Nitrogen loading rates for twenty-one seagrass inhabited bays in Nova Scotia, Canada

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ABSTRACT

Nutrient loading from anthropogenic sources has implications for the health and functioning of aquatic species and ecosystems. Using a nutrient loading model (NLM), nitrogen loading from point and non-point sources was estimated for 21 Nova Scotia bays where eelgrass (*Zostera marina*) has been documented. Overall, atmospheric deposition contributed most (73 – 100%) of the total nitrogen loading, followed by fertilizer use and discharges from seafood processing and finfish aquaculture. The highest total loading was in St. Margaret's Bay, and the lowest in Kejimkujik National Park Seaside. The maximum loading rates standardized by watershed area (yield) found in Nova Scotia were lower compared to the maximum estimates previously found in New Brunswick, and nitrogen residencies (delta-N) were much lower than in Prince Edward Island. Loading rates per estuary area were positively related to nitrogen tissue content in eelgrass leaves. This NLM can be easily updated and applied to other watersheds to compare nutrient loading rates across similar systems.

RÉSUMÉ

Nagel, E.J., Murphy, G., Wong, M.C. and Lotze H.K. 2018. Taux de chargement d'azote pour vingt et une baies habitées d'herbiers en Nouvelle-Écosse, Canada. Can. Tech. Rep. Fish. Aquat. Sci. 3260: v + 37.

La charge en éléments nutritifs provenant de sources anthropiques a des répercussions sur la santé et le fonctionnement des espèces et des écosystèmes aquatiques. À l'aide d'un modèle de charge en éléments nutritifs, on a mesuré la charge en azote provenant de sources ponctuelles et non ponctuelles pour 21 baies de la Nouvelle-Écosse où la présence de zostère (Zostera marina) a été documentée. Dans l'ensemble, ce sont les dépôts atmosphériques qui ont contribué le plus à la charge en azote totale (de 73 à 100 %), suivis de l'utilisation d'engrais et des rejets issus de la transformation des fruits de mer et de l'aquaculture des poissons à nageoires. La charge totale la plus élevée a été enregistrée dans la baie St. Margaret's. C'est au Parc national Kejimkujik Bord de mer qu'elle était la plus faible. Les taux de charge maximaux normalisés par bassin hydrographique (rendement) observés en Nouvelle-Écosse étaient inférieurs aux estimations des taux maximaux observés antérieurement au Nouveau-Brunswick, et les temps de résidence de l'azote (delta-N) étaient beaucoup plus courts qu'à l'Île-du-Prince-Édouard. Les taux de charge par estuaire étaient directement proportionnels au contenu des tissus en azote des feuilles de zostère marine. Ce modèle peut facilement être mis à jour et être utilisé pour d'autres bassins hydrographiques, afin de comparer les taux de charge en éléments nutritifs entre des systèmes similaires.

INTRODUCTION

Coastal waters receive nutrients from the surrounding watersheds from both natural and anthropogenic sources (Bugden et al. 2014, McIver et al. 2015). Nitrogen is often a limiting nutrient in coastal marine ecosystems; however, nitrogen enrichment can have negative effects on sensitive species and ecosystem structure and functioning (Howarth and Marino 2006, Dixit and Brylinsky 2008, Bugden et al. 2014). Enhanced nutrient availability is known to increase phytoplankton growth and epiphytic and benthic annual algae cover, which can shade and smother seagrasses, while increased decomposition of organic material can lead to oxygen depletion and hydrogen sulfide accumulation at the sediment-water interface (Bricker et al. 2007, Schmidt et al. 2012, Benson et al. 2013). As such, human-derived nutrient loading has been identified as one major human disturbance impacting seagrasses around the world (Short and Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009, Short et al. 2011). Depending on the magnitude of nutrient enrichment, seagrass beds can respond with reduced shoot density and biomass, increased canopy height, or overall reductions in bed cover (Bricker et al. 2007, DFO 2012, Schmidt et al. 2012).

Eelgrass (*Zostera marina*) is the only seagrass species found commonly in the bays and estuaries of Atlantic Canada. Previous studies have shown that eelgrass beds in this region provide essential habitat and food for a variety of epiphytic, benthic and mobile species and provide important ecosystem services such as fisheries maintenance, provision of biodiversity and food web support, carbon storage, and sediment stabilization (Coll et al. 2011, Schmidt et al. 2011, Wong and Dowd 2016, Namba et al. 2018, Wong 2018). Because of its unique role in soft-sediment ecosystems, eelgrass has been classified as a DFO Ecologically Significant Species (ESS) in Atlantic Canada (DFO 2009). Degradation or loss of eelgrass beds can have important ecological consequences (DFO 2012, Schmidt et al. 2012, Cullain et al. 2017), yet little is known on nitrogen loading rates in Nova Scotia. Here, we model nitrogen loading from natural and anthropogenic sources for selected bays and estuaries in Nova Scotia where eelgrass beds are found.

Previous work by McIver et al. (2015) estimated nitrogen loading to seven estuaries in New Brunswick, Canada. These estimates were generated from a modified version of a nitrogen loading model (NLM) by Valiela et al. (1997, 2004) for Waquoit Bay, Massachusetts, as part of the Waquoit Bay Land Margin Ecosystems Research project (WBLMER). Results from this work indicated that atmospheric deposition contributed the greatest amount of nitrogen to New Brunswick watersheds and bays; however, local point sources, including wastewater treatment and seafood processing plants, as well as non-point sources, including septic systems and fertilizer use, were also important (McIver et al. 2015).

In addition to the magnitude of nitrogen loading, residence time of nitrogen in estuaries or coastal bays can influence the consequences of nutrient loading in coastal ecosystems (Valiela et al. 2000, McIver et al. 2015). Nitrogen residence time is

influenced by variations in freshwater inputs as well as oceanographic variables, such as tidal flushing. In New Brunswick, bays with low tidal flushing (i.e. longer flushing times) have been found to be more susceptible to eutrophication than those with higher flushing rates (McIver et al. 2015). Nitrogen residency can be quantified by combining nitrogen loading estimates with tidal flushing and freshwater dilution parameters into an index termed delta-N (Monsen et al. 2002, Bugden et al. 2014). In Prince Edward Island (PEI), larger delta-N values increased the risk of eutrophication, and significantly higher delta-N values were found in anoxic relative to non-anoxic estuaries (Bugden et al. 2014).

Seagrasses in high-nutrient bays and estuaries typically have increased tissue nitrogen and lower C:N ratios compared to seagrasses in low-nutrient bays and estuaries (Duarte 1990, Erftemeijer et al. 1994). Nitrogen tissue content in eelgrass blades and roots have previously been suggested as an indicator of nutrient conditions in coastal bays (Lee et al. 2004). McIver et al. (2015) found strong positive relationships between nitrogen loading rates estimated from the NLM and nitrogen tissue content in eelgrass leaves, suggesting that tissue nitrogen may be useful as an indicator for human-derived nutrient loading in Atlantic Canadian bays and estuaries.

This study extends the nitrogen loading model (NLM) used by McIver et al. (2015) to 21 watersheds in Nova Scotia along the Atlantic and Gulf coasts. Using provincial and national data sources on land use, human population densities and human activities in watersheds and coastal bays, estimates of total nitrogen loading were derived for each bay, as well as standardized nitrogen loading rates per hectare watershed (yield) and per hectare estuary. In addition, information on tidal flushing and freshwater inputs was used to quantify residence time of nitrogen in each bay (delta-N; Bugden et al. 2014). Lastly, existing estimates of eelgrass tissue nitrogen content from a subset of bays were related to nitrogen loading estimates to evaluate whether they can be used as an indicator of anthropogenic nitrogen loading in Nova Scotia.

MATERIALS AND METHODS

The nitrogen loading model (NLM) used assumes steady state input of nitrogen from anthropogenic sources, including point and non-point sources (Valiela et al.1997, McIver et al. 2015). Point sources of nitrogen included direct atmospheric deposition, discharges from wastewater treatment plants and seafood processing plants, and nitrogen addition from aquaculture (Valiela et al. 1997, McIver 2015, McIver et al. 2018). Direct atmospheric deposition occurs on the surface of the estuary and bay from atmospheric sources of nitrogen (nitrate and ammonium). In addition, indirect atmospheric deposition occurs onto the watershed surface and seeps through the vadose zone and aquifer into the bay, with loss parameters occurring at each step (Valiela et al. 1997). Loss parameters include volatilization, vegetation uptake, vadose transport and aquifer transport (Valiela et al. 1997). Indirect atmospheric deposition was considered a diffuse non-point source of nitrogen along with septic systems and fertilizer addition from agriculture, lawns and fertilized turf (sports fields, cemeteries and golf courses) (Valiela et al. 1997, McIver et al. 2015).

Modifications to the original WBLMER nitrogen loading model developed by Valiela et al. (1997) for New Brunswick watersheds are described by McIver et al. (2015). This approach was adapted for Port Mouton in Nova Scotia by McIver et al. (2018), and is used here with minimal modifications since the general composition of underlying coastal substrates and soils are similar (USGS 2003, McIver et al. 2015, 2018). No large-scale peat harvesting occurred in the selected bays in Nova Scotia, and calculations for septic systems assumed seasonal residency proportional to the number of part time residences documented in areas within 200 m of the coast. Data were not as readily available for point sources such as wastewater treatment plants and seafood processing plants as in New Brunswick, so the calculations for nitrogenous effluent differed in this respect. We derived an average estimate of nitrogen loading for the years 2010–2016 to cover the period in which eelgrass tissues have been sampled (Cullain et al. 2017). Following calculations of nitrogen loading, tidal parameters (i.e. tidal volume, tidal cycle time) were used to calculate flushing times for each bay (McIver et al. 2015). To create comparable estimates of nitrogen residency between bays, delta-N (an approximation of nitrogen concentration, Budgen et al. 2014) was calculated.

The NLM only considers atmospheric deposition and human-derived sources of nitrogen entering the estuaries and coastal bays and does not quantify oceanic sources of nitrogen. Generally, eelgrass in coastal bays and estuaries on the Atlantic and Gulf coast of Canada are considered N-limited, with tissue N concentrations below the 1.8% benchmark established by Duarte (1990) (Schmidt et al. 2011, 2012); thus, anthropogenic nitrogen loading is expected to influence coastal bays and estuaries despite ambient nitrogen input from oceanic sources.

WATERSHEDS AND LAND USE

The twenty-one locations in Nova Scotia included 9 on the South Shore, 5 on the Eastern Shore, 1 in western Cape Breton and 6 on the North Shore (Northumberland Strait) (Figure 1). These locations were selected to specifically include bays where seagrass beds are present. Field sampling of eelgrass (*Zostera marina*) beds has occurred in these twenty-one bays over the past 10 years (Weldon et al. 2005, Schmidt et al. 2011, Wong et al. 2013, Cullain et al. 2017, Wong 2018), These bays also reflect a gradient of low to high terrestrial human activities. However, several bays where we would expect high human-derived nutrient loading were excluded because we do not have information on eelgrass presence (e.g., Halifax Harbour).

Watershed boundaries delineated by the Nova Scotia Department of Environment were used (NSE 2017). Given that we were interested in nutrient loading to specific bays, the watersheds required further delineation and combination of multiple tertiary watersheds

in order to include all freshwater inputs. Hydrographic data combined with a digital terrain model (Province of Nova Scotia 2015) were used to predict the watercourse drainage patterns and aid in the delineation of watershed boundaries for each bay.

The area of coastal water associated with each watershed was determined to estimate direct atmospheric deposition onto the water surface, account for point sources around and inside the bay, as well as to calculate nutrient loading rates per area (ha) of estuary or bay. Using ArcGIS, a convex hull polygon was generated around the watershed boundaries. Areas that overlapped with coastal water polygons (Province of Nova Scotia 2015) were considered part of the bay area. Population size was determined using the number of civic addresses present in each watershed (Province of Nova Scotia 2015) and multiplying this with the average number of residents per household (2.3) in Nova Scotia (Statistics Canada 2017a). Population density was considered the number of individuals in each watershed divided by watershed area.



Figure 1 Nova Scotia watersheds selected for development of a nitrogen loading model (n=21). Map generated using ArcGIS (ESRI 2017). For details on watershed names see Figures 2-8.

For each watershed, the total land use in several categories was determined as the retention of atmospheric deposition on the watershed surface and through the soil varies by land cover (Valiela et al. 1997). These categories included forest/ wetland, agriculture, turf, coastal beach and developed urban areas, which was subdivided into pervious and non-pervious surfaces. Watershed land use was determined using available spatial data from provincial and national sources. The Nova Scotia forest inventory (NSDNR 2017) was used to calculate areas covered by coastal beach, inland water, agriculture and blueberry fields. Agriculture and blueberry were separated as they were assumed to receive different rates of fertilizer application (Maqbool et al. 2016).

The 'designated areas' dataset contained spatial coverage for developed urban areas such as cemeteries, parking areas and industrial activities (Province of Nova Scotia 2015). Non-residential building footprints were calculated from the 'buildings' layer (Province of Nova Scotia 2015). Ecological Land classification data from the Nova Scotia Department of Natural Resources (NSDNR 2013) were used to determine areas covered by wetland, which included inland marshes and tidal salt marshes. Protected areas were available from the Canadian Council on Ecological Areas CARTS database (CCEA 2016). All shapefiles were clipped to the predetermined watershed areas and land use units was summed to determine the area of each type of usage per watershed.

The average area for residential properties was calculated to estimate the area covered by residential lawn or garden for turf fertilizer application. This land use type was not considered in atmospheric deposition calculations as it was a mix of forested, pervious and non-pervious surfaces that was not able to be subdivided given available data. An average residential lot size was first determined by matching civic address locations in watersheds (Province of Nova Scotia 2015) with property parcels, or lots in the Nova Scotia Property Database (Service Nova Scotia 2016). Large non-residential lots (such as provincial parks) which contained civic address points were removed from the calculation. Lots larger than 50 ha were also excluded from the residential turf calculation as these areas mostly overlapped with areas designated as farmland. The proportion of residential lots covered by turf (i.e. lawn or garden) was calculated for a random sample of 25 lots in each watershed using the measuring tool in the online map provided by Viewpoint for Nova Scotia (ViewPoint Realty 2017). For each watershed, the total area covered by turf was estimated by multiplying the average percent turf cover by the average lot size and number of lots.

ATMOSPHERIC DEPOSITION

Given the rapid increase of fertilizer use and fossil fuel consumption over the past century, nitrogen in atmospheric deposition is primarily attributed to anthropogenic sources (Grumber and Galloway 2008, Duce et al. 2008). Therefore, we consider atmospheric deposition on the watershed and bay surfaces as a human-derived source of nitrogen.

Atmospheric deposition on the watershed and bay surface was quantified using measurements of direct inorganic nitrogen (DIN) deposition of the ions NO₃⁻ and NH₄⁺ and precipitation. As measurements did not exist for dissolved organic nitrogen (DON), DIN was assumed to represent 70% of total dissolved nitrogen (TN) (Valigura et al. 2001, Valiela et al. 2004, McIver et al. 2015). Direct deposition refers to deposition directly onto the bay surface, with no loss parameters assumed (Valiela et al. 1997, McIver et al. 2015). Indirect deposition included deposition on the watershed surface, which was subject to loss parameters. Since retention of atmospheric nitrogen on the watershed surface and through the soil differed depending on the type of surface, the land uses listed above were separated into 5 categories for the indirect atmospheric deposition calculations and assigned different loss parameters (Valiela et al. 1997, McIver et al. 2015). The 5 land uses included forest/ wetland, agriculture, turf and developed urban areas, which was subdivided into pervious and non-pervious surfaces.

To calculate atmospheric nitrogen deposition, average daily ion deposition rates of nitrate and ammonium in precipitation were calculated using data from three monitoring stations across Nova Scotia representing different air zones between the years 2001 -2011 as provided by the Canadian Air Precipitation Monitoring Network (CAPMoN) and Nova Scotia Precipitation Station Network (NSPSN) (Environment and Climate Change Canada 2017a). Data from the CAPMoN Kejimkujik station were used for the Western Air Zone including sites Green Harbour, Port Joli, Port L'Hebert, Port Mouton, Kejimkujik Seaside, Crescent Beach, Second Peninsula, Sambro and St. Margaret's Bay (Environment and Climate Change Canada 2017a). Deposition data for the Eastern Air Zone from the NSPSN Sherbrooke station were used for False Passage – Cable Island, Lower Three Fathom, East Petpeswick, Musquodoboit Harbour, Taylor's Head, and Mabou. Data for the Northern Air Zone from the CAPMoN Jackson station were used for Merigomish Big Cove, Pictou, Caribou, Tatamagouche, Pugwash and River Philip (Environment and Climate Change Canada 2017a). Standardized total precipitation (rain and snow) measurements from Environment Canada (Environment and Climate Change Canada 2016) were used to calculate average yearly rainfall from monthly totals between 2000 and 2016. Years of data were omitted if more than two months of measurements were missing.

To calculate the wet atmospheric deposition for each watershed and bay, the regional nitrogen content in precipitation was multiplied by the average yearly rainfall corrected for loss from evapotranspiration (Table 1) following McIver et al. (2015). Applying this average deposition rate to the bay area accounted for mean direct wet atmospheric

deposition onto the bay surface. To also account for direct dry deposition through air, a ratio of 70% dry to wet atmospheric deposition was used according to rates of particulate nitrogen deposition calculated for Atlantic Canada and the Gulf coast of the United States and the fact that there is less three-dimensional structure on the bay surface compared to vegetation on land, where a 1:1 ratio is assumed as described below (Valigura et al. 2001, Valiela et al. 2004, McIver et al. 2015). DON was not included in the calculation for dry deposition (McIver et al. 2015). As atmospheric deposition rates were variable throughout the year, the minimum and maximum rates of deposition were calculated as well as the mean.

Indirect atmospheric deposition was calculated separately for each land use type using loss parameters in Valiela et al. (1997), which were also used by McIver et al. (2015) for watersheds in New Brunswick (Table 1). Wet atmospheric deposition was calculated as above, and a 1:1 ratio for dry to wet atmospheric deposition was used according to rates of particulate nitrogen deposition calculated for Atlantic Canada and the Gulf coast of the United States (Valigura et al. 2001, Valiela et al. 2004, McIver et al. 2015). Again, DON was not included in the calculation for dry deposition (McIver et al. 2015). In Nova Scotia, areas labelled as coastal beach were omitted from the atmospheric deposition calculation to avoid counting submerged coastal areas twice in the model. The area footprint of residential settlement was not subtracted from the natural/ forest area as surface retention on forest and pervious surfaces (such as driveways) was similar. Turf area from lawns and gardens was added to the same category as turf from cemeteries, golf courses and sports fields for atmospheric deposition. Developed areas (i.e. industrial activities and buildings) were separated into impervious (paved) surfaces and pervious (unpaved) surfaces as this affected surface retention of nitrogen and the amount of area for nitrogen directly running off into storm water, freshwater and the ocean. Areas of paved and unpaved roads may have been underestimated due to incomplete spatial data (Province of Nova Scotia 2015). Loss parameters for these surface types followed rates established in previous studies (Valiela et al. 1997, 2004, McIver et al. 2015), and retention rates were the following for transfer between the surface layer and vadose zone: agriculture (38%), turf (38%), forest/wetland (35%), developed impervious (100%, assumed to runoff into waterways) and developed pervious (38%) (Table 1). Retention from travel through the vadose zone was 39%, and retention from the aquifer to the bay was 65% (Valiela et al. 1997, 2004, McIver et al. 2015).

NON-POINT SOURCES

Septic systems

Nitrogen loading from septic systems was calculated using the number of civic addresses in watersheds and an estimate for the amount of nitrogen produced per person per year (Table 1). Civic address locations (Province of Nova Scotia 2015) for which a wastewater treatment plant was not present were assumed to run on septic

systems. Septic systems for these addresses were separated into two categories: within 200m of the coast and greater than 200m from the coast using ArcGIS, as those within 200m had fewer loss parameters applied to effluent estimates (McIver et al. 2015). Addresses ≤200m from the coast were further subdivided into seasonal or full-time residences based on the proportion of full time residences (i.e. houses) compared to seasonal residences (i.e. cottages) surveyed by Young and Menon (1998) as this affected the total yearly loading estimate (Mclver et al. 2015). Only sites on the South Shore were surveyed by Young and Menon (1998), however as other regions of Nova Scotia have not been surveyed for seasonal residency the average proportion of 66% full time residences from the South Shore was used in this study for the Eastern Shore and Northumberland Shore. This proportion may be an underestimate of full time residences in these areas. Septic calculations for this study used an estimate of 4.19 kg nitrogen person⁻¹ yr⁻¹ (USEPA 2002) following Valiela et al. (1997) and McIver et al. (2015) as these data were not available for Nova Scotia or Canada. This is similar to the rate used by Bugden et al. (2014) for PEI, which was 10.4 kg N yr⁻¹ per household or 4.5 kg N yr⁻¹ per person if divided by 2.3 persons per household.

The total number of civic addresses was multiplied by this estimate and by 2.3, the average number of persons in private households in Nova Scotia, Canada (Statistics Canada 2017a) (Table 1). Loss parameters were applied as listed in Table 1, including the percentage lost in the septic and leaching fields (40%), in the vadose zone (34%) and, for households >200m away, also in the aquifer (35%) (Valiela et al. 1997, 2004, McIver et al. 2015). For civic addresses assumed to be occupied seasonally the total loading was reduced by half.

Table 1 Selected calculations used in the Nova Scotia Nitrogen Loading Model (NLM) adapted from McIver et al. (2015, 2018) and Valiela et al. (1997). Loss parameters are applied as outlined for each nitrogen source in the text. TN = total nitrogen. Seafood processing nitrogen rates adapted from Jamieson et al. (2017).

Source of nitrogen	Equation
Wastewater treatment plant	TN (kg yr ⁻¹) Per nitrogen type monitored = Actual flow rate (L day ⁻¹) × TN concentration in effluent (kg L ⁻¹) × Days yr ⁻¹ in operation
Seafood effluent	TN (kg yr ⁻¹) Per seafood species processed = (lb species processed yr ⁻¹) × (kg N lb ⁻¹ species processed)
Septic systems	TN (kg yr ⁻¹) = (Loading \leq 200m from shore) + (Loading \geq 200m from shore); where, loading \leq 200m = (Average persons per household × number of full time residences + Average persons per household × number of seasonal residences × 0.5) × 4.19 kg N person ⁻¹ yr ⁻¹ × 0.60 × 0.66; and loading \geq 200m = Average persons per household × 4.19 Kg N person ⁻¹ yr ⁻¹ × 0.60 × 0.66 × 0.65
Agricultural fertilizer	TN (kg yr ⁻¹) = [(Area for blueberry crop (ha) × 20 kg ha ⁻¹ yr ⁻¹) + (Area for crop fertilizer application (ha) × Average recommended fertilizer application rate (kg TN ha ⁻¹ yr ⁻¹))] × 0.61 × 0.61 × 0.39 × 0.65
Turf/lawn fertilizer: for golf courses, cemeteries, sports fields and lawn turf area	TN (kg yr ⁻¹) = Area for fertilizer application (ha) × 150 kg TN ha ⁻¹ yr ⁻¹ × 0.61 × 0.61 × 0.39 × 0.65
Indirect atmospheric deposition	TN (kg yr ⁻¹) = Dry or wet deposition (kg TN yr ⁻¹) × proportion of nitrogen reaching vadose zone (dependant on watershed surface type) × 0.39 × 0.65
Direct atmospheric deposition	TN (kg yr ⁻¹) = Dry deposition (kg TN yr ⁻¹) + wet deposition (kg TN yr ⁻¹)

Fertilizer addition

As described above, the proportion of civic address lots covered by turf (lawn or garden) was calculated using the 'draw area' tool in Viewpoint (ViewPoint Realty 2017). To calculate the area of residential turf in each watershed this proportion was multiplied by the calculated average lot size and number of civic address lots. This was multiplied by 0.98 from the average proportion of households reporting having a lawn or garden in Nova Scotia between 2007 and 2015 and by 0.1675, the proportion of homes using synthetic fertilizer in Nova Scotia between 2007 and 2015 and by 0.1675 (Statistics Canada 2017b) (Table 1). Other areas for synthetic fertilizer application included sports fields, cemeteries and golf courses. To calculate fertilizer N applied to these areas a concentration of 150 kg TN ha⁻¹yr⁻¹ was used from New Brunswick as Nova Scotia estimates were not available (Government of New Brunswick 2017). Loss parameters

from volatilization (39%), vegetation (39%), vadose transport (61%) and the aquifer (35%) were applied to achieve the final estimate (Valiela et al. 1997) (Table 1).

<u>Agriculture</u>

The area of agriculture and cultivated blueberry in each watershed was calculated using the Nova Scotia Forest Inventory (NSDNR 2017). The proportion of agricultural area to which fertilizer was applied was found for three applicable major drainage areas in 2006: 15.7% for the Southeastern Atlantic Ocean (01E), 15.6% for Cape Breton (01F) and 22.2% for the Bay of Fundy and Gulf of St. Lawrence (Statistics Canada 2010). The type of agriculture occurring on lands with the agriculture designation was unknown, so information from Statistics Canada was used to list the range of crops grown in the census area closest to watersheds (Statistics Canada 2017c). From this list, the average fertilizer application rate (101 kg TN ha⁻¹yr⁻¹) was estimated using crop specific rates from New Brunswick, as none were available from Nova Scotia (NBAAF 2001). Crops grown in Nova Scotia included wheat, corn, melons, potatoes, fruit and tree nuts, mushrooms, hay and unspecified fruit and vegetables (Statistics Canada 2017c).

The fertilization rate for blueberry fields in Nova Scotia was noted to be 20 kg N ha⁻¹yr⁻¹ by Maqbool et al. (2016), although this study recommended higher application rates. Loss parameters for agricultural fertilizer and blueberry fertilizer from volatilization (39%), vegetation (39%), vadose transport (61%) and the aquifer (35%) were the same as for turf fertilizer application (Valiela et al. 1997) (Table 1).

POINT SOURCES OF NITROGEN

Point source inputs of nitrogen in Nova Scotia included effluent from wastewater treatment plants, seafood processing plants and finfish aquaculture. Not all wastewater treatment plants in Nova Scotia are required to monitor nitrogen species (i.e. nitrate, nitrite, ammonia and organic nitrogen) as approvals from the Nova Scotia Department of the Environment have different requirements for different facilities. Estimates of nitrogen loading were only made where data existed and noted as incomplete where an estimate could not be made (2 watersheds), as no relationship was found between the population serving the wastewater treatment plant and nitrogen output. Additionally, where nitrogen species were tested in effluent not all forms were always tested for. Therefore, these estimates are most likely an underestimation.

Where data were available, average concentrations of nitrogen from organic and inorganic sources were used to generate an estimate of the nitrogen discharge from a plant in one year. Measurements of total Kjeldahl nitrogen (TKN) included organic nitrogen and ammonia, and inorganic measurements included nitrite, nitrate and ammonia. Few wastewater treatment plants provided all nitrogen species

measurements, therefore estimates of total nitrogen (TN) may be underestimations. Data were available for 8 plants between the years 2010 and 2016. Data were provided directly by wastewater treatment plant operators, municipalities and the Nova Scotia Department of the Environment. Two additional plants were identified, but as nitrogen species were not monitored in effluent at these plants estimates of nitrogen loading could not be made. For these watersheds (Pictou and East Petpeswick) the total nitrogen loading is likely an underestimation. Concentrations of nitrogen in effluent (mg/L) were multiplied by the actual flow rate (L/day) and the number of days per year the plant was in operation to achieve a loading estimate and averaged across the years 2010–2016 if multiple years of data were provided (Table 1).

The NLM calculates nitrogen loading from seafood processing plants by combining estimates of nitrogen concentration in effluent, effluent flow rate, and number of days of plant operation (Table 1). There were eleven seafood processing plants currently in operation located in 6 of our 21 watersheds of interest. Wastewater effluent from seafood processing plants is currently not required to be monitored or tested for plants discharging into coastal waters, as it is for discharge into freshwater systems (pers. comm. Stefan Furey 2017), therefore we had to make several assumptions to estimate the amount of nitrogen entering the bays from the 11 seafood processing plants.

First, effluent characteristics for the species processed in each plant were compiled from Jamieson et al. (2017). We used Biological Oxygen Demand (BOD) to estimate nitrogen concentration in the effluent as BOD was the most common effluent characteristic provided for our species of interest. Total Kjeldahl Nitrogen (TKN) is generally estimated as 15-20% of BOD (Gaytan et al. 2009, Scott 2012). We estimated nitrogen concentration as 20% of the BOD for each species. In plants that processed more than one species we averaged the species-specific effluent nitrogen concentration. We assumed that species were processed in equal proportions as we were not able to obtain information on the specific processing proportions of each species.

Effluent flow rate for each processing plant was estimated based on plant square footage. We were not able to obtain actual effluent flow rates from any plants, but we assumed that effluent flow rate was roughly related to plant size based on the strong, linear relationship between plant square footage and effluent flow rate ($R^2 = 0.88$) for ten New Brunswick seafood processing plants in McIver et al. (2015). We used the regression equation for this relationship combined with plant square footage, which was measured using Google Earth, to estimate effluent flow rate in Nova Scotia seafood processing plants. Finally, we assume that all plants operated 5 days/week (260 days/year).

Estimates of nitrogen loading from finfish aquaculture were based on McIver et al. (2018) for Port Mouton. One other finfish operation was identified in False Passage – Cable Island for the period of interest, however information on stocking density could

not be obtained. As the scale of this operation was noted to be similar to that in Port Mouton (pers. comm. Inka Milewski), the same loading estimate was applied (Table 1).

TIDAL PARAMETERS

Following calculations of nitrogen loading rates, tidal statistics were calculated to examine the effects of different oceanographic conditions in each bay. Flushing times were calculated to examine residency of nitrogen inputs. To examine rates of nitrogen flux, delta-N was calculated which yields an average concentration of nitrogen in the bay considering rates of tidal exchange and freshwater recharge (Bugden et al. 2014).

Calculations of flushing time, freshwater recharge and delta-N were made using parameters from Gregory et al. (1993) and stream gauge measurements from Environment Canada (Environment and Climate Change Canada 2017b). Gregory et al. (1993) made measurements of tidal range, amplitude and bay area and volume for multiple inlets across Nova Scotia. Watersheds in this study without matching tidal data were Second Peninsula and Crescent Beach. To calculate flushing time with freshwater inflow, stream gauge measurements from stations nearest to watersheds were used to find the average flow per unit area of watershed, and extrapolated to the total size of the watershed (Environment and Climate Change Canada 2017b). Tidal flushing time calculations followed methods in McIver et al. (2015). As tidal statistics were not available for the watershed of Second Peninsula, the tidal amplitude from Mahone Bay was used instead (Gregory et al. 1993).

Bays on the Atlantic coast of Nova Scotia were assumed to have a cycle time (i.e. hours to bring in the volume of water for an average tidal cycle) of 12.42 hours due to semidiurnal tides. For bays located on the Gulf of St. Lawrence, the cycle time was assumed to be greater than 12.42 due to mixed semi-diurnal tides and was calculated following methods in McIver et al. (2015). To calculate the new cycle time, tidal height measurements from Fisheries and Oceans Canada (DFO 2017) were used to calculate the volumetric increase over 21 days which was then multiplied by the estuary surface area (Gregory et al. 1993, McIver et al. 2015). This volume was then divided by the average tidal volume in Gregory et al. (1993) to calculate the number of tidal volumes in 21 days.

Where:

 V_v is the volumetric increase over 21 days (m³):

 V_v =total flood tidal increase (m) x bay area at mid tide (m²)

 V_p is the mean tidal prism (m³)

 ω is the cycle time in hours:

 $ω = 504 \text{ hr } 21 \text{ days}^{-1} / (V_v / V_p)$

Nitrogen flux was approximated by calculating a delta-N (Δ N) value for each watershed. Delta-N represents the approximate concentration of nitrogen remaining in the bay by accounting for tidal flushing and freshwater dilution (Bugden et al. 2014). Delta-N was calculated using the following formula:

$$\Delta N \text{ (mg TN L}^{-1}\text{)} = \frac{\text{Total loading (kg TN yr}^{-1}\text{)} x 1000}{V_t + V_f}$$

Where:

V_t = volume of incoming tide per year:

 V_t (m³ yr⁻¹) = Mean tidal range (m) x bay surface area (m²) x tidal cycles yr⁻¹

 $V_f(m^3 yr^{-1}) =$ volume of freshwater discharge per year

LINKING NLM RESULTS AND EELGRASS TISSUE CONTENT

To evaluate whether enhanced nitrogen loading in the estuary or bay is reflected in the tissue content of eelgrass, we compared the nitrogen loading rate per hectare estuary (estuary loading) and delta-N to the nitrogen content (%) in aboveground eelgrass tissue using linear regression. Tissue nitrogen data (percent of nitrogen in dry eelgrass tissue) were only available for 12 of the estuaries. All samples were collected in mid-summer (July and August), with 9 sites sampled in 2013 (PLH, CB, SP, SMB, SA, EP, LTF, TH, FPCI, Cullain et al. 2017, Wong unpublished data) and 3 sites sampled in 2015 (GH, PM, PJ, Cullain et al. 2017).

RESULTS

Watershed details including population size and population density are listed in Table 2.

Table 2 Estimated area of watershed and associated estuary or coastal bay, and human population size and density for 21 watersheds in Nova Scotia selected for application of the nitrogen loading model.

Watershed name and abbreviation	Watershed area (ha)	Estuary or Bay area (ha)	Population size	Population density (persons ha ⁻¹)	
South Shore					
Port Mouton (PM)	27,375	5,560	1,736	0.063	
Port Joli (PJ)	5,886	1,714	287	0.049	
Port L'Hebert (PLH)	9,478	1,245	246	0.026	
Kejimkujik National Park Seaside (KS)	773	361	0	0.000	
Green Harbour (GH)	8,822	1,090	660	0.075	
Crescent Beach (CB)	1,428	725	575	0.403	
Second Peninsula (SP)	2,930	449	816	0.278	
Sambro (SA)	5,945	973	2,976	0.501	
St. Margaret's Bay (SMB)	69,837	13,705	20,419	0.292	
Eastern Shore					
False Passage – Cable Island (FPCI)	19,233	3,412	1,069	0.056	
Lower Three Fathom (LTF)	17,557	1,439	5,715	0.325	
East Petpeswick (EP)	5,798	1,009	1,534	0.265	
Musquodoboit Harbour (MH)	76,710	2,378	5,352	0.070	
Taylor's Head (TH)	16,660	2,766	708	0.042	
Cape Breton Island					
Mabou (MA)	37,621	1,097	1,810	0.048	
Northumberland Shore					
Merigomish Big Cove (MBC)	49,098	3,002	4,020	0.082	
Pictou (PI)	112,067	3,206	40,843	0.364	
Caribou (CA)	14,711	1,906	2,127	0.145	
Tatamagouche (TA)	89,715	11,818	9,995	0.111	
Pugwash (PU)	17,449	1,003	1,559	0.089	

River Philip (RP)	71,229	777	5,395	0.076

LAND USE

The watershed with the fewest human impacts was Kejimkujik National Park Seaside, as this area contained no civic addresses or point sources of nitrogen (Figure 2). Many South Shore watersheds contained a high proportion of forest and wetland areas, with civic addresses mostly located close to the coast (Figure 2, Figure 3).



Figure 2 Land use in Nova Scotia watersheds for South Shore sites including Green Harbour (GH), Port L'Hebert (PLH), Port Joli (PJ), Kejimkujik Seaside (KS) and Port Mouton (PM).



Figure 3 Land use in Nova Scotia watersheds for South Shore sites including Second Peninsula (SP) and Crescent Beach (CB).

Both St. Margaret's Bay and Sambro had a mixture of civic addresses next to the coast and in the watershed interior, with the remainder of the watershed area mostly made up of forest (Figure 4).



Figure 4 Land use in Nova Scotia watersheds for South Shore sites including St. Margaret's Bay (SMB) and Sambro (SA).

Eastern Shore sites also were mainly composed of forest (Figure 5). Musquodoboit Harbour had low population density (Table 2), and civic addresses scattered along the coast and watershed interior (Figure 5).



Figure 5 Land use in Nova Scotia watersheds for Eastern Shore sites including Lower Three Fathom harbour (LTF), East Petpeswick (EP), Musquodoboit Harbour (MH), False Passage – Cable Island (FPCI) and Taylor's Head (TH).

Agricultural area in Mabou followed the same pattern as the location of civic addresses, which were scattered mainly in the watershed interior (Figure 6). Relatively large areas of blueberry cultivation are also apparent (Figure 6).



Figure 6 Land use in Nova Scotia watersheds for Mabou (MA), located in Cape Breton.

Land use in Northumberland shore sites was dominated by civic addresses, with some areas of wetland remaining closer to the coast (Figure 7). The Pictou watershed had the highest population density (Table 2).



Figure 7 Land use in Nova Scotia watersheds for Northumberland Shore sites including Tatamagouche (TA), Caribou (CA), Pictou (PI) and Merigomish Big Cove (MBC).

Both Pugwash and River Philip had multiple areas for blueberry cultivation (Figure 8). Areas of other agriculture are spread throughout the watershed and follow settlement patterns (Figure 8).



Figure 8 Land use in Nova Scotia watersheds for Northumberland Shore sites including River Philip (RP) and Pugwash (PU).

POINT SOURCES

Watersheds with point sources of nitrogen are shown in Table 3. Point sources included wastewater treatment plants, seafood processing plants and finfish aquaculture sites operating between the years 2010–2016. Tatamagouche had the highest total number of point sources with 3 seafood processing plants and 1 wastewater treatment plant (Table 3).

Watershed	Operational wastewater treatment plants	Operational seafood processing plants	Operational finfish aquaculture sites
Port Mouton	-	-	1
Sambro	-	3	-
False Passage – Cable Island	-	-	1
St. Margaret's Bay	1	1	-
Musquodoboit Harbour	1	-	-
Mabou	1	-	-
Merigomish Big Cove	-	2	-
Pictou	3	-	-
Caribou	-	1	-
Tatamagouche	1	3	-
Pugwash	1	-	-
River Philip	1	1	-

Table 3 Watersheds in Nova Scotia with point sources of nitrogen effluent operational between 2010 – 2016, and the number of each source type.

NITROGEN LOADING ESTIMATES

Total nitrogen loading included all point and non-point sources of natural and anthropogenic nitrogen (Table 4). The standardized loading rate per ha watershed (yield) was calculated by summing all nitrogen sources except for direct atmospheric deposition to the bay surface, to only reflect the footprint per ha watershed, and dividing this by the watershed area. The standardized loading rate per ha estuary (estuary loading) was calculated by dividing the total loading by the bay surface area, to reflect the footprint in the coastal environment. The highest total loading was found in St. Margaret's Bay, which also had the longest flushing time (Table 4). Sambro had the highest yield, and River Philip had the highest loading rate per ha estuary, while also having the shortest flushing time. The lowest total loading and estuary loading rate was found in Kejimkujik Seaside, and Mabou had the lowest yield (Table 4). Table 4 Total nitrogen loading and standardized loading rates from 21 Nova Scotia watersheds to associated bays, as well as tidal flushing parameters and delta-N values (ΔN). Total loading includes the mean \pm minimum and maximum loading for each site based on minimum and maximum atmospheric deposition rates, while other values only list the mean. Yield does not include direct atmospheric deposition to the bay surface. Flushing time includes freshwater inflow.

Watershed	Total Loading (kg TN yr ⁻¹)	Yield (kg TN ha watershed ⁻ ¹ yr ⁻¹)	Loading rate (kg TN ha estuary ⁻¹ yr ⁻¹)	Flushing time (h)	ΔN (mg/L)
Port Mouton	211,816 ± 92,202	3.74	48.57	113.75	0.0036
Port Joli	58,374 ± 24,919	2.62	34.05	53.03	0.0033
Port L'Hebert	$55,833 \pm 23,800$	2.60	44.81	31.48	0.0065
Kejimkujik Seaside*	11,002 ± 4,740	2.51	30.43	24.00	0.0065
Green Harbour	50,550 ± 21,422	2.63	46.37	48.52	0.0056
Crescent Beach	23,106 ± 9,588	3.19	31.83	NA	0.0028
Second Peninsula	20,971 ± 8,264	3.23	46.63	NA	0.0044
Sambro	56,262 ± 17,790	5.20	57.77	68.38	0.0078
St. Margaret's Bay	598,365 ± 234,287	3.46	43.66	296.95	0.0038
False Passage–Cable Island	134,977 ± 55,255	3.63	39.56	57.40	0.0128
Lower Three Fathom	86,545 ± 29,297	3.03	60.14	123.95	0.0291
East Petpeswick**	39,778 ± 14,464	2.83	39.41	45.29	0.0060
Musquodoboit Harbour	258,222 ± 92,885	2.65	108.56	27.04	0.0524
Taylor's Head	86,655 ± 33,275	2.03	31.32	76.57	0.0027
Mabou	77,318 ± 26,901	1.65	70.45	86.63	0.0225
Merigomish Big Cove	168,383 ± 52,863	2.31	56.08	45.82	0.0086
Pictou**	340,894 ± 117,666	2.52	106.31	71.00	0.0164
Caribou	76,340 ± 22,545	2.81	40.04	43.75	0.0058
Tatamagouche	409,268 ± 124,397	2.39	34.63	56.07	0.0040
Pugwash	56,436 ± 17,874	2.29	56.27	30.04	0.0124
River Philip	167,522 ± 47,653	2.17	215.50	16.56	0.0768

*Flushing time from Wong et al. (2013), post bridge/causeway removal

**Indicates the actual total nitrogen loading is likely higher than the reported values due to effluent data from municipal wastewater treatment plants not being available or not having been measured to generate an estimate. The delta-N value reported is for the estimated loading only.

The proportion of total nitrogen loading from each source is shown in Figure 9. Direct and indirect atmospheric deposition formed the majority of total loading, with fertilizer and waste from seafood processing and finfish aquaculture formed the next highest proportion in most watersheds. Seafood processing and finfish aquaculture formed a large portion of total loading in Port Mouton, Sambro and False Passage–Cable Island. Septic systems and municipal wastewater treatment plants were a small proportion of total loading in most watersheds (Figure 9).



Figure 9 Percentage of total nitrogen loading from 21 Nova Scotia to associated bays from direct and indirect atmospheric deposition, fertilizer, septic systems, municipal wastewater and waste from seafood processing and finfish aquaculture. Watersheds include Green Harbour (GH), Port Mouton (PM), Port Joli (PJ), Port L'Hebert (PLH), Kejimkujik Seaside (KS), Crescent Beach (CB), Second Peninsula (SP), Sambro (SA), St. Margaret's Bay (SMB), False Passage–Cable Island (FPCI), Lower Three Fathom (LTF), East Petpeswick (EP), Musquodoboit Harbour (MH), Taylor's Head (TH), Mabou (MA), Merigomish Big Cove (MBC), Pictou (PI), Caribou (CA), Tatamagouche (TA), Pugwash (PU), and River Philip (RP).

Across the 21 watersheds, there was significant positive linear relationship between total nitrogen loading and watershed size (p<0.001, R^2 =0.65) (Figure 10), reflecting the strong contribution of indirect atmospheric distribution onto the watershed surface in addition to human activities.



Figure 10 Total nitrogen loading compared to watershed area for 21 Nova Scotia watersheds. Adjusted $R^2 = 0.65$, p<0.001.

Standardized nitrogen loading rate per ha watershed (yield) was positively related to population density (p=0.002, $R^2 = 0.37$, Figure 11), reflecting the footprint of human activities in the watershed.



Figure 11 Population density and standardized measures of nitrogen loading rate per hectare of watershed (yield) for 21 Nova Scotia watersheds.

FLUSHING TIME AND DELTA-N

Flushing times could not be calculated for Crescent Beach and Second Peninsula due to missing hydrographic data. St. Margaret's Bay had the longest flushing time, at 296.95 hours (Table 4). All other flushing times were <100 hours except for Port Mouton and Lower Three Fathom. River Philip had the shortest flushing time at 16.56 hours. Delta–N values were all less than 0.08, with River Philip having the highest value. The lowest delta-N values were found at Crescent Beach and Taylor's Head (Table 4).

LINKING NLM RESULTS AND EELGRASS TISSUE CONTENT

For the 12 sites where eelgrass tissue was sampled and analyzed (Cullain et al. 2017, Wong unpublished data), aboveground tissue percent nitrogen content was compared to nitrogen loading rates per hectare estuary. The mean value across sites for blade tissue collected in mid-summer (July-August) was 1.40% (±SD 0.26%) nitrogen, and the large majority of samples had tissue N contents of <1.8%, indicating N limitation (Duarte 1990). There was a significant positive trend between tissue percent nitrogen and estuary loading rates (p<0.001, R² = 0.11, Figure 12). Unfortunately, we did not have tissue nitrogen data from those estuaries with the highest estuary loading (e.g. River Phillip, Musquodoboit Harbour, and Pictou; Table 4), which may have made this relationship stronger. Delta-N was also compared to tissue percent nitrogen with a linear regression and showed a trend towards a positive relationship (p=0.01, R² = 0.06).



Figure 12 Relationship between nitrogen loading rates per ha estuary and above ground eelgrass tissue percent nitrogen for 12 Nova Scotia sites including Green Harbour, Port Mouton, Port Joli, Port L'Hebert, Crescent Beach, Second Peninsula, St, Margaret's Bay, Sambro, East Petpeswick, Lower Three Fathom, Taylor's Head and False Passage – Cable Island.

SUMMARY AND CONCLUSIONS

Estimates of nitrogen loading, flushing time and delta-N were made for 21 Nova Scotia estuaries and bays for the years 2010-2016, the time period in which eelgrass beds were also sampled in several of the bays. Baseline estimates of nitrogen loading in Nova Scotia are useful to compare to those in other regions across Atlantic Canada, including New Brunswick (McIver et al. 2015) and Prince Edward Island (Bugden et al. 2014), that have in parts different land uses and human activities, and different watershed and estuary/bay configurations. In addition, linking nitrogen loading estimates to the nitrogen content found in eelgrass tissue samples can help identify whether enhanced nitrogen loading rates are reflected in biotic components of coastal ecosystems (McIver et al. 2015).

The majority of total nitrogen loading at all sites resulted from atmospheric deposition, both indirect deposition onto the watershed surface as well as direct deposition onto the bay surface (Figure 9). This is comparable to previous nutrient loading model results for New Brunswick, where atmospheric deposition was the main contributor to nitrogen loading in most bays except one, Laméque Bay, where seafood processing was the biggest source of nitrogen (McIver et al. 2015). However, due to underlying uncertainties in atmospheric deposition rates and variations in rainfall amounts and ion deposition rates, the variance for total nitrogen loading estimates (Table 4) is high. Overall, the highest total nitrogen loading was in St. Margaret's Bay, followed by Tatamagouche, Pictou, Musquodoboit Harbour and Port Mouton. In St. Margaret's Bay, atmospheric deposition was the main driver of the high nitrogen loading. Despite having a moderate watershed area, St. Margaret's Bay had the largest estuary/bay surface and the highest atmospheric nitrogen deposition rates per hectare in western Nova Scotia. In Tatamagouche and Pictou, high loadings were related to the relatively large watersheds combined with higher human population sizes and fertilizer use (Table 2, Figure 9). In Musquodoboit Harbour, high loadings resulted from the large watershed size, while in Port Mouton finfish aquaculture played a significant role. In comparison, overall nitrogen loading was lowest in Kejimkujik National Park Seaside, followed by Second Peninsula and Crescent Beach, all on the South Shore of NS. These three sites are characterized by small watershed areas, small estuary/bay areas and small human populations (Table 2).

Standardized loading rates per ha watershed (yield) provide a good measure of the footprint of human activities in the watershed, since indirect atmospheric deposition is largely related to watershed size. In New Brunswick, there was a strong positive relationship between nitrogen yield and population density (McIver et al. 2015). This relationship was also present and significant for Nova Scotia sites (Figure 11); however, the relationship was less strong due to the much smaller range in yield values. Whereas the highest yield in New Brunswick was 20.7 kg TN per ha watershed in Laméque (McIver et al. 2015), the highest yield in Nova Scotia was 5.2 kg TN per ha watershed in Sambro (Table 4). This high yield in Sambro can be explained by the relatively small

watershed area, leading to less indirect atmospheric deposition, as well as the high human population density and contributions from seafood processing plants. In comparison, the lowest yield was in Mabou (1.65 TN kg per ha watershed), which had a human population density ten times lower and a watershed 6 times larger than in Sambro (Table 2). Aside from these two sites, other watersheds had relatively consistent yields of between 2.0 - 3.7 kg TN per ha watershed. This is in contrast to New Brunswick watersheds, where yields ranged between 1.5 and >20 kg TN ha watershed/ yr, with the highest yield driven mostly by seafood processing effluent in Laméque and the lowest yield found in Kouchibuguac National Park (McIver et al. 2015). New Brunswick estimates of yield did include direct deposition onto the bay surface in their calculation (McIver et al. 2015), whereas this was omitted in this study to remove the variation from large differences in bay surface areas and only account for the watershed nitrogen loading footprint.

Nitrogen loading rates that are standardized to the area of the estuary or bay (estuary loading) are useful for assessing the potential footprint of the received loading in the bay environment and are influenced by the total loading as well as the watershed to bay ratio (McIver et al. 2015). Estimates for estuary loading were highest in River Philip, followed by Musquodoboit Harbour and Pictou (Table 4), all of which have high total loading and large watershed to bay ratios. In turn, estuary loading rates were lowest for Kejimkujik National Park Seaside, which had much lower total loading and watershed to bay ratio.

As an indicator of nitrogen loading in the estuary or bay, the tissue nitrogen content in eelgrass blades was significantly and positively related to the estuary loading rates (Figure 12), similar to those found in New Brunswick (McIver et al. 2015). Since the majority of eelgrass tissue samples indicated N limitation (<1.8%; Duarte 1990), anthropogenic N loading is likely responsible for this positive relationship. Unfortunately, eelgrass tissue data were missing for those estuaries with the highest estuary loading rates (River Philip, Musquodoboit Harbour and Pictou, Table 4), which may have made this relationship stronger. The high variability of tissue content within and between sites likely reflects that nitrogen concentrations are not equally distributed throughout an eelgrass bed and estuary/bay, as assumed in our estuary loading estimate. In Massachusetts estuaries, for example, variation was seen in habitat quality between eelgrass beds, and healthy beds existed in areas where average nitrogen concentrations of water column samples were less than 0.34 mg/L (Benson et al. 2013). Intensified eelgrass tissue sampling in different parts of the estuary or bay, as well as at different distances from the river mouths, from shore and from point sources of nutrients might result in better estimates of nitrogen content and relationships with nitrogen loading in Nova Scotia and elsewhere.

Flushing times varied widely among bays, ranging from short flushing times of just 16.56 hours in River Philip to long flushing times of 296.95 hours in St. Margaret's Bay (Table 4). The flushing times calculated in this study improve upon those previously

reported in Gregory et al. (1993) as they account for the difference in tidal cycle time (the time for recharge of the average tidal prism) for bays with mixed semi-diurnal tides. This method to calculate flushing time was also used by McIver et al. (2015) for sites in New Brunswick. In River Philip, the short flushing time may help to counteract the otherwise highest estuary loading rates. In St. Margaret's Bay, the very large bay area helps to dilute the otherwise highest total nitrogen loading combined with longest flushing time.

As a combined measure of nitrogen loading estimates together with tidal flushing and freshwater dilution, delta-N estimates the nitrogen residency in an estuary or bay (Monsen et al. 2002, Bugden et al. 2014). Delta-N values for the 21 Nova Scotia bays were an order of magnitude lower than those calculated for Prince Edward Island by Bugden et al. (2014) (median delta-N for NS = 0.006, median delta-N for PEI = 0.062). In Prince Edward Island, larger delta-N values were associated with an increased risk of eutrophication, and a strong relationship was found between the frequency of anoxic events and high delta-N values (Bugden et al. 2014). Most delta-N values calculated for Nova Scotia bays were well below the threshold of 0.06 used to predict the likelihood of anoxic events in Prince Edward Island (Bugden et al. 2014). However, these rates may not always be representative for smaller areas of the bays or for individual seagrass beds as delta-N calculations assume even mixing of the entire bay (Monsen et al. 2002).

Gathering data for anthropogenic sources of nitrogen loading in Nova Scotia posed a challenge due to the differences in regulations for monitoring wastewater effluents compared to other provinces. Unlike in New Brunswick, seafood processing plants in Nova Scotia are not required to monitor and report on nitrogen species in discharged effluent to coastal waters, and regulations vary for wastewater treatment plants depending on customized reporting requirements from the Department of the Environment that depend on the plant size and when it became operational (pers. comm. Stefan Furey 2017). We therefore made some conservative assumptions as to the plant size and effluent rates (see methods above), resulting in a likely underestimation of the total nitrogen loading of a watershed. Fertilization rates were also unavailable for Nova Scotia crops, therefore the assumption was made that rates would be similar to those published for New Brunswick (NBAAF 2001). With better monitoring and reporting of wastewater effluents, aquaculture production activities, fertilizer applications and other nitrogen uses in the future, the NLM derived estimates could be updated and improved.

Overall, applying the nutrient loading modal (NLM) to 21 watersheds and bays in Nova Scotia proved useful in understanding the magnitude of nitrogen loading from different point and non-point sources as well as the footprint per area of watershed and in the receiving estuary or bay. In addition, the estimates of flushing time and delta-N help to understand potential mitigating factors for the receiving estuaries or bays. This NLM can be easily updated and applied to other watersheds in Nova Scotia or Atlantic Canada to compare nutrient loading rates across similar systems.

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