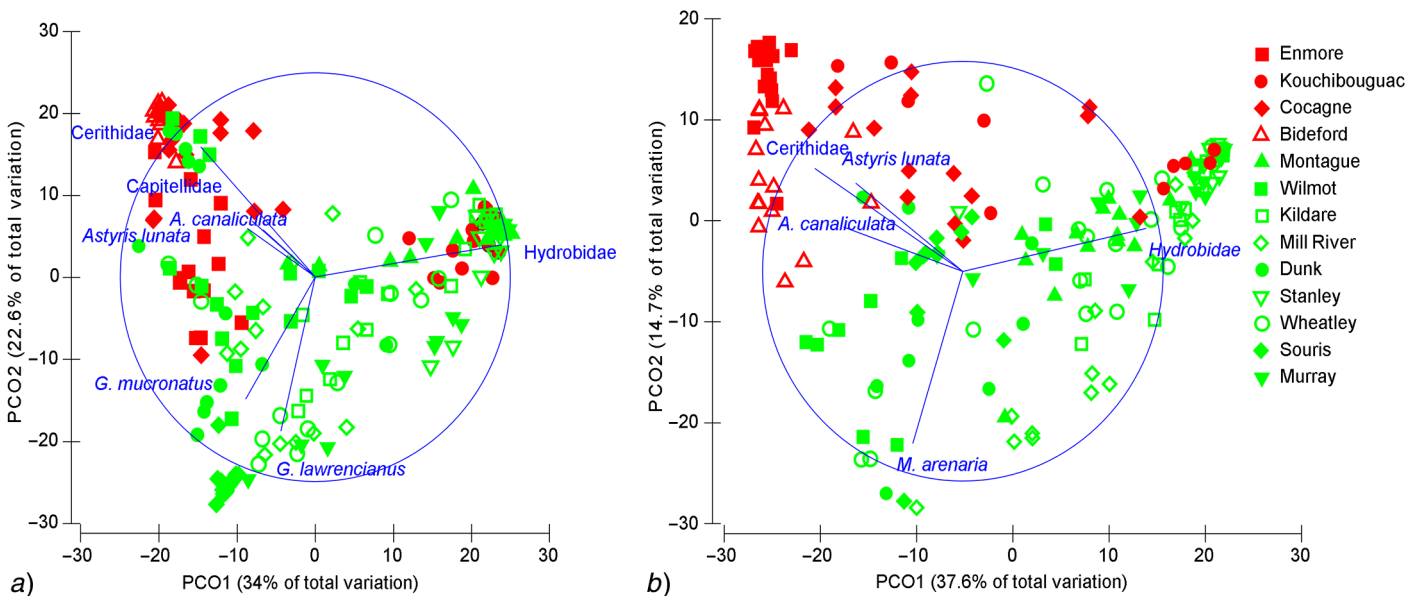

Fig. 3. Principal coordinate ordinations for epifauna (*a*) and infauna (*b*) from all estuaries, *Zostera marina* habitats are in red and those of *Ulva* spp. are in green. Estuaries draining into the Gulf of St. Lawrence are open and those draining into the Northumberland Strait are filled. All sampling times are pooled. Vector length corresponds to the correlation ($r < 0.4$) of a particular species.

Table 3. The results of the similarity percentages analysis describing the top taxa contributing to the dissimilarity between habitat types.

Taxon	Average relative abundance		Average dissimilarity	Contrasting taxa (contribution %)
	Z. marina	*Ulva*		
Epifauna				
Hydrobidae	0.19	0.34	17.49	21.83
Cerithidae	0.27	0.06	13.48	16.82
Gammarus mucronatus	0.08	0.16	8.18	10.20
G. lawrencianus	0.01	0.15	7.47	9.31
Capitellidae	0.10	0.00	4.85	6.05
Mya arenaria	0.05	0.04	3.43	4.28
Corophidae	0.02	0.05	2.77	3.46
Littorina spp.	0.01	0.04	2.54	3.16
Nereidae	0.04	0.01	2.44	3.04
Astyris lunata	0.03	0.02	2.29	2.86
Acetocina canaliculata	0.04	0.00	2.04	2.55
Tritia obsoleta	0.01	0.04	2.04	2.54
Chironomidae	0.03	0.01	1.80	2.25
Infauna				
Hydrobidae	0.15	0.43	19.64	23.92
Cerithidae	0.35	0.02	16.99	20.69
M. arenaria	0.06	0.15	8.12	9.89
Capitellidae	0.10	0.07	6.50	7.91
A. canaliculata	0.09	0.01	4.56	5.56
Nereidae	0.04	0.05	4.15	5.05
G. mucronatus	0.03	0.05	3.89	4.73
L. obtusata	0.01	0.07	3.55	4.33
A. lunata	0.07	0.00	3.39	4.14
N. obsoletus	0.00	0.04	2.14	2.60
Chironimidae	0.02	0.02	2.03	2.47

Note: The similarity cut off threshold was 2% for both epifauna and infauna.

average dissimilarity between the invertebrate communities found in the two plant habitats for both epifauna and infauna. Epifauna were further distinguished by *Gammarus mucronatus* Say, 1818, *Gammarus lawrencianus* Bousfield, 1956, capittelid polychaetes, and the softshell clam *Mya arenaria* Linnaeus, 1758. Infauna were distinguished by *M. arenaria*, capittelid polychaetes, *Acteocina canaliculata* (Say, 1826), and nereid polychaetes (Table 3). Nested PERMANOVAs revealed that invertebrate communities were significantly different between vegetation types and sites but not sampling times (Table S1). There was a significant interaction between Site (vegetation type) × Month; a

posteriori tests revealed that each site was significantly different from all other sites for every month (results not shown).

For both sampling methods, pooled across time and site, there was significantly higher species richness in *Z. marina* than *Ulva* habitat (ANOVAs for epifauna and infauna: $F_{1,37} = 20.182$, $p < 0.001$ and $F_{1,35} = 7.744$, $p < 0.009$, respectively; Table 4). The different sampling methodologies precluded a statistical comparison of abundances which were higher in *Ulva* habitat. Gammarid amphipod abundances, for example, differed by up to two orders of magnitude (1000s vs. 10s) in *Ulva* relative to *Z. marina* sites. Overall, hydrobid snails, several species of *Littorina* Ferussac, 1822, *Tritia obsoleta* (Say, 1822) (previously *Ilyanassa obsoleta*), *G. mucronatus*, *G. lawrencianus*, and corophid amphipods were more abundant in *Ulva* habitat, whereas cerithid snails, *A. canaliculata*, *Astyris lunata* (Say, 1826), and capitellid polychaetes were more abundant in *Z. marina* (Table 3).

Table 4. Faunal species presence and absence at all study sites.

	Enmore	Kouchibouguac	Cocagne	Bideford	Murray	Montague	Wilmot	Kildare	Mill River	Dunk	Stanley	Wheatley	Souris
Gastropods													
Tritia obsoleta	Z	—	Z	Z	U	U	U	U	U	U	U	U	—
Hydrobidae	Z	Z	Z	—	U	U	U	U	U	U	U	U	U
Cerithidae	Z	—	Z	Z	—	U	U	U	U	U	U	U	—
Pyramidellidae	Z	Z	Z	Z	U	U	U	U	U	U	—	U	—
Acteocina canaliculata	Z	—	Z	Z	U	U	U	U	U	U	U	U	—
Littorina spp.	Z	Z	Z	Z	U	U	U	U	U	—	—	U	—
Astyris lunata	Z	—	Z	Z	—	—	U	—	U	U	—	—	—
Bivalves													
Mya arenaria	Z	—	Z	Z	U	U	U	U	U	U	U	U	U
Gemma gemma	Z	Z	—	Z	—	U	U	U	U	—	U	U	U
Cerastoderma pinnulatum	—	—	—	Z	—	—	—	—	—	—	—	—	—
Macoma calcarea	—	Z	—	—	—	—	—	—	—	—	—	—	—
Mytilidae	Z	—	Z	Z	U	U	—	U	U	—	U	—	U
Crassostrea virginica	Z	—	—	Z	—	—	—	—	U	U	—	—	—
Crustacea: Amphipod–Tanaid–Isopod–Decapod													
Gammarus mucronatus	Z	Z	Z	Z	U	U	U	U	U	U	U	U	U
G. lawrencianus	Z	Z	—	Z	U	U	U	U	U	U	U	U	U
G. oceanicus	—	—	Z	—	U	U	U	—	U	—	—	U	—
G. tigrinus	—	Z	—	—	—	—	—	—	—	—	—	—	—
Ampithoidae	Z	Z	—	Z	—	—	U	—	—	U	—	—	—
Corophidae	Z	—	—	Z	U	U	U	U	U	U	U	U	U
Tanaidacae	Z	—	—	—	—	—	—	—	—	—	U	—	—

(continued)

Table 4. (*concluded*)

	Enmore	Kouchibouguac	Cocagne	Bideford	Murray	Montague	Wilmot	Kildare	Mill River	Dunk	Stanley	Wheatley	Souris
Jaera sp.	Z	—	Z	—	—	U	U	—	U	U	U	—	U
Palaemon spp.	Z	—	Z	—	—	—	U	—	—	U	—	U	U
Crangon crangon	—	Z	Z	Z	—	U	U	—	U	—	—	—	—
Insecta													
Chironominae	Z	Z	Z	—	U	—	—	—	U	—	U	U	—
Orthocladiinae	Z	—	—	—	—	—	—	—	—	U	—	—	—
Annelida: Polychaeta–Clitellata													
Nereidae	Z	Z	Z	Z	U	U	—	U	U	—	U	U	U
Glyceridae	—	Z	—	Z	—	—	—	—	U	—	—	—	—
Capitellidae	Z	Z	Z	Z	U	—	U	U	U	U	U	U	U
Nephtys spp.	Z	Z	Z	Z	U	—	U	U	U	—	U	U	U
Orbiniidae	Z	Z	Z	Z	U	—	—	—	U	—	U	—	—
Spionidae	Z	Z	—	Z	U	—	—	U	—	—	U	U	—
Pectinariidae	Z	—	—	Z	—	—	—	U	U	—	—	—	—
Terebellidae	Z	Z	Z	Z	U	—	U	—	—	—	—	—	—
Polynoidae	—	—	—	Z	—	—	—	U	U	U	—	U	—
Naididae	—	—	—	—	—	U	—	—	—	U	—	—	—
Nemertea and Hemichordata													
Nemertea	Z	—	Z	—	—	—	—	—	—	—	—	—	—
Hemichordata	—	Z	—	—	—	—	—	—	—	—	—	—	—
Echinodermata													
Asterias sp.	—	—	Z	Z	—	U	—	U	—	—	—	—	—

Note: *Z. marina*-dominated habitat is indicated with the letter "*Z*" and *Ulva*-dominated habitat by the letter "*U*". Only species occurring in more than one sample per site are shown.

Within-habitat differences

Z. marina

Epifaunal and infaunal communities both differed significantly between *Z. marina* sites, but there was no significant effect of sampling time. The interaction Month × Site was significant in both cases and indicates that changes over time were not consistent between the sites (Table S2). It should be noted here that the interaction term is a test of the "generality" of whether sampling time effects vary between sites and, if significant, does not preclude interpretation of the main effects. When assemblage data were analyzed further using DISTLM and visualized with a dbRDA ordination, the site differences become apparent (Fig. 4). Over 88% of the overall fitted variation was explained by the first two axes for both epifauna and infauna (Fig. 4). The adjusted R^2 was 0.81 using three variables for epifauna and 0.60 with four variables for infauna (significant variables from the model are bolded in Fig. 4).
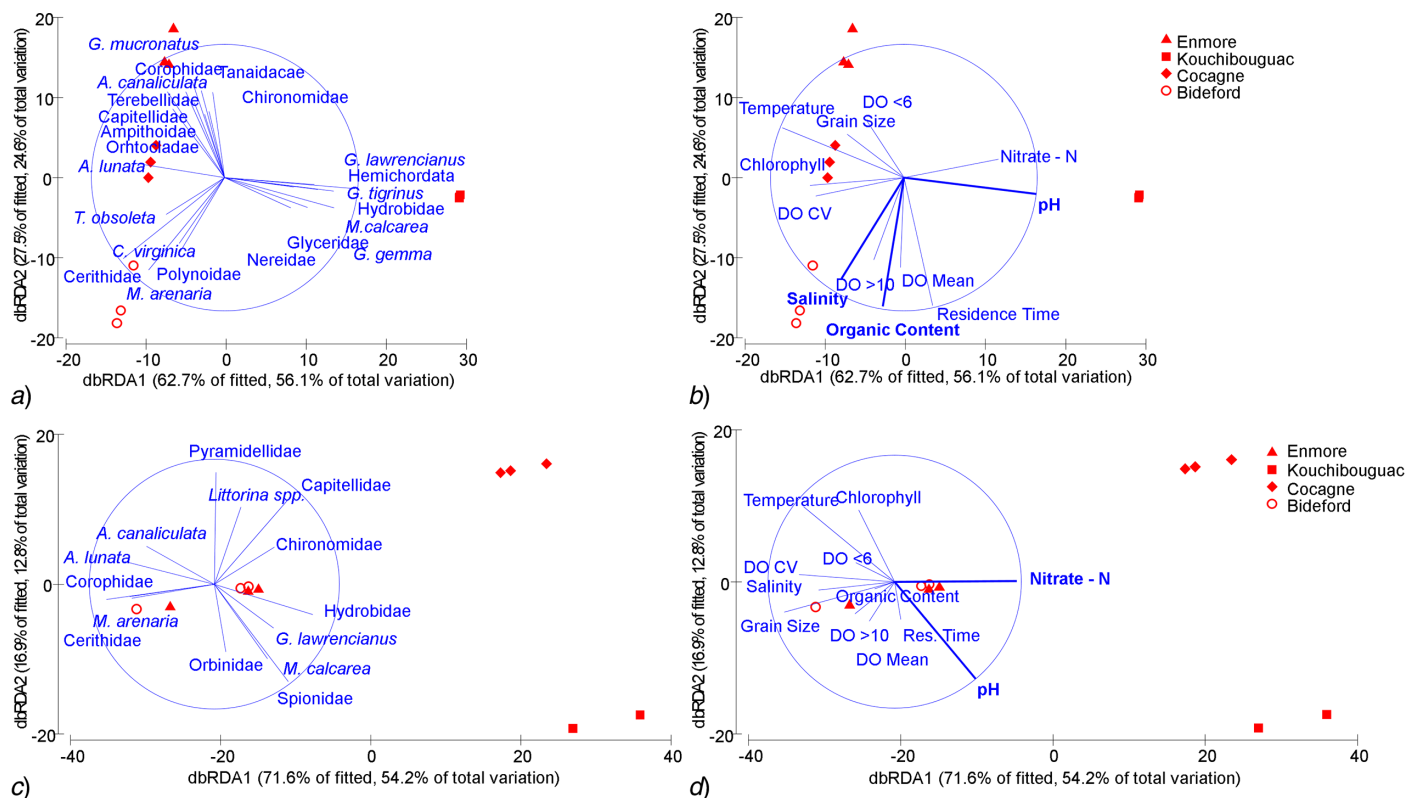
Fig. 4. Distance-based redundancy analysis (dbRDA) ordination plot of species (*a*, *c*) and environmental variables (*b*, *d*) of epifauna (*a* and *b*) and infauna (*c* and *d*) communities in *Zostera marina*. Vector length corresponds to a Pearson's correlation of $r < 0.5$. Significant predictor variables from the distance-based linear models are bolded in the ordinations. Sites are represented by the symbols used in previous figures with samples representing the average abundance of a species between all samples per sampling time (month). DO, dissolved oxygen; CV, coefficient of variation.

Predictor variables unrelated to eutrophication were most important for discriminating between *Z. marina* sites. Kouchibouguac shared many of the same species as *Ulva* sites, but it also had high polychaete richness like the other *Z. marina* sites (**Fig. 4**). Furthermore, Kouchibouguac had several species not found elsewhere such as *Gammarus tigrinus* Sexton, 1939, *Macoma calcarea* (Gmelin, 1791), and Hemichordata (**Table 3**). With respect to environmental variables, Kouchibouguac had higher pH and nitrate-N loading, whereas the remaining three sites were dispersed along the *y*-axis that was driven by residence time, dissolved oxygen, and salinity (for epifauna, **Fig. 4b**).

Ulva

Like in *Z. marina*, epifaunal and infaunal communities at *Ulva*-dominated sites varied significantly between sites but not through time (**Table 4**). The interaction term was significant, indicating that changes within sites were not consistent through time. When visualized using dbRDAs, more than 71% of the overall fitted variation is explained by the first two axes for both epifauna and infauna (**Fig. 5**). The adjusted R^2 of the DISTLM is 0.57 using 11 variables for epifauna and 0.64 with 8 variables for infauna (significant variables are shown in bold in **Fig. 5**). For both epifauna and infauna, two distinct axes occur whereby variables related to eutrophication (e.g., organic content, water residence time, and oxygen-related metrics) dominate the primary axis, and nitrate-N loading, grain size, and some of the aforementioned variables related to eutrophication load heavily onto the secondary axis (**Fig. 5**). Wilmot and Dunk are close to one another in the ordination
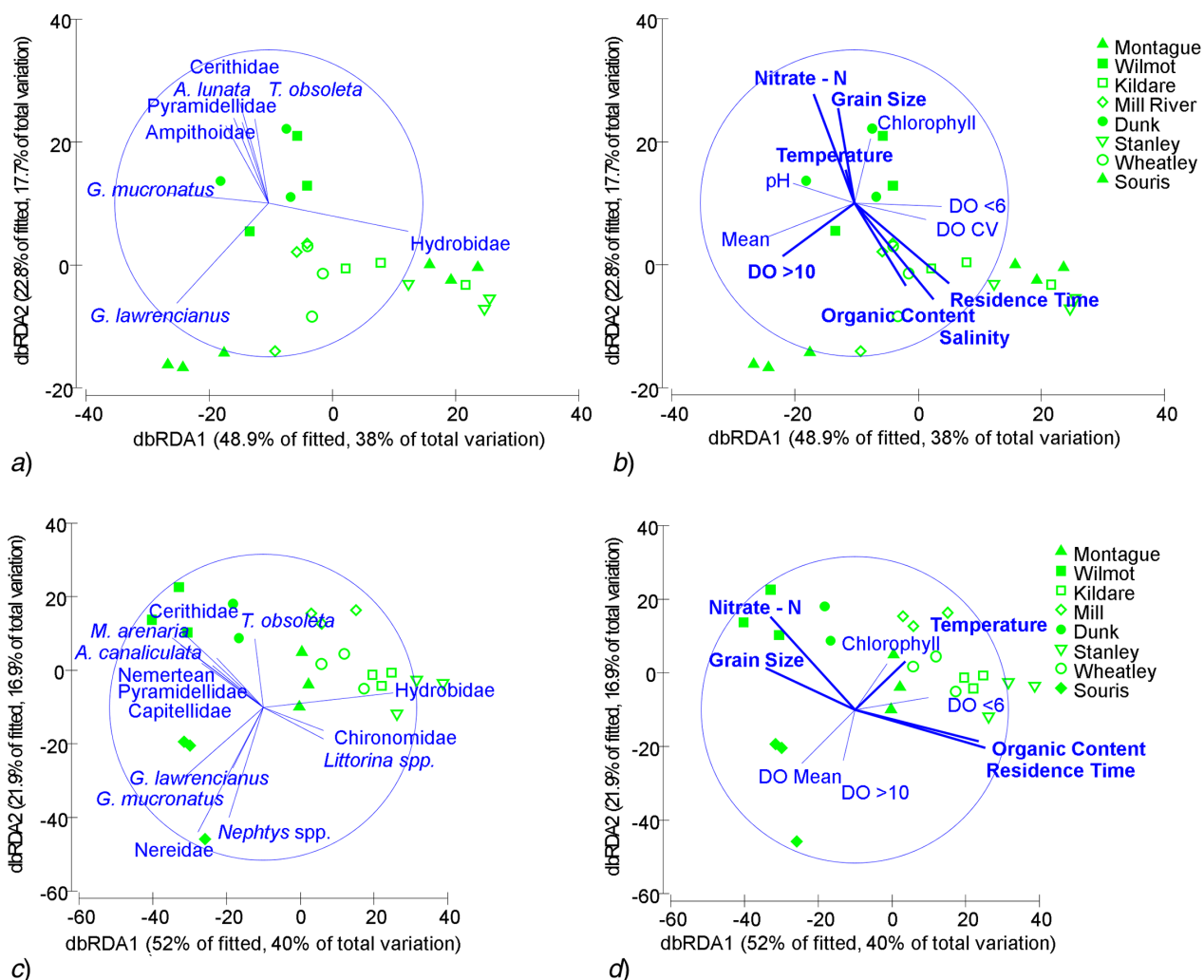
Fig. 5. Distance-based redundancy analysis (dbRDA) ordination plot of species (*a*, *c*) and environmental variables (*b*, *d*) of epifauna (*a* and *b*) and infauna (*c* and *d*) communities in *Ulva* spp. Vector length corresponds to a Pearson's correlation of *r* < 0.5. Significant predictor variables from the distance-based linear models are bolded in the ordination plot. Sites are represented by the symbols used in previous figures with samples averaged within month for fauna. DO, dissolved oxygen; CV, coefficient of variation.

and with respect to geography (**Fig. 1**); they both have a relatively large grain size, high nitrate-N loading, and shorter residence time relative to the other sites. Souris has shorter water residence time and higher dissolved oxygen concentration than any other site, and it has low species richness: primarily *G. lawrencianus*, *G. mucronatus*, and nereid polychaetes. Mill, Stanley, Wheatley, and Kildare are all north-shore estuaries with relatively long water residence times and are closely associated in the ordinations for both epifauna and infauna. These sites experience greater dissolved oxygen variability, hypoxia, and dissolved oxygen supersaturation that are also generally correlated with long water residence time and high organic content. Although hypoxia was not as strongly correlated in the linear model with the species assemblages as organic content, water residence time, nitrate-N, or substrate grain size, it was negatively correlated with amphipod abundances and positively correlated with snail abundances.

Discussion

Ulva-dominated sites differed from *Z. marina* sites in that they tended to have longer water residence times, higher nutrient loads and organic content, and more variable dissolved oxygen. Invertebrate communities differed between *Ulva*- and *Z. marina*-dominated sites, with hydrobid and cerithid snails and gammarid amphipods contributing most to their dissimilarity. Results suggested an effect of nutrient-induced impacts on the *Ulva* habitat faunal community. There was a gradient among our nutrient-related variables, with a higher relative abundance of hydrobids corresponding to hypoxia and high organic content, and high gammarid abundance corresponding to elevated dissolved oxygen concentration.

Fauna may be exposed to more variable conditions in *Ulva* than *Z. marina*. *Ulva* and *Z. marina* are both well adapted for shallow, estuarine conditions, and *Ulva* was sometimes observed at low abundances under oligotrophic conditions within *Z. marina*-dominated habitat. Unlike *Z. marina*, which has strict substrate requirements, *Ulva* grows ephemerally, in nutrient-enriched systems, and is therefore not limited by grain size (Webster et al. 1998; Gamito et al. 2012). In this study, average substrate particle size (D50) had a greater range at *Ulva* sites (22–235 μm) compared with *Z. marina* sites (36–89 μm), presumably due to some combination of higher levels of fine sediment input from agriculture (Alberto et al. 2016) and differences in local lithology (van der Poll 1983) and hydrology. Furthermore, *Ulva*-dominated sites experience greater dissolved oxygen variability than *Z. marina*-dominated sites (Coffin et al. 2018), which may limit the number of taxa capable of inhabiting them. Like dissolved oxygen, the other environmental parameters measured were also more variable in *Ulva*-dominated estuaries. As *Ulva* mats are ephemeral they may experience an even greater range of conditions, by traveling up- or down-stream, which could stress its inhabitants. Conversely, the relative stability of *Z. marina* may promote a more stable and diverse faunal community.

Zostera marina was found to have higher species richness than *Ulva* in the present study, but that is not always the case in other areas (Drake and Arias 1996; Norkko et al. 2000), particularly when macroalgae is well oxygenated (Cebrian et al. 2014). When *Z. marina* and macroalgae co-occur the combination of greater food resources, structural complexity, and below-ground habitat availability can have a positive effect on diversity (Boström et al. 2002). *Zostera marina* is composed of long, thin shoots above ground and a complex rhizome network below ground, whereas *Ulva* generally occurs as large, ephemeral sheets that smother the substrate. The aforementioned stability of *Z. marina*, and the loose and anoxic substrate associated with *Ulva*, may result in fewer niches for epibenthic invertebrates. In this study, estuarine sites dominated by *Ulva* tended to have higher nitrate-N loading, longer water residence time, and were more prone to experience hypoxia and dissolved oxygen supersaturation than sites dominated by *Z. marina*, all of which may have contributed to reduced species richness as much or more than the plant community itself.

Differences in food type and availability between *Z. marina* and *Ulva* may be responsible for differences in snail distributions and are also important factors structuring invertebrate assemblages. Living *Z. marina* is not particularly palatable for invertebrates; mobile grazers generally feed on epiphytes growing on the plant itself (Moore and Wetzel 2000) or on decomposing shoots after they have been shed (Hemminga and Duarte 2000). Conversely, *Ulva* is more palatable, alive or as detritus, and is also substrate for epiphytes (Andersson et al. 2009; Van Alstyne et al. 2009). Cerithid snails, *Astyris lunata*, *Acteocina canaliculata*, and pyramidellids were common in *Z. marina* but not in *Ulva*, which was dominated by hydrobids and littorinids. Cerithids feed on epiphytes of *Z. marina* (Larkum et al. 2006), *A. lunata* are carnivorous and feed on ectoprocts and tunicates (MacDonald et al. 2012), *A. canaliculata* feed on bivalves, particularly *Gemma gemma* (Totten, 1834), and foraminferans (Chester 1993), and pyramidellids are generalist predators (Robertson and Mau-Lastovicka 1979).

Hydrobid and littorinid snails occurred in both habitats but far outnumbered other snail species in *Ulva* and are known to feed on diatoms and (or) detritus that is common there (Fenchel and Kofoed 1976; Pollock 1998; Brookes and Rochette 2007). Thus, the increased diversity of prey available in *Z. marina* habitat results in more snail species that occupy higher trophic levels than in the more homogenous *Ulva* habitat, despite greater food resources overall. These findings are consistent with reports that eutrophic systems are less diverse and dominated by small-bodied species (Hemminga and Duarte 2000; Rosenberg 2001; Levin et al. 2009; Amundrud et al. 2015).

Community assemblages were remarkably similar across sampling methodologies, and indeed, all of the 11 most important species for distinguishing between *Z. marina* and *Ulva* for infauna were also important for distinguishing between epifauna. A study by Quintino et al. (2011) used two methods to test for a transition in community assemblage from freshwater to saltwater and found that both methodologies successfully detected the transition but that different species were responsible for these differences. The present study also used two methods, with the intention of sampling different faunal groups, but generally found community assemblages to be similar regardless of sampling method. Like the work of Nestlerode and Diaz (1998) and Riedel et al. (2014) on invertebrates and hypoxia/anoxia, we observed that many benthic taxa (e.g., polychaetes, corophidae, chironomidae, and certain bivalves) were at shallow depth in *Ulva* habitat. In this study, benthic invertebrates were sampled within epibenthic mats of *Ulva*, implying they may be surfacing from their burrows to acquire oxygen. Thus, a potential explanation for the similarity between sampling methodologies within *Ulva* habitat may be that infauna are forced out of the loose and anoxic sediment (Vaquer-Sunyer and Duarte 2010) into *Ulva* mats.

The potential stress of dissolved oxygen fluctuations may limit the occupancy of *Ulva* habitat. This variability, sometimes ranging from anoxic to supersaturated over a single day, means that dissolved oxygen at the time of sampling may not reflect the conditions that the invertebrate community recently experienced. It has been established that dissolved oxygen concentration can alter animal behaviour through avoidance (Levin et al. 2009), feeding cessation (Nestlerode and Diaz 1998; Riedel et al. 2014), reduction in activity (Riedel et al. 2014), and death (Miller et al. 2002; Nedergaard et al. 2002; Landman et al. 2005; Howarth 2008). Previous work by Coffin et al. (2017) in this region showed evidence of short-term response to sustained hypoxia by amphipods. In that study, amphipod occupation of surface-floating *Ulva* mats, relative to submerged mats, increased with the duration of hypoxia. The implication from that work was that amphipods that occupy surface floating mats benefit from the temporary refuge and that they may also be transported from the area of impact and (or) return to the impacted area using this method. In the present study, crustaceans were the second most-dominant taxa, after gastropods, and they are known to be more sensitive to hypoxia than gastropods or annelids (Levin et al. 2009). The combination of gammarids being mobile and hypoxia-intolerant (Cook and Boyd 1965) means they are likely to be responsive to dissolved oxygen and potentially useful as a species indicative of degraded environments in estuaries. In *Ulva*, gammarid amphipod abundance was best correlated with dissolved oxygen either due to their sensitivity to oxygen or their ability to avoid low dissolved oxygen.

Within *Ulva*, nutrient-related variables were correlated with a relative increase in gastropods and a concomitant decline in crustaceans. Despite epibenthic and infaunal invertebrate assemblages being sampled with different methodologies similar patterns were observed, likely because epifauna and infauna co-occupy *Ulva* mats. Continuous monitoring of dissolved oxygen concentration, in conjunction with detailed information about the environment, was helpful in explaining the observed decrease in epifauna immediately after hypoxia/anoxia. Given the transient nature of hypoxia and its potential impact on fauna, we suggest that similar methods be employed for future work in

eutrophic estuaries, whether hypoxia is the factor of interest or not. Continuous assessment of stressors in severely impacted systems, coupled with more frequent sampling of the community, will improve our understanding of community response.

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Author contributions

MRSC, SCC, and MRVDH conceived and designed the study. MRSC and CCP performed the experiments/collected the data. MRSC, SCC, KMK, and MRVDH analyzed and interpreted the data. SCC and MRVDH contributed resources. MRSC, SCC, KMK, CCP, and MRVDH drafted or revised the manuscript.

Competing interests

The authors have declared that no competing interests exist.

Data accessibility statement

All relevant data are within the paper and in the Supplementary Material.

Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2017-0044.

Supplementary Material 1

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