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Dissolved oxygen as a Marine Environmental Quality (MEQ) measure in upper estuaries of the southern Gulf of St. Lawrence: Implications for nutrient management and eelgrass (*Zostera marina*) coverage

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Eutrophication, the biological response to nutrient enrichment, is a well-studied process that poses a threat to coastal ecosystems around the world. Despite an understanding of the mechanisms that contribute to nutrient impacts, there is no universal method for their quantification or monitoring. Herein, a monitoring indicator is presented and evaluated for estuaries that empty into the southern Gulf of St. Lawrence. Two monitoring indicators were assessed, the first evaluating responses of the regionally dominant seagrass, eelgrass (*Zostera marina*), and the second examining changes in dissolved oxygen (DO). Eelgrass coverage as a percentage of available habitat was previously quantified for each estuary and related to a suite of environmental variables. The only variable for which a significant relationship was found was nitrogen loading which explained 53.0% of the variability in eelgrass coverage using a non-linear response function. The relationship shows that ≈63% of available habitat occupied is the maximum for estuaries within the region. Interpolations using that relationship show eelgrass declines correspond to estuarine external nitrate-N. Evidence is also presented that DO is suited to be a primary indicator of nutrient impacts in systems with high levels of both benthic and pelagic primary production. DO loggers were deployed in the upper estuary, defined by a salinity range of 15-25 PSU and an average water depth of 1-2.5 m of 27 distinct estuaries from 2013-2020 across the southern Gulf of St. Lawrence. Time series data were parsed into biologically relevant metrics that captured symptoms of eutrophication: hypoxia, dissolved oxygen supersaturation, and coefficient of variability. These metrics were analyzed using multivariate statistics and distinct groups formed around habitat type, with algae-dominated habitat reflecting these eutrophication symptoms. The combination of hypoxia and dissolved oxygen supersaturation metrics led to a new metric termed “Eutrophic Time” (the percentage of time an estuary spends <4 mg/L and >10 mg/L dissolved oxygen) which effectively captured estuaries with a tendency towards hypoxia and those towards supersaturation. This new measure was then analyzed through multiple regression with nitrate-N loading and water residence time as predictor variables which were ultimately well correlated (R^2 = 0.81). This relationship can be exploited to provide estuary-specific nitrate-N loading targets for nutrient impacted systems at management’s discretion.

INTRODUCTION

Establishing indicators for assessing ecosystem habitat quality is critical for developing cost-effective monitoring programs that can inform management decision-making. Consequently, effective indicators must have characteristics for them to be initially adopted, and to persist as part of a sustained monitoring effort (Rice and Rochet 2005). Ideally, such indicators should be biologically relevant and quantitatively measurable to provide a meaningful and useful tool for monitoring. Most importantly, there must be a mechanistic relationship between the indicator and the ecosystem stressor such that changes to the indicator are the result of changes in the stressor (i.e., that the indicator is sensitive and specific to changes with the stressor).

Eutrophication, defined by increased primary production (usually algae) due to nutrient loading, is a threat to coastal ecosystem health (Lotze et al. 2006). Despite nutrient effects being well-understood there is no universally accepted method for quantifying eutrophication in estuaries. This challenge is in part because nutrient loading alone is not sufficient to predict impacts, as nutrient uptake by plants is affected by a variety of factors including (but not limited to) salinity, light availability, temperature, and water velocity. For these reasons, among others, it has been recommended that monitoring programs be developed at the regional scale to inherently account for the wide swath of modifying factors that contribute to biological response variability (Duinker and Greig 2006).

In coastal systems, there is a well-established biological continuum from oligotrophic to eutrophic conditions (Pearson and Rosenberg 1978). Oligotrophic estuaries along the north Atlantic coast are characterized by having extensive eelgrass (*Zostera marina*) meadows. Eelgrass has been successful in estuaries, in part, because its rhizomes support symbiotic bacterial fauna that fix nitrogen, providing a competitive advantage under oligotrophic conditions. Eelgrass is important to estuarine ecosystems as it provides three dimensional epibenthic structure, acts as a nursery for economically important fisheries, is a carbon sink, attenuates wave action that limits coastal erosion, and provides food to invertebrates and migratory birds (Hemminga and Duarte, 2000). Given this myriad of ecosystem services, eelgrass has been recognized as an Ecologically Significant Species by the Department of Fisheries and Oceans (DFO 2009). However, at least 29% of the known areal extent of seagrasses (including eelgrass) has been lost globally, and the rate of loss has increased over the past three decades (Waycott et al. 2009). As such, the protection of eelgrass in our region is imperative.

The mechanisms by which eelgrass is impacted by nutrient enrichment are not fully understood. For example, in the early stages of eutrophication, eelgrass may be affected by the direct toxicity of nitrogen species (van Katwijk et al. 1999, Touchette and Burkholder, 2000, Touchette et al. 2003), by light limitation due to phytoplankton and epiphytic growth (Dennison et al. 1993), and by toxic sulfide intrusion due to sediment anoxia (Fraser and Kendrick, 2017, Ugarelli et al. 2017). In the more severe stages of eutrophication, it is evident that benthic and/or pelagic algal species become dominant and have a competitive advantage over eelgrass even without water column light attenuation (Valiela 1997). As a result of these changes to habitat type, the biotic community also experiences a shift towards tolerant species, typically losing species richness and becoming dominated by high densities of animals, typically invertebrates, that are able to thrive in macroalgal-dominated environments (Schein et al. 2012).

Increases in primary production result in spikes of dissolved oxygen (DO) during the day via photosynthesis and declines at night due to respiration in the absence of photosynthesis, both of which are symptoms of eutrophication (Kemp and Boynton, 1980). In severe cases, increased oxygen demand from the decomposition of organic matter can exceed the importation of oxygen from the atmosphere, tidal and freshwater, and its production from photosynthesis. This results in sustained periods of low or no oxygen (anoxia) that can affect marine biodiversity and reach lethal levels to biota (Miller et al. 2002, Landman et al. 2005, Vaquer-Sunyer and Duarte 2008, Riedel et al. 2014).

Given the potential severity of nutrient impacts, it is imperative that any monitoring program be capable of accurately evaluating the trophic status of estuaries and that there is a quantitative link between nutrient loading and the response indicator. In this report a combination of DO and eelgrass distribution is used to assess trophic status of estuaries within the southern Gulf of St. Lawrence in relation to nitrogen loading.

EUTROPHICATION INDICATORS WITH RESPECT TO SPECIFICITY AND SENSITIVITY

Endpoints to quantify trophic status tend to vary with location based on regional-scale influences (physical, chemical, biological, geological, etc.). While the underlying biological processes and progression of estuaries from oligotrophic to eutrophic conditions are well understood (Pearson and Rosenburg 1978, Valiela 1997), predicting where and when shifts from oligo- to eutrophic (and vice versa) occurs is more difficult. The inherent variability within estuaries is further complicated by differences in regional climate, freshwater loading, estuarine morphology, substrate, and tidal regime, which can make efforts at reproducibility or transferability problematic.

While nutrient loading is ultimately the factor directly affecting trophic status, it is not practical as an indicator. Nutrient loading is not always quantifiably linked to impacts, as nutrients only impact systems if they are available to be utilized by plants (e.g., organic forms of nutrients are not immediately available; Priha 1994). Furthermore, the modifying factors of eutrophication mentioned above can dampen or attenuate responses (Coffin et al. 2018). Because increased primary production defines eutrophication, α-chlorophyll concentration in water is the classic endpoint from the limnological literature (OECD 1982) and has also been employed in estuaries (Meeuwig et al. 1998). α-Chlorophyll (also referred to as chlorophyll a) concentration, a proxy for phytoplankton biomass, is well established as an indicator of eutrophication (see Meeuwig 1999). However, there are a number of issues with using this measure in shallow estuaries that render it ill-suited as a generalized indicator of nutrient impact. Unlike in deep lake systems, in shallow estuaries, α-Chlorophyll only represent the fraction of primary production that is pelagic and does not account for benthic production, which can be substantial depending on estuary depth (Valiela 1997, Schein et al. 2012). Further, the shallow, turbid nature of some estuaries results in probe interference and inaccurate results, which limits confidence in high-frequency chlorophyll probes (James 2013).

Seagrass monitoring is among the most common methods for examining impacts, including those of nutrients, in estuaries. However, seagrass monitoring endpoints are not a measure of increasing primary production and thus are only indirectly related to eutrophication. Seagrass metrics represent the culmination of nutrient impacts spanning annual scales, meaning that they are less sensitive on shorter time-scales. Furthermore, seagrasses are sensitive to many stressors including temperature, salinity, sedimentation, mechanical disturbance, ice-scour, aquaculture and other fisheries. Light penetration has long been regarded as the limiting factor of eelgrass distribution as it requires between at least 11% (Olesen and Sand-Jensen 1993) to 34% (Ochieng et al. 2010) of the light reaching the surface to survive. Light penetration can be limited by organic or inorganic particulate matter, as well as dissolved organic matter.

The basis of eelgrass monitoring is that when nutrients are readily available, plant species other than seagrasses have a competitive advantage, frequently resulting in the extirpation of eelgrass (Valiela 1997). This succession is well documented, and the proliferation of macroalgae is considered the first step in this succession (Valiela 1997). This complicates monitoring efforts, as quantifying primary production would necessitate data collection on multiple plant species that vary temporally and spatially within estuaries. Macroalgae would need to be quantified, requiring geospatially intensive surveys of biomass of these heterogeneously distributed, temporally variable, non-rooted, bottom dwelling plant species (Schein et al. 2012). This added dimension of primary production makes it costly and logistically difficult as an indicator.

In contrast to measures of primary production, DO may represent the best indicator of nutrient impact, as it is easily measured and reflects both the production of DO via photosynthesis, as well as DO consumption through respiration (Kemp and Boynton 1980). DO also represents an endpoint that can be continually monitored, capturing the full temporal variability in estuarine responses. Furthermore, given that hypoxia/anoxia negatively affects the majority of fauna, it is sensible to measure it directly to determine if those fauna might be affected (Miller et al. 2002, Landman et al. 2005, Vaquer-Sunyer and Duarte 2008, Riedel et al. 2014). Technological advances and the low cost of DO loggers make them a reliable tool that provide accurate data with minimal field maintenance. Coffin et al. (2018) investigated the usefulness of DO loggers in the latest iteration of DO monitoring in the sGSL (Bugden et al. 2014). Therein, Coffin et al. (2018) found independent responses for hypoxia and oxygen supersaturation, and were able to discriminate between seagrass and algae-dominated habitats through the use of integrated metrics. Furthermore, a hypoxia metric (i.e., proportion of time at <4 mg/L) showed high correlation with the two primary factors affecting nutrient uptake, nitrate-N loading and water residence whereby over 70% of the variability in hypoxic conditions was explained.

DESCRIPTION OF STUDY AREA

The study area within the southern Gulf of St. Lawrence (sGSL), Canada, characterized here as river estuaries flowing into the Northumberland Strait from New Brunswick (NB) and Nova Scotia (NS) and all of Prince Edward Island (PEI) (Figure 1), is an ecologically and economically significant area. Land-use among watersheds in this study area were quite variable, ranging from primarily forested watersheds throughout much of NB and NS, to watersheds with high levels of row-crop agriculture on PEI, where nutrient impacts are more prevalent. Estuaries included in this study were selected across a geographic and nutrient loading gradient to capture the full range of estuarine conditions within the region. So far, data for dissolved oxygen have been collected in over 32 sites among the three provinces, though only 27 sites had data ready for analysis for this report. However, of those 27 sites, there are 59 year-long dissolved oxygen time series, as certain sites were monitored over multiple years (2013-2020). Eelgrass data presented here have been evaluated in 16 of those estuaries but has been collected in 16 additional systems for which the data are also not yet available for analysis.

In general, estuaries and bays in this region are lagoons, or coastal embayments, with barrier islands (Glibert et al. 2010). Mean tidal amplitude and periodicity differ between sites (0.3 – 1.6 m), with most exhibiting micro- or mesotidal, mixed semi-diurnal, or diurnal tides (Pingree and Griffiths 1980, Godin 1987). Defining estuarine boundaries can be complex, as there are differing definitions of boundary conditions (Elliott and McLusky 2002). Herein, the estuaries are defined by salinity at the uppermost area (< 0.5 PSU), and by geographical boundaries at the outermost point, either where the estuary opens into a larger bay and/or where it enters the sGSL or Northumberland Strait directly, where fresh and salt water are fully mixed. Estuaries within this region range from 17 to 386 km2, and of shallow depth (mean depth 1 - 5 m). Because of their relatively shallow depth, small tidal amplitude, and low freshwater input, the transition zone between fresh and marine water is relatively short and characterized by well-mixed water (i.e., a general lack of vertical stratification). Furthermore, this transition zone is where land-derived nutrients are first available in the marine environment, and typically where nutrient impacts are most severe. Therefore, this is where the oxygen monitoring in this study occurred, in the upper estuary and defined by a salinity range between 15-25 PSU, with the intention of capturing the worst water quality conditions. Areas more impacted by nutrients are typically dominated by *Ulva* spp. whereas those that are not are dominated by eelgrass. Thus, eelgrass monitoring, in contrast, was within the full spatial gradient of the estuary. Altogether, the shallow depth and relatively low freshwater input to estuaries in the sGSL are similar to many other estuaries in the North Atlantic, though the effects of winter ice coverage does make them distinct.

PREVIOUS RESEARCH AND MONITORING PROGRAMS

Nitrogen loading to PEI rivers and estuaries has been increasing concomitant with the increase in land-use of row-crop agriculture, particularly between 1960 and 1999 when the peak of potato production occurred (PEI Department of Environment, Water, and Climate Change 2020). This peak in potato production has resulted in a plateau in surface water nitrate concentration (PEI Department of Environment Water, and Climate Change 2020). Agricultural land use in some watersheds exceeds 60%, which is predominantly potato rotations (Jiang et al. 2015, Grizard et al. 2020). Given that the varieties of potatoes planted on PEI have a high nitrogen demand, fertilization application rates, and subsequently leaching to groundwater are higher than for other crops (Jiang et al. 2015, Grizard et al. 2020). Excess nitrogen not taken up by the plant is water-soluble and tends to leach into groundwater through the sandy-loam substrate characteristic of PEI (Jiang et al. 2015). This process operates on a multi-year scale such that there is a delay between when nitrogen is applied to fields and when it enters streams and rivers (Liang et al. 2019). In PEI, stream baseflow is mostly groundwater in summer, with a relatively stable nitrogen/nitrate concentration (nitrate leaches through the soil into groundwater, which later reaches streams and rivers, resulting in a lag that can be multiple years), which simplifies nitrogen loading calculations, as it can safely be assumed that concentrations are not over- or under-estimated based on flow rate (Jiang et al. 2015).

The first iteration of relating DO monitoring to nitrogen loading in the sGSL (Bugden et al. 2014) assumed that the majority of nitrogen enters the estuary from land via freshwater, and that the risk of developing low oxygen conditions increases with water residence time. This model resulted in the derivation of delta-N for each estuary, a value that incorporated both the nutrient loading and tidal flushing. This was based on the premise that nitrogen entering the estuary would be flushed out by tidal exchange before incorporating into plants. Bugden et al. (2014) treated estuaries as homogenous (i.e., nutrient concentrations are equal throughout) and publicly reported anoxic events were initially used as the endpoint. Several shortcomings of this methodology were noted, including the use of a binary endpoint representing the worst-case scenario for nutrient impact (anoxia), and not accounting for system-specific bathymetry or spatial gradients of nutrient impact. Also, the presumption that incoming nutrients are flushed out by tides before uptake by plants has long been known to be incorrect. The tidal model employed did not account for freshwater input. In fact, nutrients are efficiently removed by plants in the first few hundred meters of an estuary, resulting in relatively low nutrient concentrations in most of the estuary (Valiela 1997). Nevertheless, this exercise had predictive value and further work was motivated by the consistent use of better DO endpoints, rather than by deficiencies in the predictive value of the model.

Improved DO monitoring for the development of a more refined DO model was subsequently developed by the Northumberland Strait Environmental Monitoring Partnership (NorSt-EMP) – a group consisting of Indigenous groups, stakeholders, academics, and provincial (PEI and NB) and federal governments. Their approach was to use optical DO loggers that were deployed in the upper portion (i.e., where nutrient impacts are typically most severe at approximately the upper 10% of the area of a given estuary) of 15 estuaries across NB, NS, and PEI. These data enabled greater discrimination between sites of varying levels of nutrient impact. Estimates of water residence time were also improved over the Bugden et al. (2014) model through the development of harmonic tidal models for all estuaries, and a tidal prism model that accounted for freshwater flow, tidal flow, and estuarine volume (see Methods section below). Finally, and most importantly, this work resulted in a quantitative model linking nitrate loading and water residence time with DO concentration, that was more mechanistically based than the previous model (importation of oxygen rather than export of nutrients).

Eelgrass was also a priority focus of NorSt-EMP. Prior to this research effort, there had been no consistent monitoring, nor attempts at models relating stressors with eelgrass endpoints. The general question was focussed on the nature of the eelgrass endpoints, how to best measure them, and determining if they could be related to nutrient loading or other estuarine variable at all (Hitchcock et al. 2017, van den Heuvel et al. 2019). Work initially focused on a suite of eelgrass health endpoints at four locations from the uppermost to outmost extent of eelgrass in four estuaries (Hitchcock et al. 2017; Wheatley River, PEI; Bideford, PEI; Kouchibouguac, NB; and Bouctouche, NB). Endpoints included canopy height, above and below ground biomass, leaf nitrogen, carbon, and sulfur content and stable isotopes thereof. All endpoints demonstrated strong spatial variance within the estuary that was most strongly associated with the salinity gradient (See Hitchcock et al. 2017 for a breakdown of variance parameters). As this provided little or no variance and therefore statistical power to resolve these endpoints between estuaries, the likelihood of developing predictive models relating stressors and endpoint was low. However, these endpoints can have utility if measured at the same points through time within an estuary. SeagrassNet (www.seagrassnet.org), a global program that utilizes such an approach has been implemented by the Southern Gulf of St. Lawrence Coalition on Sustainability in the sGSL region. However, the lack of potential to develop predictive models that managers can use, and the already existing SeagrassNet led to recommendations against including such monitoring as part of the Marine Environmental Quality (MEQ) program. In contrast, the use of measures of estuarine eelgrass coverage resulted in the development of predictive models using nitrogen loading as a stressor (van den Heuvel et al. 2019) as are described herein.

While other endpoints were examined by NorSt-EMP, oxygen and eelgrass were chosen for the MEQ program because they were technically, logistically, and financially feasible, ecologically important, and were most likely to produce stressor-response models that could be used by managers. This latter point is particularly important as MEQ was re-launched nationally in 2017 to establish thresholds for managing stressors affecting the quality of the marine environment. While DFO is the lead for this program, it would not be possible to execute research of this scope without collaboration from multiple partners. The provincial government of PEI spearheaded the earliest investigations of oxygen-nutrient relationships leading to the present program, has an outstanding nutrient monitoring program dating back decades, and has been applying the NorSt-EMP/MEQ methodology for several years and have been freely sharing the data they collect to inform the MEQ program. The province of NB is a more recent addition to the partnership and successfully monitored NB estuaries for the 2020 field season. Agriculture and Agrifood Canada is also a partner, and the MEQ program benefits from frequent collaboration with the Southern Gulf of St. Lawrence Coalition on Sustainability, and academic partners, most notably the University of Prince Edward Island and University of Waterloo. While the current iteration of this monitoring program uses many elements developed under NorSt-EMP, it is important to verify their results given that project was only tested at a limited number of sites and over a single field season (Coffin et al. 2018). The original NorSt-EMP project also suffered from deficiencies in the quantity of nutrient data from New Brunswick and Nova Scotia that has been subsequently improved to facilitate the MEQ program. Furthermore, methods that are developed for the purposes of academic advances are not always easily transferable to real-world monitoring scenarios. This document represents an opportunity to evaluate NorSt-EMP operations and adjust MEQ indicators to better reflect trophic status of estuaries. Data presented herein were collected from 2013-2020, which includes the original dataset contained in Coffin et al. (2018) and van den Heuvel et al. (2019).

METHODS

DISSOLVED OXYGEN

In this study, dissolved oxygen (DO) was measured in 31 estuaries in the southern Gulf of St. Lawrence with Onset Hobo® optical dissolved oxygen loggers intermittently from May-November in 2013, 2014, 2018 and 2019, and July-October in 2020 (Table 1). Analyses conducted in Coffin et al. (2018) were on the entire period when DO loggers were deployed, whereas analyses contained herein were on the time period of July to September, which is when most anoxic events occur. The previous study showed the full duration of open-water oxygen monitoring is not critical as there was a high degree of correlation between the full season dataset and the July to September monthly metrics (Coffin et al. 2018). Of the 31 study estuaries, four are monitored annually: Wheatley (PEI), West (PEI), Cocagne (NB), and Pugwash (NS). The original monitoring plan was to have 20 sites on PEI and 10 each on NB and NS on a three-year monitoring cycle, with 12-15 sites being monitored each year (Table 1). In each estuary, loggers were deployed in the upper estuary, hereby defined as upstream 10% of the total estuarine area where salinity ranged from 15-25 PSU, and recorded both DO (mg/L) and temperature (°C) at either 30 or 60 minute intervals. The loggers were fixed 0.5 m from the substrate in an area where average water depth was between 1.0-2.5 m, where benthic production can occur. To minimize the potential effects of biofouling, anti-fouling guards comprised of copper wire were affixed near the probe’s sensor. Data were downloaded and loggers were cleaned of fouling organisms bi-weekly, though this schedule was not consistently maintained due to limitations with personnel (2018) and the reality of the COVID-19 pandemic (2020).

Exploration of the DO time series allowed for the derivation of metrics that reflected oxygen concentrations symptomatic of eutrophication (high or low DO) based on the duration, variability, frequency, and/or timing of a given event (Table 1, see Coffin et al. (2018) for the full list). These metrics allowed for the documentation of estuarine oxygen regimes while avoiding confounding effects of serial autocorrelation (Daigle et al. 2011). The original analysis in Coffin et al. (2018) discriminated among estuaries along a nutrient loading gradient but was specifically tailored to nutrient impact. According to their Principle Component Analysis (PCA), the most important metrics for discriminating sites were all frequency measures; hypoxia (proportion of time <4 mg/L), oxygen supersaturation (proportion of time >10 mg/L), and coefficient of variation. While there was very high correlation between a number of metrics, we selected thresholds of 4 and 10 mg/L to represent deviations from normoxia in order to avoid zero-inflation by ensuring that data were available for all sites. Hypoxia and oxygen supersaturation were orthogonal to each other on the PCA, meaning they are not correlated with each other. Coffin et al. (2018) interpreted that lack of correlation to mean that some estuaries tended toward hypoxia while other tended toward oxygen Supersaturation. As a consequence, the sum of these two values created a metric for the proportion of time < 4 mg/L and proportion of time > 10 mg/L, which was termed “Eutrophic Time” to represent nutrient impact more holistically. As some sites had multiple years of data, the DO metrics of each site were averaged to limit a disproportionate influence of those sites on the model.

EELGRASS

The eelgrass dataset is comprised of 16 representative estuaries in the sGSL (Figure 1) that were completed in 2014 and previously published (van den Heuvel et al. 2019). An additional estuary, the Barbara Weit was evaluated by UPEI in 2018 and this was added to the dataset as the only estuary examined so far that had no eelgrass at all. Since MEQ was initiated, 16 estuaries have been surveyed using sonar by Coalition-SGSL. Unfortunately, the analysis of these data is not sufficiently advanced to include here. Methods are presented for both the UPEI and Coalition-SGSL collections to provide methodological comparisons.

At the time the original data from 16 estuaries was collected, it was unknown whether nutrient loading could be related to eelgrass coverage. In fact, given the plethora of potential influences on eelgrass, it seemed naïve to think that a single variable could predict coverage. Thus, coverage was compared to a group of environmental variables including water residence time, tidal amplitude, total and inorganic nutrient load (methodology described below), light attenuation, turbidity, temperature, salinity, and chlorophyll. As the only one of those variables that was significantly related to eelgrass coverage was nutrient load (van den Heuvel et al. 2019), these variables are not considered further in this document as further refinement of the originally published model is the objective.

The 16 estuaries surveyed in 2014, with the addition of Barbara Weit estuary in 2018, were evaluated with a Knudsen model 1612 portable echosounder equipped with a Knudsen KEL-291 210 kHz transducer with a 3° beam width. The transducer was mounted to the front of a 5.2 m Carolina Skiff. While the Knudsen unit produced excellent quality data, subsequent work was completed by the Southern Gulf of St. Lawrence Coalition on Sustainability in partnership with DFO Gulf region using BioSonics MX Aquatic Habitat Echosounder single-beam 204.8 kHz transducer (8.7°width) mounted to a 3 m aluminum boat. The primary reason to change to BioSonics was both the cost, and the integration of components, including software, with the specific purpose of monitoring submerged aquatic vegetation.

Positioning for the 2014 and 2018 surveys was achieved with a Trimble® GeoXT differential GPS to generate real-time high resolution (0.5 m) geographic coordinates using the Can-Net Virtual Reference Station Network for dGPS corrections. The BioSonics unit has a built-in GPS that has the capability of dGPS to an accuracy of <3 m. Knudsen software was used to acquire sonar data for the 2014 and 2018 surveys used. While in subsequent surveys, the echogram was visually monitored in real time using BioSonics Visual Acquisition software. While generally eelgrass is apparent from the surface, occasionally a drop camera was used for confirmation. During transects notes were taken to identify submerged aquatic vegetation for confirmation with the echogram.

NITRATE-N LOADING

Coffin et al. (2018) demonstrated that inorganic nitrate-N loading was a superior predictor for dissolved oxygen compared to total nitrogen loading, presumably because nitrate-N is an immediately bioavailable form of nitrogen, while total nitrogen, which is likely dominated by organic forms of nitrogen. While nitrate concentration values are abundant on PEI, nutrient water analysis is sparse for NB and NS streams.

The original oxygen-nitrogen models were based on only two samples collected by Grizard et al. (2020) in NB and NS. Furthermore, summer nitrate concentrations in some NB and NS streams are very low and detection limit issues in the previously collected dataset may have resulted in elevated nitrate levels at some of these locations. The previously determined nitrate-N concentrations were not used in the present analysis for this reason. To expand on available data for NB and NS, water was sampled monthly at 42 locations in those provinces in 2018. Sites were chosen at the closest upstream road access to the head of tide in order to avoid any tidal influence. This encompassed all watersheds between Tabusintac in the west, to the Margaree on Cape Breton Island in the east. Water was analyzed for nitrate, phosphate, total nitrogen, total phosphorus using anion chromatography as per methods previously described (Schein et al. 2012). Analysis of annual trends in nitrate-N concentration revealed strong seasonal patterns between summer and winter, with winter nitrate-N levels often being 10-fold greater than those measured in the warmer months. Since eutrophication responses are more strongly manifest in summer, only the May to October mean nitrate-N concentrations were used (n=6 for all estuaries).

Surface water nitrate-N concentrations for PEI nitrate loading were obtained from the PEI provincial nutrient monitoring database. Surface water concentrations were available for every year from 2013 to 2020. Two exceptions were noted, Enmore and Bideford, that are not in the regular collection conducted by the PEI provincial government, and therefore, pre-2013 data were used instead. While PEI nitrate-N does not show the same strong seasonal cycles as for NB and NS, nitrate concentrations for samples collected between May 1 and October 31 were also used. Nitrate-N measurements between 2013 and present (the period when all oxygen data were collected) were averaged for this study.

Given that a significant proportion of systems have multiple tributary streams with different N concentrations, each watershed was delineated to account for the proportional input of nitrate-N from each tributary. The tributaries chosen were only those that entered the estuary upstream of, or in the vicinity of the 10% estuary sampling site. Flow rate for each tributary/estuary was based on the modelled relationship between area and flow rate described in Coffin et al. (2018), whereby Environment and Climate Change Canada provided daily discharge data within 20 regional gauging stations. More specifically, a linear relationship between the log-transformed average daily flow rate (measured in m^3/s), and log-transformed watershed area above the gauging stream were modeled for each year that data were available (2013-2018; Figure 1). The average R^2 value across years was 0.97, indicating that the vast majority of variance in flow, averaged over the May-October period, can be described by watershed area for a given year. Since flow rates for the two most recent years were not available (2019 and 2020), flow rates were averaged among all years from 2013-2018 for each of the 27 watersheds. Further, because gauging stations were sometimes far from the estuary, flow rate had to be prorated to the full extent of watershed area contributing to the upper estuary (where the DO logger was located). Where possible, flow was determined for each tributary entering the estuary. There were some exceptions where the watershed sub-areas have not yet been delineated and individual tributary flow could not be calculated; in these cases, the total watershed flow estimate was used instead.

Nitrate-N loading was computed by multiplying the nitrate-N concentration by modelled flow, for each tributary contributing to the overall freshwater discharge, and then prorated to the DO logger location. By calculating flow and nitrate-N loading using this method, the assumption is that flow rate isn't especially variable and therefore nitrate-N loading is relatively stable (see Jiang et al. 2015). Further, it is assumed that land-use and therefore nitrate-N concentration is proportional downstream of wherever it was collected for a given watershed. Ultimately, there is confidence in this approach to estimating nutrient loading as it has been validated (Jiang et al. 2015) and is robust to potential over- or underestimations of nutrient loads. In cases where sub-watershed had not been delineated, the total watershed area and the average nitrate-N concentration was used to calculate the loading. Nitrate-N loading was adjusted to the total area of the estuary and expressed as if that loading occurred throughout the year (kg N ha^{-1} estuary area yr^{-1})

WATER RESIDENCE TIME

Water residence time was estimated using a whole-estuary tidal prism model. This model computed water residence time as:

$$WRT = \frac{V_{estuary}}{V_{freshwater} + V_{tidal}}$$

where WRT is water residence time, $V_{estuary}$ is the total estuarine volume at mean tide, $V_{freshwater}$ is the freshwater volume entering the estuary over one tidal cycle and V_{tidal} is the tidal volume. To obtain the total volume of each estuary ($V_{estuary}$), an onboard sonar with a 210 kHz transducer (Knudsen for data collected from 2013-2016 and Biosonics thereafter) was used to record bathymetry along parallel transects spaced with a target of ≈50-100 m apart. For areas not explicitly mapped using the depth sounder, spline interpolation was applied to interpolate the estuarine bathymetry using ArcGIS 10.3 (van den Heuvel et al. 2019). ArcGIS 10.3 was used to calculate $V_{estuary}$ from the obtained bathymetric raster. Freshwater volumes (i.e., $V_{freshwater}$) were modeled using the prorated linear model method as described above for nitrate-N loading. For tidal volumes (i.e., V_{tidal}), Onset Hobo Water Level Titanium® pressure loggers were deployed for ≥30 days in each estuary to collect pressure data at 10 min intervals. To measure barometric pressure (in air), a logger was placed in the general vicinity of estuaries where data were being collected or was taken from the nearest airport gauging station. Once collected, depth measurements were computed from the pressure data and harmonic tidal models were built based off the t_tides program in either Matlab or Python (Pawlowicz et al. 2002). Tidal simulations for the entire period of DO logger deployment were created from the models and tidal amplitude calculated for each day (difference of maximum and minimum water lever on each day). Using these daily tidal amplitudes, the mean for the May to October period was computed and multiplied by the estuary surface area to get the tidal volume.

DATA ANALYSES

Dissolved oxygen

While DO data were collected from 32 estuaries in the southern Gulf of St. Lawrence, Cardigan River, Foxley River, Tabusintac River, and Vernon River estuaries were removed from the multiple regression analysis due to a lack of available bathymetric data for the most recent year to calculate water residence time.

Dissolved oxygen data from July-September were used to calculate the aforementioned DO metrics (Eutrophic Time [<4 + >10 mg/L]; hypoxia [<4 mg/L]; oxygen supersaturation [>10 mg/L]; and coefficient of variation from each of the 27 study estuaries (Table 1; averaged by years with multiple years of data). Plymouth Routine in Multivariate Ecological Research (PRIMER package v7; Clarke and Gorley 2006) was used to analyze the oxygen data using PCA on standardized data (mean of zero and standard deviation of one).

Multiple regression was used to relate each of the above dissolved oxygen metrics with nitrate-N loading and water residence time. Note that the poor fit of α-Chlorophyll with nitrate-N loading and water residence time from Coffin et al. (2018) informed our decision to exclude it from any analysis herein. For significant models, the standardized Beta coefficients, which are measures of the standard deviation change in the predictor, were compared to determine the relative contribution of each independent variable to the overall model, as in Coffin et al. (2018).

Eelgrass

The sonar files were processed using either Sonar5-Pro (third party software for the Knudsen data) or using BioSonics Visual Habitat/Visual Aquatic software. Automatic bottom and vegetation depth methods were initially used; however, manual methods were used to improve the accuracy of benthic surface and vegetation delineation. A file of eelgrass height and bottom elevation for each distinct sonar ping was imported to a spreadsheet for further analysis. Due to the very high resolution of data, duplicate latitudinal points were eliminated.

Data were imported into ArcGIS 10.5-10.7 for further analysis. For interpolation analysis, regular kriging, co-kriging, and inverse distance weighted interpolation were initially evaluated but were unsuitable because eelgrass data were non-normal and zero inflated. Instead, indicator kriging was employed (see van den Heuvel et al. 2019 for details).

Suitable eelgrass habitat area was defined as regions with depths of less than 3 m, depths greater than the mean low tide, and salinities greater than 10 PSU that did not contain aquaculture. The estuary polygons originally created to define the extent of the estuary for oxygen analysis were modified by truncating the upper end of the estuary corresponding to 10 PSU mean salinity (interpolated from observations). The suitable habitat was further refined by subtracting the area above mean low tide, below 3 m depth, and aquaculture area. The percentage of habitat occupied by eelgrass was obtained by dividing the area of the interpolated eelgrass polygons by the total area of suitable habitat, referred to as percent coverage henceforth.

RESULTS

DISSOLVED OXYGEN

PCA analysis on the most pertinent DO metrics identified in Coffin et al. (2018) for the 66 DO time series collected for the MEQ program resulted in similar ordinations (Figure 2). The first two components of the ordination explained 66.1 and 31% of the total variation for PC1 and PC2, respectively, and accounted for 97% of the overall variation between sites. Similar to Coffin et al. (2018), hypoxia and coefficient of variation were orthogonal to supersaturation. Eutrophic Time is positively correlated with *Ulva* spp. dominated sites, whereas eelgrass dominated sites were negatively correlated (Figure 2). Presenting sites in ascending order according to Eutrophic Time revealed that there was an inflection point around 0.35 where plant habitat changed from eelgrass to *Ulva* spp. (Figure 3).

In this analysis, Eutrophic Time was best predicted by nitrate-N loading and water residence time (Table 2). This relationship had an overall R^2 of 0.81, an improvement from 0.55 in Coffin et al. (2018), and similar contributions from each of the predictors as indicated by beta coefficients: nitrate-N = 0.54 and water residence time = 0.69. This finding is in contrast to Coffin et al. (2018), where the metrics for hypoxia and coefficient of variation had the strongest relationship with nitrate-N loading and water residence time. However, caution should be applied to this conclusion as hypoxia deviates from a planar relationship. Fortunately, Eutrophic Time has a linear relationship with our predictors which facilitates interpretation (Figure 4).

The multiple regression model created for Eutrophic Time was applied to provide estimates of nutrient reduction to reach the approximate point where habitat shifts from eelgrass-dominated to *Ulva* spp. dominated (i.e., Eutrophic Time is <0.35). This exercise, intended to be of use for watershed managers, revealed that some estuaries require substantial reduction in nutrient loading (Table 1).

EELGRASS

The eelgrass and nitrate-N loading relationship improved marginally compared to van den Heuvel (2019) from explaining 50.4% of the variation to explaining 53.0% of the variation (Figure 5). The relationship showed that ≈66% of available habitat occupied is the maximum for estuaries within the region. Interpolations using that relationship show eelgrass declines of 10%, 25%, 50%, 75%, and 90% correspond to estuarine external nitrate-N loads of 3, 12, 57, 274, and 1317 kg/ha/yr, respectively. This range of values was broader than the previous analysis due to the addition of the Barbara Weit estuary that contained no eelgrass (nitrate-N loading of 1188 kg/h/yr). Overall, nitrate-N loading inputs were improved from the original study (van den Heuvel et al. 2019) except for two sites, Enmore and Bideford, which used the original dataset. Souris River remains an outlier for unknown reasons, though there has been speculation that Green crab may have contributed to the lower than predicted coverage.

DISCUSSION

DISSOLVED OXYGEN AS AN INDICATOR OF TROPHIC STATUS

The primary purpose of this study was to quantify the relationship between dissolved oxygen (DO) alongside eelgrass coverage within estuaries in the sGSL, and nitrate-N loading and water residence time to inform MEQ measures. The results herein suggest that DO can serve as a reliable indicator for assessing where along the eutrophic continuum an estuary might be at a given point in time. A correlational relationship between a DO metric that reflects symptoms of eutrophication (i.e., Eutrophic Time) and the two primary factors that determine the severity of eutrophication (water residence time and nitrate-N Loading) was established. This relationship was explored to obtain a target nitrate-N loading for each estuary with a Eutrophic Time that is over the empirically observed threshold where eelgrass habitat transitions to algae habitat (i.e., Eutrophic Time > 0.35). Though this relationship is not mechanistic, it does provide a “ballpark” estimate for nitrate-N loading reduction that should theoretically lead to a shift back to water quality conditions that favour eelgrass habitat, thereby improving ecosystem quality.

Our results indicate that the model employed in this study is applicable across the southern Gulf of St. Lawrence region. Model results from Coffin et al. (2018) indicated an R^2 of 0.55 when Eutrophic Time was modelled against water residence time and nitrate-N Loading, whereas the analysis herein with additional years and sites increased the R^2 to 0.81. While the sites in our study are restricted to the sGSL, the tenets of this model should apply to shallow estuaries with watershed areas within the same range in the North Atlantic where nutrients are primarily land-derived. While Eutrophic Time was the best performing variable using linear multivariate relationships, this is largely because it follows a linear trend more so than the other variables. Non-linear models can be considered instead, but they greatly complicate the estimation of nutrient targets and are not explored herein.

Conventional proxies of trophic status (e.g., α-Chlorophyll, nutrient loading) do not reflect the severity of eutrophication in estuaries of the southern Gulf of St. Lawrence. Indeed, Filgueira et al. (2015) revealed that α-Chlorophyll values observed in Malpeque Bay were far lower than expected based on nitrogen loading. Further study revealed that a substantial amount of these nutrients ended up as benthic production of *Ulva* spp. (Lavaud et al. 2020). In the same vein, the extirpation of eelgrass in the upper portions of estuaries in this study reflects both the gradient of nutrient and the dominant role of benthic production in the upper estuary, as nutrient impacts diminish with distance from it (Hitchcock et al. 2017, van den Heuvel et al. 2019). Given that *a priori* knowledge is required to know if eelgrass is currently within its historical range (i.e., its range prior to local nutrient impacts), it is difficult to assess change, recovery, or further perturbation, thus diminishing the usefulness of eelgrass distribution as a stand-alone indicator of estuarine trophic status. van den Heuvel et al. (2019) attempted to resolve this issue by estimating "potential" eelgrass habitat (which approximates historical distribution). Less spatially intensive data on eelgrass distribution from 1967 was available for one estuary (Hughes and Thomas 1971), the Dunk River, and this was evaluated against 2014 sonar surveys (van den Heuvel et al. 2019). Eelgrass in the Dunk River in 1967 extended approximately 2 km further upstream than it presently does, which likely represents a 50% decline in eelgrass area concomitant with nutrient loading increase from 1967 to 2014 (van den Heuvel et al. 2019). DO and its derived metrics, however, are a much simpler indicator than eelgrass that are directly related to ecosystem metabolism and organismal health (Kemp and Boynton 1980, Vaquer-Sunyer and Duarte 2008, Riedel et al. 2014). Furthermore, interpretation of eelgrass metrics is only applicable at the annual time-step and can be confounded by multiple stressors over many years (e.g., Souris River where green crabs may have affected eelgrass distribution).

When modeled with water residence time and nutrient loading, DO can indicate trophic status and determine what is potentially required for a trophic shift (i.e., degree of nutrient reduction needed to reach a predicted target). As eelgrass decline is still of considerable concern, there is value in comparing the quantitative estimates of Eutrophic Time and hypoxia with eelgrass coverage to put these values into context. As DO varies significantly with estuary residence time, and eelgrass coverage does not, this complicates the comparison. However, this comparison can be performed for a hypothetical 'average' estuary by using the mean of the residence time for all estuaries evaluated to date. We thus calculated the mean residence time for estuaries studied herein, revealing a mean residence time of 1.59 days. We then plotted Eutrophic Time, hypoxia, and eelgrass coverage with this mean water residence time against an ascending log-scale for nitrate-N loading. This exercise revealed that as nitrate-N load increased, eelgrass coverage decreased; likewise, there was a concomitant increase in symptoms of eutrophication with increased nitrate-N loading (i.e., Eutrophic Time and hypoxia, Figure 6).

In our model, Eutrophic Time was defined as the proportion of time a given estuary spent at DO concentrations <4 mg/L and >10 mg/L. Given that Eutrophic Time was strongly linked with both water residence time and nitrate-N loading, it is suggested that MEQ guidelines adopt the approach of characterizing trophic status using the frequency of these symptoms of eutrophication. Furthermore, Eutrophic Time can be indicative of ecosystem quality, as 27 of 29 DO time series with a Eutrophic Time <0.35 were characterized by eelgrass (except for Tryon River in 2013 and Covehead in 2019, Figure 2). Likewise, 34 of 35 DO time series with a Eutrophic Time >0.35 were characterized by *Ulva* spp. (except for Bideford River 2014, Figure 2). The model functions at the watershed and estuary-scale, thus local effects are unaccounted for, and can lead to deviations from our predictions, which is perhaps what occurred for the mischaracterizations apparent in Figure 2. This ultimately provides MEQ with a simple method of defining estuarine trophic status and an approximate target to manage toward.

The degree of nutrient reduction required for a return to a more desirable trophic state (i.e., ≤0.35 eutrophic time) for eutrophic estuaries was also determined. Interestingly, coefficients from the model revealed that the relative contribution of water residence time and nitrate-N loading to Eutrophic Time was 56:44% in favor of water residence time (derived from Beta coefficients). Given that water residence time cannot be altered without drastic management action, only nitrate-N loading can be adjusted through remediation efforts. This is reflected in our nitrate reduction estimates, as estuaries with longer water residence times generally require the greatest reduction in nitrate-N loading (Table 1). While the target Eutrophic Time of 0.35 may be reasonable to attain through reducing nitrate-N loading for some systems, it is not possible to estimate the length of time it would take for recovery to occur using our methods though there are examples of seagrass recovery following nutrient reduction (e.g., Tomasko et al., 2018). For this reason, it is recommended that estuaries of interest be monitored on a cycle. Currently MEQ estuaries are monitored on a 3-year rotation.

KNOWLEDGE GAPS AND CONCLUSIONS

An increase to the number of study sites and years resulted in an improvement in the relationship between the predictor variables (nitrate-N loading and water residence time) and our dissolved oxygen metric (Eutrophic Time). With this new data acquisition, 81% of the overall variation between estuaries was explained by this model. Furthermore, the relatively strong relationship between nitrate-N loading and eelgrass distribution, the biological indicator of interest, is also encouraging. Despite this success, the researchers associated with this project recognize the limitations of the work and are currently attempting to refine the predictor variables (i.e., nitrate-N loading and water residence time).

This model is driven using only external nutrient inputs and water residence time. However, it is quite likely that internal nutrient availability also contributes to the DO values observed in this study and is perhaps even dominant. Indeed, it is suspected that external nutrient loading may be acting as an imperfect proxy for internal nutrient production, which would explain the weaker relationship with external nutrient loading than residence time. Eventually, nutrient loading in our region may be divided into internal and external components, as has been calculated for other systems (Valdemarsen et al. 2015). Once these parameters are better understood, predictions on how systems will respond to reductions in external nutrient loading may improve and provide more detailed advice to MEQ in the future. However, actively removing internal nutrients may accelerate remediation of nutrient impacts beyond limiting their entry to the system.

In this study, a tidal prism model was used to calculate water residence time. While the output of that equation yielded results that functioned well in our model, more accurate methodology would be of benefit. To this end, the authors of this report are helping to develop a regional hydrodynamic model that would provide more accurate estimates for water renewal time within the upper portion of sGSL estuaries. Such a model could be exploited to better track nutrients throughout a given estuary and identify zones outside of the upper estuary that may be at risk of nutrient impacts.

While the models used in this study provide a solid tool for identifying trophic status for sGSL estuaries, there is room to improve the relationship between predictor variables and dissolved oxygen/eelgrass distribution. However, whether the continued refinement of the model is necessary to inform decision-making is unclear, as the current model clearly identifies estuaries requiring nitrogen reduction from those that do not. Ultimately, such a decision regarding the usefulness of model refinement will be dictated by whether or not more detailed information is needed to advise further iterations of the MEQ measure. Dissolved oxygen and eelgrass monitoring are complementary tools that provide a comprehensive assessment of the trophic status of estuaries in the southern Gulf of St. Lawrence. While eelgrass is an Ecologically Significant Species and its distribution is impacted by nutrients, high-frequency DO monitoring provides detailed information about the worst symptoms of eutrophication and appears to be equally sensitive to nutrients.

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TABLES

*Table 1. Summary table of estuaries included in this study. Dominant vegetation is indicated by the letters Z or U to represent eelgrass (*Zostera marina*) or* Ulva *spp. habitat, respectively. Temperature data were collected from the dissolved oxygen probe deployed in the upper estuary, and presented as "average (min-max)", from July-September for the year in bold. Salinity data were either point measures (YSI V2 6600) and in square brackets or from a Star Oddi salinity logger and are presented as "average (min-max)", from July-September for the year in bold. See methods for calculations for Eutrophic Time, Water Residence Time and nitrate-N (kg/ha/yr; calculated for May-October). Nitrate-N target is the theoretical reduction in nitrate-N loading to shift from Ulva spp to eelgrass habitat, % nitrate-N change is this reduction in percent. Note that Covehead and Hunter River (highlighted in gray) both have Eutrophic Times above the 0.35 threshold but the predicted Eutrophic Time is underestimated by the model and therefore no nitrate-N target is calculated.*

Site	Years Sampled	Watershed area (km^2)	Dominant Vegetation	Temperature (°C)	Salinity (PSU)	Eutrophic Time	Water Residence	Nitrate-N kg/ha/yr	Nitrate-N target	% nitrate-N Change
New Brunswick										
Bouctouche	2013, 2018, **2020**	376.7	Z	21 (12-28)	15 (0-18)	0.05	1.17	4.61	-	-
Cocagne	2018, **2019**, 2020	248.8	Z	21 (11-30)	19 (4-24)	0.21	1.10	1.48	-	-
Kouchibouguac	2013, **2019**	385.7	Z	18 (12-25)	17 (0-23)	0.15	1.41	8.88	-	-
Kouchibouguacis	**2019**	330.5	Z	20 (11-28)	14 (0-22)	0.02	1.14	0.80	-	-
Tabusintac*	**2020**	-	Z	21 (9-31)	15 (0-20)	0.06	-	-	-	-
Nova Scotia										
Pictou East	**2019**	186.6	Z	20 (14-26)	18 (14-24)	0.07	1.50	0.29	-	-
Pictou West	**2019**	170.8	Z	20 (14-25)	21 (18-25)	0.26	2.50	0.19	-	-
Pugwash	2018, **2019**	112	Z	21 (13-28)	21 (0-27)	0.05	0.61	0.29	-	-
River John	**2018**	274.3	Z	22 (13-30)	[20 (7-25)]	0.10	0.94	0.61	-	-

Site	Years Sampled	Watershed area (km^2)	Dominant Vegetation	Temperature (°C)	Salinity (PSU)	Eutrophic Time	Water Residence	Nitrate-N kg/ha/yr	Nitrate-N target	% nitrate-N Change
Tatamagouche	**2013**, 2018	225.6	Z	21 (12-27)	[19 (3-26)]	0.08	0.35	21.67	-	-
Prince Edward Island										
Bideford	**2013**, 2014	19.3	Z	21 (15-27)	[26 (21-27)]	0.49	2.13	10.01	2.84	72
Cardigan*	**2020**	-	Z	19 (10-24)	[28 (27-28)]	0.12	-	-	-	-
Covehead	**2018**, 2019	33.3	U	21 (13-26)	[22 (21-22)]	**0.37**	2.12	3.94	-	-
Dunk	**2013**, 2014, 2019, 2020	161.1	U	21 (12-30)	[27 (26-29)]	0.46	0.77	296.87	199.75	33
Enmore	**2013**, 2014	36.6	Z	22 (12-31)	[17 (5-25)]	0.17	0.56	6.65	-	-
Foxley*	**2020**	-	U	21 (13-27)	[23 (21-25)]	0.50	-	-	-	-
Grand River	**2018**	79.6	U	21 (13-26)	[25 (24-27)]	0.47	2.11	4.71	3.02	36
Hunter River	2019, **2020**	62.4	U	20 (12-27)	[19 (17-23)]	**0.56**	1.76	8.37	-	-
Kildare	**2013**, 2014, 2015, 2018	17.4	U	22 (16-28)	[22 (13-26)]	0.85	3.48	70.33	0.04	99
Mill	**2013**, 2014, 2015, 2019	88.3	U	21 (14-27)	[23 (15-25)]	0.61	2.72	96.73	0.45	99
Montague	**2013**, 2018, 2020	163.8	U	18 (12-25)	[23 (17-28)]	0.53	2.16	76.91	2.59	99

Site	Years Sampled	Watershed area (km^2)	Dominant Vegetation	Temperature (°C)	Salinity (PSU)	Eutrophic Time	Water Residence	Nitrate-N kg/ha/yr	Nitrate-N target	% nitrate-N Change
Murray	**2019**	57.3	U	20 (12-25)	[24 (6-27)]	0.39	2.31	9.51	1.62	83
North River	**2019**	65	Z	20 (11-27)	[24 (23-26)]	0.18	0.65	13.66	-	-
Souris	**2013**, 2019	31.6	U	20 (16-25)	[25 (17-28)]	0.56	1.85	42.96	6.82	84
Southwest	**2018**	19	U	22 (14-27)	[27 (25-28)]	0.43	2.05	9.66	3.65	62
Stanley	**2013**, 2020	39.2	U	20 (10-28)	[24 (15-28)]	0.67	3.80	67.88	0.02	99
Tryon	**2013**	41.9	U	21 (13-27)	[25 (20-28)]	0.20	0.24	226.14	-	-
Vernon*	**2020**	-	Z	22 (11-28)	[26 (23-28)]	0.07	-	-	-	-
West	**2013**, 2018, 2019, 2020	113.6	Z	19 (11-25)	[19 (7-22)]	0.08	0.82	27.40	-	-
Wheatley	2013, 2014, 2018, **2019**, 2020	42.1	U	21 (11-25)	21 (13-27)	0.52	1.74	154.11	9.61	94
Wilmot	**2013**, 2019	71.6	U	21 (12-31)	[21 (14-25)]	0.50	0.73	451.50	226.37	50
Winter*	**2020**	-	U	21 (14-27)	[24 (7-27)]	0.35	-	-	-	-

* Dissolved oxygen data are available but not bathymetric or flow data

Table 2. Results of multiple linear regressions for dissolved oxygen against nitrate-N loading and water residence time. Beta coefficients are a standardized measure of effect size that indicates the standard deviation change of the dependent variable in response to a 1 standard deviation change in the predictor, partial r^2 indicates the proportion of overall variability explained by the individual model parameter. Significant regressions are bolded.

Response Variable	**Adjusted r^2**	**Overall p**	**N loading (Beta, partial r^2)**	**Water residence (Beta, partial r^2)**	**Nitrate-N loading p**	**Water residence p**
Nitrate-N Load						
Coefficient of Variation	**0.61**	**<0.0001**	0.38, 0.14	0.68, 0.46	**0.005**	**<0.0001**
Hypoxia	**0.63**	**<0.0001**	0.33, 0.10	0.71, 0.45	**0.01**	**<0.0001**
Eutrophic time	**0.81**	**<0.0001**	0.54, 0.30	0.69, 0.50	**<0.0001**	**<0.0001**
Supersaturation	**0.19**	**0.03**	0.43, 0.06	0.21, 0.18	**0.02**	**0.24**

FIGURES

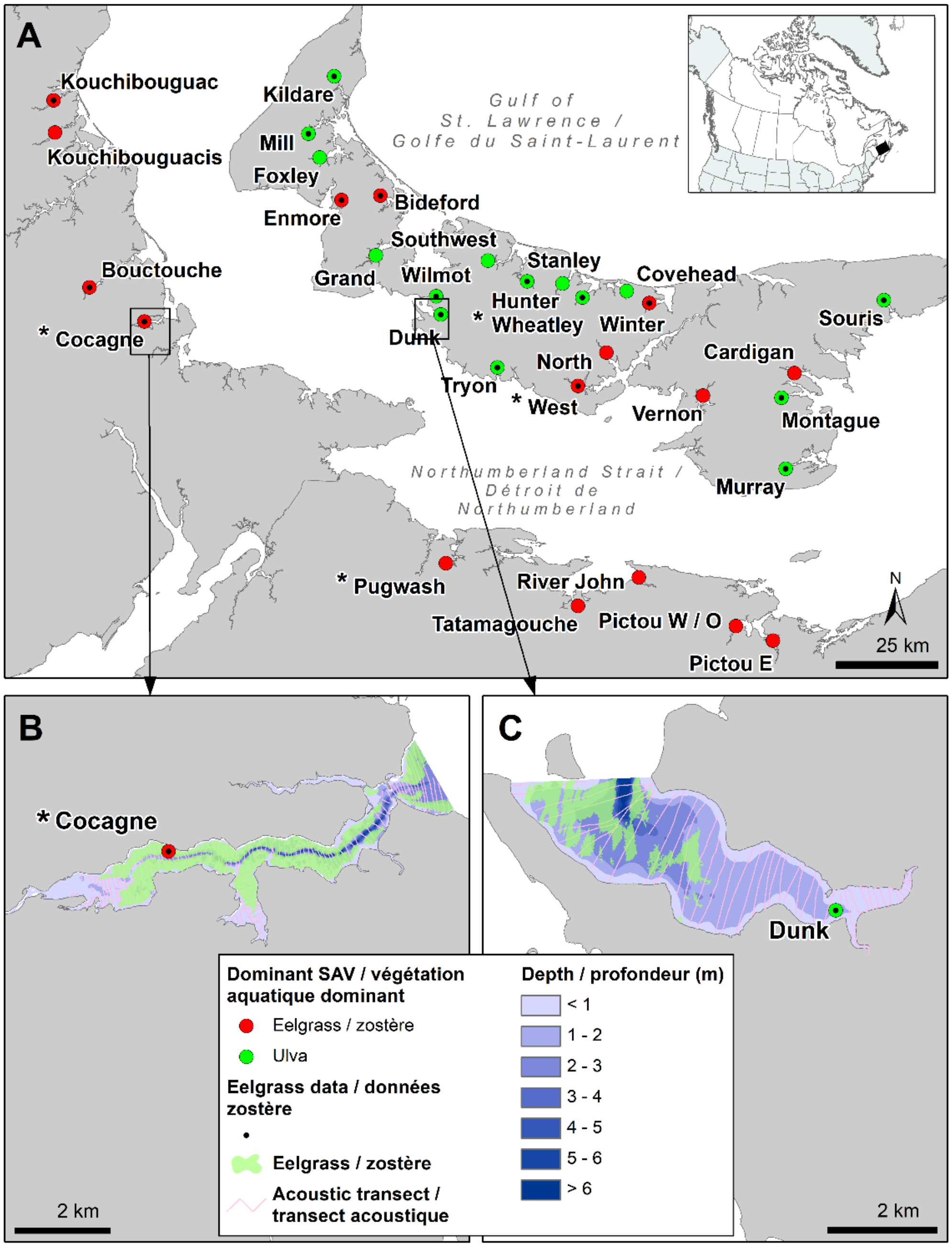

Figure 1. Study area in the context of eastern North America. A) estuaries that were studied in the southern Gulf of Saint Lawrence; Ulva *spp. dominated estuaries are denoted by green circles, and Z. marina dominated estuaries by red circles (a convention that is maintained throughout the document). Estuaries where eelgrass data were collected denoted by a black dot. Estuaries with an asterisk represent MEQ monitoring sites where data are collected annually, B) and C) insets demonstrate the approximate location of the dissolved oxygen probes, and show acoustic transects and modelled eelgrass distributions at two representative sites.*

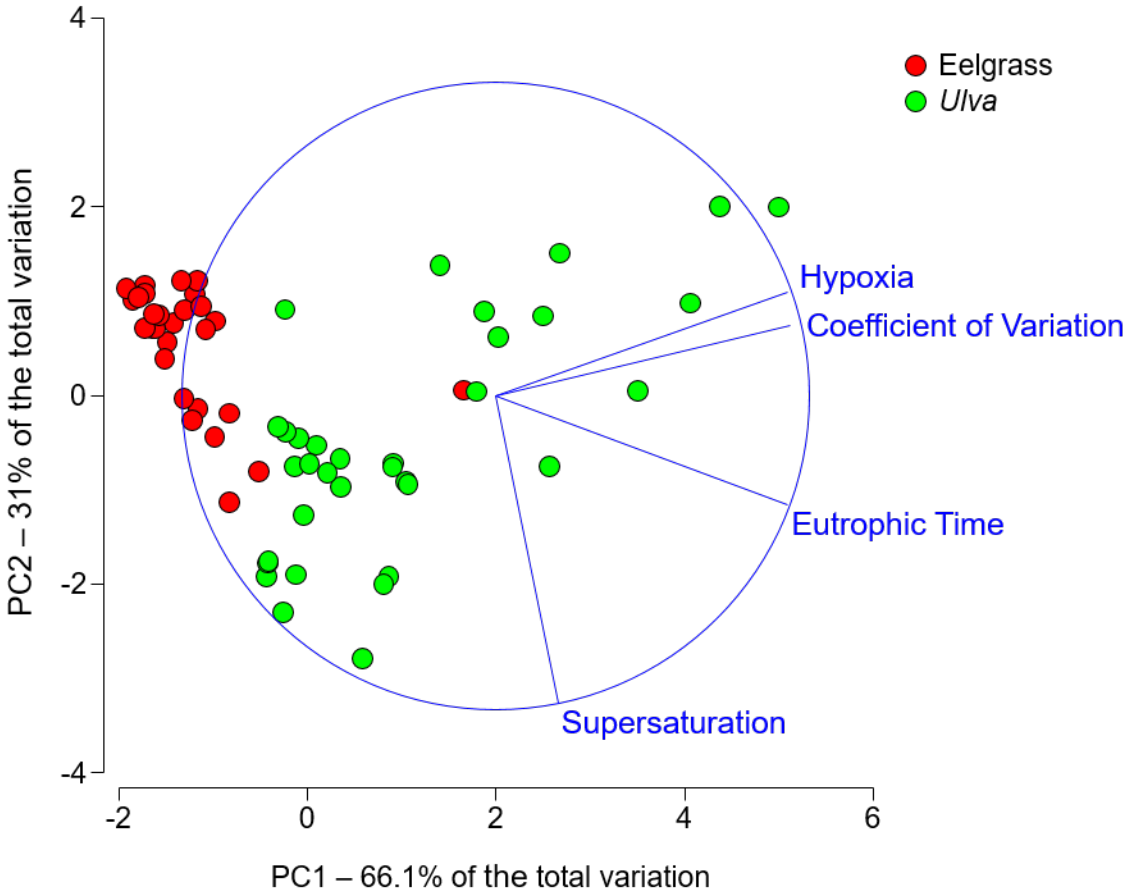

Figure 2. Principal Component Analysis using the strongest dissolved oxygen correlates identified in Coffin et al., 2018. Overall, 97% of the total variation is explained by the first two axes, 66.1% and 31% respectively. Sites are indicated by dominant vegetation with Z. marina *dominated estuaries indicated by red circles and* Ulva *spp. dominated estuaries by green circles.*

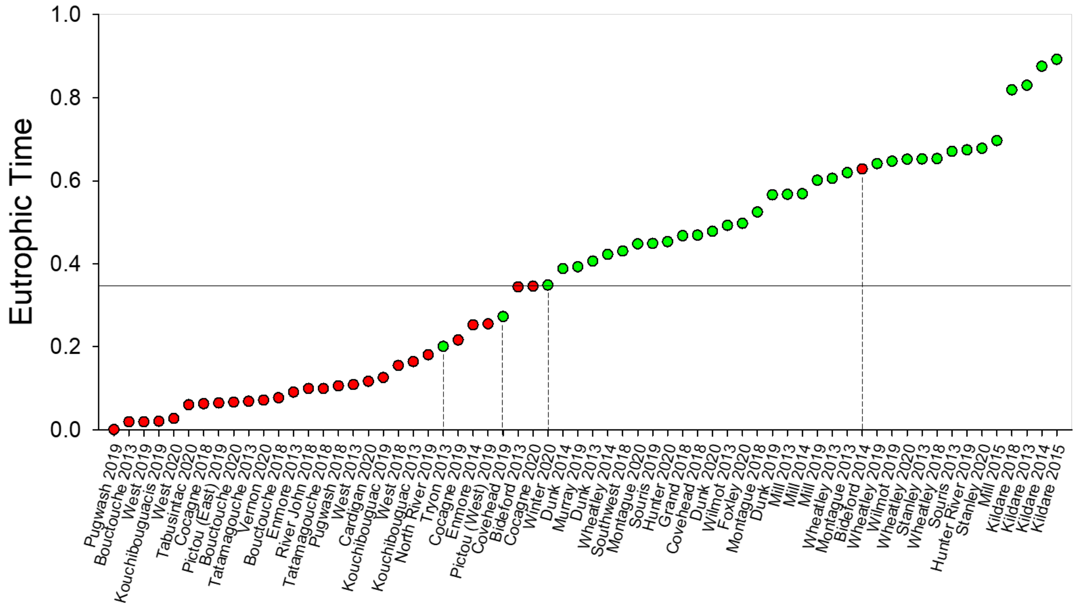

Figure 3. Eutrophic time at each site for each year sampled. Sites are presented in ascending order for Eutrophic Time and are coloured according to dominant vegetation with Z. marina dominated estuaries indicated by red and Ulva *spp. dominated estuaries by green. The domination vegetation shift (horizontal line) threshold is at approximately 0.35. Of note, the outlier systems Bideford 2014, an eelgrass (*Z. marina*) site grouped with* Ulva *spp. sites and Tryon 2013 and Covehead 2019, Ulva spp. sites grouped with eelgrass sites.*

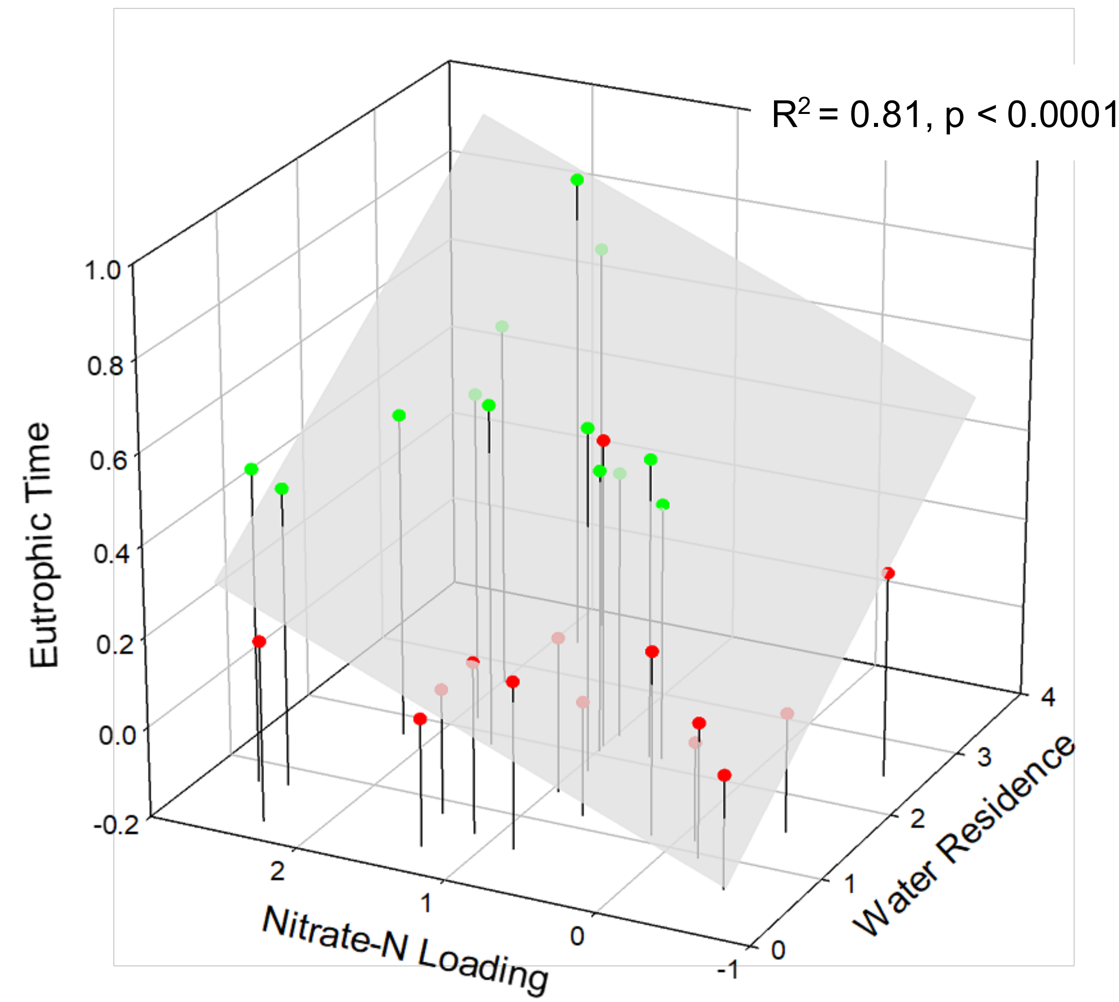

*Figure 4. Representation of the planar regression for the effect of water residence time and nitrate-N Loading (log-transformed) on Eutrophic time (proportion of time under 4 mg/L and over 10 mg/L). The plane is a visual representation of the best-fit regression, with the proportion of variation explained (R^2), and the significance level (p) noted. Estuaries dominated by Ulva spp. are denoted by green circles, and Z. marina by red circles. Note that the best-fit plane is transparent and that points above the plane are underestimated by the model and those below are overestimated (equation of the line: y = 0.1714*water residence time + 0.1262*nitrate-N Loading – 0.0723).*

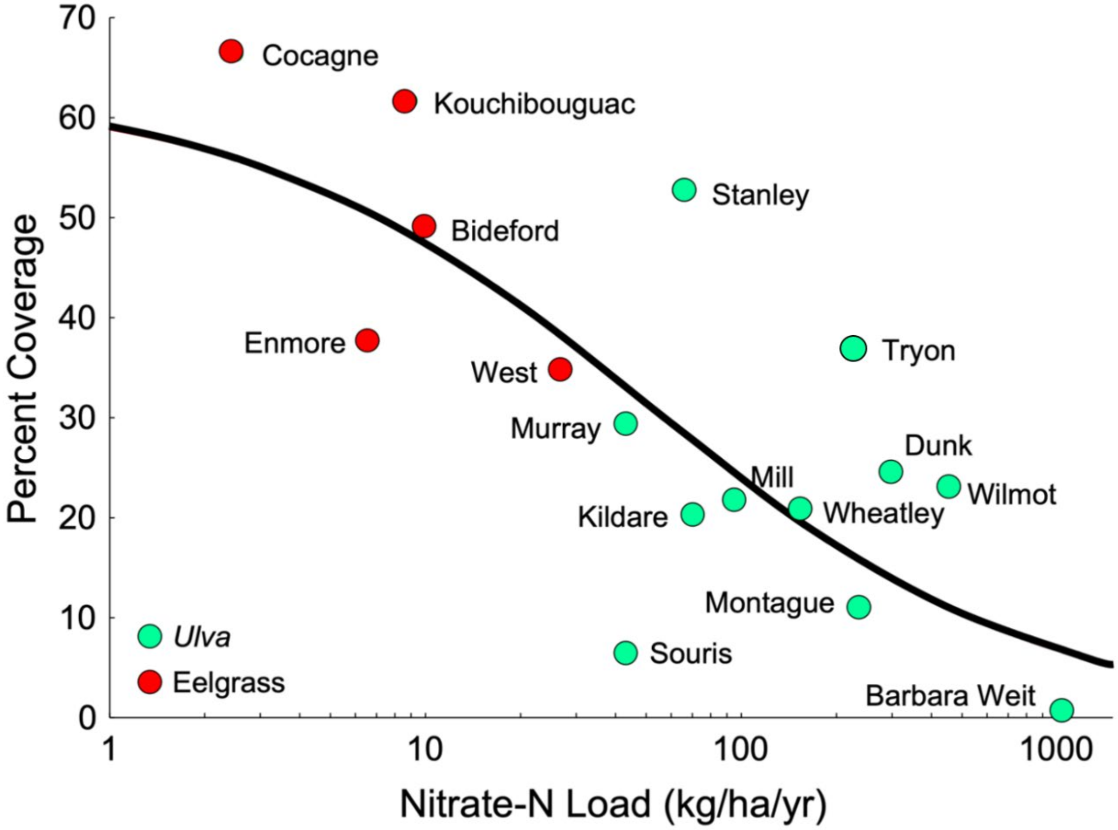

*Figure 5. Logistic regression between the percent coverage of eelgrass and nitrate-N loading (log scale) across sites in PEI and NB. Eelgrass coverage data were collected in 2014, except for Barbara Weit, which was measured in 2018. Green circles denote sites that are dominated by Ulva spp. in the upper estuary, whereas red points denote sites that are dominated by eelgrass (*Z. marina*) in the upper estuary. The black line represents the logistic regression line of best fit.*

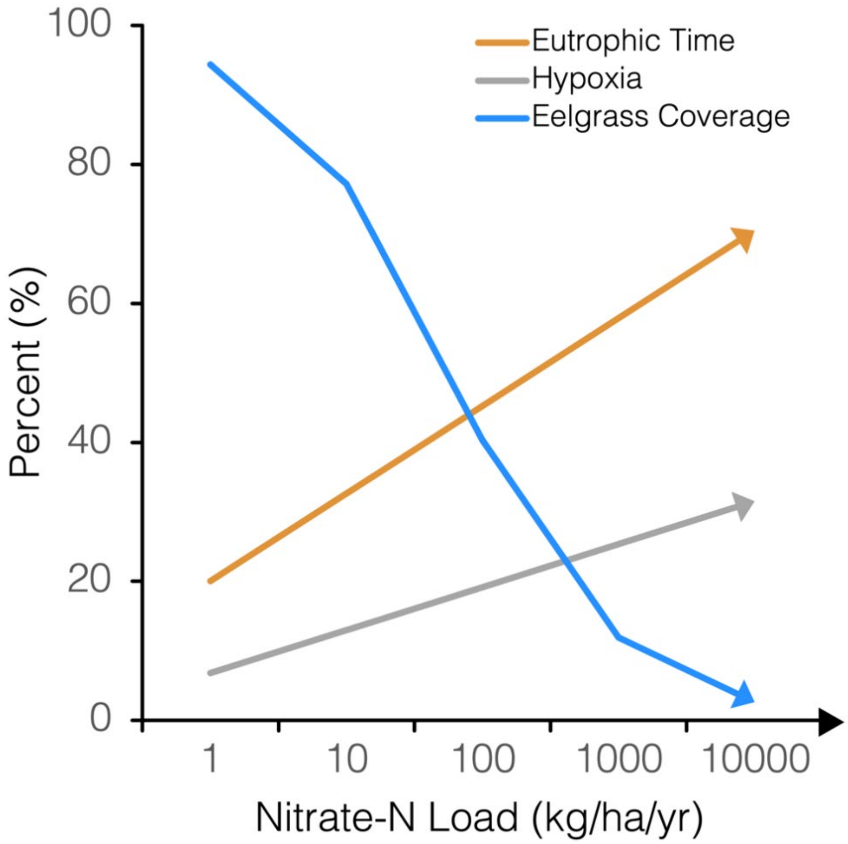

Figure 6. Theoretical relationship between changes in Eutrophic Time, hypoxia (DO) and eelgrass coverage for a hypothetical estuary (computed using the average water residence time among all sites) and log-scale ascending nitrate-N loading. Note that eelgrass coverage and the metrics representing symptoms of eutrophication are inversely correlated.