

PALEOLIMNOLOGICAL INVESTIGATION OF
THE ST. LOUIS RIVER ESTUARY TO INFORM
AREA OF CONCERN DELISTING EFFORTS

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Abstract

The St. Louis River Estuary (SLRE) has a long history of human development since Euro-American settlement ~200 years ago. Due to degradation from logging, hydrologic modification, industrial practices, and untreated sewage in the region including two developing cities (Duluth, MN and Superior, WI), the Environmental Protection Agency (EPA) designated the SLRE as an Area of Concern in 1987. Prior to 1987, actions had begun to restore water quality including the enactment of the Clean Water Act in 1972 and startup of the Western Lake Superior Sanitary District (WLSSD) in 1978 to help remove some beneficial use impairments (BUIs). A better understanding of the historical significance of these contributions over the years is necessary to help document both progress and knowledge gaps related to water quality. Therefore, a paleolimnological study of the SLRE was initiated. Various paleolimnological indicators (pigments, diatom communities, and diatom-inferred phosphorus) were analyzed from six cores taken throughout the estuary and western Lake Superior. Reductions in eutrophic diatom taxa such as *Cyclotella meneghiniana* and *Stephanodiscus* after 1970 in certain cores suggest a recovery of water quality over the last 40 years. However, in cores taken from estuarine bay environments, persistence of eutrophic taxa such as *Cyclostephanos dubius* and *Stephanodiscus binderanus* indicate continuing nutrient loading and increased production. Sedimentary pigments indicate increases in cyanobacteria in some bays over the last two decades. Diatom model-inferred phosphorus and contemporary monitoring data suggest some of the problems associated with excess nutrient discharges have been remediated, but modern conditions (internal phosphorus loading, changing climate) may be contributing to ongoing water quality problems in some locations. The integrated biological, chemical, and physical indicators from the sediments will aid agencies in determining where to target resources and BUI removal efforts.

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Introduction

Natural history

The St. Louis River (SLR) flows 288 km through northeastern Minnesota draining an area of 9412 square kilometers. On average, it delivers 73.3 m³/s of water to Lake Superior, making it the largest tributary to Lake Superior in the United States. The geology of the drainage basin is most influenced by glaciers. The headwaters consist of coarse soil and glacial till. Going downstream soil type transitions to glacial outwash and fine-grained, iron-stained clay. The drainage basin land cover consists of forests (36%), wetlands (30%), grasslands (19%), and the basin is largely undeveloped. The developed land supports residential communities, mining, and forestry (MPCA 2013). The estuary became a drowned river mouth as a result of isostatic rebound following the last deglaciation.

The SLR empties into Lake Superior's most western point. The most downstream portion (after Minnesota Highway 23) of the river before it joins Lake Superior is the St. Louis River Estuary (SLRE) (Figure 1). In contrast to a mostly rural upstream, the dominant land use surrounding the SLRE is urban. Here, depending on the seiche of Lake Superior, water can flow downstream or upstream and the lake and river water mix. The estuary is bordered by two major cities—Duluth, MN and Superior, WI—which have a combined population of around 113,000 people (MDNR and WDNR 1992).

European settlement

Evidence suggests the first human settlement of the region occurred around 2950 BP. Since then, various Native American tribes including the Dakota Sioux (1600s) and Ojibwa (1700s to present) inhabited the region (ARBC 1976). The French explorers Radisson and Groseilliers were the first European settlers to visit the estuary, arriving in 1659. Soon after, the estuary became an important stop along fur trade routes, especially after the Hudson Bay Company established a trading post in 1689 in what is now Superior, Wisconsin. After the fur trade declined the region was occupied briefly by

copper miners in the early 1800s. Because of the lack of high-quality copper ore the industry was short-lived (Flower 1890).

Proximity to water, especially a body of water that provides access to the Eastern United States and the world, expansive valuable timber stands, and iron-rich rock provided ample opportunity for industry and settlement of the region. Since European settlement the SLRE became one of the most impacted ecosystems in the Laurentian Great Lakes because of the industries and communities it supports (Hartig and Vallentyne 1989).

In the late 1890s the SLRE became a major shipping port. With the opening of the Soo Locks at the eastern end of Lake Superior in 1855, the port gained access to the rest of the Great Lakes and beyond. This was accelerated when the Lake Superior and Mississippi Railroad was built in 1870. Now both goods and people could travel in and out of Duluth (ARBC 1976), increasing the potential for urban development. The shipping port helped supply the Midwest with essential goods such as grain and coal. It was also a way to distribute the northland's timber and taconite that was used in the steelmaking industry. The estuary was first dredged in 1867 and the Duluth Shipping Canal was completed by 1871 (MPCA and WDNR 1992). Approximately 1600 hectares of open water and shoreline were filled in with dredged material since 1861 (Devore 1978). Dredging is still routine in order to maintain the port for large ships.

Logging also impacted the SLRE. The "Cutover" period from the 1800s to early 1900s had a dramatic impact on the northern Great Lake States, including the portion of Minnesota drained by the SLR. During this time virtually all forested areas were cleared. The change in land cover of the once heavily forested drainage basin led to increased runoff of both water and soils. The SLR itself was essential to the logging industry as a means of transporting the logs to more populous areas. In order to control the movement of logs downstream, approximately 50 to 100 dams were installed on the river. The SLRE was home to several sawmills, pulp mills, and paper mills using lumber from the region.

These were a major source of pollution to the estuary, discharging large quantities of sawdust and slab wood (MPCA and WDNR 1992).

Because of its proximity to iron-ore mines in northeastern Minnesota, Duluth and Superior became home to the iron industry. Several operations existed in the late 1800s in Superior and Duluth producing pig iron, coke, and other iron products. This included Duluth Blast Furnace Co (est. 1873), Standard Iron Works (est. 1888), and West Superior Iron and Steel Company (est. 1888). In 1890, Duluth Iron and Steel Company was built three miles upstream from the mouth of the SLR and later became Interlake Iron (MPCA and WDNR 1992). Duluth Tar Company (later Barrett, Dominion and American Tar Company) was built adjacent to the Interlake site, utilizing the coking byproducts to produce tar products in 1916 (EPA 2012). Tar production stopped in 1948 and Interlake closed in 1961. Discharge from both plants over the time of their operation led to contaminated soil and sediments. Contaminants consisted of several carcinogenic and noncarcinogenic pollutants: polycyclic aromatic hydrocarbons (PAHs), tar, heavy metals, and PCBs. The presence of these contaminants compelled the EPA and MPCA to place the site on Minnesota's Superfund list in 1982 (MPCA 2016a).

Built in 1907 and located in the Morgan Park neighborhood of west Duluth eight miles upstream from Lake Superior, the U.S. Steel Duluth Works plant manufactured coke and steel. While in operation, especially in its earlier less-regulated past, the plant disposed of various harmful wastes into the river. PAHs from coal tar were discharged into a settling pond that eventually emptied into the SLRE. Ammonia, cyanide, and phenols were discharged directly into the river. In 1979, the plant closed and in 1984 it was designated a Minnesota Superfund site (MPCA 2016b).

The vitality of these combined industries quickly drew settlers to the region. The population of Duluth and Superior grew from an estimated 600 in 1865, to 53,000 in 1900, and reached a population of 100,000 by 1930 (MPCA and WDNR 1992). Population growth also meant an increase in impacts to the estuary. The region lacked

proper stormwater and wastewater infrastructure. The untreated wastewater inputs included human waste and high-phosphorus detergents that together introduced nutrients and organic matter into the estuary, leading to episodic hypoxia in some areas of the estuary (Hargis et al. 1983; Carlson and Thomas 1984). However, monitoring data documenting eutrophication from earlier in the 20th century are sparse.

Early water quality and AOC listing

As early as 1928, the Minnesota State Board of Health noticed degradation of the SLR and after a study declared the river as “pollutional” (MSBH 1929) Another assessment twenty years later confirmed untreated sewage and other sources of pollution were causing hypoxia, sludge deposits, and a bad taste in fish (MSBH 1948).

In 1966, the Federal Water Pollution Control Administration Great Lakes Regional Office made recommendations on strategies to reduce pollution. These included installing wastewater and stormwater treatment facilities to reduce nutrient loading (FWPCA 1966). In response, the Minnesota legislature created the Western Lake Superior Sanitary District (WLSSD) in 1971 and the facility was completed in 1978. The plant treats wastewater from a 530 square mile region that includes Duluth, Cloquet, Carlton, Hermantown, and other smaller surrounding communities (WLSSD 2007). A study was completed comparing concentrations of various metals, nutrients, and physical parameters before and after 1978. The results showed significant improvement in condition of the SLRE including a decrease in total phosphorus, turbidity, total coliform, lead, and copper and an increase in dissolved oxygen (McCollor 1990). Despite the improvement, issues such as fish tissue contamination and degraded fish and macrophyte habitat persisted.

The SLRE was not alone in its polluted state. Across the Great Lakes resource managers and community members were growing more aware of the environmental degradation that had taken place since European settlement. The International Joint Commission, initially formed by the 1909 Boundary Waters Treaty to address issues facing boundary

waters shared between Canada and the United States, wrote and passed the Great Lakes Water Quality Agreement in 1972. The purpose of the agreement was “to restore and maintain the chemical, physical, and biological integrity of the Waters of the Great Lakes” (IJC 1972). The legislation was amended in 1987 to designate degraded areas throughout the Great Lakes as Areas of Concern (AOC). Each AOC was made up of one or more of 14 beneficial use impairments (BUIs) indicating the type of impairment present such as loss of fish and wildlife habitat, degradation of aesthetics, and fish tumors (IJC 1987). The SLRE was listed as one of 43 (26 in the US) AOCs in the Great Lakes and was defined by 9 BUIs: restrictions on fish and wildlife consumption, degraded fish and wildlife populations, fish tumors and other deformities, degradation of benthos, restrictions on dredging, excessive loading of sediments and nutrients, beach closings and body contact restrictions, degradation of aesthetics, and loss of fish and wildlife habitat. As with all AOCs, history of the SLRE accompanied by an analysis of each BUI was detailed in a Remedial Action Plan (RAP) (MPCA and WDNR 1992).

Restoration of the estuary

After designation as an AOC, a task force led by the Minnesota Pollution Control Agency (MPCA) and the Wisconsin Department of Natural Resources and accompanied by representatives from other agencies (e.g., MN and WI Sea Grant, Lake Superior National Estuarine Research Reserve, Minnesota Department of Natural Resources, Minnesota Department of Health, City of Superior, City of Duluth) was created to address the issues facing the SLRE. In 1992, the first Remedial Action Plan was released outlining the degradation of the SLRE, with descriptions of the BUIs (MPCA and WDNR 1992). Since 2010 when funding from the Great Lakes Restoration Initiative became available, a plan has been created for each BUI detailing the extent of the degradation, restoration goals, and timelines. With one BUI already removed (degradation of aesthetics) agencies hope to have all BUIs removed and the AOC delisted by 2025 (MPCA and WDNR 2013). In order to achieve this, there has been an estuary-wide effort including \$420 million invested so far to improve environmental conditions (MPCA and WDNR 2013).

Agencies have developed removal targets for BUI 6 (excessive loading of sediment and nutrients). Legislation restricting the use of phosphorus detergents, the installation of WLSSD, and development of best management practices within the watershed have helped to reduce the flux of nutrients to the estuary (MPCA and WDNR 2015) and improve legacy-related BUIs, but understanding the extent of recovery is complex. A phosphorus dataset beginning in 1953 exists for the estuary and consists of monthly measurements taken from two locations. A more comprehensive study of phosphorus in the estuary began in 2012. These data, though limited until recently, show a recovery in phosphorus concentrations (Bellinger et al. 2016). Still, measurements of nutrient concentrations and sediment loading, and biological responses to these changes, are absent for most of the estuary's past, especially pre-impact conditions.

Paleolimnology

A paleolimnological study of the SLRE was initiated to help to better understand temporal data gaps. Paleolimnology is a tool that provides insight into changing conditions typically through the analysis of sedimentary records. By assessing the biological, physical, and chemical properties of sediment cores, changes in conditions of the basin can be inferred (Smol 1992). Analyzing cores from the SLRE has the potential to provide insight on long-term environmental impacts and remediation while considering the known human history of the catchment.

Diatoms (Bacillariophyceae) are important indicators of water quality. As primary producers they are sensitive to changes in the water column including nutrient concentrations, salinity, and turbidity. Their silica cell wall (frustule) provides for fossil preservation in the sedimentary record and taxonomic identification (Dixit et al. 1992). Environmental characteristics of the individual species can be used to provide past environmental information. For instance, diatoms have been used to infer past anthropogenic conditions in Lake Superior (e.g. Stoermer et al. 1985a; Reavie and Allinger 2011; Chraïbi et al. 2014). In order to examine the historical environmental conditions of the SLRE, diatom communities, and relative abundances of diatom flora

before, during, and after European settlement in the SLRE were characterized. Changes in diatom assemblages, and inferred environmental information were correlated with anthropogenic activities and validated with additional data, including fossil pigments and organic content. The extent of degradation and recent remediation of the AOC was clarified, and management recommendations relevant to AOC BUI removal are made herein.

Methods

Site Selection

In order to have suitable material for analysis cores were taken from undisturbed sediments and continuous depositional environments. For the SLRE, maps and hydrological data were used along with consultation with the Army Corps of Engineers in order to avoid areas previously impacted by dredging or shipping activities. Sites were chosen to represent a variety of environments including different hydrologic regimes (bays, harbor, Lake Superior) and varying anthropogenic impacts (formerly polluted versus purportedly less impacted). A total of seven cores were collected; six cores were from discrete locations in the lower SLRE and one core was from western Lake Superior (Table 1; Figure 1).

Coring and Extruding

At each coring location we attempted to collect at least 200 years of sediment in order to evaluate the recent anthropogenic history of the estuary. SLRE cores were collected the winter of 2014. A piston corer designed for sampling recent lake sediments was used (Glew et al. 2001). Three cores were taken at each location to have sufficient material for analyses; however, only one core was used for the analyses included in this paper. For each core, approximately one meter of sediment was collected and sectioned into 1-cm intervals (0.25-cm intervals for Lake Superior). The core taken from western Lake Superior was collected in May 2014 from the EPA's research vessel *Lake Guardian* by

use of a multicorer (methods are described by Chraïbi et al. 2014). Samples were extruded in intervals using a close-sectioning extruder and kept refrigerated for later analyses.

Sediment chronology

Lead-210 (^{210}Pb) isotopic analysis of alpha emissions was used to determine chronological profiles of sediment cores. The Science Museum of Minnesota's St. Croix Watershed Research Station completed all ^{210}Pb analyses. Methods followed Schelske et al. (1994) including calculations from Appleby and Oldfield (1978). Subsamples of cores were dried, weighed, and sealed in a vial with a layer of epoxy resin. Samples were left to equilibrate for two weeks. After equilibration, a stet detector was used to count isotopic decay. ^{210}Pb counts were then used to calculate sediment accumulation rates (Appleby and Oldfield 1978, 1983). Counting of the gamma emissions from the isotope cesium-137 (^{137}Cs) was also performed for one core that had ambiguities in the ^{210}Pb record.

Inorganic and Organic Chemistry

Loss on ignition analysis to determine inorganic and organic quantities followed Dean (1974). Water content was determined by heating sediment samples to 100 °C for 24 hours. Samples were heated to 550 °C for two hours to determine organic content and the remaining material was brought to 1000 °C to estimate clastic composition.

Pigments

The Laboratory of Chemical Extractives at the Natural Resources Research Institute completed pigment extraction and analysis. Eight pigments (carotenoids and chlorins) were analyzed to examine historical algal communities according to methods (outlined in Appendix 1) based on Reuss (2005) and Reuss and Conley (2005). The pigments analyzed represented total algae (chlorophyll *a*, pheophytin *a*, and β -carotene), diatoms (diatoxanthin and fucoxanthin), and dinoflagellates (fucoxanthin), cryptophytes (alloxanthin), and cyanobacteria (aphanizophyll and myxoxanthophyll). Pigments were extracted from the freeze-dried sample material using acetone. After extraction, the

material was quantitatively analyzed using a Shimadzu High Performance Liquid Chromatographer equipped with a photodiode array detector.

Diatom Preparation

For each core interval analyzed for diatoms, approximately 1 g of wet sediment was subsampled and digested with strong acid to remove all organic material and isolate siliceous microfossils. Samples were then rinsed with distilled water to remove acid and applied to coverslips quantitatively using the Battarbee (1986) method. This method allowed for the quantitative analysis of diatom abundance and accumulation rates. Coverslips were mounted to microscope slides for identification and enumeration.

Diatom identification and enumeration

Diatoms were identified and enumerated by use of oil immersion on a light microscope (1,250X magnification). Diatoms on each slide were identified along random transects until at least 400 diatom valves were enumerated. Each diatom was identified to species level according to Reavie and Kireta (2015), Hofmann et al. (2011), Krammer and Lange-Bertalot (1989 - 1991), and Patrick and Reimer (1966 - 1975). Phytoliths, sponge spicules, chrysophyte scales and stomatocysts, and testate amoebae plates were also counted when observed. These siliceous remains, though rare, are also used to infer environmental conditions and may provide additional insight on the ecological condition of the estuary (Smol et al. 2001).

Cluster analysis

Relative abundance was calculated for each species for all core intervals. For common taxa (at least five occurrences with at least 5% abundance in one or more samples), a depth-constrained cluster analysis was done using the “chclust” function in R using the “rioja” package (Juggins 2014) to identify temporal zones containing unique diatom assemblages. The CONISS algorithm (Grimm 1987) was used to perform clustering constrained to vertical stratigraphy, based on dissimilarity in squared Euclidian distances among samples. The embedded function “bstick” was used to perform a broken-stick

analysis and determine the minimum number of significant clusters (Bennett 1996). Transitions between zones may reflect historical events leading to reorganization of the diatom community.

Nonmetric multidimensional scaling

In order to better assess the similarities among cores and to track temporal trajectories, several non-metric multidimensional scaling (NMDS) analyses were performed. NMDS is an ordination technique that allows for visualization of highly dimensional data in lower dimensional space. Multidimensional scaling examines the distances between observations (e.g. samples or species); shorter distances indicate similarity. The statistical software R with the vegan package (R Core Team 2014; Oksanen et al. 2015) was used to create an NMDS plot from diatom relative abundance data. Species with a maximum relative abundance less than 5% were omitted to reduce analytical artifacts from rare species. Because the core taken in western Lake Superior had very different diatom assemblages than the cores taken in the estuary, three analyses were done: all cores together, SLRE cores, and Lake Superior by itself.

Diatom-inferred modeling

Diatom-inferred (DI) modeling translates fossil diatom data into quantitative profiles of water quality variables. When there are changes in nutrient concentrations (e.g. phosphorus and nitrogen), salinity, turbidity, pH, or other parameters of interest, the algal community shifts, and diatom species assemblages respond predictably to environmental change. In development of a DI model, diatom species in a training set of samples are related to an analyte of interest (e.g. phosphorus) and species coefficients (e.g. phosphorus optima) are calculated. These species-specific coefficients are applied to the diatom assemblages found in cores, and the variable of interest is inferred based on the relative abundances of fossil diatom taxa. Two models were developed for the Great Lakes: open water (Reavie et al. 2014; used for the Lake Superior core) and coastal embayments, wetlands, and high-energy areas (Reavie et al. 2006; used for the six

estuary/harbor cores). Both have shown robust reconstructions of phosphorus in the Great Lakes and were deemed appropriate for use in the SLRE and Lake Superior.

DI Model Validation

A set of analyses verified the efficacy of both models' ability to reconstruct phosphorus. An analog analysis determined the similarities between diatom assemblages in the models and fossil assemblages. Using the R package analogue (Simpson and Oksanen 2015) assemblages from the model were matched to the fossil assemblages following Flower et al. (1997) and Simpson et al. (2005). Analogs were determined using Bray-Curtis dissimilarity (Bray and Curtis 1957). Dissimilarities between fossil and modern samples were examined to determine how well fossil assemblages were represented in the model assemblages. A constrained canonical correspondence analysis (CCA) was done to examine the relationship between modern phosphorus and diatom assemblages, and then fossil samples were ordinated passively to determine the goodness of fit. Using the R packages vegan and analogue (Oksanen et al. 2015; Simpson and Oksanen 2015) a CCA defined residual distances of fossil assemblages (i.e. sample scores) and the total phosphorus (TP) gradient (i.e. constrained CCA axis 1). Fossil residual distances within the 95 % confidence interval of the modern sample distances were considered to have good fit to TP.

Analyses according to Reavie et al. (2014) were completed to determine if the analyte of interest, TP, was related to changes in fossil species assemblages. Using the R package vegan (R Core Team 2014; Oksanen et al. 2015), each set of fossil data in a given core was distilled using principal components analysis (PCA) to derive axis scores representing the primary gradient of variation in the diatom assemblage data (Juggins and Birks 2012). A correlation coefficient (r) was calculated for historical diatom inferred total phosphorus (DI-TP) versus the axis 1 PCA scores. If $|r|$ was high and significant, it was likely that changes in fossil diatom assemblages in cores were at least in part determined by phosphorus, and so use of the DI-TP model was considered appropriate.

Results

Sediment chronology

Exponential decay of ^{210}Pb with sediment depth was used to determine the validity of chronological profiles. With the exception of North of Clough Island, cores showed a consistent record of sediment accumulation, and were dateable (Figure 2). ^{210}Pb data from the North of Clough Island core suggested recent disturbance, likely due to increased sedimentation from a 500-year flood that affected the SLRE in 2012 (Czuba et al. 2012). Unsupported (excess) ^{210}Pb data were relatively monotonous with depth, aside from an uppermost section above ~35 cm depth with higher concentrations. Supplementary dating using ^{137}Cs characterized high concentrations of that isotope around 1963 due to nuclear weapons testing (Krishnaswami and Lal 1978). Based on a peak in ^{137}Cs at 60 cm depth, we assigned a rough, recent chronology based on knowing the 1963 interval, acknowledging that dates since 1963 are highly uncertain.

With the exception of North of Clough Island, cores showed increased sedimentation rates in the early 1900s or just prior (Figure 3, left-most panels). Four of the cores demonstrated a recovery in sedimentation rates: Allouez Bay and Billings Park had peaks around 1920 - 1930 and subsequently fell to sedimentation rates of 0.35 to 0.15 $\text{g}/\text{cm}^2/\text{y}$ and 2.0 to 0.2 $\text{g}/\text{cm}^2/\text{y}$ respectively. In Western Lake Superior, sedimentation rates peaked around 1970 at 0.12 $\text{g}/\text{cm}^2/\text{y}$ with a secondary peak at 0.11 $\text{g}/\text{cm}^2/\text{y}$ in 1900; rates recovered to near pre-settlement conditions around 2000. This trend is similar to North Bay where there was a secondary peak in 1930 (0.3 $\text{g}/\text{cm}^2/\text{y}$) and a peak at 1970 (0.4 $\text{g}/\text{cm}^2/\text{y}$). Rates declined to 0.2 $\text{g}/\text{cm}^2/\text{y}$ by 2000, but rates remained higher than pre-settlement. In contrast, sedimentation rates continued to increase in cores from Minnesota Point and Pokegama Bay. At Minnesota Point, accumulation rates rose from less than 0.02 $\text{g}/\text{cm}^2/\text{y}$ to 0.14 $\text{g}/\text{cm}^2/\text{y}$, with the greatest rate of change occurring in the last 40 years. Sedimentation rates at Pokegama Bay increased from 0.05 $\text{g}/\text{cm}^2/\text{y}$ to 0.25 $\text{g}/\text{cm}^2/\text{y}$ with two peaks occurring at 1960 and 1990 (both around 0.35 $\text{g}/\text{cm}^2/\text{y}$). The accumulation profile for North of Clough Island was based on a single ^{137}Cs date, so we

have great uncertainty about the recent accumulation rates. Overall, differences in average sediment accumulation rates among cores reflect their physical settings, such as the lower rates in the more lacustrine areas (Minnesota Point and Lake Superior).

Sediment Content

All cores indicated decreasing water content with depth due to compaction (Figure 3). Cores from Allouez Bay and North Bay had the most distinct changes in organic content with a peak in the 1930s and a concomitant increase in % inorganic material. An increase in organic content was also seen at North of Clough Island since 2000, and due to uncertainty in dating may reflect a depositional layer from the 2012 flood (Czuba et al. 2012).

Accumulation rates of organic, inorganic, and carbonate components largely followed total sedimentation rates, although there were some anomalies. There was a period of very low carbonate accumulation from ~1970 through ~1995 in North Bay, and from 1910 – 1940 in Lake Superior. In North of Clough Island, there was lower accumulation of organic material from 1970 – 2000 and heightened accumulation from 2000 until the present.

Pigments

Pigments in four estuary cores (North Bay, Billings Park, North of Clough Island, and Minnesota Point; Figure 4) tracked historical shifts in algal groups. Pigments representative of total algae (chlorophyll *a*, pheophytin *a*, and β -carotene) in all cores showed temporary heightened productivity in recent sediments since ~1990 in North Bay and Billings Park. Fucoxanthin and diatoxanthin were higher in North Bay and Billings Park. Fucoxanthin (diatoms and dinoflagellates) increased rapidly in more recent intervals, since 1990 in North Bay and 2005 in Billings Park, whereas diatoxanthin (diatoms) showed a gradual increase since 1980. Pigments from cyanobacteria (aphanizophyll and myxoxanthophyll) have increased in both North Bay and Billings Park in the last 20 years. Although there was a strong peak in alloxanthin (representing

cryptophytes) around 1970, since ~1980 pigment concentrations in MN Point and North of Clough Island cores remained relatively low and steady. To account for the possibility of some pigments degrading with time (e.g. chlorophyll *a* tends to have low stability; Leavitt and Hodgson 2001), we note that recent increases occur in pigments with known reliability in long-term preservation in sedimentary records (pheophytin *a*, fucoxanthin, diatoxanthin, aphanizophyll, and myxoxanthophyll, and especially β -carotene).

Diatoms

A total of 654 diatom taxa were observed from 88 genera. In estuary cores, both benthic and phytoplanktonic diatoms were common whereas the species composition in Lake Superior was mostly phytoplankton. Diatom accumulation rates in North Bay, Pokegama Bay, Billings Park, and Allouez Bay all peaked in the mid-20th century (Figure 5); however, Superior, Allouez Bay, and North of Clough Island had higher accumulation after 1970. North Bay and Allouez Bay show shifts to centric-dominated (i.e. phytoplanktonic) assemblages (~1900 and ~1940 respectively) and had mostly consistent proportions of pennates to centrics. Chrysophyte stomatocysts had the highest proportion of the siliceous microfossil assemblage in earlier intervals (North Bay, Pokegama Bay, Billings Park, Minnesota Point, and Allouez Bay). Because chrysophytes are more competitive in oligotrophic environments, higher ratios of chrysophyte cysts to diatoms are associated with lower nutrients (Smol 1985), so we infer from this trend a long-term increase in nutrient load. In North Bay, Allouez Bay and Billings Park it appears this ratio is continuing to decline, while it has stabilized in Minnesota Point and Pokegama Bay. Stomatocysts were not sufficiently abundant in the Superior core to present these data.

In several estuary cores (North Bay, Pokegama Bay, Minnesota Point, and Allouez Bay) there was a transition (~1850 - 1900) from benthic diatoms (*Staurosira* and *Staurosirella*) to communities dominated by the planktonic *Aulacoseira*. This suggests a transition to a more lacustrine environment resulting from damming (associated with the lumber industry) taking place during this time period.

Because of the uniqueness of assemblages among cores, we present diatom results separately for each core. Stratigraphic zones representing periods of major assemblage changes, as determined by cluster analysis, are used to guide historical trends.

North Bay

North Bay had two significant zones determined by the broken stick analysis, pre and post-1900. However based on apparent shifts in diatom assemblages, we delineated three diatom assemblage zones in the core: (A) pre-1900, (B) 1900 - 1945, and (C) post-1945 (Figure 6). The core was made up of mostly planktonic diatoms (*Aulacoseira*, *Fragilaria*, *Stephanodiscus*), but was also accompanied by some benthic genera (*Staurosira*, *Achnantheidium*, *Cocconeis*, and *Navicula*). Steady populations of *Staurosira construens* var. *venter* and *Staurosirella pinnata* dominated Zone A (combined ~20 %), epipsammic and epipelagic diatoms indicating a low-nutrient, benthic-dominated community (Estep and Reavie 2015; Morales 2010a). In the early 1900s (Zone B) many species comprising the modern assemblage increased in abundance while *S. construens* var. *venter* and *S. pinnata* declined. Higher-nutrient indicators *Aulacoseira ambigua*, *Stephanodiscus parvus*, *Cyclotella meneghiniana*, and *Stephanodiscus hantzschii* (Stoermer et al. 1985b; Stoermer and Yang 1970; Stoermer and Håkansson 1984) appeared in greater abundance in Zone C. Since their initial increase, there has been some decline in the last decade in some species (*C. meneghiniana* and *S. hantzschii*), although a few nutriphilic taxa (*Aulacoseira granulata* and *S. parvus*) became more abundant during the last decade. Also in Zone C, benthic and epiphytic taxa like *Cocconeis placentula* (Round et al. 1990), *Navicula gregaria* (Round et al. 1990), *Fragilaria vaucheriae* (Morales 2010b), and *Fragilaria mesolepta* (Potapova and Spaulding 2013) increased in abundance, reflecting a probable, local increase in macrophyte habitat.

Pokegama Bay

Phytoplanktonic centric diatoms dominated the core from Pokegama Bay (Figure 7). While only four zones were determined to be significant by broken stick analysis, we

delineated five zones based on the cluster analysis and major shifts in diatom assemblages: (A) pre-1830, (B) 1830 - 1910, (C) 1910 - 1970, (D) 1970 - 1980, and (E) post-2000. The historical community in Zone A consisted of *S. pinnata* (benthic; Estep and Reavie 2015), *Achnantheidium minutissimum* (epiphytic; Potapova 2009), and *Aulacoseira pusilla* (planktonic; Potapova 2010). In Zone B, *A. pusilla*, joined by *A. granulata* and *A. ambigua* grew to dominate the assemblage. *Aulacoseira ambigua* and *A. granulata* continued to grow in Zone C until their peak (35 % in 1940, 45 % in 1960) after which they generally declined. *Stephanodiscus parvus*, a eutrophic indicator (Stoermer and Håkansson 1984), also increased in this period until it declined in Zone D, though its abundance was still higher than pre-settlement. In Zone E, *Aulacoseira* still dominated (~40 %) but was partly replaced by another eutrophic diatom, *Cyclostephanos dubius* (Hickel and Håkansson 1987), whose abundance grew to around 10 % of the diatom assemblage.

North of Clough Island

The diatom record from the core taken at North of Clough Island only extended back to 1940 so pre-impact conditions cannot be determined (Figure 8). The broken stick analysis determined that at least three temporal zones were significant. In Zone A (pre-1970) the eutrophic indicators *C. meneghiniana* and *A. granulata* peaked in the late 1960s (20 % abundance) and then rapidly returned to earlier conditions (1 - 4 %) in Zone B (1970 - 1990). During Zone B *S. parvus* also increased, but abundance decreased around 1990 (the start of Zone C). The modern assemblage had high diversity and consisted of both pelagic and benthic diatoms. Common genera were *Cyclotella*, *Cocconeis*, *Aulacoseira*, *Achnantheidium*, *Staurosira*, and *Staurosirella*. We again note great uncertainty in the timing of changes in this core due to the recent flood, which may have deposited allochthonous material in an undetermined layer near the core surface. We have confidence that the uppermost ~2 intervals represent post-flood deposition, and that assemblage (*S. pinnata*, *A. minutissimum*, and *A. ambigua*) indicates lower nutrients than pre-1970 taxa.

Billings Park

The core from Billings Park was dominated by planktonic diatoms, especially species from the genus *Aulacoseira* (Figure 9). The core had three significant zones, however we determined five zones showed important changes in assemblages: (A) ~1900 - 1940, (B) 1940 - 1970 (C) 1970 - 2000, (D) 2000 - 2010, and (E) post-2010. The assemblage was made up of largely *A. pusilla*, *A. granulata*, and *A. ambigua* in Zone A, but shifted to a *A. granulata* and *A. ambigua* dominance in Zone B. Nutrient-tolerant diatoms, *S. parvus*, *S. hantzschii*, *S. binderanus*, and *C. meneghiniana* increased in relative abundance in Zone C, followed by a partial decline as they were replaced by small, benthic species (e.g. *Staurosira construens* var. *venter*, *S. pinnata*, *F. vaucheriae*, and *Pseudostaurosira brevistriata*) in Zone D. In Zone E there was a shift back to an *Aulacoseira*-dominated assemblage similar to before ~1970.

Minnesota Point

The core from Minnesota point was largely made up of centric and araphid planktonic diatoms with a smaller proportion of benthic species (Figure 10). Three zones (two were determined significant by broken stick analysis) were identified: (A) pre-1850, (B) 1850 - 1980, and (C) post-1980. Zone A was dominated by *S. construens*, *S. pinnata*, *S. construens* var. *venter*, *A. granulata*, and *A. ambigua*, which existed as far back as ~1700. In Zone B, *Staurosira* and *Staurosirella* decreased and there was some growth in the already dominant *Aulacoseira* population. Eutrophic *S. parvus* increased and reached a maximum abundance (<10 %) in ~1965 and returned to near pre-settlement abundances (<5 %) in Zone C. Also in Zone C *A. granulata*, *A. ambigua*, and *P. brevistriata* increased.

Allouez Bay

Allouez Bay consisted of mostly planktonic diatoms (Figure 11). Although the broken stick analysis only found two significant zones, we delineated four zones based on apparent changes in diatom assemblages. The historical assemblage (Zone A, pre-1880) was very diverse, including the phytoplankton *Aulacoseira subarctica*, *A. pusilla*, *A.*

ambigua, *A. granulata*, *Stephanodiscus* sp. #10, the epiphytic *Achnantheidium minutissimum* and *Eunotia incisa*, and the benthic *S. construens* var. *venter* (each ~5 %). In Zone B (1880 - 1940) *A. ambigua* and *A. granulata* grew to dominate the assemblage, indicating greater planktonic dominance and probable nutrient enrichment. They continued to rise and reached a maximum (together 40 % of the assemblage) in Zone C (1940 - 1960). In Zone D (post-1960), eutrophic indicators *S. binderanus*, *C. meneghiniana*, and *C. dubius* (Stoermer et al. 1987; Hickel and Håkansson 1987) began to increase in abundance, each occupying ~5 – 10 % of the assemblage in the upper intervals.

Lake Superior

The species assemblage in Lake Superior was dominated by planktonic centric diatom species (*Lindavia*, *Cyclotella*, *Stephanodiscus*, and *Aulacoseira*) (Figure 12). Zone A (pre-1910) was dominated by *S. sp. #10*, *Lindavia ocellata*, *Cyclotella atomus* var. 1, and *Lindavia comensis*, taxa generally reflecting low nutrients. *Aulacoseira subarctica*, *A. islandica*, and *A. ambigua*, mesotrophic diatoms indicating higher nutrients in oligotrophic Lake Superior (Stoermer 1993) increased in Zone B (1900 - 1970) but decreased in Zone C (1970 - 1985). Small centric diatoms, *L. comensis* and *L. comensis* var. “rough center with process” (*Lindavia* cf. *delicatula*; Reavie and Kireta 2015) began increasing in Zone C and grew to a combined abundance of ~40 % in Zone D (post-1985). These low-nutrient taxa have been suggested to be related to climate-driven physical changes in the lake (Chraïbi et al. 2014).

Nonmetric multidimensional scaling

Based on an initial ordination of diatom samples from all cores, Lake Superior was highly dissimilar to the cores taken from the estuary, indicating substantial differences in common taxa between the lake and estuary (Figure 13A). Because of this the analysis was repeated to examine (1) all cores, (2) Lake Superior alone, and (3) the estuary cores to allow for better visualization of historical trajectories in NMDS ordinations.

NMDS of Lake Superior (Figure 13B) reflected a constant reorganization of the diatom assemblage, from a pre-1900 assemblage dominated by *S. sp. #10*, *L. ocellata*, and *L. comensis*, followed by an increase in higher nutrient taxa (e.g. *A. subarctica* and *A. islandica*) in the upper right quadrant. Migration to the left reflects current conditions dominated by small centrics such as *Lindavia* species like *L. comensis* and *L. cf. delicatula*.

With the exception of Billings Park, the oldest intervals of each estuary core fell within the lower, right quadrant (Figure 13C), indicating consistent assemblage baselines of *S. construens*, *S. construens* var. *venter*, and *S. pinnata*. Into the 20th century the assemblages migrated to the upper, left quadrant, representing assemblage shifts associated with higher nutrients (e.g. *C. meneghiniana* and *S. parvus*). The most recent sample scores in Billings Park, Pokegama Bay, and Allouez Bay are especially constrained to the left of the ordination in accordance with higher relative abundances of *C. dubius*, *S. binderanus*, and *Aulacoseira*. In general, the fossil assemblages exhibited consistent reorganization, and there is little evidence these primary producers are returning to a pre-impact state.

Diatom-inferred modeling

Based on model validation, there was a significant relationship between changes in TP and diatom assemblages in all cores; i.e. DI-TP had a strong correlation with the primary gradient of variation in assemblages in each core (Appendix 2). Further, analog analyses showed good fit between fossil assemblages and model training sets in all cases.

DI-TP results (Figure 14) indicated that Lake Superior had much lower concentrations of TP (3 - 6 µg/L) than the SLRE (15 - 80 µg/L). The open water cores (Lake Superior, Minnesota Point, and North of Clough Island) had increased DI-TP during the mid-20th century followed by a decline (Superior and North of Clough Island) or stabilization (Minnesota Point). The cores taken from bays (North Bay, Billings Park, Allouez Bay, and Pokegama Bay) showed, in general, increasing DI-TP since the mid-20th century.

DI-TP in Lake Superior suggested phosphorus loading in the early 1900s contributing to a maximum of TP of 5.5 $\mu\text{g/L}$ around 1930, and a secondary peak (5 $\mu\text{g/L}$) around 1970. After 1970, TP quickly decreased and stabilized around pre-settlement concentrations (~ 3 $\mu\text{g/L}$), much like that observed in other Lake Superior cores (Chraïbi et al. 2014).

Of the estuary cores, the cores taken from more open-water environments (Minnesota Point and North of Clough Island) showed stabilization or restoration of TP. The DI-TP from North of Clough Island showed an increase from 25 $\mu\text{g/L}$ to 65 $\mu\text{g/L}$, peaking around 1970. Recovery to lower concentrations occurred later and the earlier concentration of ~ 25 $\mu\text{g/L}$ was reached in the upper intervals. Because North of Clough Island's record did not extend before 1940, it was not possible to compare pre- and post-settlement conditions. The North of Clough Island reconstruction indicated an increase in the late 1900s from 25 $\mu\text{g/L}$ to 35 $\mu\text{g/L}$ TP. After a peak in 1980, DI-TP stabilized around 30 $\mu\text{g/L}$. Again, due to uncertainty in accumulation, we inferred higher nutrients in the 1960s, and lower nutrients today, but the timing of transitions are ambiguous.

Cores from bay environments showed modern conditions of increasing DI-TP. The cores from Allouez Bay and Pokegama Bay both remained at near-constant concentrations of DI-TP (30 and 45 $\mu\text{g/L}$, respectively) until ~ 1950 , after which both experienced increases in P concentration as high as ~ 80 $\mu\text{g/L}$. DI-TP began to increase around 1920 at North Bay and rose from ~ 50 to ~ 60 $\mu\text{g/L}$ in modern intervals. In Billings Park DI-TP increased from ~ 1950 to ~ 2000 (from ~ 20 to 38 $\mu\text{g/L}$), followed by two modern intervals with lower DI-TP (~ 20 $\mu\text{g/L}$).

Compared to historical measured TP from a location in the lower estuary (at Blatnik Bridge; Bellinger et al. 2016), DI-TP concentrations were lower (Figure 14); however, the general trend of declining TP in recent decades was similar to that for DI-TP from the North of Clough Island and Lake Superior cores. The monitoring dataset spanning 1953 to 2014 showed a peak of TP in ~ 1980 (~ 180 $\mu\text{g/L}$) and afterward a steady decrease in

TP concentration to approximately 40 $\mu\text{g/L}$ (based on the lowest smoothing), which is a fair match with modern DI-TP of $\sim 30 \mu\text{g/L}$ from the Minnesota Point and North of Clough Island cores.

Discussion

These paleolimnological data describe the history of anthropogenic influence on the estuary and western Lake Superior and reveal where remediation may be occurring. As previously detailed by Reavie and Edlund (2010), paleolimnology in lotic environments can be challenging. We believe we have overcome these limitations through application of multiple fossil indicators and core locations. Pigments confirm the diatom record, DI-TP, and to some degree, monitoring data.

Early impacts from logging and subsequent modifications of the drainage basin and the SLR were prevalent in the paleorecord. When logging was at its peak ($\sim 1850 - 1900$), a transition in estuary diatom communities from benthic genera (*Staurosira* and *Staurosirella*) to centric, planktonic diatoms (e.g. *Aulacoseira*) suggested a physical transformation to a more lacustrine (but still fluvial) system as a result of hydrological manipulation by damming and dredging of the SLR and the estuary.

By the 1930s, with growing industries and a growing population to support them, the estuary's ecology changed. Increased sedimentation rates, more eutrophic diatom species, and higher DI-TP dominated the paleorecord. This was likely due to the combined effects of untreated wastewater and runoff from a landscape transformed by logging. With the installation of the Fond du Lac dam (upstream of all core locations) in 1924, one might have expected sedimentation rates to decrease due to the retention effect of the new reservoir. However, it is clear that other factors (algal production and watershed disruptions leading to increased erosion) have contributed to an increase in sediment loads at some locations. Since 1970, sedimentation decreased in all cores with the

exception of Minnesota Point and Pokegama Bay, and nutrient trajectories varied among locations. The cores from open-water environments suggested a remediation or stabilization of environmental quality, while in the bay areas phosphorus loading may be continuing. Fossil pigments corroborate this recent trend, with heightened pigments from total algae and those from cyanobacteria in two of the bay locations.

Changes in legislation like the Clean Water Act accompanied by the restoration efforts to improve the condition the estuary are associated with the recovery we observed in some cores. The recovery is defined partly by a decrease in nutrient-tolerant diatoms—*Aulacoseira* and *S. conspicueporus* in Lake Superior, *Aulacoseira* and *C. meneghiniana* in North of Clough Island, and *S. parvus* in Minnesota Point. This was affirmed by a decrease in DI-TP—a reduction in Lake Superior and North of Clough Island and apparent stabilization in Minnesota Point.

The results from these cores mostly agreed with the monitoring data from Bellinger et al. (2016). Though the overall trend in measured TP matched DI-TP (especially North of Clough Island), the concentrations found by Bellinger et al. were much higher than those inferred by the model. This discrepancy may be due to the natural variability in the estuary as the nearest coring location (Minnesota Point) is ~3.5 km away. Additionally, DI-TP produces values representing a more integrated dataset (spatially and temporally) rather than the episodic TP measurements reported by Bellinger et al., so DI-TP may integrate nutrient information from unmonitored times, such as winter.

Despite some remediation, fossil data from the four cores taken from bay environments suggest continued high nutrients in these parts of the estuary. Higher populations of all algae groups (notably cyanobacteria), a growth in abundance of nutrient-tolerant diatoms (*C. dubius*, *C. meneghiniana*, *S. parvus*, and *S. binderanus*), and increased DI-TP all support this conclusion. The recent persistence of high concentrations of nutrients in parts of the estuary may be due to more localized nutrient sources, potentially due to recent development and the continued presence of industry. But, contemporary anthropogenic

issues facing other water bodies like those reported in Lake Erie—internal phosphorus loading and higher runoff from high-intensity rain events associated with climate change (Kane et al. 2009; Reavie and Allinger 2011)—may also be responsible. Such possible drivers need additional study in the estuary.

There is little doubt that management to remediate the estuary has reduced the flux and concentrations of nutrients in the estuary. To meet BUI removal targets, the portion of Lake Superior in the AOC must have TP concentrations below 10 µg/L (the upper limit for oligotrophic designation) and the estuary must be below 30 µg/L, the upper limit for mesotrophic designation (MPCA and WDNR 2013). According to DI-TP, Lake Superior easily falls within the passing criteria. Minnesota Point, North of Clough Island, and Billings Park (at least according to the most recent interval) have stabilized TP concentrations around or below 30 µg/L, whereas North Bay, Pokegama Bay, and Allouez Bay far exceed desired concentrations. In fact, pre-impact concentrations of DI-TP at North Bay and Pokegama Bay (40 - 45 µg/L) surpass the delisting criteria, so a criterion of 30 µg/L may be unrealistic for these areas. Ultimately, North Bay, Pokegama Bay, and Allouez Bay appear to require focused remediation efforts for nutrients. Contemporary nutrient monitoring data from these areas would be beneficial to support our findings and to better determine policy programs that should be the focus of continued restoration work in the SLRE (e.g. National Pollutant Discharge Elimination System, Total Maximum Daily Loads).

Four of the core locations (Lake Superior, North of Clough Island, Minnesota Point, and Billings Park) meet nutrient BUI delisting criteria. However, those not meeting phosphorus criteria (North Bay, Pokegama Bay, Allouez Bay) appear to be naturally productive (more than 30 µg/L TP during pre-European times). Because of this, delisting goals may need reconsideration in order to accommodate the natural state of the estuary. The higher inferred TP at these locations may be due to legacy pollution, but it is also possible that these recent increases are instead a result of more modern stressors like climate change and internal phosphorus loading. These phenomena were not well

understood at the time of the AOC listing and RAP development. Managing agencies may choose to remove the nutrient BUI with the intention of addressing these modern issues driving water quality in the estuary.

Presently, only four American and three Canadian AOCs have been delisted, leaving 36 between the two countries remaining. Although there have been paleolimnological studies in AOCs in the past (e.g. Warwick 1980; Reavie et al. 1998; Yang et al. 1993), there have been few studies done intentionally to advise AOC programs. In a similar study to ours, Dixit et al. (1998) examined metals, accumulation rates, and diatom taxa to understand the anthropogenic influence in the Spanish Harbor of Lake Ontario to inform a Remedial Action Plan. Retrospective studies such as these will be valuable for scientists and resource managers because revealing environmental history is essential in developing management plans. As demonstrated here, paleolimnological investigations can be useful in not only developing RAPs, but also in gauging the extent of remediation in AOCs to aid in their eventual delisting.

Tables

Table 1. List of cores with information regarding collection and coring location.

Core Name	Date	Water Depth (m)	County, State	Latitude	Longitude	Core Length (cm)	Note
North Bay	1/11/14	1.68	Saint Louis, MN	46.654	-92.237	86	gyttja with silty layering
Pokegama Bay	3/18/14	2.48	Douglas, WI	46.695	-92.171	97	gyttja with silty layering
North of Clough Island	3/18/14	1.92	Saint Louis, MN	46.713	-92.181	86	gyttja with silty layering
Billings Park	1/10/14	2.67	Douglas, WI	46.71	-92.147	86	gyttja with silty layering
Minnesota Point	3/17/14	3.55	Saint Louis, MN	46.732	-92.065	58	gyttja with silty layering
Allouez Bay	1/11/14	1.94	Douglas, WI	46.691	-91.998	86	gyttja with silty layering
Lake Superior West	5/16/14	50	Saint Louis, MN	46.816	-91.852	60	0-10 cm silt; 10-50 cm gyttja; 50-70 cm gyttja (banding at 59 & 63 cm and transition at 50 cm)

Figures

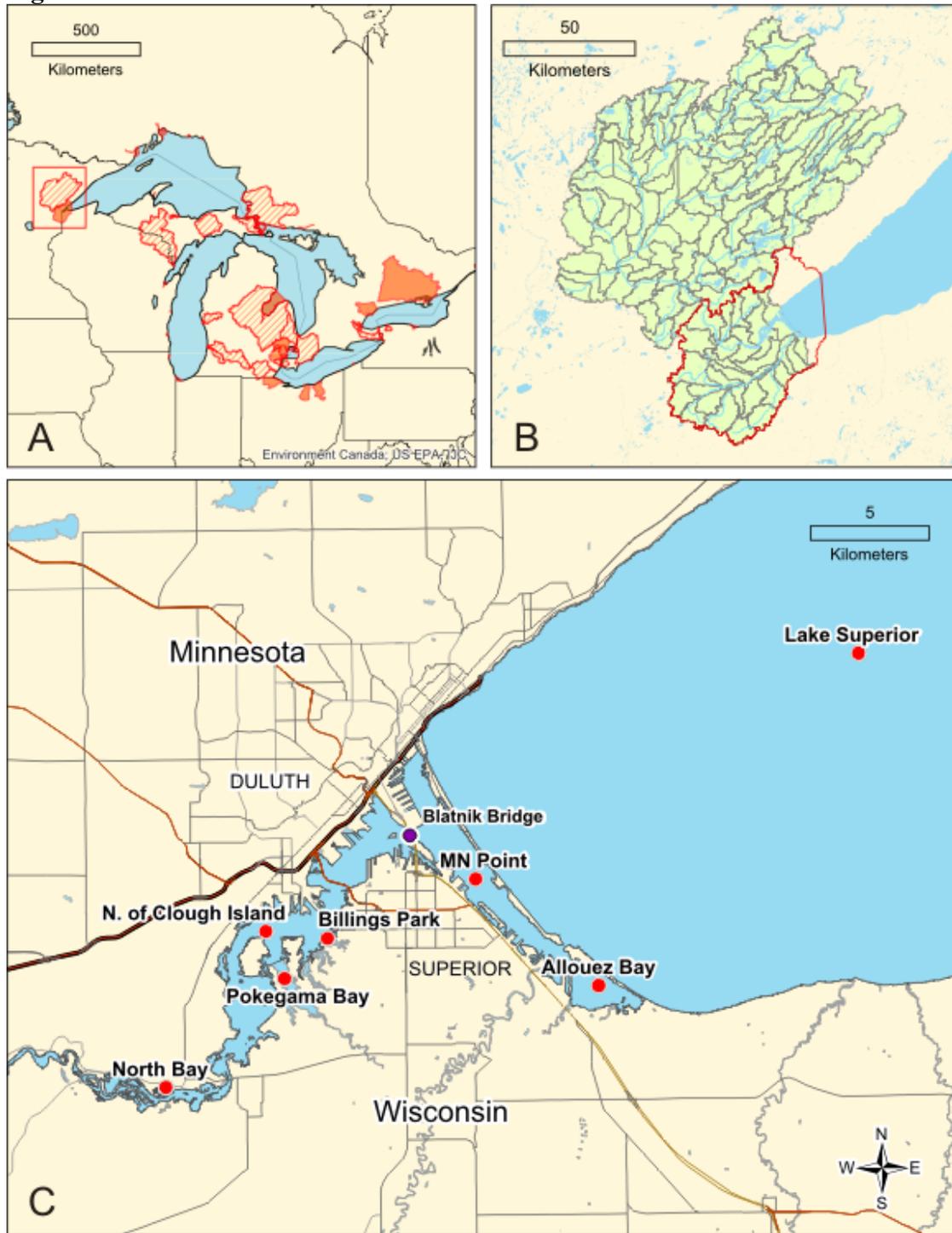


Figure 1. (A) Map indicating the location of the St. Louis River estuary (SLRE) area of concern (AOC) relative to all Great Lakes AOCs (orange) and their associated watersheds (red hash) (Environment Canada, EPA, IJC 2013) (B) Map of the St. Louis River drainage basin and the boundary (red) of the AOC (C) Map of coring locations in the SLRE.

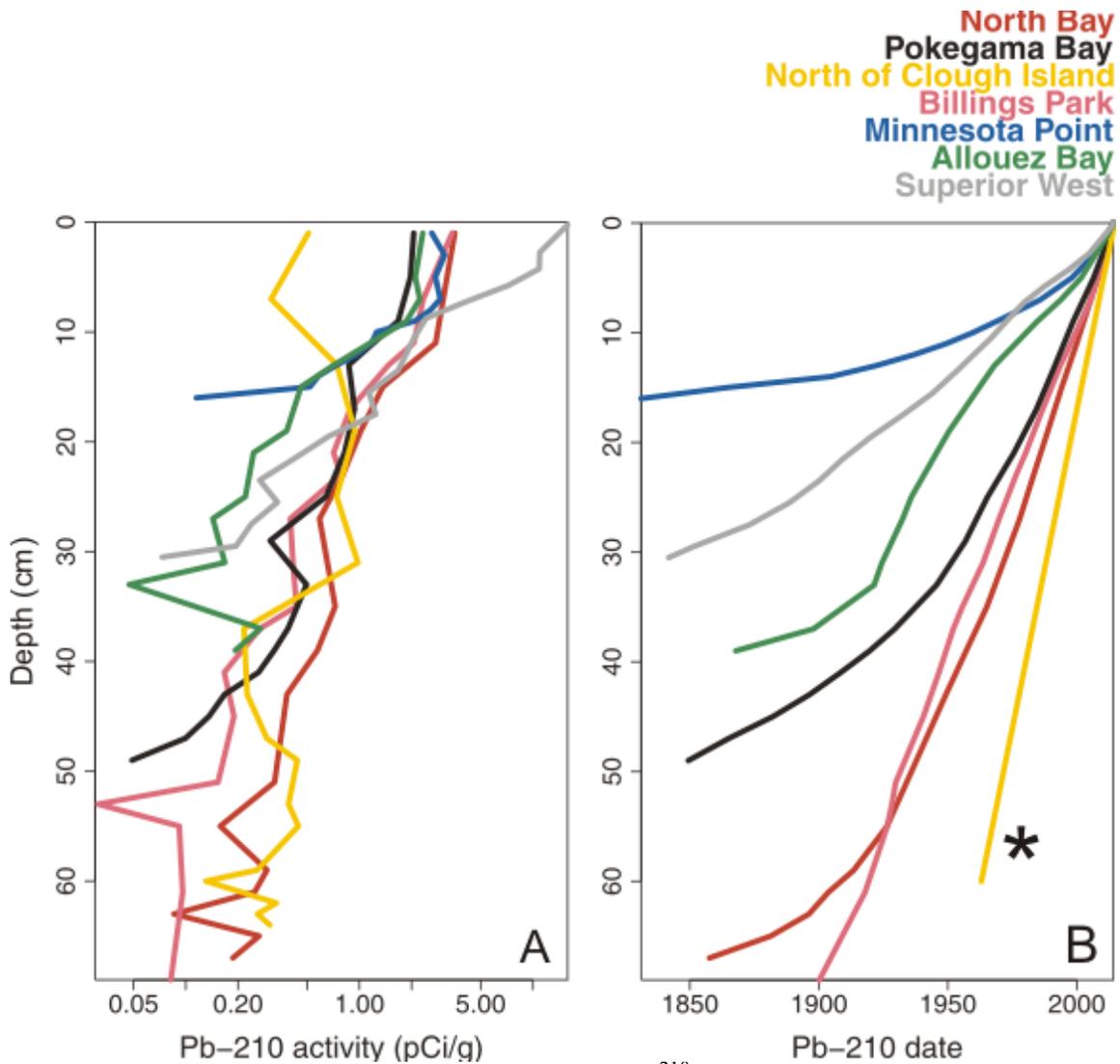


Figure 2. (A) The magnitude of supported lead-210 (^{210}Pb) activity versus depth in the core. (B) ^{210}Pb inferred dates of the cores versus sediment depth. *The North of Clough core demonstrated a poor ^{210}Pb record, so we provide a very rough estimate of dates and accumulation rates based on ^{137}Cs data that indicated the ~1963 interval.

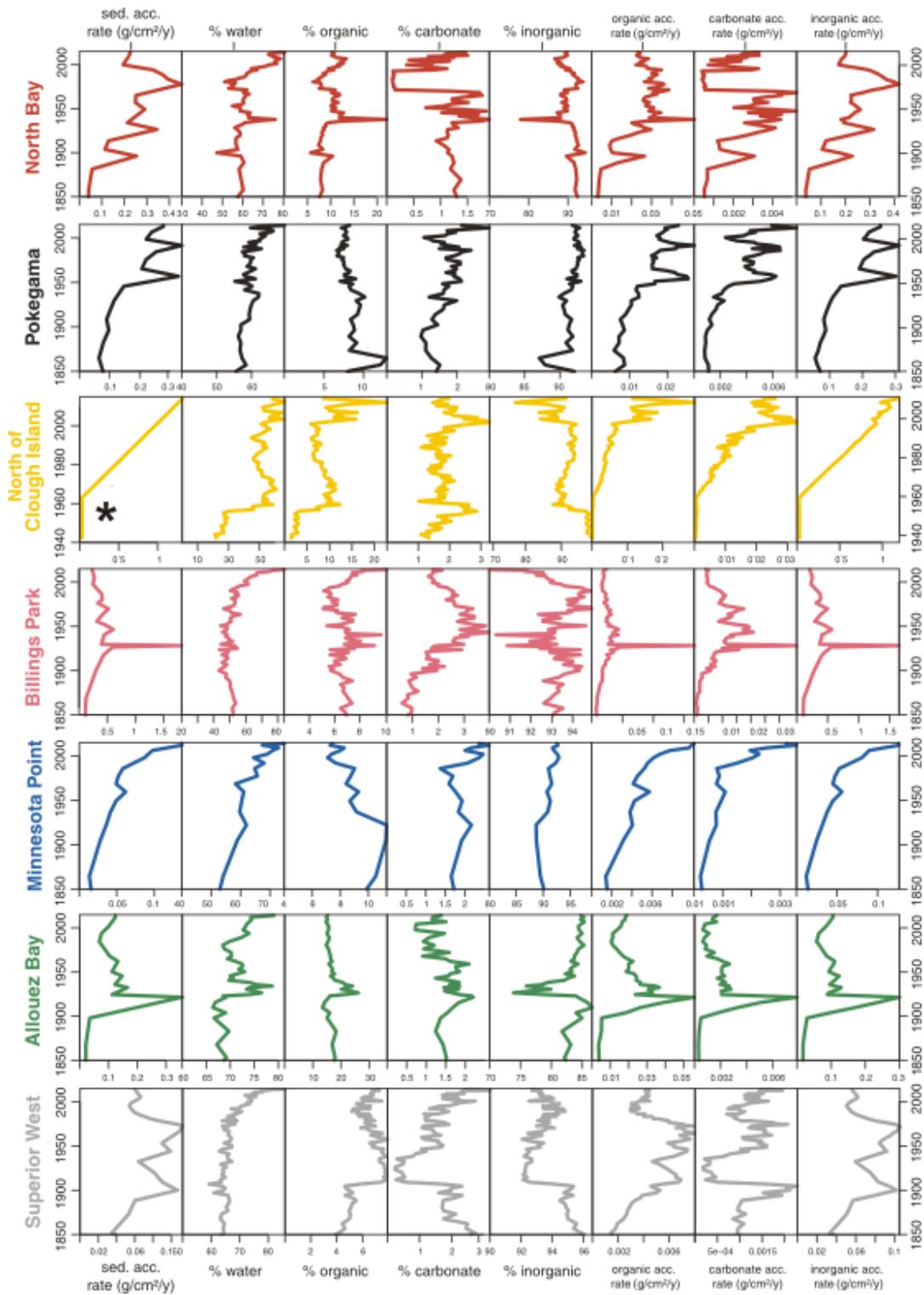


Figure 3. Results from of inorganic and organic content analyses of seven sediment cores from the SLRE and Lake Superior. *The North of Clough demonstrated a poor ^{210}Pb record, so we provide a very rough estimate of dates and accumulation rates based on ^{137}Cs data that indicated the ~1963 interval. Note the scales are different for each core.

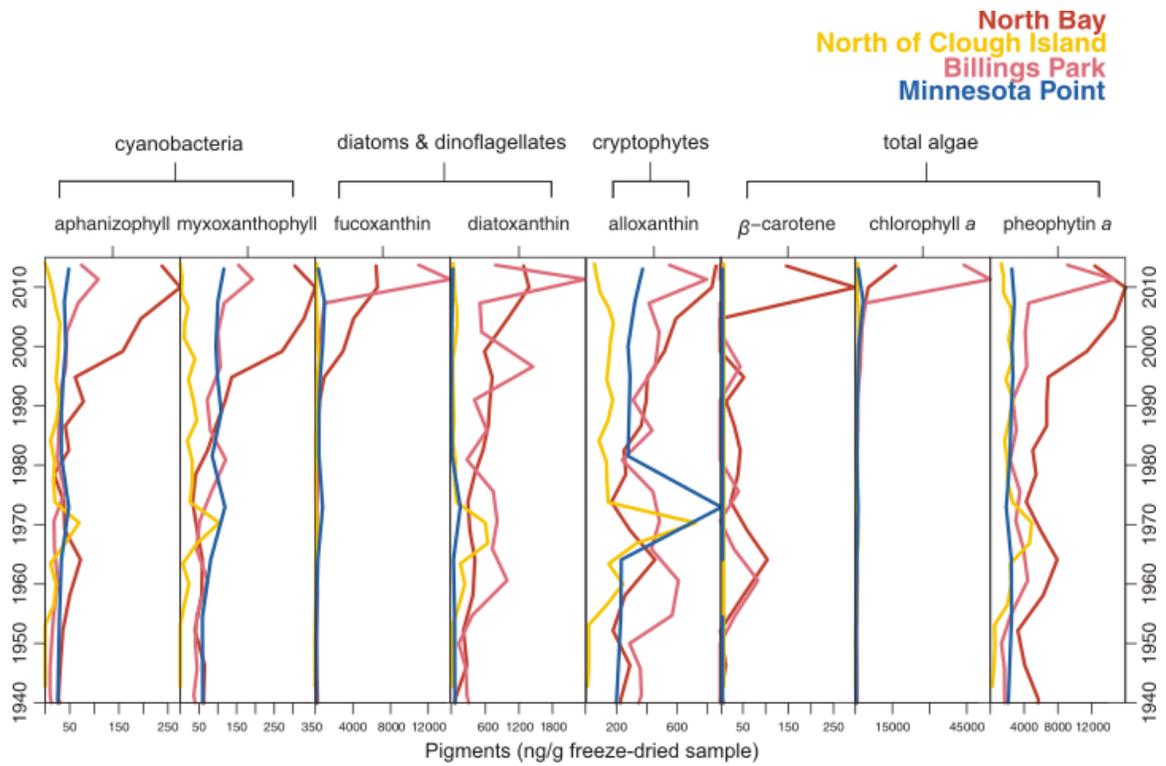


Figure 4. Concentrations of various algal pigments determined by HPLC in four estuary cores.

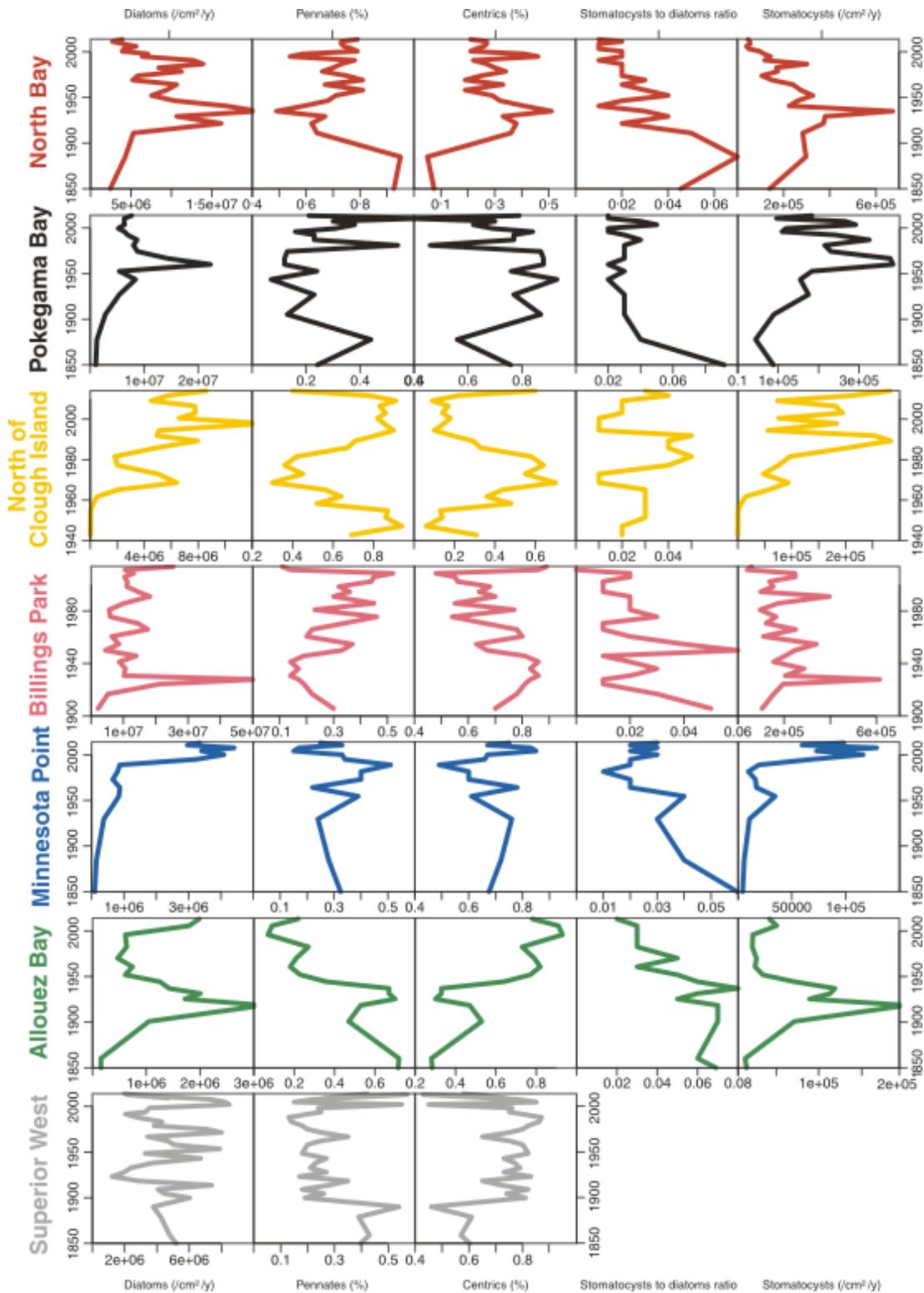


Figure 5. Diatom accumulation rates, % pennates, % centrics, ratio of chrysophyte stomatocysts to diatoms, and chrysophyte stomatocyst accumulation rates of seven cores.

in the SLRE and Lake Superior. Chrysophyte stomatocysts were not in great enough abundance in Lake Superior to be plotted.

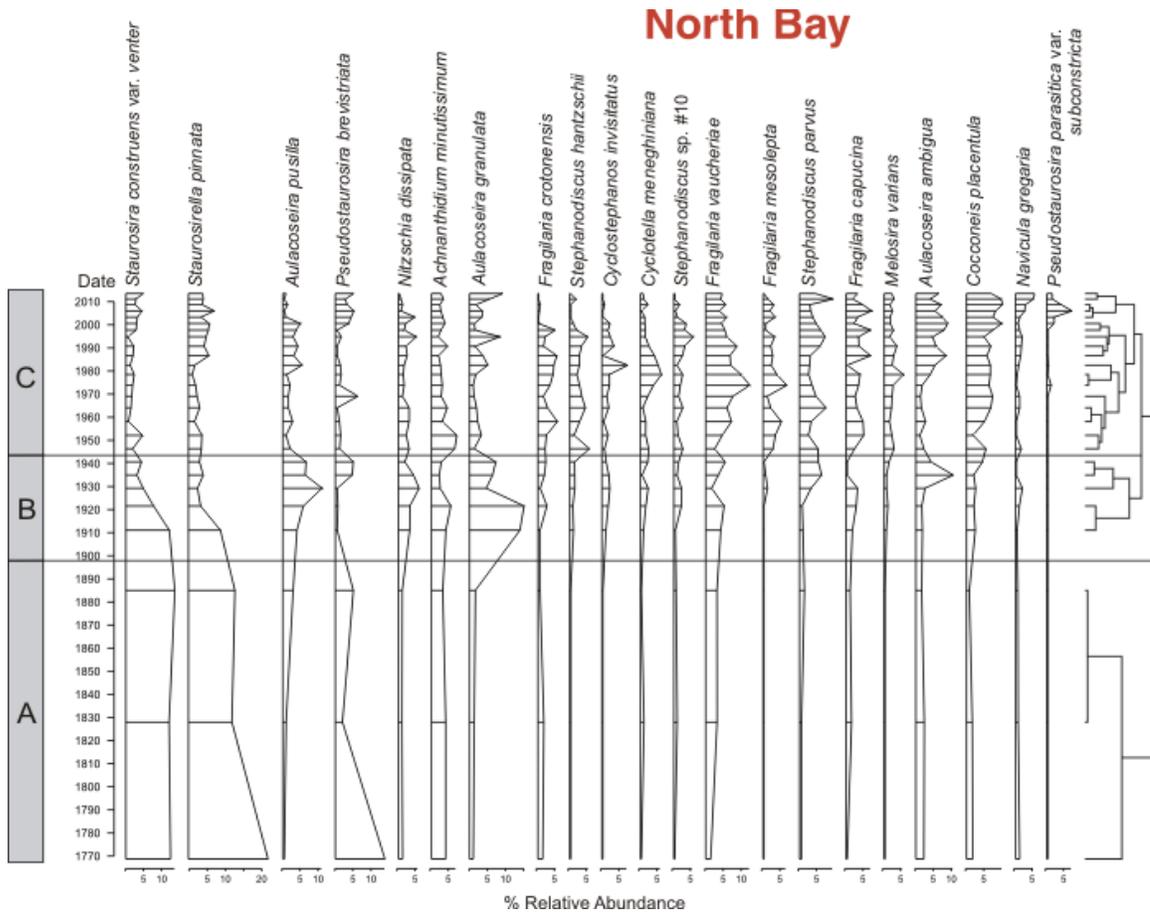


Figure 6. Relative abundances of the most common taxa in the core taken from North Bay. The labeled zones represent changes in assemblages determined by the clustering analysis.

Pokegama

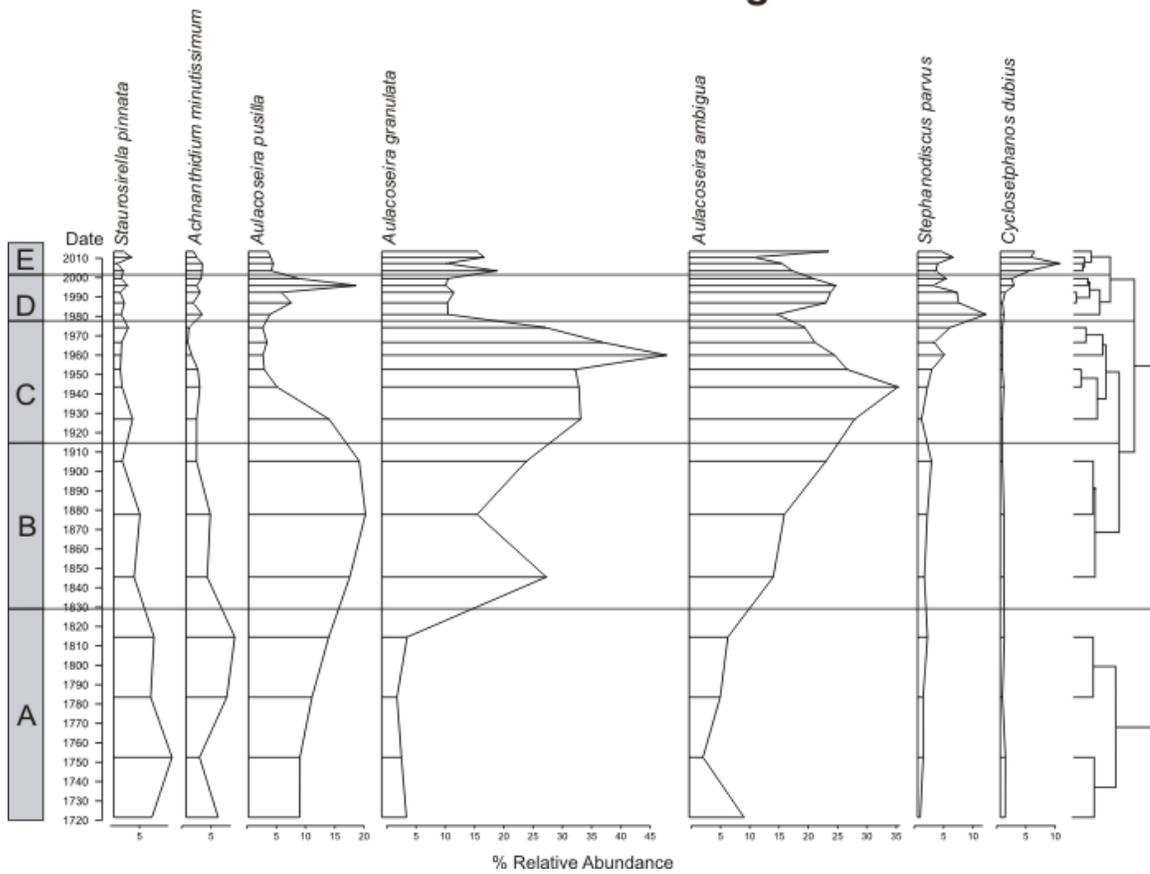


Figure 7. Relative abundances of the most common taxa in the core taken from Pokegama Bay. The labeled zones represent changes in assemblages determined by the clustering analysis.

North of Clough Island

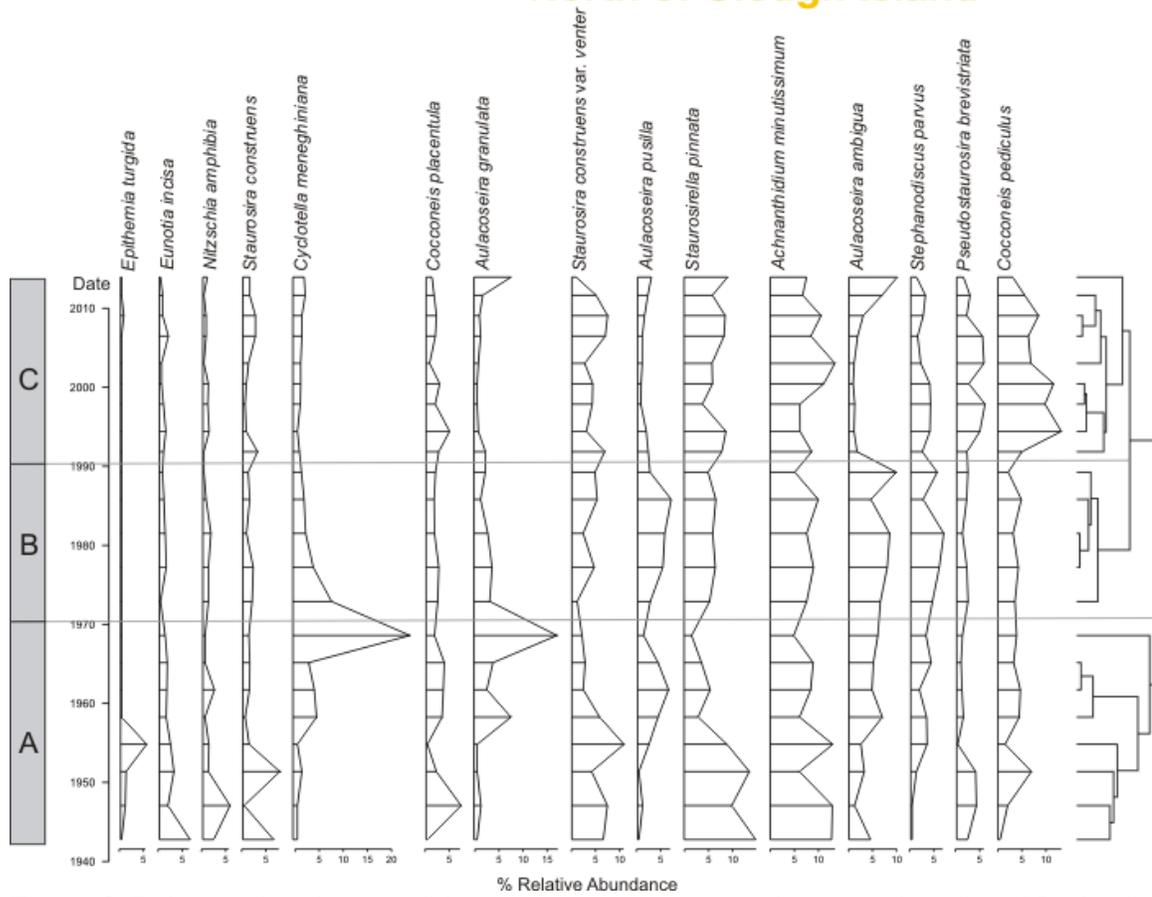


Figure 8. Relative abundances of the most common taxa in the core taken from North of Clough Island. The labeled zones represent changes in assemblages determined by the clustering analysis.

Billings Park

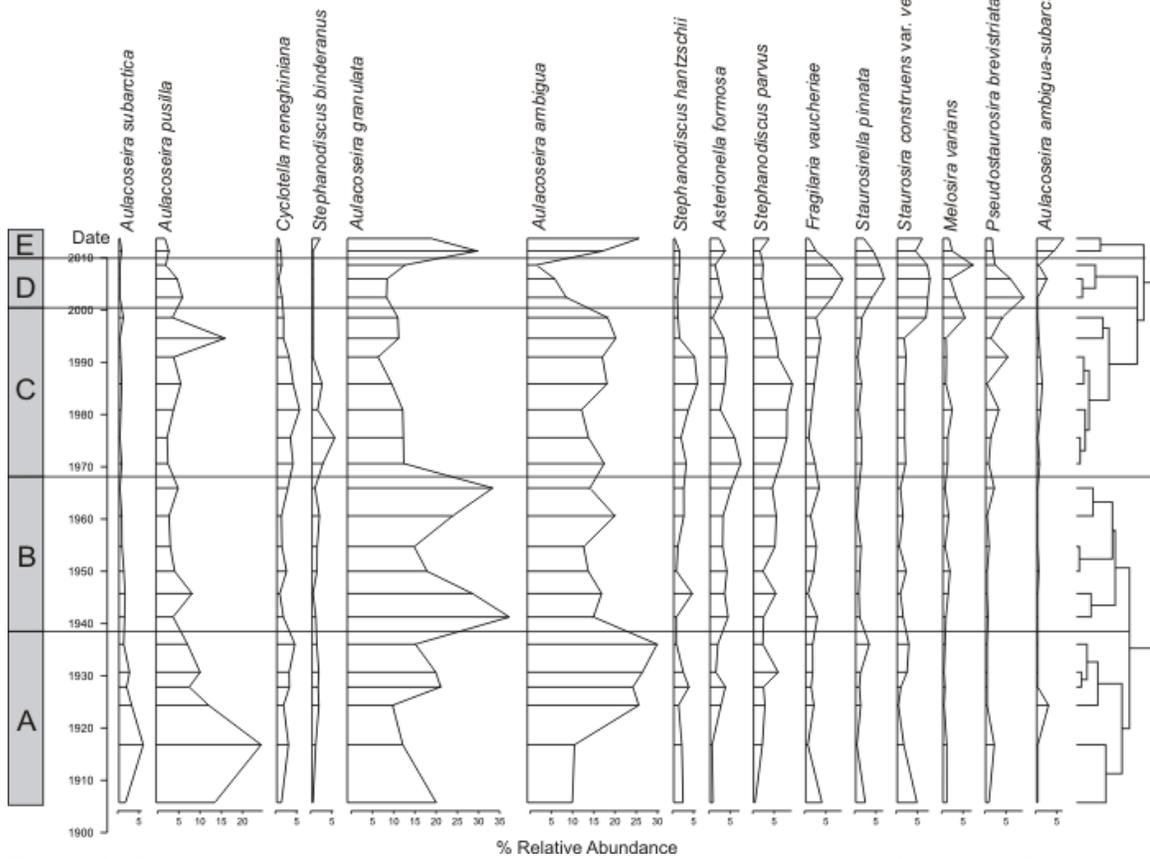


Figure 9. Relative abundances of the most common taxa in the core taken from Billings Park. The labeled zones represent changes in assemblages determined by the clustering analysis.

Minnesota Point

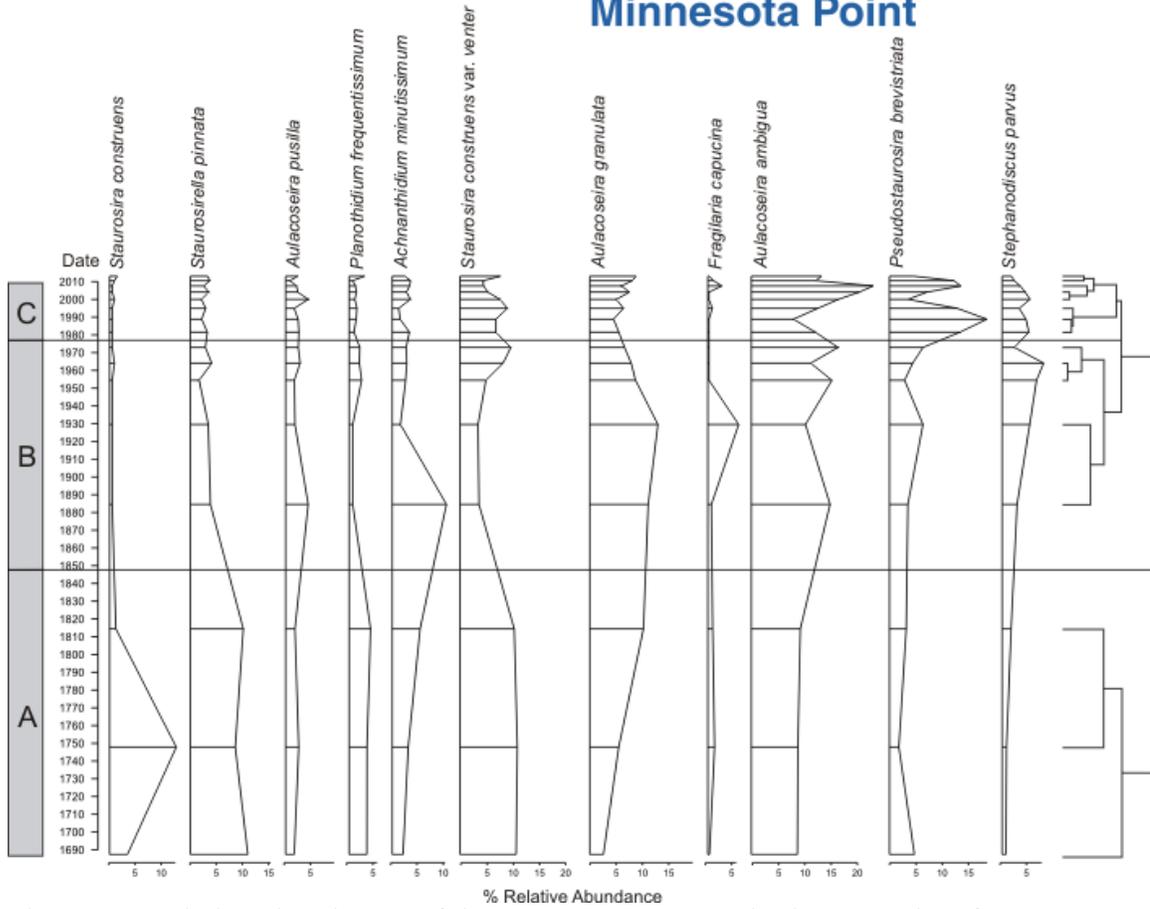


Figure 10. Relative abundances of the most common taxa in the core taken from Minnesota Point. The labeled zones represent changes in assemblages determined by the clustering analysis.

Allouez Bay

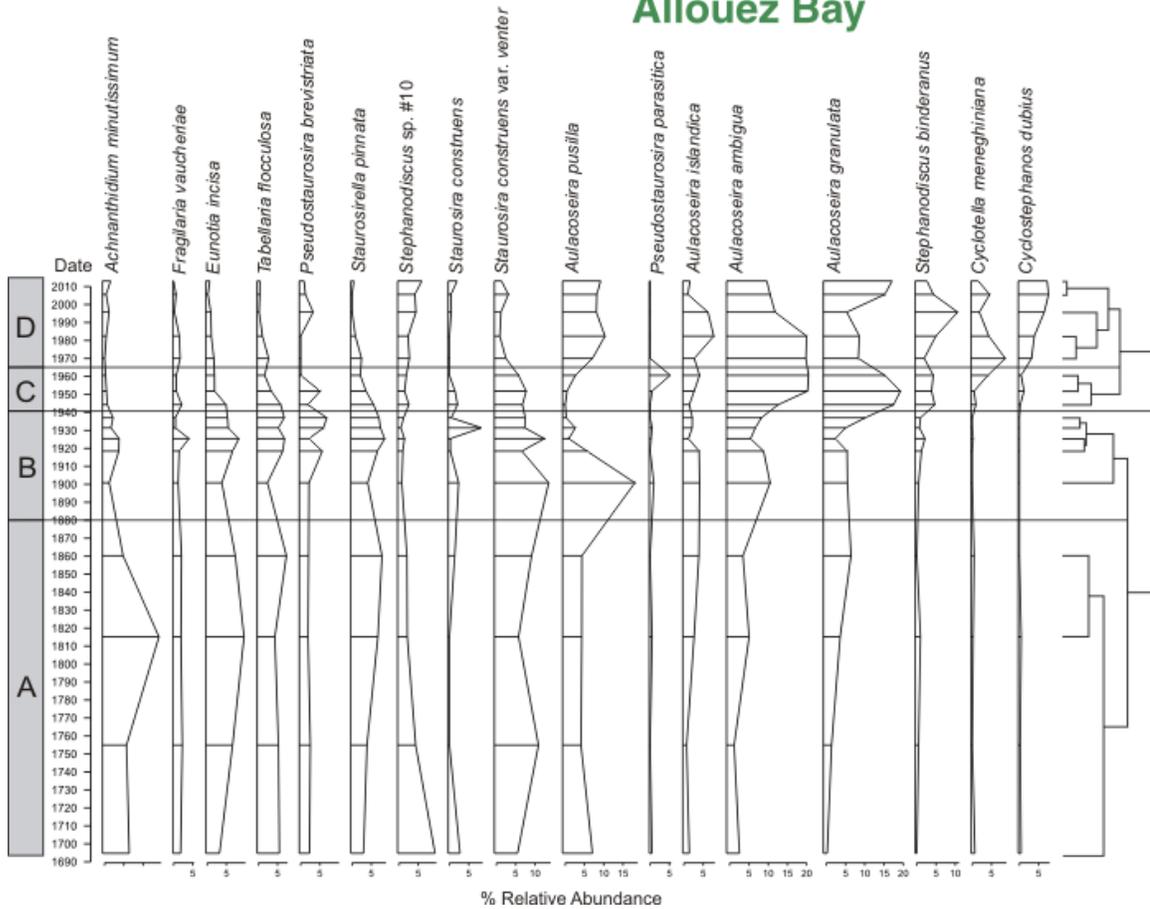


Figure 11. Relative abundances of the most common taxa in the core taken from Allouez Bay. The labeled zones represent changes in assemblages determined by the clustering analysis.

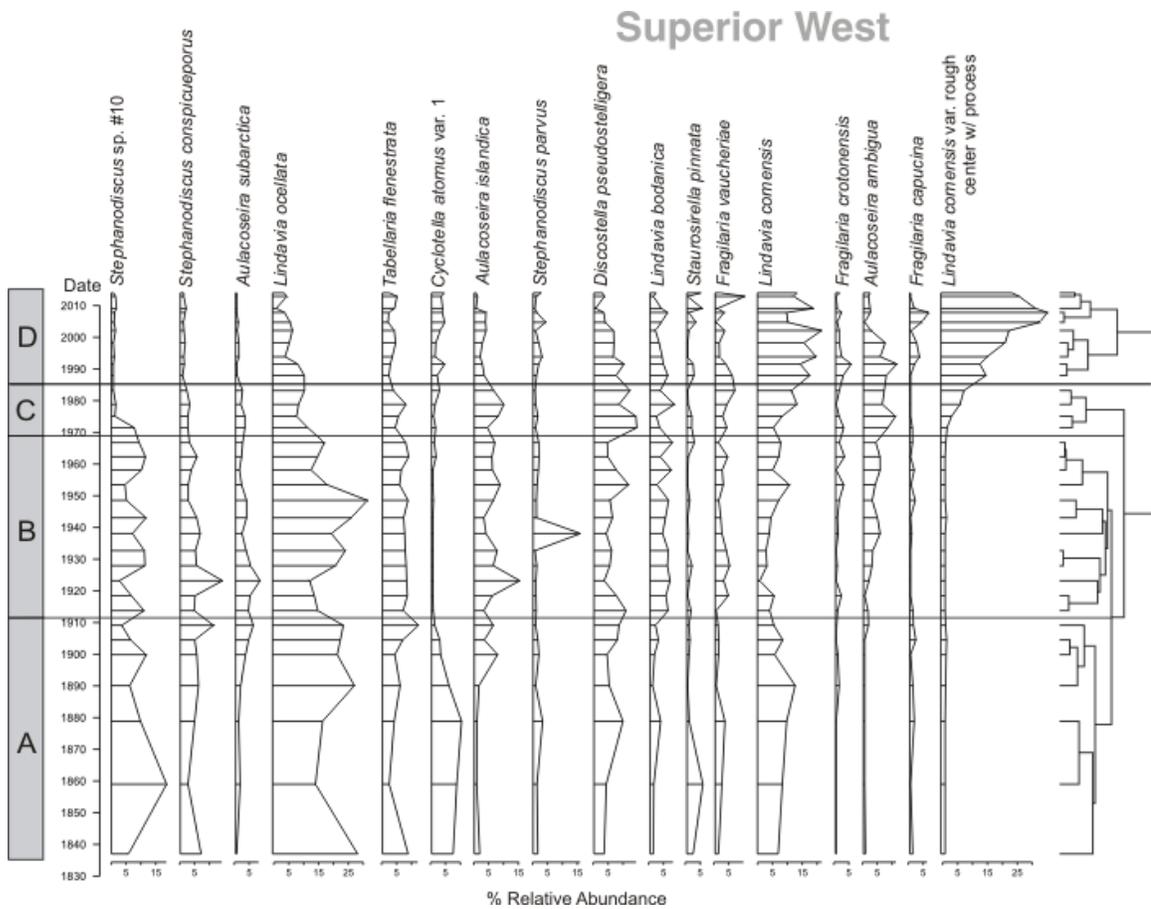


Figure 12. Relative abundances of the most common taxa in the core taken from Western Lake Superior. The labeled zones represent changes in assemblages determined by the clustering analysis.

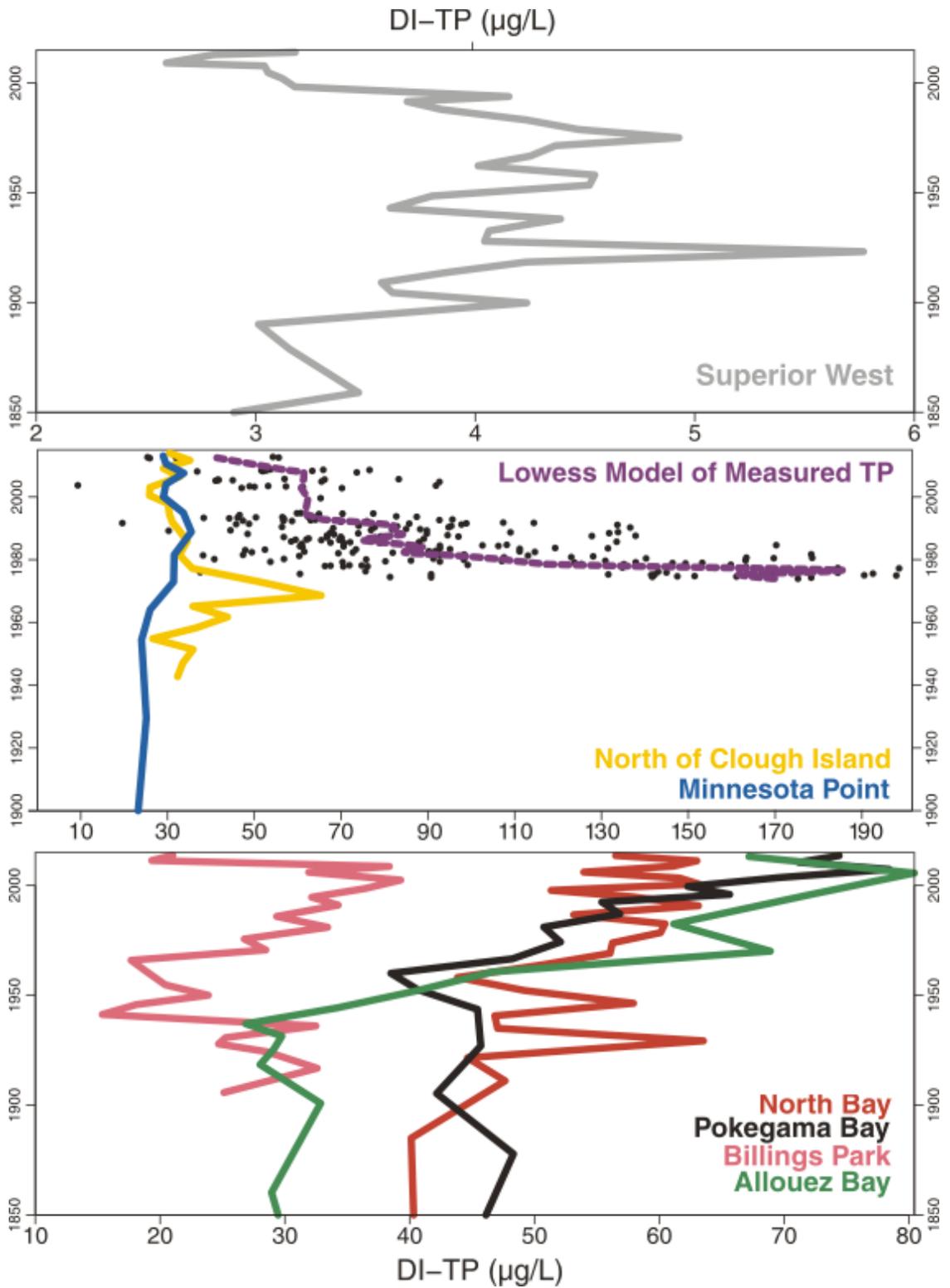


Figure 14. Diatom-inferred total phosphorus from all cores. The purple line represents a lowess model of total phosphorus measurements (black dots) from the Blatnik Bridge from 1958 to 2012 as reported in Bellinger et al. (2016).

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Appendix 1

University of Minnesota Duluth
Natural Resources Research Institute
Center for Applied Research and Technology Development
Laboratory of Chemical Extractives

Extraction and Pigmentary HPLC Analysis for Freshwater Sedimentary Samples

General Procedure

Introduction

This is a general procedure for extraction of freshwater sedimentary samples and their analysis for pigment content.

All sediment samples should be frozen at -20°C or colder as soon as possible after sampling and stored at this temperature without additional treatment (*e.g.*, freeze-drying) until just before analysis of pigments. If dictated by other proxy analyses, raw sediment can be stored at 3°C for up to 6 months, whereas freeze-dried sediment should always be stored frozen.

Freeze-dried samples should be provided/used for the extraction and further HPLC analysis. Eight pigments (carotenoids and chlorins) chosen for the analysis in this study represent total algal abundance (chlorophyll a, pheophytin a, and β -carotene), the dominating phytoplankton community of diatoms and dinoflagellates (fucoxanthin and diatoxanthin), cryptophytes (alloxanthin), and cyanobacteria (aphanizophyll and myxoxanthophyll)¹ (**Figure 1**).

Materials

Apparatus:

- Shimadzu SCL-10Avp system controller, Shimadzu LC-10ATvp solvent delivery module for high performance liquid chromatograph, Shimadzu DGU-14A solvent degasser, Shimadzu FCV-10ALvp solvent mixer, Shimadzu SIL-20A autosampler, Shimadzu CTO-20A column oven, Supelcosil LC-18 HPLC column (Aldrich 58298, $5\ \mu\text{m}$ particle size, $L \times \text{I.D. } 25\ \text{cm} \times 4.6\ \text{mm}$) with Supelcosil LC-18 Supelguard cartridge (Aldrich 59564, $5\ \mu\text{m}$ particle size, $L \times \text{I.D. } 2\ \text{cm} \times 4.0\ \text{mm}$), Shimadzu SPD-M10Avp UV/VIS photodiode array detector, PC

with MS Windows 2000 Pro SP4, and EZSTART v7.3 SP1 Chromatography Software

- near • Calibrated analytical balance capable of weighing to the est
0.1 mg
- Sonicator (Fisher Scientific FS140)
- Freezer (-20°C)
- Volumetric flasks and pipettes (assorted sizes)
- Vials (preferably amber glass, appropriate sizes) with screw caps (for extraction, extract storage, and HPLC autosampler)
- Plastic syringes and syringe filters (appropriate sizes)

Chemicals and solvents:

- Alloxanthin, aphanizophyll, β -carotene, chlorophyll a, diatoxanthin, fucoxanthin, myxoxanthophyll, pheophytin a, and mixed pigments (pigment standard solutions from DHI Laboratory (Hørsholm, Denmark))
- *trans*- β -Apo-8'-carotenal (Sigma Aldrich)
- Ammonium acetate (Sigma Aldrich)
- Acetone, water, methanol, acetonitrile, ethyl acetate (HPLC grade solvents (Fisher Scientific))

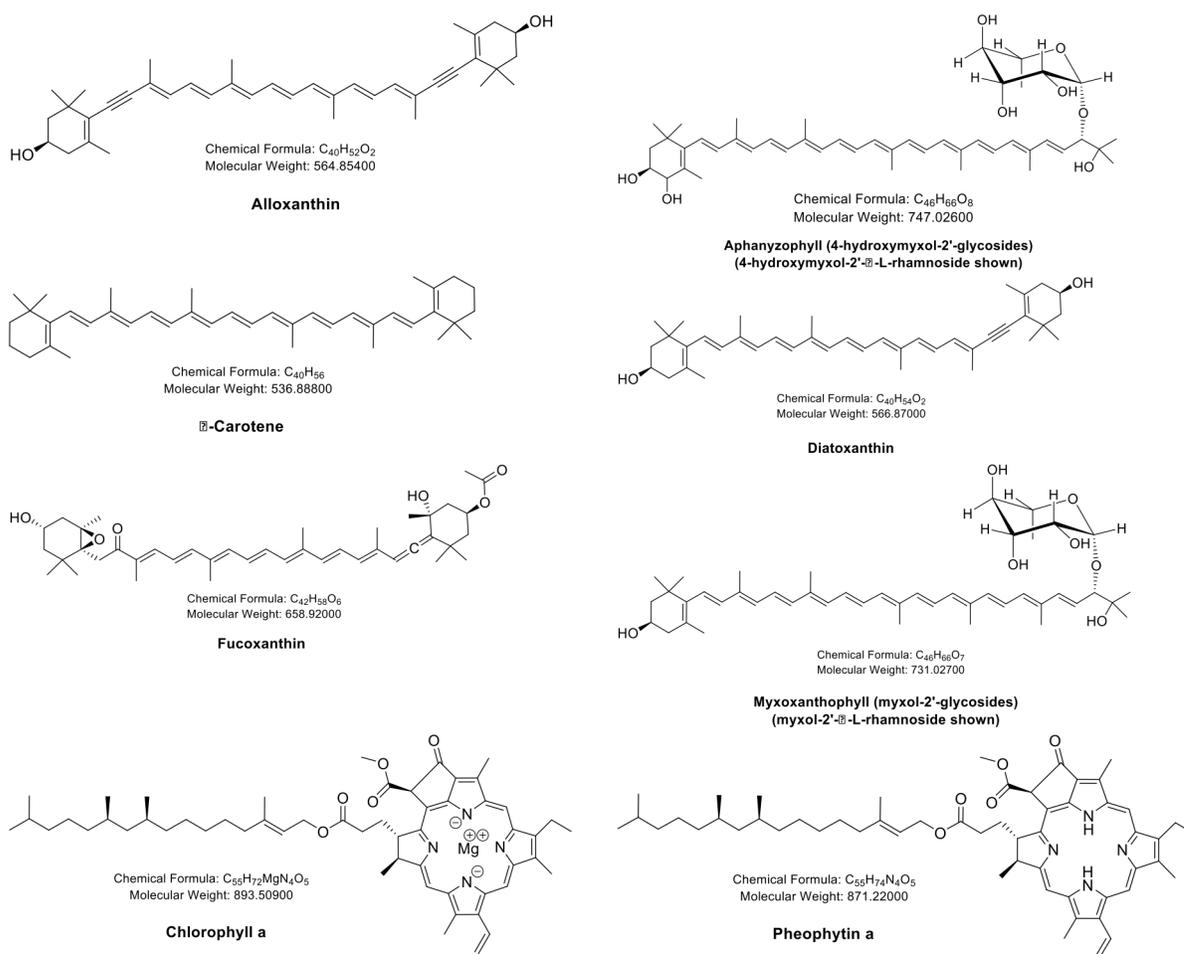


Figure 1 Pigments for HPLC analysis

Extraction

HPLC grade acetone/water (90/10, 5 mL, volumetric pipette, room temperature) is added to the powdered sample (1 g, weighed to nearest 0.1 mg) in 22 mL amber glass vial (Aldrich 27004) with screw cap (PTFE liner). The mixture is sonicated (Fisher

FS140 sonicator) in an ice bath (3°C) for 20 min and left for extraction in a freezer (-20°C) for 24 h (shaken occasionally). The extract is filtered through a 0.45 µm, 17 mm CAMEO syringe filter (PTFE membrane, Aldrich S7187, 10 mL plastic syringe Fisher 03-377-23) in 15 mL amber glass vial (with screw cap (PTFE liner) Aldrich 27003) and used immediately (unused part is kept at -20°C in the dark in freezer). Extract (0.5-1.0 mL) is transferred to 1.5 mL vial for autosampler (Shimadzu 228-15652-92 clear glass with silicone septum and PTFE liner caps or Aldrich 854172 Supelco amber glass (SU860076 caps with silicone septum and PTFE liner)) for immediate injection (30 µL). The vial is transferred to the freezer again after injection and kept in there till the next injection. Extracts can be kept in the dark in freezer at -20°C for a few days without significant changes in the pigment content, though they should be analyzed as soon as possible. Actual injection volume (30-60µL) (as well as freeze-dried sample weight (1-2 g) and extraction solvent volume (4, 5 mL) if necessary) may be changed in order to obtain chromatogram with satisfactory response and resolution for each individual case.

HPLC analysis

Quantitative analyses of all pigments are conducted on a Shimadzu HPLC equipped with photodiode array detector (SPD-M10Avp). Injection (0-100 µL) of the pigment solution is performed using a Shimadzu SIL-20A autosampler. A reverse phase Supelcosil LC-18 column² with a guard column is used for separation.

Eluents: A 80/20 MeOH/0.5 M NH₄OOCMe aq., B 90/10 MeCN/H₂O, C 100% EtOOCMe, D 90/10 MeCOMe/H₂O (washing line). All solvents are HPLC grade (Acetone Fisher A9494, Acetonitrile Fisher A9984, Ethylacetate Fisher AC610060040 (Acros), Methanol Fisher A4524, Water Fisher W54). Ammonium acetate is received from Aldrich (431311).

Two injections using different gradient programs are performed for each sample. First program for aphanizophyll and myxoxanthophyll (49 min, 1.00 mL/min flow): 0 min 100% A, 9 min 0% C, 22 min 70% B, 34 min 30% B and 60% C, 42 min 50% B and 50% C, 44 min 100% A, 49 min stop. Second program for fucoxanthin, alloxanthin, diatoxanthin, chlorophyll a, pheophytin a, and β-carotene (49 min, 1.00 mL/min flow): 0 min 100% A, 4 min 100% B, 20 min 65% B and 35% C, 39 min 40% B and 60% C, 43 min 100% B, 47 min 100% A, 49 min stop.

Column oven temperature: 30°C (max set to 35°C).

UV-VIS PDA settings: 380-700 nm band, 1.5625 Hz acquisition frequency, 0.64 sec time constant.

Peaks are identified based on retention time and spectral matches with pigment spectra obtained from authentic pigment standards (DHI, Denmark). Response factors used for quantification of each pigment are based on HPLC calibration runs of the standards. First chromatogram is analyzed at 508 nm (4 nm bandwidth) for quantification of aphanizophyll and myxoxanthophyll. Other pigments are better resolved and quantified in second chromatogram. Second chromatogram is analyzed at 449 nm (4 nm bandwidth) for quantification of fucoxanthin, alloxanthin, diatoxanthin, and β-carotene and at 666 nm (4 nm bandwidth) for quantification of chlorophyll a and pheophytin a.

HPLC calibration

The photodiode array detector is calibrated using a multipoint calibration procedure for a range of injection volumes (0-100 μ L) of pigment standards (DHI, Denmark). Regressions are performed using pigment peak area (y) vs. its quantity (ng, x , calculated on the basis of injection volume of solution with known pigment concentration) and are of the form $y = ax$; where a is the slope. All regressions have a coefficient of determination $R^2 > 0.99$. The slope of the fitted line is used as a response factor ($RF_p = a$) for the following calculation of pigment concentration in the sample.

Calibrations for aphanizophyll and myxoxanthophyll are performed at 508 nm (4 nm bandwidth) using first gradient program. Calibrations for fucoxanthin, alloxanthin, diatoxanthin, and β -carotene are performed at 449 nm (4 nm bandwidth) using second gradient program. Calibrations for chlorophyll a and pheophytin a are performed at 666 nm (4 nm bandwidth) using second gradient program.

HPLC validation

Carotenal blanks (*trans*- β -apo-8'-carotenal in acetone/water, 90/10) are run before the sample runs to verify peak time reproducibility, peak area precision, and instrument performance. Long term quality control is achieved by analyzing pure and mixed standards supplied by DHI, Denmark.

HPLC data handling

The concentration of targeted pigment (aphanyzophyll, myxoxanthophyll, fucoxanthin, alloxanthin, diatoxanthin, β -carotene, chlorophyll a, and pheophytin a) in freeze-dried sediment sample is calculated using equation:

$$c_p = \frac{A_p V}{RF_p V_p m} 1000;$$

where c_p is pigment concentration in ng per g freeze-dried sedimentary sample (ng/gFDS), A_p is pigment peak area obtained by integration of appropriate chromatogram, V is volume of solvent used for extraction (mL), RF_p is response factor for the pigment obtained from calibrations at appropriate waveband using appropriate gradient program, V_p is injection volume (μ L), and m is weight of freeze-dried sedimentary sample taken for extraction (g).

Note

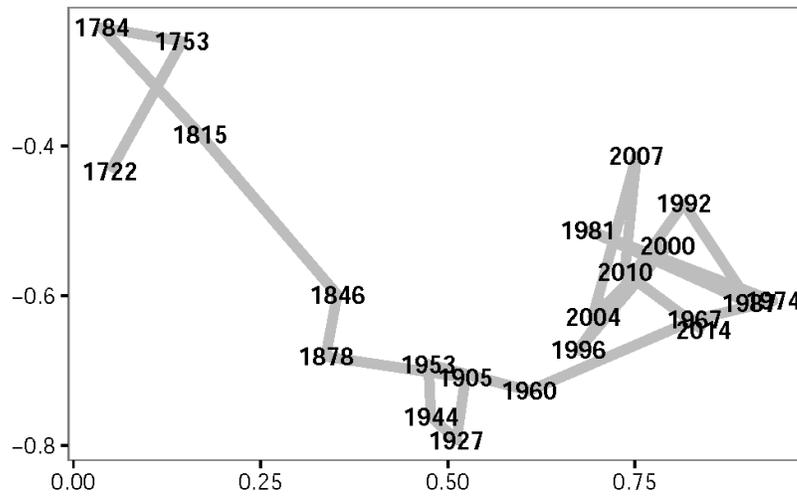
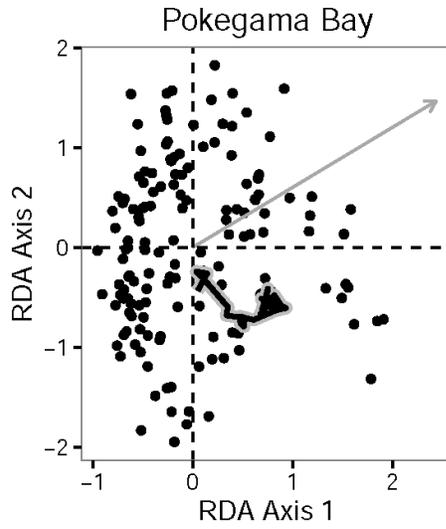
Found pigment concentrations in the samples are presented as ng per g freeze-dried sedimentary sample (ng/gFDS). Data on percentage of organic carbon or organic matter in the freeze-dried sedimentary samples should be provided in order to calculate pigment content in μ g per g organic carbon or organic matter (μ g/gOC or μ g/gOM) if necessary.

References

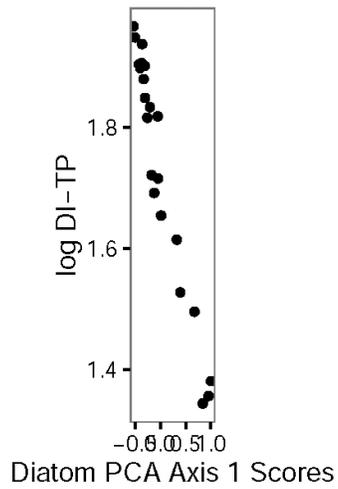
1. Nina Reuss. Sediment pigments as biomarkers of environmental change. PhD Thesis. National Environmental Research Institute, Ministry of the Environment, Denmark, 2005.
2. Nina Reuss and Daniel J. Conley. Effects of sediment storage conditions on pigment analyses. *Limnol. Oceanogr.: Methods* 3, 2005, 477–487.

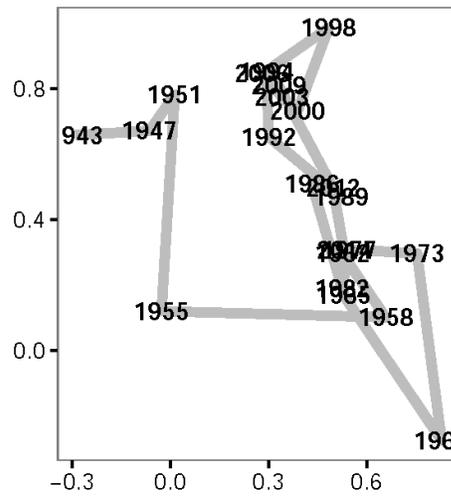
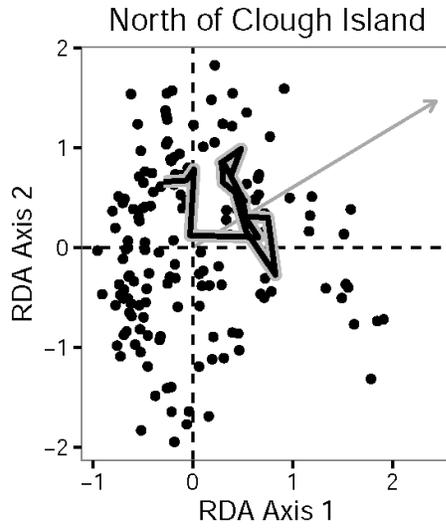
Appendix 2

Analysis to determine if total phosphorus is responsible for fossil species assemblages for 6 SLRE cores. From top to bottom plots show (A) redundancy analysis of model assemblages (points) constrained to measured TP with passive ordination of the fossil assemblages (black line with gray outline), (B) trajectory of the fossil assemblages, and (C) a plot of DI-TP versus the PCA axis 1 scores of the of the fossil diatom assemblage. R-values with asterisk indicate significant correlation ($P < 0.05$).

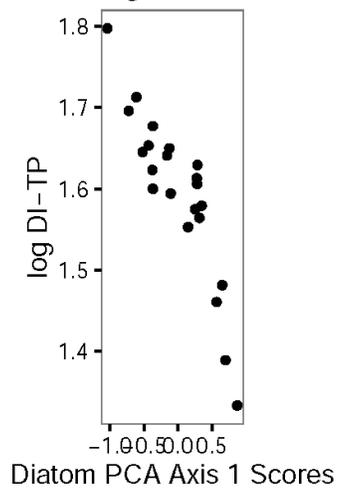


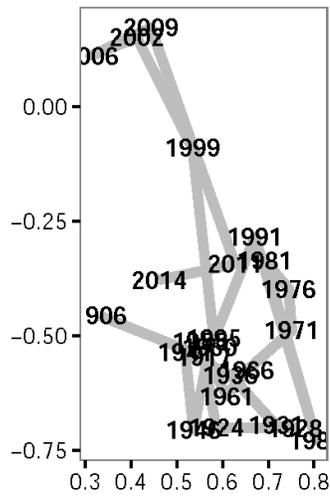
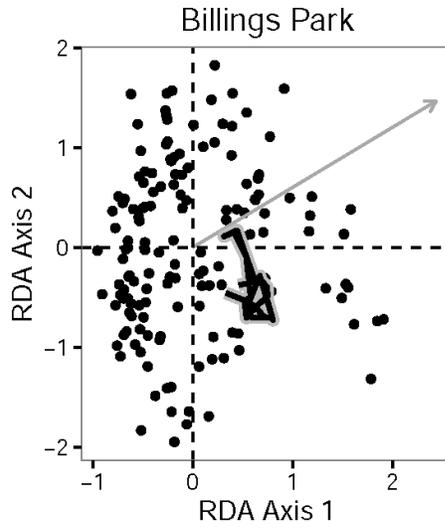
Pokegama Bay $r = -0.98^*$



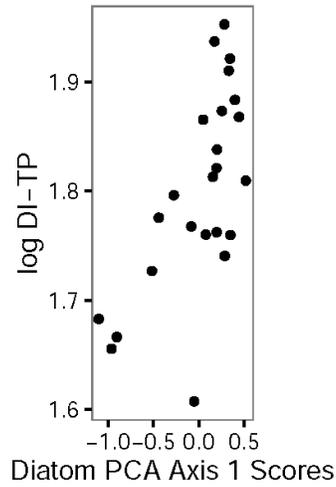


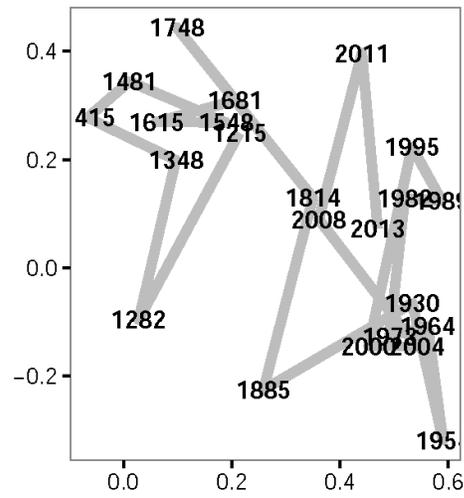
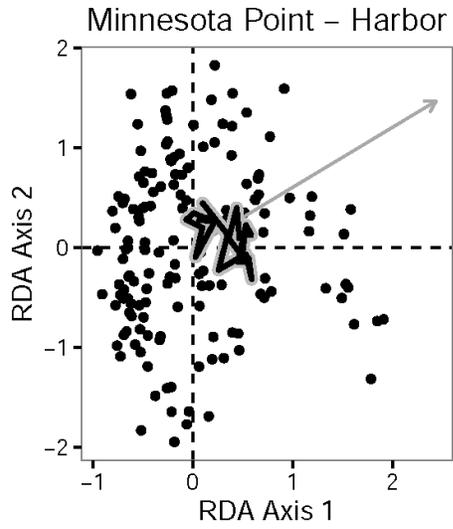
North of Clough Island $r = -0.89^*$



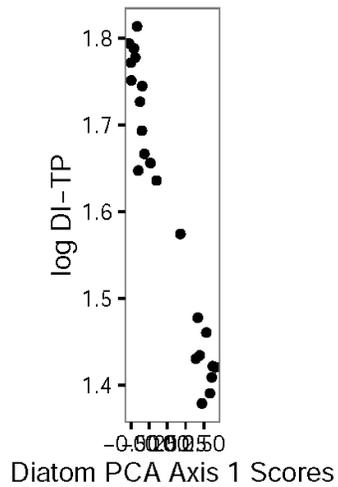


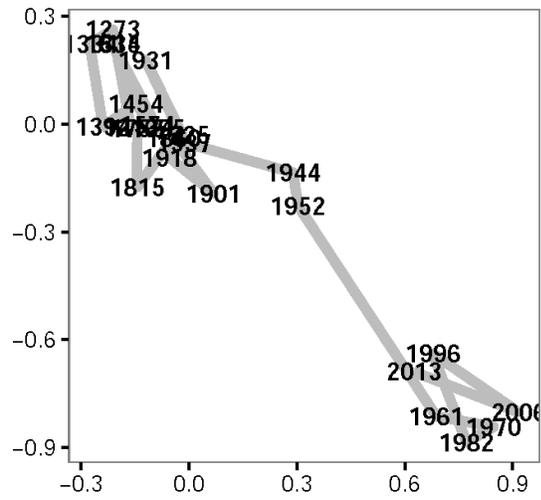
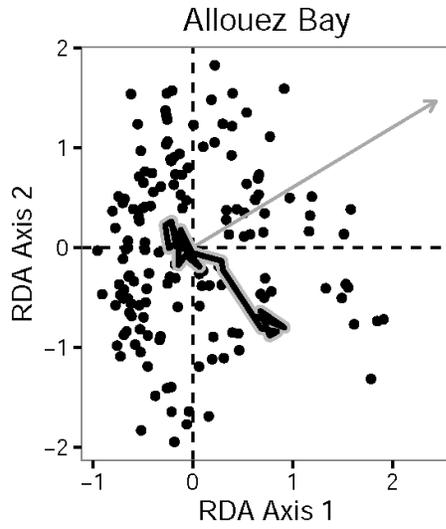
Billings Park $r = 0.67^*$





Minnesota Point – Harbor $r = -0.97^*$





Allouez Bay $r = -0.96^*$

