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Effect of nutrient pollution on dinoflagellate cyst assemblages across estuaries of the NW Atlantic

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Abstract

We analyzed surface sediments from 23 northeast USA estuaries, from Maine to Delaware, and nine estuaries from Prince Edward Island (PEI, Canada), to determine how dinoflagellate cyst assemblages varied with nutrient loading. Overall the abundance of cysts of heterotrophic dinoflagellates correlates with modeled nitrogen loading, but there were also regional signals. On PEI cysts of *Gymnodinium microreticulatum* characterized estuaries with high nitrogen loading while the sediments of eutrophic Boston Harbor were characterized by high abundances of *Spiniferites* spp. In Delaware Bay and the Delaware Inland Bays *Polysphaeridium zoharyi* correlated with higher temperatures and nutrient loading. This is the first study to document the dinoflagellate cyst eutrophication signal at such a large geographic scale in estuaries, thus confirming their value as indicators of water quality change and anthropogenic impact.

Keywords

Nutrient loading; plankton; Prince Edward Island; environmental indicators; water quality; palynology

1. Introduction

With increasing urbanization and growth of agriculture, nutrient pollution has become a major concern in many coastal regions (e.g., Carpenter et al., 1998; Rabalais, 2002). Excess nutrients (eutrophication) can result in increased phytoplankton production, harmful algal blooms, higher production of organic matter, depleted dissolved oxygen levels (hypoxia or

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anoxia), mass mortality of marine organisms, and changes in community structure (e.g., Turner and Rabalais, 1994; Turner et al., 1998; Glasgow and Burkholder, 2000; Anderson et al., 2008). These changes can be detrimental to marine ecosystems and affect the economic value of waters through loss of tourism, fishing, and aesthetic enjoyment. Eutrophication is becoming more widespread in the US (Scavia and Bricker, 2006), with approximately 65% of assessed estuaries now moderate to highly eutrophic (Bricker et al., 2007), while at least 40 estuaries of Canada's Prince Edward Island (PEI) have experienced one or more anoxic events in the last ten years (Bugden et al., 2014).

Understanding relationships between nutrient inputs and biological responses in estuaries are useful to determine how much nitrogen is required to change from one trophic status to another. Yet nutrient concentrations in coastal waters do not always reflect the total amount of nutrients entering a water body, as they can be rapidly taken up by phytoplankton and aquatic vegetation, such that even a heavily degraded water body may have low nutrient concentrations (e.g., Cloern, 2001; Rabalais et al., 2002; Dodds, 2003; Howarth and Marino, 2006; Bricker et al., 2007). There are two primary methods for evaluating nutrient input into, or effects on, coastal waters: 1) using nutrient loading models (e.g., Frink, 1991; Latimer and Chapentier, 2010; Seitzinger et al., 2010; Moore et al., 2011) and 2) using indicators to act as early warning signs of water quality degradation (e.g., Bricker et al., 2007; Castro and Freitas, 2011). The eutrophication condition of an estuary is commonly assessed using a combination of indicators such as chlorophyll-*a*, macro-algae, low bottom water dissolved oxygen, nuisance/toxic blooms, and submerged aquatic vegetation such as seagrasses (Short et al., 2006; Bricker et al., 2007). While incorporating multiple indicators of eutrophication, i.e., a tiered approach, is best for describing trophic status (e.g., Bricker et al., 1999, 2007; Bugden et al., 2014), acquiring data on multiple parameters is often challenging. Furthermore, many of these variables temporally vary, e.g., daily to seasonally, and thus require data collection to occur at frequent intervals to capture their annual range. Using sediment-bound fossilized microplankton as indicators of eutrophication alleviate many of the aforementioned shortcomings of alternative sampling methods. Organic-walled dinoflagellate cysts, which represent an integrated assemblage over time, have successfully been used as indicators of eutrophication in coastal waters (e.g., Dale and Fjellsa, 1994; Matsuoka et al., 2003; Pospelova et al., 2004; Pospelova and Kim, 2010; Lu et al., 2017) and provide biological data not available through conventional plankton surveys.

Dinoflagellates are an important component of planktonic communities. Approximately 50% of dinoflagellate species are heterotrophic and the rest are autotrophic or mixotrophic (Dale, 2009). Many dinoflagellates produce a resting cyst which, once produced in the water column, sinks to the sea-floor. Organic-walled dinoflagellate cysts are resistant to biological, chemical and physical degradation (e.g., Dale, 1996; Fensome et al., 1996a), unlike the motile stage which is easily degraded. Dinoflagellate cyst assemblages reflect the sea-surface conditions under which they were produced, including nutrient availability (e.g., Thorsen and Dale, 1997; Matsuoka et al., 2003; Pospelova et al., 2005).

Dale and colleagues first noted that dinoflagellate cysts indicated eutrophic conditions in Oslofjord, Norway (Dale and Fjellsa, 1994; Dale et al., 1999; Dale, 2009), where total cyst concentrations increased, as well as the relative abundance of *Lingulodinium*

machaerophorum. There, *Lingulodinium polyedrum* (the dinoflagellate producing *L. machaerophorum* cysts) blooms in the late summer, and its increase reflects increased nutrient availability at a time when they have historically been limiting (Dale, 2009). Increased abundances of *L. machaerophorum* also were documented by Sangiorgi and Donders (2004) in the northwestern Adriatic Sea and have been linked to cultural eutrophication. Near the Po River (Italy), Zonneveld et al. (2012) found that *L. machaerophorum* relative and absolute abundances increased around the time of the industrial revolution in Italy and the first production of ammonia in Europe.

In other regions, cysts produced by heterotrophic dinoflagellates become more abundant in eutrophic waters, specifically Protoperidinioid, Polykrikaceae, and diplopsalid species (e.g., Thorsen and Dale, 1997; Matsuoka, 1999; Kim et al., 2009; Krepekevich and Pospelova, 2010; Pospelova and Kim, 2010). Heterotrophic dinoflagellates have varied feeding strategies and prey preferences (e.g., Jacobson and Anderson, 1986; Hansen, 1991; Hansen and Calado, 1999; Naustvoll, 2000), thus the abundance and species composition of their prey influences the response of heterotrophic dinoflagellates to eutrophication. In addition, estuaries have different physical and environmental characteristics, therefore the response of dinoflagellate cysts to eutrophication in coastal waters may also depend on the type of estuary that is being investigated (Pospelova et al., 2002). A recent study by Price et al. (2016) has shown that dinoflagellate cyst assemblages in estuaries vary by biogeographic province, and that within a biogeographic province samples from the same estuary type generally had more similar assemblages compared to a different estuary type.

The primary objective of this study was to assess the relationship between nutrient loading and dinoflagellate assemblages across a gradient of eutrophication statuses in estuaries of the northwest Atlantic. For the first time modeled nutrient loading values were compared to cyst assemblages to determine if a relationship was present. We also investigated how the eutrophication signal varied by biogeographic province and estuary type. A previous study by Pospelova et al. (2002) in New Bedford Harbor and Apponagansett Bay (Massachusetts, USA) revealed an increase in the number of dinoflagellate cyst taxa from oligotrophic to mesotrophic conditions, and then a decline in taxa richness when highly eutrophic conditions occurred. We hypothesized that a similar pattern would be found among estuaries; that species diversity would increase with increasing nutrient loading, until a threshold is reached (highly eutrophic), followed by decreased diversity.

2. Study area

We examined 23 estuaries from the northeast USA, from Maine to Delaware, and nine estuaries from Prince Edward Island (PEI, Canada) (Fig. 1). This region encompasses the Acadian and Virginian biogeographic provinces (Hale, 2010), with the boundary between the two occurring at Cape Cod.

Prince Edward Island has over 100 estuaries, and for this study five estuaries were selected that display eutrophic symptoms (i.e., anoxic events, *Ulva* blooms) and four estuaries that did not exhibit these symptoms (Table 1). On PEI estuaries are either coastal embayments or lagoons. Many estuaries on the north shore are lagoons with spits or barrier islands partially

restricting tidal exchange. These estuaries also have lower tidal ranges (<1.5 m). The south shore is characterized by coastal embayment-type estuaries and greater tidal ranges (~1.25–2.5 m). The water depth in the studied estuaries is generally <10 m, with many <3 m. While winter estuarine sea-surface conditions have been only sparsely or not documented at all, some broad generalizations can be made regarding the study area. On PEI sea-ice typically is present for 1–3 months of the year, forming in estuaries in December or January and breaking up in February to March (Canadian Ice Service, 2016).

In the northeast US, 23 estuaries were selected from different estuary types (fjord, coastal embayment, river dominated, lagoon) and different eutrophic conditions (low to high) (Table 2). From Maine to Cape Cod, the dominant estuary types are fjords and coastal embayments. Highest tidal ranges occur in Maine (2.7–3.8 m) and are lowest near Cape Cod (2.4–2.9 m). Winter SSTs are ~2–4°C, with sea-ice forming during the coldest winters in waters north of Rhode Island Sound. In the Virginian province, estuaries are either coastal embayments, river-dominated or lagoons. The most southerly estuaries (e.g., New Jersey Inland Bays, Delaware Inland Bays) have a tidal range of <2 m and are generally <3 m deep.

The degree of seasonality differs within the study area. Many PEI estuaries have an average summer (June–September) SST of >20°C and maximum SSTs >28°C. Estuaries in Maine to Cape Cod have average summer SSTs ranging from 11 to 21°C, and in the Virginian province average summer SSTs range from 20 to 25°C. The Virginian province and PEI experience the greatest seasonal range in SSTs.

2.1 Land use in coastal watersheds and nutrient sources

Coastal watersheds are a major contributor to nutrients in coastal waters and play a key role in eutrophication (e.g., Bricker et al., 2007; Billen et al., 2011). Nutrients derived from urban and agricultural landscapes contribute significantly to cultural eutrophication. Nutrients released from different land use types may also have different seasonal patterns. For example, in some watersheds fertilization of crops is highly seasonal, whereas sewage discharge in urban centers is more consistent. Thus, as the dominant land use in NW Atlantic watersheds varies, the source of nitrogen (N) and phosphorous (P) from these watersheds is also variable. Agricultural land (2279%) and forests (11–72%) are the largest land use type in PEI, with urban or developed land contributing less than 8% (Table 1). In Maine, the watersheds are predominantly (>70%) forested, with smaller areas of wetland (1–13%), agriculture (1–12%), and urban cover (1–19%; Bricker et al., 2007). Of the nitrogen entering Maine estuaries 29–60% is derived from wet atmospheric deposition and 15–38% forested land (Alexander et al., 2008). Watersheds of Massachusetts Bay and Boston Harbor are more heavily populated and 75–80% of the drainage basin is urban. Greater than 90% of both nitrogen and phosphorus delivered to these two estuaries are derived from urban sources (Alexander et al., 2008). Cape Cod is primarily forested (60%) and urban (27%). Gardiners Bay, Delaware Bay and Delaware Inland Bays have the largest proportion of agricultural land (24–38%) in the northeast US (Bricker et al., 2007) (Table 2).

2.2. Nutrient loading

Nitrogen loading estimates for PEI estuaries were calculated according to Bugden et al. (2014). It is a simple loading model based on the principle of a Continuously Stirred Chemical Reactor (Bugden et al., 2014), henceforth called the Bugden model. The Bugden model assumes that nitrogen from runoff is limited on PEI and thus the N load is calculated for the groundwater component only. The steps in the model development can be summarized as followed. First, land use within each watershed was identified and categorized. Second, the annual leached nitrate concentration for each land use category was defined, and together with the land use areas, was used to calculate an area-weighted concentration. Third, nitrate loading from septic systems was estimated and converted to an equivalent leached nitrate concentration. Forth, the area-weighted concentrations and septic system equivalent leached nitrate concentrations were integrated with the average annual groundwater recharge to yield an annual groundwater nitrate load (Bugden et al., 2014). Recent N loading estimates using this model were provided by the PEI Department of Communities, Land and Environment (Cindy Crane, personal communication). They are listed in Table 1.

Nitrogen and phosphorus loading estimates for US estuaries were obtained from the 1992 national SPARROW Decision Support System (Alexander et al., 2008; SPARROW, 2012). This model adopts 1992 as the base year for analysis and the spatial infrastructure is provided by the enhanced Reach File 1 (E2RF1) stream network (Nolan et al., 2002). The nutrient load of each reach is determined by three main types of variables: source variables, land-to-water variables and removal rate variables. Nutrient sources include mean atmospheric deposition (N model only); urban pollution; 1992 National Land Cover Data estimates of shrub land, barren land and forested land; fertilization of corn/soy, alfalfa, wheat (N model only) and other crops; and manure fertilization. Land-to-water variables are those that mediate the amount of a contaminant that is transported from its source to streams, and removal rate variables are those that determine how much of the contaminant is transported through streams (Schwartz et al., 2011). The model uses long-term mean annual loads computed from monitoring data collected at 425 stations in the US from 1975–1995, detrended to the 1992 base year. The Estuary Drainage Area (EDA) code was used to obtain the nutrient loads from all the reaches within each drainage basin (Table 2). The incremental load from each reach was added together to obtain the total load entering each estuary. A full description of the model is provided by Schwarz et al. (2006) and the supplementary material of Alexander et al. (2008).

2.3 Estuarine eutrophication status

On PEI one parameter that is commonly used to diagnose the eutrophication status of an estuary is oxygen levels, where periodic anoxic events are an extreme symptom of eutrophication. When oxygen levels are depleted, some estuaries experience discolored and/or sulfur smelling water (Bugden et al., 2014). Other eutrophic symptoms experienced on PEI are the proliferation of *Ulva* and periodic microalgae blooms. Estuaries that are dominated by *Ulva* are more likely to experience anoxic events, compared to those that are dominated by eelgrass (Table 1). Bugden et al. (2014) classified PEI estuaries using a binary method, where estuaries that experienced one or more anoxic events were classified as

anoxic, while those that had not were classified as non- anoxic. Five anoxic and four oxic estuaries were selected from different estuary types. For purposes of comparing the eutrophication status among all studied NW Atlantic estuaries, we consider PEI estuaries that are dominated by *Ulva* and that have experienced one or more anoxic events to be moderately high to highly eutrophic.

Bricker et al. (1999) report the eutrophication status of northeast US estuaries, and assign an overall eutrophic condition of low, moderately low, moderate, moderately high, or high to each estuary. They use a combination of primary symptoms (e.g., high chlorophyll *a* and macro-algae abundance) and secondary symptoms (e.g., low bottom water dissolved oxygen, the presence of toxic or nuisance blooms, and loss of submerged aquatic vegetation) to determine the overall eutrophic condition of each estuary. The eutrophic status of US estuaries in this study is listed in Table 2.

2.4 Sediment collection and hydrographic and sediment chemistry data

Sediment collection and measurements of sea-surface parameters are described fully by Price et al. (2016). Sediment samples from PEI were collected using an Ekman dredge the summer of 2013, and 18 samples with surface salinities >20 psu were chosen for this study (Fig. 1). Northeast US surface sediments were collected using a 0.04 m² Young-modified Van-Veen grab or similar sampler, by the US Environmental Protection Agency (EPA) in summer 2000 (Strobel, 2000). A total of 61 samples were selected from sites also with a summer sea surface salinity (SSS) >20 psu (Fig. 1). At all sites the top 2 cm of sediment was retained for analysis, which represents approximately 6 to 20 yrs of accumulation based on sedimentation rates $\sim 0.1\text{--}0.3\text{ cm yr}^{-1}$ (Fitzgerald, 1980; Sonshine, 2012). The number of samples per estuary ranges from one to nine.

Sea-surface temperature and salinity from PEI estuaries were measured biweekly during summer 2013, using a YSI VD 6600 multi-parameter sonde (Yellow Springs, OH, USA). Longterm hydrographic data from the US were obtained from sites located near the sediment sampling locations, that were collected by numerous estuary interest groups and citizen scientists. Only summer (June-September) measurements were used as data from other seasons were not consistently collected.

Residence times for the US estuaries were obtained from Bricker et al. (2007) and have units of days. A proxy for residence time was calculated for PEI estuaries using a tidal prism model employing estuarine volume, mean tidal amplitude and freshwater input (Table 1). Increased residence time corresponds to increased estuarine volume with respect to freshwater input plus tidal exchange.

Biogenic silica (BSi), a proxy for diatom productivity, was measured using the standard wet-alkaline procedure (Mortlock and Froelich, 1989) at the Pacific Center for Isotopic and Geochemical Research at the University of British Columbia. Only relative abundances are reported as the sedimentation rate is not well constrained.

2.5 Palynological sample preparation and microscopy

Sediments were treated using a standard palynological method (Pospelova et al., 2005; Price et al., 2016). In brief, a known volume of sediment was rinsed, oven dried, and weighed. *Lycopodium clavatum* spores were added prior to sample treatment to enable the determination of cyst concentrations. Room temperature hydrochloric and hydrofluoric acid were used to remove carbonates and silicates. The residue was sieved using 15 µm and 120 µm nylon mesh to remove both coarse and fine fractions, and sonicated for less than 90 sec. Aliquots of the residue were mounted in glycerine jelly, and cysts were counted at 400x to 600x magnifications. A minimum of 111 cysts were counted in each sample, with an average of 327. Cyst concentrations were calculated using the dry weight of the sample.

The motile stage is not known for some of the dinoflagellate cyst species found in this study, therefore the paleontological nomenclature is used. Dinoflagellate cysts were identified according to the taxonomy described by Lentin and Williams (1993), Rochon et al. (1999), and Zonneveld and Pospelova (2015). We identified *Lejeunecysta olvia* as depicted in Pospelova et al. (2004; 2005). Cysts of *Gymnodinium microreticulatum* were identified based on their small size (~20–28 µm) and reticulation. Our category of spiny brown cysts (SBCs) also includes cysts of cf. *Archaeoperidinium minutum* sensu Yamaguchi et al. (2011). Cysts of cf. *Biecheleria* sp. were enumerated but not included in total cyst counts due to their small size (~8–30 µm) and potential loss through the 15 µm sieve. Cysts were identified to the species level where possible and photos of most taxa are included in the supplementary material (Plates 1–4). The cyst taxa recorded in this study and their corresponding motile stage is found in Table 3. Total cyst counts and relative abundance data are provided in the supplementary material (Tables S1 and S2).

2.6 Statistical analyses

Constrained ordination was performed on dinoflagellate cyst relative abundances and environmental data using CANOCO 5.0 for Windows (ter Braak and Šmilauer, 2012). First, the nature of variability within the cyst assemblage was tested using Detrended Correspondence Analysis (DCA) to determine whether the assemblage show unimodal (length >2) or linear (length <2) variation. The length of the first gradient was 2.19 standard deviations, indicating unimodal variation and justifying the use of Canonical Correspondence Analysis (CCA). Canonical Correspondence Analysis is a direct gradient analysis that uses a set of response variables (species data) and the explanatory variables (environmental data) to show if there is a relationship between them. Only taxa with relative abundances >1% were included in the analysis. Dinoflagellate cyst relative abundances were logarithmically transformed to increase the statistical weight of species that occur at lower abundances. Nitrogen loading (not normalized) was included in all analyses. Phosphorus loading (not normalized) was unavailable for PEI estuaries and thus was only included in the US Acadian and Virginian CCAs. Variables that are statistically significant ($P < 0.05$) in explaining variation in the cyst assemblage were determined using a forward selection Monte Carlo permutation test, with 499 unrestricted permutations.

Correlations between N loading, species diversity and the abundance of cysts of heterotrophic taxa were investigated using regression analysis in IBM SPSS. Species

diversity indices were calculated for each sample, including the species or taxa richness (S), Shannon-Weiner index (H), Simpson index of diversity (1-D) and the evenness (E). Calculating different diversity indices is advantageous as some only consider the number of taxa (S), while others also include abundance data (H, 1-D, E). Henceforth we refer to species richness as taxa richness, as it was not possible to identify all cysts to the species level. These calculations and their descriptions are found in the supplementary material. Regressions between the species diversity indices and SSS, the percentage of heterotrophic taxa, and nitrogen loading were performed on all samples, and then separately for each biogeographic province. Simple and partial regressions were performed using Minitab Express and IBM SPSS to determine if a statistically significant relationship between nitrogen loading and cysts produced by heterotrophic taxa is present. Normality was tested with Anderson-Darling. Modeled N loading values were normalized by the estuary area, estuary volume and watershed area. They were subsequently logarithmically transformed to meet the assumption of normality. To avoid pseudoreplication the relative abundance of heterotrophic taxa were averaged from each entire estuary (n=29). In addition, to determine if the sampling location within the estuary was important, averages were calculated using samples from the upper (n=17), middle (n=25), or lower (n=24) sections of each estuary. The number of estuaries included in each analysis varied, as some estuaries did not have samples in, for example, the upper section, in which case, they were not included in that particular analysis.

3. Results

Dinoflagellate cyst assemblages varied by region. PEI had the greatest proportion of cysts produced by heterotrophic taxa (45–93%) compared to the US Acadian province (4–70%) and the Virginian province (3–71%). The most common heterotrophic taxa overall were *Brigantedinium* spp., *Islandinium? cezare*, and *I. minutum*. Cysts produced by autotrophic taxa, in particular *Spiniferites* spp. and *Operculodinium centrocarpum*, dominated in the northeast US.

Cyst concentrations were variable throughout the study, ranging over three orders of magnitude from 93 to 337387 cysts g⁻¹. This can be explained in part by differences in grain size throughout the study region, with the proportion of silt ranging from 3.7% to over 90%, as well as probable differences in sediment accumulation rates. For these reasons this study focuses on relative abundance data.

The relationship between the relative abundance of heterotrophic taxa and nitrogen (N) loading was investigated. Statistically significant relationships were found when N loading was logarithmically transformed. In general, the correlations were stronger, with higher R and lower p-values, when quadratic or cubic regressions were used instead of linear regressions (Table 4). Nitrogen loading was normalized to the estuary area, estuary volume and watershed area. The relationship between the relative abundance of heterotrophic taxa and N loading normalized to the estuary area was statistically significant, while N loading normalized to the estuary volume yielded less significant results. The highest degree of correlation occurred when N loading was normalized to the watershed area (Table 4). To determine if the type of estuary had impact on the relationship between heterotrophic taxa

and N loading, partial correlations were conducted, controlling for estuary type. However, the partial correlation values when the type of estuary was controlled for were similar to the Pearson correlation coefficients (Table 4). The same statistics were performed for heterotrophic taxa averaged from the upper, middle and lower sections of each estuary. The most statistically significant correlations in all three sections occurred when N loading was normalized to the watershed area (Tables S3-S5).

Canonical correspondence analysis of all samples showed that samples from the Acadian and Virginian biogeographic provinces formed two distinct clusters, and that, within the Acadian province, samples from PEI and the US could be distinguished. Regional differences appeared to overwhelm the nutrient signal as eutrophic estuaries showed no discernible pattern, thus each region was analyzed independently in subsequent analyses. Similarly, diversity indices regressions were weaker when all samples were included, thus only the results of the regional analyses are shown in Table 5. The CCA and regression analyses demonstrate that N loading correlates to cyst taxa, but that regional signals are present. Cyst assemblage descriptions are also presented at the regional level to better understand how they vary between regions, and to be able to establish indicator species of nutrient enrichment.

3.1 Acadian biogeographic province - Prince Edward Island

Cyst assemblages were dominated by *Brigantedinium* spp. (7–43%), *Islandinium? cezare* (0.6–26%), *I. minutum* (0.5–26%), SBCs (3.8–44.8%), *Spiniferites* spp. (0–37%), *S. ramosus* (0.5–30.9%), cyst of *P. dalei* (0–11.6%), and *Dubridinium* spp. (0–8.7%). A total of 42 taxa were recorded, with taxa richness ranging from 13 to 26. Some estuaries or individual samples were dominated by one species. For example, samples from Mill estuary were composed of 4 to 22% cysts of *G. microreticulatum*. Total cyst concentrations were generally low and ranged from 230 to 11,940 cysts g⁻¹, averaging 3360 cysts g⁻¹ (SD 3785 cysts g⁻¹). Samples with summer salinities below 22 psu had much higher concentrations (6440–14,290 cysts g⁻¹) of cysts of cf. *Biecheleria* sp. compared to the remaining PEI samples (<1500 cysts g⁻¹).

All samples in this study were selected from sites with average summer SSSs > 20 psu; however, a greater number of PEI sites had lower (21.5–28.9 psu) average summer SSSs, compared to NE US estuaries which commonly had salinities over 30 psu (Price et al., 2016). Statistically significant linear and quadratic relationships were found between salinity and the Simpson Index of Diversity and Evenness (Table 5).

Residence time and nitrogen loading were statistically significant variables influencing the cyst assemblages, explaining 18.6% and 9.5% of the variation, respectively. Summer SSS and tidal range were nearly significant with p-values of 0.064 and 0.066, and explain 8.6% and 7.6% of the variation respectively. Cysts of *G. microreticulatum* showed the highest degree of correlation to N loading (Fig. 3 A). Cysts of *P. oblongum*, cysts of *P. kofoidii*, and *N. labyrinthus* correlated to higher summer SSTs, greater residence times, lower tidal range, and lower summer SSS. As expected, samples from lagoons generally correlated with parameters characteristic of such estuaries: greater residence times, lower tidal ranges, lower SSS, and higher summer SST (Fig. 3B). Coastal embayment samples were spread around

the bottom left-periphery of the CCA diagram and correlated better to higher tidal ranges and SSSs (Fig. 3B). Eutrophic estuaries were located primarily on the right side of the diagram and correlated with greater residence times, lower tidal range, lower SSS, warmer SSTs (Stanley and Wheatley) or high nitrogen loading (Mill) (Fig. 3B).

3.2 Acadian biogeographic province - Cape Cod to Maine (USA)

In the US part of the Acadian biogeographic province the taxa richness ranges from 13 to 29, and while no relationship was found between species diversity and salinity, a statistically significant linear and quadratic relationship was found between the relative abundance of heterotrophic taxa and the Shannon-Wiener index, Simpson Index of Diversity, and Evenness (Table 5). Cyst assemblages were dominated by the autotrophic *O. centrocarpum* (0.9–72.4%), cyst of *P. dalei* (0.8–23.7%) and *Spiniferites* spp. (2.4–30.5%) as well as the heterotrophic *Brigantidium* spp. (0.5–24.1%), *Dubridinium* spp. (0.2–29.1%), and *Selenopemphix quanta* (0–8.9%).

In this region, average summer SST and annual N loading are statistically significant parameters, and explain 8.2% and 9.3% of the variation respectively. Most of the species are clustered near the center of the CCA diagram (Fig. 3C) except *O. israelianum* which is located in the lower right hand side of the diagram, correlating with warm SSTs, higher SSSs and nutrient loading, and low residence time. Eutrophic estuaries are generally ordinated in the lower half of the diagram (Fig. 3D), with those from fjord type estuaries clustering together near high N and P loading, lower SSTs, and shorter residence times. Boston Harbor is the only coastal embayment type estuary in this region that is eutrophic. It is ordinated in the far lower right hand side of the diagram, and correlates to high N and P loading, and low water depth and residence time. The other samples from coastal embayments cluster together in the upper part of the diagram near higher SSTs and lower nutrient loading, while most fjord type estuaries are located in the lower part of the diagram correlating to lower SSTs, higher nutrient loading and lower residence time.

3.3 Virginian biogeographic province - Delaware to Cape Cod

Taxa richness is highest in the Virginian biogeographic province and ranges from 16 to 35. A statistically significant linear and quadratic relationship was found between the relative abundance of heterotrophic taxa and diversity (Table 5). Samples were dominated by autotrophic taxa, in particular *Spiniferites* spp. (7.5–50%), *O. centrocarpum* (1–50%), *Achomosphaera* spp. (0.8–23%), and cysts of *P. dalei* (0–21%). In the two most southerly estuaries, Delaware Bay and Delaware Inland Bays, *P. zoharyi* contributes 3–46% of the total assemblage. Heterotrophic taxa such as *I. minutum* and *Lejeunecysta* spp. are most abundant in Narragansett Bay.

In the Virginian province tidal range, N loading, P loading and summer SST are all statistically significant variables and explain 7.9%, 10.3%, 6.2% and 5.4% of the variation, respectively. Most autotrophic taxa, especially *P. zoharyi*, are ordinated on the right side of the diagram near lower tidal range, higher SSTs and increased nutrient loading. Estuaries that are moderately high to highly eutrophic (Delaware Inland Bays, Great South Bay, Gardiners Bay) are all located on the right hand side of the diagram towards low tidal range

(Fig. 3E). Delaware Inland Bays correlates strongly with higher SSTs and nutrient loading, while samples from Great South Bay and Gardiners Bay are ordinated closer to higher SSSs and lower residence times (Fig. 3F).

4. Discussion

Our results indicate that cyst assemblages reflect nutrient loading, but that the dominant taxon varies by region and estuary. When analyzed together the biogeographic signal dominates, prompting independent analyses within each biogeographic province. Our discussion focuses on the eutrophication signal at both the regional and inter-estuary scale.

4.1 Eutrophication signal

Nitrogen loading statistically relates to cyst assemblages in all three regions - PEI, Maine to Cape Cod, and Cape Cod to the Delaware Inland Bays. The species that best correlate with nutrient loading differed between estuaries, demonstrating region specific eutrophication signals. This may be partially attributed to differences in SSSs and SSTs between regions, which influence species assemblages in addition to nutrient and prey availability. Furthermore, estuaries display spatial heterogeneity (e.g., Kennedy et al., 2017) which can make it challenging to determine relationships between nutrient loading and estuarine cyst assemblages as nutrient loads are calculated at the watershed scale, and are not site-specific. For example, an estuary may have high nutrient loading, but if the water residence time is short or if nutrients are removed through processes such as denitrification (Nixon et al., 1996), sites away from the main source of nutrients may not display eutrophication symptoms (e.g., Tyron). In contrast, if an estuary has low nutrient loading, but a long residence time, nutrients and organic matter may accumulate and result in eutrophication symptoms (e.g., Stanley). Water residence times can therefore be an important consideration, especially for eutrophic estuaries. In some cases, environmental parameters and cyst assemblages display more intra-estuary variation than inter-estuary variation.

Many studies around the world have shown an increase in heterotrophic taxa associated with both cultural eutrophication (Thorsen and Dale, 1997; Matsuoka, 1999; Pospelova et al., 2002; Krepakevich and Pospelova, 2010; Pospelova and Kim, 2010) and with naturally occurring upwelling of nutrient rich water (e.g., Radi and de Vernal, 2004; Pospelova et al., 2006; Bouimetarhan et al., 2009; Bringue et al., 2013). The heterotroph signal includes taxa such as *Brigantedinium* spp., *Dubridinium* spp., cysts of *Polykrikos*, and *Selenopemphix quanta* found in subpolar to equatorial regions (e.g. Zonneveld et al., 2013). In sediment traps these species can be found year round in many regions and become more abundant when biogenic silica, a proxy for diatom abundance, increases (e.g., Pospelova et al., 2010; Zonneveld et al., 2010; Price and Pospelova, 2011; Bringue et al., 2013). These heterotrophic taxa appear to be more responsive to changes in prey availability (e.g., diatoms) than temperature (e.g., Zonneveld et al., 2010). The heterotrophic signal is evident in regions where the following three conditions are met: (1) the heterotrophic dinoflagellates are limited by prey composition and/or abundance, (2) their prey are nutrient limited and (3) the autotrophic taxa do not increase more or outpace the heterotrophic taxa. If any of these

conditions are not met, an increase in heterotrophic taxa with nutrient loading should not be expected to occur.

To determine if the heterotrophic signal is present in NW Atlantic estuaries we compared cysts of heterotrophic taxa to N loading and BSi. No relationship was found between heterotrophic taxa and BSi% ($R = 0.077$), however if we had flux data this relationship might improve. A relationship was found between heterotrophic taxa and N loading when N loading was logarithmically transformed ($R = 0.556$, $p = 0.002$) and further improved when nitrogen loading was normalized by the watershed area ($R = 0.739$, $p < 0.001$). Nitrogen loading normalized to the watershed area or nitrogen “yield” provides an indication of the relative intensity or concentration of N occurring within a watershed (e.g., Lloyd et al., 2016). Nitrogen yields are typically higher in watersheds that are predominately urban (Kinney and Valiela, 2011) or agricultural land (Heggie and Savage, 2009). Similar to Heggie and Savage (2009) we find N yields increase with the proportion of agricultural land ($R = 0.823$, $p < 0.001$). PEI has the largest N yields (Fig. 2) due to small watershed size and a high percentage (42–78%) of agricultural land in many watersheds (Table 1). The greater correlation between heterotrophic taxa and N yields compared to N loading suggests that heterotrophic cysts better reflect the relative N concentration than the total N loading.

Multivariate analysis showed that eutrophic estuaries tended to cluster together in CCA (Fig. 3), suggesting they have similar assemblages; however, the eutrophic estuaries are not all characterized by a greater abundance of heterotrophic taxa. On PEI heterotrophic taxa such as cysts of *G. microreticulatum* and cysts of *P. kofoidii* are more abundant in four of the five eutrophic estuaries. In contrast, the autotrophic *P. zoharyi* correlates with both higher SSTs as well as nutrient loading in the Virginian biogeographic province.

It appears there is no distinct cyst assemblage that characterizes estuaries with a low eutrophic status. The estuaries with the lowest N loading on PEI, Enmore and Murray, do not have similar assemblages. Enmore is characterized by high abundances of *O. israelianum* (19%) while Murray has high abundances of *Lejeunecysta* (up to 33%). Three fjord-type estuaries in the US section of the Acadian biogeographic province have a eutrophic condition of low (Penobscot) or medium-low (Blue Hill and Muscongus), while in the Virginian biogeographic province four estuaries (Buzzards Bay, Narragansett, New Jersey Island Bays, Delaware Bay) have a eutrophic condition of medium-low (Bricker et al., 2007). These US estuaries also do not appear to have a unique set of taxa.

4.1.1 Prince Edward Island - Acadian Biogeographic province—There is no single dinoflagellate cyst taxon, or group, that correlates with nitrogen loading on PEI. Instead, a few taxa (cysts of *G. microreticulatum*, cysts of *P. kofoidii*, *N. lahyrinthus*, *Spiniferites* spp., and *S. mirabilis*) appear to reflect nutrient enrichment (Fig. 3 A). On PEI, Mill, West, and Wilmot estuaries all have high levels of nitrogen loading (Table 1), yet only Mill and Wilmot display eutrophic symptoms. This can be partially explained by differences in residence time. West has a shorter residence time compared to Mill, which has a residence time that is approximately three times greater (Table 1). On PEI, cysts of *G. microreticulatum* are only present in these three estuaries (Mill, West and Wilmot). This suggests *G. microreticulatum* may be an indicator of high nutrient loading, at least under

conditions similar to those on PEI. Stanley, Wheatley and Souris estuaries also are eutrophic (Table 1). Stanley and Wheatley estuaries experience moderate nitrogen loading (84497 and 65617 kg N yr⁻¹, respectively) and tend towards lower oxygen conditions, while Souris has lower nitrogen loading (49791 kg N yr⁻¹) and greater flushing. These three (Stanley, Wheatley, and Souris) eutrophic estuaries also have greater residence times than many of the non-eutrophic PEI estuaries (Table 1). While they do not contain cysts of *G. microreticulatum*, they are each characterized by a unique set of taxa. Stanley has a higher abundance of *N. labyrinthus* (16.8%), cysts of *P. kofoidii* (54%), and cysts of *P. oblongum* are present. Cysts of *P. kofoidii* is often abundant in nutrient rich waters (e.g., Matsuoka et al., 1999; Pospelova et al., 2005) and therefore its higher abundance in the eutrophic Stanley estuary is not surprising. Wheatley was characterized by higher abundances of *S. mirabilis* (10.4%) and *Spiniferites* spp. (37.8%). *Spiniferites* spp. is often abundant in estuaries (e.g., Pospelova et al., 2002, 2005; Price and Pospelova, 2011) and is known to tolerate a large range in SSSs (e.g., Pospelova et al., 2002, 2005) and highly eutrophic conditions (Pospelova et al., 2002).

High abundances of *I. minutum* were found on PEI and correlate with N loading normalized to the watershed area. On PEI, where sea-ice is present <3 months per year, the commonly cited “sea-ice indicator” *I. minutum* comprises up to 26% (average 12%) of the assemblages. Such high abundances are not often observed in regions with <7 months of ice cover (Radi and de Vernal, 2008); however, similar abundances have been reported in Hudson Strait (Heikkila et al., 2014) where sea-ice coverage is 2–3 months. Instead of the duration of ice cover, Heikkila et al. (2014) found that fluxes of *I. minutum* correlated with fluxes of BSi (diatom production), but we did not. However, we do not have flux data (absolute values) for either BSi or cysts. Instead we find a positive correlation between the relative abundance of *I. minutum* and nitrogen load (kg N ha⁻¹ yr⁻¹) (linear regression R = 0.760, p<0.001) further supporting that nutrients/prey are an important factor influencing their abundance.

Bates and Strain (2006) also found no clear relationships between individual phytoplankton species or groups and nutrient concentrations in PEI estuaries. There are many factors that may partially explain the lack of relationships between plankton (or cysts) and nutrients on PEI. First, Bates and Strain (2006) found that the limiting nutrient(s) on PEI varied temporally within an estuary, as well as between estuaries. Whether an estuary is limited by P, N, Si, or experiences co-limitation will have an influence on the dinoflagellate community as well as their prey (i.e., diatoms) composition and abundance. Second, the time of year and frequency with which nutrients are delivered may be important. Most of the nitrate delivered to PEI estuaries is through groundwater discharge (Bugden et al., 2014), but if a major runoff event were to occur shortly after crop fertilization for example, additional nutrients may enter both the surface and groundwater runoff (Smith et al., 1990). Watersheds may experience nutrient pulses at different times of the year. The taxa capable of exploiting nutrient pulses may vary according to other factors, such as temperature. Thus, the timing of a nutrient pulse can have different responses. This has been observed in Oslofjord (Norway) where *Lingulodinium polyedrum*, which blooms in the late summer, was found to increase in response to nutrients added to the system at a time when they would otherwise be limiting (Dale, 2009). Third, factors other than nutrients may more strongly affect the cyst signal. Salinity and temperature are known to be significant parameters influencing cyst

assemblages in many regions (Zonneveld et al., 2013). The large range in salinity and temperature may be more important parameters influencing the cyst assemblages in these estuaries. Last, the environmental data and nutrient loading estimates may not be detailed enough. Lack of water quality data from spring and fall, as well as only having one nutrient loading estimate per estuary may contribute to the unclear relationship between the cyst assemblages and eutrophication.

4.1.2. Maine to Cape Cod - Acadian biogeographic province—In the US part of the Acadian biogeographic province Sheepscot Bay, Casco Bay, Englishman Bay, Narraguagus Bay and Boston Harbor all have a eutrophic condition that is deemed moderately high (Bricker et al., 1999). Of these, only Boston Harbor is a coastal embayment, while the other four are fjord type estuaries. The watershed that drains into Boston Harbor is over 75% urban and contains over one million people, the highest population in the Acadian province (Bricker et al., 2007). As a result, over 90% of the N and P loading entering the Harbor is derived from urban sources and thus is consistent through the year. The cyst assemblage from the Harbor is characterized by high abundances of *Spiniferites* spp. (30.5%) and the presence of *O. israelianum*. Both the sample and *O. israelianum* correlate with high nutrient loading and warm summer SSTs (Fig. 3D). The presence of *O. israelianum* (3%) is important for distinguishing Boston Harbor from other estuaries but it is unlikely a good indicator of eutrophication as it is found at greater abundances (5–19%) at oligotrophic sites (e.g., Enmore and Tryon, PEI). Indeed, its presence may be more indicative of warm SSTs (Pospelova et al., 2004).

The four fjords that are considered eutrophic - Sheepscot Bay, Casco Bay, Englishman Bay and Narraguagus Bay (Bricker et al., 1999) all have watersheds that are predominately forested (>70%), with most of the nitrogen coming from nitrate wet atmospheric deposition and from forested land. These bays are dominated by *O. centrocarpum*, which is a cosmopolitan species (Zonneveld et al., 2013). *Operculodinium centrocarpum* has been found at high abundances in other fjord type estuaries. For example, Radi et al. (2007) found high relative abundances of *O. centrocarpum* in Effingham and Seymour-Belize Inlets (British Columbia, Canada), both of which experience seasonal upwelling and have high primary productivity. It does not appear that there are any specific taxa that characterize these eutrophic fjord-type estuaries. Despite this, samples from Sheepscot Bay, Casco Bay, Englishman Bay and Narraguagus Bay are clustered together, suggesting there may be small differences in the cyst assemblages that characterize these eutrophic estuaries.

4.1.3. Cape Cod to Delaware Inland Bays - Virginian biogeographic province—In the Virginian province Gardiners Bay, Great South Bay, Delaware Inland Bays and Long Island Sound are considered eutrophic (Bricker et al., 1999). Delaware Inland Bays is the only U.S. estuary with an overall eutrophic condition of high. It has been classified as such due to the abundance of macro-algae, the presence of nuisance toxic blooms, as well as moderate levels of chlorophyll *a* (Bricker et al., 1999). The watershed surrounding the Delaware Inland Bays has the largest percentage (38%) of agricultural land in the Virginian province. Delaware Inland Bays are characterized by high abundances of *P. zoharyi* and correlates with nutrient loading. *Pyrodinium bahamense*, the motile stage dinoflagellate

responsible for producing *P. zoharyi* cysts, can cause paralytic shellfish poisoning (e.g., Rouse, 2007). The correlation of *P. zoharyi* with high nutrient loading supports previous studies (e.g., Usup et al., 2012) which suggest the potential importance of nutrients in encouraging bloom formation in coastal waters. The nutrient load delivered to the Bays during summer months is likely to be most significant to this warm water species, as it only forms blooms in waters with SSTs >20°C.

Both the eutrophic Gardiners Bay and Great South Bay have relatively high abundances of *Spiniferites* spp., which has been found in high abundances in other eutrophic NW Atlantic estuaries (Boston Harbor, Wheatley - this study; Buzzards Bay - Pospelova et al., 2005). However, *Spiniferites* spp. have a large tolerance to salinity and estuary trophic condition, and also are found in high abundances in non-eutrophic estuaries (e.g., Pospelova et al., 2004; 2017). Only one sample is from Long Island Sound and was collected on the very eastern edge of the Sound, away from the main source of nutrients and therefore likely does not adequately represent the state of the estuary. This may partially explain why it does not plot near the other samples from eutrophic estuaries (Fig. 3F).

4.2 Variation in species diversity

On PEI, a decrease in Simpson's index of diversity and Evenness was found with decreasing salinity (Table 5). Previous studies in the NW Atlantic estuaries have identified low SSSs (Pospelova et al., 2004) and/or a high degree of eutrophication and pollution of xenobiotics (Pospelova et al., 2002) as correlates to decreased or low taxa richness in New England (USA) estuaries. In New England lagoons where summer SSSs varied from 3.6 to 29 psu, taxa richness was positively correlated to mean summer salinity (Pospelova et al., 2004). In contrast, estuaries in the Acadian province from Maine to Cape Cod show only a low correlation ($R^2 = 0.175$, $p = 0.034$) between SSS and taxa richness, and no correlation ($R^2 = 0.002$, $p = 0.820$) in the Virginian zone. The low correlation is likely due to the small range in SSS (20–33.1) and may explain the lack of relationship between salinity and diversity. However, there is a linear or quadratic correlation between diversity indices (Shannon-Wiener, Simpson Index of Diversity, and Evenness) and the relative abundance of heterotrophic taxa. Of the diversity indices used in this study, those that include abundance information (H, 1-D, E), were found to better correlate to SSS than taxa richness.

No relationship was found between species diversity and nutrient loading (Table 4), but the most highly eutrophic estuary in the NE US, the Delaware Inland Bays, had the lowest species richness in the Virginian province. Most estuaries in this study have a eutrophic status of medium- low to medium-high (Table 2). If additional estuaries from the extremes, either oligotrophic or highly eutrophic, were included in the analysis perhaps a relationship would be found.

5. Conclusions

Cyst assemblages reflect nitrogen loading in northwest Atlantic estuaries. A positive relationship was found between the proportion of heterotrophic taxa and logarithmically transformed nitrogen loading, and further improved when nitrogen loading was normalized by the watershed area. Our study also shows that eutrophication signals vary geographically.

In the Virginian biogeographic province, the warmest in the study region, *Polysphaeridium zoharyi* was indicative of both warmer summer sea-surface temperatures and high nutrient loading. In the US region of the Acadian biogeographic province eutrophic Boston Harbor stands out due to the abundance of *Spiniferites* spp. and the presence of *Operculodinium israelianum*. While *O. israelianum* correlates with nutrient loading in Boston Harbor this result may be confounded by high SSTs, as it is also observed in Enmore (PEI), a low nutrient estuary. On PEI, eutrophic estuaries are characterized by a large increase in the abundance of cysts of *Gymnodinium microreticulatum* (Mill), or *Nematosphaeropsis labyrinthus* and cysts of *Polykrikos kofoidii* (Stanley), or *Spiniferites* spp. and *S. mirabilis* (Wheatley).

There is no single species that characterizes eutrophic estuaries or high nutrient loading in NW Atlantic estuaries; but there one or two regionally specific taxa that are dominant. Differences in the temperature and salinity between the eutrophic estuaries may explain some of the response of different taxa to eutrophication. Further studies should examine the timing of nutrient delivery and the ratio of N:P, as an overabundance of N might cause P limitation in some estuaries (and during certain seasons). Nutrients and SSS are sometimes coupled, making it difficult to separate the signal (Pospelova et al., 2004; 2005).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Dinoflagellate cyst eutrophication signal was studied in 32 NW Atlantic estuaries
- Nutrient loading is a significant predictor of cyst assemblages
- Abundance of cysts of heterotrophic dinoflagellates increased with N loading
- Indicator taxa varied regionally

Figure 1.

(A) Map of the study area. The dashed line shows the boundary between the Acadian and Virginian biogeographic provinces. B-E) Insets of the study area. Solid dots indicate sampling locations. (D) 1. Chatham Harbor. 2. Popponesset Bay. 3. Waquoit Bay. 4. Vineyard Pond. (Figure modified from Price et al. (2016)).

Figure 2.

Linear regression between logarithmically transformed nitrogen loading normalized to the watershed area (kg N/ha/yr) and the proportion of heterotrophic taxa averaged from each estuary (n=29). The symbols differentiate between the estuary types, and the colors distinguish the eutrophication status of each estuary (red = medium high or high, yellow = medium, green = medium low or low). Samples from PEI are located in the grey oval.

Figure 3.

Canonical Correspondence Analysis (CCA) ordination diagrams of logarithmically transformed dinoflagellate cyst relative abundances and environmental variables. Statistically significant variables ($P < 0.05$) environment variables are shown with solid arrows. Average summer sea-surface temperature (SST), average summer sea-surface salinity (SSS), residence time, water depth, tidal range, biogenic silica % (BSi), and annual nitrogen load (N load). Only US estuaries have measurements of annual phosphorus load (P load). Samples from eutrophic estuaries are shown by solid red symbols. Species

abbreviations and full names can be found in Table 3. (A) and (B) Prince Edward Island species and sample scores. (C) and (D) the US part of the Acadian biogeographic province species and sample scores. (E) and (F) Virginian biogeographic province species and sample scores.

Table 1.

Edward Island estuaries and their residence time, watershed land use, annual nitrogen load, and if they have experienced an anoxic event in the past years. All embayments are coastal.

Estuary	Estuary Code	Estuary type	Residence time (Estuary volume/ tidal volume + freshwater input)	Total watershed area (Ha)	Agriculture (%)	Developed land (%)	Forest (%)	Salt marsh (%)	Sand dunes (%)	Freshwater wetland (%)	Annual N load (x10 ³ kg·yr ⁻¹)	Anoxic event in the last 10 years	Dominant vegetation
MUR		Lagoon	2.31	7027	21.6	6.2	66.9	0.1	0.1	5.0	36	No	Elgrass
ENM		Embayment	0.56	4586	15.6	3.5	71.8	0.3	0.0	8.8	17	No	Elgrass
WES		Embayment	0.82	18576	51.4	5.9	41.2	0.7	0.0	0.8	155	No	Elgrass
COL		Lagoon	1.85	5251	42.7	7.6	46.4	0.2	0.2	2.9	50	Yes	<i>U/va</i>
TRY		Embayment	0.24	5249	68.4	4.4	24.0	0.8	0.0	2.5	105	No	none
WIL		Embayment	0.73	8291	78.7	7.4	11.4	0.2	0.0	2.3	222	Yes	<i>U/va</i>
MIR		Lagoon	2.72	12039	42.1	5.6	48.1	0.0	0.0	4.1	213	Yes	<i>U/va</i>
NLB		Lagoon	3.80	9222	51.2	5.4	42.0	0.1	0.0	0.6	84	Yes	<i>U/va</i>
RUS		Lagoon	1.74	5972	74.4	4.7	20.0	0.2	0.0	0.8	66	Yes	<i>U/va</i>

Northeast US estuaries, estuary drainage area (EDA) code, estuary type where all embayments are coastal, residence time, land use, annual nitrogen and phosphorous loads, and eutrophic condition where L = low, ML= moderately low, M = moderate, MH = moderately high, and H = high.

Table 2.

	Estuary	EDA	Estuary type	Residence time (days)	Watershed area (km ²)	Urban (%)	Agriculture (%)	Forest (%)	Wetland (%)	Range (%)	Barren (%)	Total N Load (×10 ³ kg. year ⁻¹)	Total P Load (×10 ³ kg year ⁻¹)	Eutrophic Condition
Acadian biogeographic province	Englishman Bay	N020x	Fjord	5	2297	1.0	4.2	83.9	9.8	1.1	0	1053	56	MH
	Narraguagus Bay	N030x	Fjord	4	1228	1.3	6.5	75.5	12.7	4.0	0	491	23	MH
	Blue Hill Bay	N040x	Fjord	28	1803	4.0	1.3	83.3	7.2	4.2	0	673	34	ML
	Penobscot Bay	N050x	Fjord	29	22106	2.2	3.6	82.4	11.3	0.5	0	10572	722	L
	Muscongus Bay	N060x	Fjord	10	969	2.7	11.5	81.3	2.4	2.1	0	546	33	ML
	Sheepscot Bay	N080x	Fjord	23	647	1.2	10.4	84.4	2.8	1.2	0	433	28	MH
	Casco Bay	N100x	Fjord	10	2201	16.6	11.3	70.9	1.2	0.0	0	1419	112	MH
	Great Bay	N130x	Riverine	1	2486	19.2	8.1	70.0	2.6	0.1	0	1563	139	M
	Massachusetts Bay	N170w	Embayment	60	2103	76.8	1.2	21.2	0.7	0.0	0	5836	198	M
	Boston Elarbor	N170a	Embayment	2	1611	78.9	1.4	19.6	0.0	0.0	0	1627	713	MH
Virginian biogeographic province	Cape Cod Bay	N180x	Embayment	34	528	27.0	1.0	60.3	8.8	2.9	0	410	41	M
	Waquoit Bay	N190x	Embayment	4	84	18.3	1.9	38.4	41.4	0.0	0	83	8	N/A
	Buzzards Bay	M010x	Embayment	42	1168	22.6	7.5	64.5	4.4	0.9	0	1213	125	ML
	Narragansett Bay	M020x	Riverine	24	4134	41.5	5.5	51.0	1.7	0.4	0	6378	700	ML
	Gardiners Bay	M030x	Embayment	37	673	34.2	23.8	38.5	1.5	1.9	0	420	34	MH
	Long Island Sound	M040w	Embayment	56	40753	16.3	11.2	70.8	1.6	0.1	0	21371	2157	MH
	Great South Bay	M050x	Lagoon	2	1683	92.5	0.9	4.2	2.5	0.0	0	5859	706	MH
	New Jersey Island													
	Bays	M080x	Lagoon	1	3331	19.3	8.8	47.0	24.9	0.0	0	1630	131	ML
	Delaware Bay	M090x	Riverine	8	32681	19.9	32.1	44.4	3.4	0.3	0	46562	4241	ML
Delaware Inland														
	Bays	M100x	Lagoon	39	750	19.3	38.3	20.3	18.3	2.3	1.6	881	47	H

Table 3.

Taxonomic citation of dinoflagellate cyst taxa and their corresponding biological names where known.

Abbrev.	Cyst species (paleontological name)	Dinoflagellate theca (biological name)
Autotrophic		
Achom	<i>Achomosphaera</i> spp.	? <i>Gonyaulax</i> sp. Indet.
Alex	----	<i>Alexandrium</i> spp.
Atax	<i>Ataxodinium choane</i>	<i>Gonyaulax spinifera</i> complex
Biche	----	cf. <i>Biecheleria</i> sp.
Btep	<i>Bitectatodinium tepikiense</i>	<i>Gonyaulax spinifera</i>
Impag	<i>Impagidinium</i> spp.	<i>Gonyaulax</i> sp. Indet.
Lmac	<i>Lingulodinium machaerophorum</i>	<i>Lingulodinium polyedra</i>
Nlab	<i>Nematosphaeropsis labyrinthus</i>	<i>Gonyaulax spinifera</i> complex
Ocent	<i>Operculodinium centrocarpum sensu</i> Wall & Dale 1966	<i>Protoceratium reticulatum</i>
Oisr	<i>Operculodinium israelianum</i>	? <i>Protoceratium</i> sp.
Pdal	----	<i>Pentapharsodinium dalei</i>
Pzoh	<i>Polysphaeridium zoharyi</i>	<i>Pyrodinium bahamense</i>
Selong	<i>Spiniferites elongatus</i>	<i>Gonyaulax elongata</i>
Smem	<i>Spiniferites membranaceus</i>	<i>Gonyaulax spinifera</i> complex
Sbel	<i>Spiniferites belerius</i>	<i>Gonyaulax scrippsae</i> , <i>G. spinifera</i> complex
Sram	<i>Spiniferites ramosus</i>	<i>Gonyaulax scrippsae</i> , <i>G. spinifera</i> complex
Sbent	<i>Spiniferites bentorii</i>	<i>Gonyaulax digitalis</i> , <i>G. spinifera</i> complex
Sdel	<i>Spiniferites cf. delicatus</i>	<i>Gonyaulax spinifera</i> complex
Shyp	<i>Spiniferites hyperacanthus</i>	<i>Gonyaulax spinifera</i> complex
Sbull	<i>Spiniferites bulloideus</i>	<i>Gonyaulax scrippsae</i> , <i>G. spinifera</i> complex
Smir	<i>Spiniferites mirabilis</i>	<i>Gonyaulax spinifera</i> complex
Spin	<i>Spiniferites</i> spp.	<i>Gonyaulax</i> complex
Stype1	<i>Spiniferites</i> type 1.	<i>Gonyaulax</i> complex
Tpell	<i>Tectatodinium pellitum</i>	<i>Gonyaulax spinifera</i> complex
Tvan	<i>Tuberculodinium vancampoe</i>	<i>Pyrophacus steinii</i>
Heterotrophic		
Dubr	<i>Dubridinium</i> spp.	Diplopsalid group
Gymn	----	<i>Gymnodinium</i> spp.
Gymn	----	<i>Gymnodinium microreticulatum</i>
Pkof	----	<i>Polykrikos kofoidii</i>
Psch	----	<i>Polykrikos schwartzii</i>
Poblong	----	<i>Protoperidinium oblongum</i>
Poblong	----	<i>Protoperidinium paraoblongum</i>
Poblong	----	<i>Protoperidinium quadrioblongum</i>
Brig	<i>Brigantedinium</i> spp.	? <i>Protoperidinium</i> spp.
Brig	<i>Brigantedinium cariaeoense</i>	<i>Protoperidinium avellana</i>
Brig	<i>Brigantedinium simplex</i>	<i>Protoperidinium conicoides</i>
Cyro	<i>Cyrodinium</i> sp.	Unknown

Abbrev.	Cyst species (paleontological name)	Dinoflagellate theca (biological name)
Edel	<i>Echinidinium karaense</i>	<i>Protoperidinium</i> sp. indet.
Etran	<i>Echinidinium transparantum</i>	<i>Protoperidinium</i> sp. indet.
Ezon	<i>Echinidinium cf. zonneveldii</i>	<i>Protoperidinium</i> sp. indet.
Ech	<i>Echinidinium</i> spp.	<i>Protoperidinium</i> sp. indet.
Imin	<i>Islandinium minutum</i>	<i>Protoperidinium</i> sp. indet.
Icez	<i>Islandinium? cezare</i>	<i>Protoperidinium</i> sp. indet.
Ibrev	<i>Islandinium brevispinosum</i>	<i>Protoperidinium</i> sp. indet.
Lejeun	<i>Lejeunecysta marina</i>	<i>Protoperidinium</i> sp. indet.
Lejeun	<i>Lejeunecysta oliva</i>	<i>Protoperidinium</i> sp. indet.
Lejeun	<i>Lejeunecysta sabrina</i>	<i>Protoperidinium</i> sp. indet.
Lejeun	<i>Lejeunecysta</i> spp.	<i>Protoperidinium</i> sp. indet.
Pmin	-----	<i>Peridinium minutum</i> sensu Wall and Dale 1968
Pam	-----	<i>Protoperidinium americanum</i>
Squan	-----	<i>Protoperidinium nudum</i>
Poblong	---- sensu Wall and Dale 1968	<i>Protoperidinium oblongum</i>
Proto	<i>Protoperidinium</i> spp.	<i>Protoperidinium</i> spp. indet.
Qconc	<i>Quinquecupis concreta</i>	<i>Protoperidinium leonis</i>
Sneph	<i>Selenopemphix nephroides</i>	<i>Protoperidinium</i> sp. indet.
Squan	<i>Selenopemphix quanta</i>	<i>Protoperidinium conicum</i>
Stella	<i>Stelladinium reidii</i>	<i>Protoperidinium compressum</i>
Tapp	<i>Trinovantedinium applanatum</i>	<i>Protoperidinium pentagonum</i>
Vcal	<i>Votadinium calvum</i>	<i>Protoperidinium oblongum</i>
Vspin	<i>Votadinium spinosum</i>	<i>Protoperidinium claudicans</i>
CystPEI	Cyst type PEI	Unknown
CystE	Cyst type E	unknown
CystP	Cyst type P	unknown
SBC	Spiny brown cysts	? <i>Protoperidinium</i> sp. indet.

Table 4.

Regressions and partial linear correlations between nitrogen (N) loading and the average proportion of heterotrophic taxa (H%) in each estuary (n=29). Statistically significant ($p < 0.05$) relationships are in bold.

	Regression type	R	P value
H% and N loading	Linear	0.157	0.416
H% and Log(N loading)	Linear	0.556	0.002
	Quadratic	0.647	0.001
	Cubic	0.653	0.001
H% and Log(N loading/estuary area)	Linear	0.549	0.002
	Quadratic	0.610	0.002
	Cubic	0.632	0.005
H% and Log(N loading/estuary area) - controlling for estuary type	Partial	0.505	0.006
H% and Log(N loading/estuary area) - controlling for estuary type and watershed area	Partial	0.534	0.004
H% and Log(N loading/estuary volume)	Linear	0.406	0.029
	Quadratic	0.420	0.080
	Cubic	0.442	0.136
H% and Log(N loading/estuary volume) - controlling for estuary type	Partial	0.333	0.083
H% and Log(N loading/estuary volume) - controlling for estuary type and watershed area	Partial	0.330	0.093
H% and Log(N loading/watershed area)	Linear	0.739	<0.001
	Quadratic	0.794	<0.001
	Cubic	0.819	<0.001
H% and Log(N loading/watershed area) - controlling for estuary type	Partial	0.735	<0.001
H% and Log(N loading/watershed area) - controlling for estuary type and estuary area	Partial	0.723	<0.001

Table 5.

Linear and quadratic regressions between average summer sea-surface salinity (SSS), the relative abundance of cysts produced by heterotrophic taxa, annual nitrogen (N) loading and the species diversity. H = Shannon-Wiener index, S = Species Richness, 1-D = Simpson Index of Diversity, E = Evenness. Statistically significant ($p < 0.05$) relationships are in bold.

	Regression type	Acadian - PEI		Acadian - US		Virginian	
		R ²	P value	R ²	P value	R ²	P value
SSS and H	Linear	.262	.030	.041	.321	.048	.234
	Quadratic	.370	.031	.049	.562	.049	.492
SSS and S	Linear	.003	.820	.175	.034	.002	.820
	Quadratic	.108	.424	.176	.108	.002	.972
SSS and (1-D)	Linear	.442	.003	.039	.331	.049	.231
	Quadratic	.559	.002	.074	.411	.049	.494
SSS and E	Linear	.415	.004	.012	.591	.080	.123
	Quadratic	.436	.014	.023	.766	.080	.310
% Heterotrophs and H	Linear	.013	.650	.650	<.001	.549	<.001
	Quadratic	.117	.394	.701	<.001	.775	<.001
% Heterotrophs and S	Linear	.064	.313	.030	.395	.113	.052
	Quadratic	.086	.511	.060	.488	.281	.006
% Heterotrophs and (1-D)	Linear	.000	.944	.656	<.001	.484	<.001
	Quadratic	.038	.750	.715	<.001	.642	<.001
% Heterotrophs and E	Linear	.001	.929	.726	<.001	.494	<.001
	Quadratic	.078	.546	.768	<.001	.632	<.001
N loading and H	Linear	.009	.706	.000	.932	.037	.298
	Quadratic	.009	.931	.082	.374	.133	.136
N loading and S	Linear	.005	.785	.004	.756	.053	.214
	Quadratic	.028	.808	.062	.478	.053	.465
N loading and (1-D)	Linear	.003	.824	.004	.770	.052	.218
	Quadratic	.003	.974	.048	.569	.158	.090
N loading and E	Linear	.029	.503	.002	.837	.016	.495
	Quadratic	.039	.741	.068	.446	.165	.080