

## Ecology and assessment of the benthic diatom communities of four Lake Erie estuaries using Lange-Bertalot tolerance values

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### Abstract

Diatom composition of four Lake Erie estuaries was related to seasonal factors, year, location within the estuaries, and water quality parameters including nutrient and metals concentrations. Canonical correspondence analysis (CCA) revealed seasonality as the most important factor determining variability in diatom species composition among sites and dates. Alkalinity, pH, silicate, orthophosphate, and nitrite concentrations were water chemistry parameters correlated with diatom community composition. Eigenvalues for the first two CCA axes of nutrient/physical data and species data were higher than the first two CCA axes of metals data and species data. In addition, the water quality of these estuaries was evaluated using an index composed of Lange-Bertalot pollution tolerance values. The Lange-Bertalot index scores indicated that the Ashtabula estuary had the best water quality of the study sites. Lange-Bertalot index scores were highly correlated with a gradient of disturbance represented by the first axis of a principle components analysis of sites and nutrient data (Spearman  $\rho=0.7$ ). The Lange-Bertalot tolerance values could be useful for discriminating ‘good’ sites from ‘bad’ sites among the Lake Erie estuaries.

### Introduction

Anthropogenic changes in the ecology of the Laurentian Great Lakes have been occurring at least during the last century. These physical, chemical, and biological changes have resulted in a loss of biologic integrity in offshore and near shore habitats such as estuarine areas throughout the Lakes. Changes in the biological communities of these habitats have included long term alteration of diatom community structure (Davis, 1964; Stoermer et al., 1985; Herdendorf, 1989).

Attempts to restore the quality of the estuarine habitats along Lake Erie’s southern shore have included regulation of point source loadings, riparian improvements to abate non-point source

loadings, and habitat restoration (OEPA, 1991; Ohio Department of Natural Resources, 1997). Measurements of the extent of degradation and restoration of the environment in these habitats often involves monitoring the biota supported by the habitat and employing indices such as the Index of Biotic Integrity-Lacustrine (IBI-L) (Thoma, 1998) and the Invertebrate Community Index (ICI) (OEPA, 1987) which relate attributes of biota at study sites with reference sites.

An index of biologic integrity based on algae does not yet exist for these estuarine habitats. Phytoplankton has been used to assess offshore areas of the lakes (Makarewicz, 1985); however, the efficacy of phytoplankton as an assessment tool in near shore habitats such as the estuarine

areas of Lake Erie's tributaries is questionable because of mixing with lake and river water, water currents, and patchiness of the plankton. Benthic diatoms would be better for assessment because they attach to substrates and integrate conditions in that local area for the life of the organism (Stevenson & Lowe, 1986; Porter et al., 1993).

Many indicators have been developed to infer environmental conditions using benthic diatoms including community composition and autecological indices which evaluate changes in the diatom assemblages due to sensitivity and tolerance of the species to environmental variables (see review in Sgro & Johansen, 1995). Precise indices can be constructed using the weighted average inference methods of ter Braak & van Dam (1989). However, no attempts have yet been made to use benthic diatoms to assess the relative health of Lake Erie's estuaries. Nor have the benthic diatoms in most of Lake Erie's estuaries been studied. How similar are the benthic diatom communities in these systems to each other? What natural seasonal and habitat factors regulate the species composition of diatom communities in these estuaries? How do environmental impacts affect the integrity of these benthic communities?

The objectives of this study were to investigate the relationship between diatom composition and the impacts of environmental variables at four Lake Erie estuaries and to test the ability of the LBI (Lange-Bertalot Index, see Lange-Bertalot, 1979) to comparatively assess the quality of the sites. We used canonical correspondence analysis (CCA) of species and two categories of environmental variables to detect which of the measured variables were most important in impacting the integrity of the diatom communities. Fifty-seven samples were collected over a 2 year period from upstream and downstream sites in these estuaries were scored using the LBI. Principal components analysis (PCA) of sites and variables enabled a construction of a gradient of disturbance for these sites. We then evaluated the LBI by comparing its assessment of the habitat quality of the sites with the gradient of disturbance established from the PCA. This paper will demonstrate the merit of using the LBI, an index based on European rivers, for assessing impacted estuary sites in Lake Erie.

## Material and methods

### *Site description*

Benthic diatoms were assessed in four estuaries of Lake Erie, the Cuyahoga, Black, and Ashtabula Rivers and Old Woman Creek. The Cuyahoga River has the largest drainage basin and Old Woman Creek drains the smallest area (Table 1).

Two sampling sites in each estuary were established to characterize diatom composition within the estuary. One was chosen upstream where lake water makes furthest encroachment, the other downstream near the river mouth. Downstream sites may be subjected to greater pollution impacts in the estuaries where urban land use occurs between the upstream and downstream sites. Lake water might have a greater influence on river mouth sites, especially during seasons of low river flow (Trebitz et al., 2002)

Agriculture is the predominant land use in the Old Woman Creek watershed (Brant & Herdendorf, 1972). Silts and clays have accumulated in the estuary as agricultural activities have increased over the past 100 years (Buchanan, 1983). Flow through the estuary is controlled by storm events as well as the presence or absence of a barrier beach. The presence of the beach creates lentic conditions with water residence time measured in weeks; the absence of the beach creates lotic conditions with water residence time measured in hours (Klarer, 1988).

Land use of the Cuyahoga River basin varies from predominantly agricultural in the upper basin to a densely populated urban and industrial area in the lower basin. Habitat has been severely modified in the navigation channel. The morphology of the navigation channel contributes greatly to the extended residence time of water in the estuary. Discharges from combined sewer

Table 1. Size of estuaries (Brant & Herdendorf, 1972)

Estuary	Length (km)	Area (km <sup>2</sup> )	Drainage (km <sup>2</sup> )
Old Woman Creek	2.1	0.3	69
Black River	6.6	0.9	1217
Cuyahoga River	7.2	1.0	2095
Ashtabula River	2.8	0.3	355

overflows (CSO) and sanitary sewer overflows (SSO) are major sources of sediment oxygen demand. Metals and inorganic compounds reach the river from non-point and point sources. Sewage treatment plants and CSO/SSO's are the major sources of nitrogen and phosphorous loadings (Ohio Environmental Protection Agency, 1994). The Cuyahoga River estuary is exposed to chronic pollution and is designated an Area of Concern (AOC) by the International Joint Commission (Cuyahoga River Community Planning Organization, 1992, unpublished).

The Black River Basin is largely agricultural. Soil erosion from agricultural land is believed to be a major source of sediment in the estuary. Only 10% of the basin is urban. Major industrial areas are located between river miles 5.4 and 3.3. The dredged shipping channel reduces dissolved oxygen (DO) in much of the estuary, while both point and non-point sources of pollution contribute loadings of heavy metals, toxic organics, conventional pollutants and contaminated sediments. The Black River is a designated AOC (Black River Remedial Action Plan Coordinating Committee, 1994, unpublished).

The Ashtabula River basin is 87% rural and agricultural and much of this activity is confined to the upper reaches of the river. The shipping channel (originally dredged to 6 m) was not dredged for 30 years due to the high PCB content of the sediment. The sources of organic contaminants are the abandoned hazardous waste sites in the Strong Brook and Fields Brook tributaries. Fields Brook is a Superfund site, due to the fact that the sediments are so PCB rich that they qualify as toxic waste. This estuary is also in an AOC (Ohio Environmental Protection Agency, 1991).

#### *Sampling and analysis*

Benthic diatoms on artificial substrates were collected quarterly from November, 1992 through September, 1994. Artificial substrates were used to reduce variability in microhabitat features among sites sampled and suitable, stable natural substrates for diatom colonization were rare. A floating diatometer containing six glass slides was placed at each site with slides submerged just below the water surface. The diatometers were positioned in similar conditions off stream banks

and remained in position for approximately two weeks before sampling.

Periphyton on three selected diatometer slides from each site was processed independently to make three permanent diatom slides for counting. Diatometer slides that were under colonized or uneven in colonization were eliminated, and the three slides processed were chosen at random from those remaining. Permanent diatom slides were prepared by cleaning with nitric acid and potassium dichromate and mounting in Naphrax diatom mountant (Sgro & Johansen, 1995).

Diatoms were examined at 1250 $\times$  using an Olympus BH2 photomicroscope with Nomarski DIC optics. Nine hundred frustules were identified and counted for each site and date (three hundred from each processed slide). No attempt was made to distinguish living from dead organisms, though proportions of dead algae are probably small on diatometer slides (Stevenson & Lowe, 1986). Seven diatometers were lost and consequently no data is available for the following site/date combinations: November 1992, Black and Cuyahoga downstream; April 1993, Old Woman Creek downstream and Cuyahoga upstream; September 1993, Cuyahoga downstream; April 1994, Cuyahoga downstream; September 1994, Cuyahoga upstream. This resulted in 57 characterizations of diatom assemblages for the 2 years, 4 seasons, 2 locations per estuary, and 4 estuaries.

At all sites temperature and pH were measured in the field with a Barnant 30 pH meter and DO was measured with a YSI 51B DO meter. Water samples were collected in polypropylene bottles rinsed with DI water for analysis of the following parameters: silicate, nitrate-nitrite, nitrite, ammonia, orthophosphate, sulfate, alkalinity, and conductivity. Standard methods were used (American Public Health Administration, 1989) for all the above analyses, except for a modification of the ammonia assay first recommended by Zaborojny et al. (1973). A Unicam UV 2-100 photospectrometer and a Radiometer TTT 80 autotitrator were used in these analyses.

Additional water samples were collected in polypropylene bottles and filtered in the field with a 0.45  $\mu$ m filter and treated with metals-grade nitric acid to pH <2. From these samples cation and heavy metal concentrations were determined using several techniques depending upon time and

availability of instruments. Concentrations of Fe, Ca, Mg, Cr, and Ti were determined in the samples from November 1992 to April 1993 by ICP emission spectrometry using a Jarrel-Ash 61 Spectrophotometer. Concentrations of Cu, Se, Cd, and Pb in these samples were determined using an ELAN 250 inductively coupled plasma mass spectrometer (ICP/MS). Concentrations of Mg, Ca, Fe, Cr, T, Cu, Se, Cd, Pb, and Zn in samples collected from June 1993 through April 1994 were determined using ICP/MS as described above. Concentrations of Mg and Fe were determined by flame atomic absorption analysis in samples from June to September 1994 using an Instrumentation Laboratory Smith-Hieftje Model 11AA/AE Spectrophotometer. Concentrations of Ca, Ti, Cr, Zn, Se, Cd, and Pb in these samples were determined using ICP/MS as described above. The analyte and internal standard spectral lines monitored in this work were as follows: Mg 24, Ca 44, Ti 47, Cr 52, Fe 56, Cu 65, Zn 68, Se 82, Cd 111, Pb 206, Pb 207, and Pb 208. Hg was not detected in any sample by the methods of sample collection and analysis used in this study (Sgro & Johansen, 1998).

Detrended correspondence analysis (DCA) (ter Braak, 1987, 1990) was used to determine unimodal character of species responses. Gradient lengths for the first four axes of DCA with species/nutrient data ranged from 2.7 to 3.7 and with species/metals data from 2.8 to 3.6, thus we selected a CCA ordination model (Jongman et al., 1995) using CANOCO v3.0 (ter Braak, 1987, 1990) to analyze algal community response to water chemistry gradients. CCA ordines both sites and taxa on axes that are linear combinations of environmental variables.

Two different sets of environmental variables were used for two separate analyses. One set of environmental variables included temperature, pH, DO, conductivity, alkalinity, and drainage basin size, plus the six nutrient parameters collected for the study (Sgro & Johansen, 1998). The other set was of metals data (Sgro & Johansen, 1998). We wanted metals to have equal importance as the nutrient variables in examining pollution impact on diatom distribution in these sites and not analyzed simply as co-variables with nutrients. Therefore, we chose to keep the data sets separate for these analyses.

The data were screened using CALIBRATE v.86 (Juggins & ter Braak, 1992) and CANOCO

v3.0 (ter Braak, 1987, 1990) to eliminate species and taxa which would not provide information in the analysis, for example, rare species and variables that are correlated and thus redundant. There were 57 diatom samples used in these analyses. Taxa which were not equal to or greater than 1% relative abundance in at least three samples were eliminated. There were 73 species retained for the analysis. We did not perform square root transformations or downweighting of rare species in this analysis.

Forward selection was used to remove redundant environmental variables and determine which variables accounted for the greatest amount of variance in the diatom distribution (ter Braak, 1987, 1990). Monte Carlo permutation tests (999 permutations) were used to test the significance of each variable added by this procedure. Variables were eliminated which provided approximately less than 0.11 extra fit in the analysis. Seven variables from the nutrient data set were retained for the analysis (temperature, pH, alkalinity, orthophosphate, nitrite, silicate, and drainage). Five variables from the metals data set were retained (Se, Ti, Ca, Mg, and Cd). Histograms of variable values were generated with the program CALIBRATE v.86 (Juggins & ter Braak, 1992) to identify variables with skewed distributions. The variables with skewed distributions were either log or square root transformed to satisfy the statistical assumptions of ordination (Jongman et al., 1995).

A LBI was calculated for each sample (Lange-Bertalot, 1979). The LBI was chosen because it is used to assess rivers in at least two other states in the USA (Bahls et al., 1992; Kentucky Department of Environmental Protection, 1993). Species tolerance values for the LBI were determined from observations in the Main and Rhine Rivers and based on species tolerance to organic pollution (Lange-Bertalot, 1979).

An indicator value of 3 is assigned to the least pollution tolerant species, a value of 2 is assigned to more tolerant species and a value of 1 is assigned to the most tolerant species. The index was calculated by multiplying the indicator value assigned to a species by its relative density in the sample. Therefore, theoretically, a calculated index score of 3 for a sample would indicate excellent water quality and a score of 1 would indicate poor water quality.

In the present study, about a third of the 270 species was assigned an indicator value and was used in calculating the pollution index for these samples. The remaining species (Sgro & Johansen, 1998) in this study were not included in the LBI and were not used for calculating the pollution index for these samples.

To compare LBI to a gradient of human disturbance characterized with PCA of nutrient data measured in the study (ammonia, orthophosphate, nitrate, nitrite, and alkalinity). Statistical software (Manugistics Corp., 1992) was used for PCA. A separate analysis was performed on cool season (November/December and April) and warm season (June and September) samples. An additional warm season analysis was performed on the metals parameters retained in the CCA (Ca, Cd, Mg, Se, and Ti). The influence of outliers was removed prior to PCA by calculating the natural logarithm for the variables and in the case of orthophosphate by adding 2 to the values before calculating the natural logarithm. One was added to the natural log of the metals. All data were standardized. PCA constructs theoretical axes which are linear combinations of the variables (Jongman et al. 1995). The first axis explains the greatest amount of variance within the variables and for our purpose represents a gradient of disturbance estimated from the variables for the sites. A cool season gradient for the metals was not determined due to the large number of undetected or unreported values for Se and Ti in the cool seasons. Ti concentrations were mostly below detection levels in the cool seasons by the methods used. Se concentrations were also below detection levels in cool seasons, while in December, 1993 and April, 1994 samples were not reported due to an  $R^2$  value  $< 0.995$  for the calibration curve. Spearman's rank correlation coefficient (Spearman's  $\rho$ ) was determined the relationship between LBI and the gradients of disturbance represented by the component scores along the first PCA.

## Results

We identified 270 diatom taxa from all 57 diatometer samples (Sgro & Johansen, 1998). *Navicula lanceolata* (Ag.) Ehr., *Achnanthydium minutissimum* Kütz., and *Gomphonema parvulum*

Kütz., were the most common pennate species in the samples. *Melosira varians* Ag. was the most common centric diatom in these samples.

In the species-nutrient CCA analysis (Fig. 1) eigenvalues for axes 1–4 were 0.509, 0.349, 0.208, and 0.167, respectively. The species environmental correlations were high, being 0.897 and 0.828 for axes 1 and 2, respectively. The first two axes explained 57.0% of the variance in the data. We interpreted the first ordination axis to be a temperature gradient with warm weather samples on the right and cool weather samples on the left. In this analysis seasonality (temperature) was the most important factor influencing the diatom community structure in these estuaries.

Relatively low nutrient enrichment samples occurred above the horizontal axis and relatively high enrichment samples fell below the horizontal axis (Fig. 1). In our study, the Ashtabula River was consistently low in nutrients, while the Cuyahoga and Black Rivers were consistently high in enrichment. Old Woman Creek varied over time, and had both high and low nutrient samples, although the majority was high enrichment.

In the CANOCO analysis of diatometer samples using the metals analysis data, the first ordination axis delineated an environmental gradient with samples associated with high concentrations of Se and Ti on the right (Fig. 2). The second, or vertical axis, depicted a gradient with samples associated with Ca and Ti above the horizontal axis and higher Mg, Se, and Cd concentrations below the horizontal axis. However, the eigenvalues for this analysis were low (0.378, 0.281, 0.140, 0.071, axes 1–4 respectively), indicating that the diatom assemblages do not appear, in this analysis, to be strongly impacted by metals concentrations.

The highest LBI scores among all samples were obtained from the Ashtabula samples on all sampling dates except in April when Old Woman Creek had the highest score (Table 2). Old Woman Creek had generally higher scores than the Black and Cuyahoga systems except in summer. Generally, highest scores were obtained among all samples in the November/December sampling period. The least separation of sites by index scores occurred during the cool seasons. Over the 2 year period the cool-season scores ranged from 1.97 for the April, 1993, Black River upstream sample to



Table 2. LBI pollution index values for diatometer samples from the four estuaries in the study (OWC=Old Woman Creek, BR=Black River, CR=Cuyahoga River, AR=Ashtabula River, U=up, D=down). Absence of index value signifies loss of diatometer for that sample

Site	Nov./Dec.	Apr.	June	Sep.
<i>1992-93</i>				
OWC-U	2.12	2.02	1.51	1.84
OWC-D	2.46		1.13	1.66
BR-U	2.07	1.97	1.74	1.77
BR-D		2.02	1.59	1.18
CR-U	2.02		1.27	1.63
CR-D		2.02	1.26	
AR-U	2.38	2.17	2.94	2.94
AR-D	2.24	2.12	2.77	2.52
<i>1993-94</i>				
OWC-U	2.11	2.22	1.47	2.30
OWC-D	2.04	2.35	1.95	2.18
BR-U	2.0	2.05	1.52	1.40
BR-D	2.10	2.13	1.38	1.57
CR-U	2.16	2.03	1.32	
CR-D	2.12		1.58	1.68
AR-U	2.28	2.05	2.99	2.81
AR-D	2.32	2.18	2.59	2.70

variability in diatom species composition among sites and dates in these Lake Erie estuaries. Distinct cool season and warm season diatom assemblages were observed in these habitats, as in other studies of changes of community structure with temperature (Klarer & Hickman, 1975; Squires et al. 1979; Vinson & Rushforth, 1989). Variability among diatom communities was less during the cool seasons (November/December and April) than warm seasons (June, September). This may reflect effects of higher flows from watersheds during cool seasons that homogenize conditions among sites and estuaries. During low flow periods (typically warm seasons), local factors may be more important determinants of water quality and increase variability among sites and estuaries.

Alkalinity, pH, silicate, orthophosphate, and nitrite concentrations also were correlated with diatom community composition. Phosphorous, nitrogen, and pH are usually the most important determinants of algal growth and community composition in streams (Pan et al., 1999). This study revealed a relationship between drainage basin size and orthophosphate, alkalinity, and nitrite. Other studies have shown that water chemistry variables in rivers may be functions of

drainage basin size (Hynes, 1960; Harrel & Doris, 1968) and land use (Omernik, 1976; Osborne & Wiley, 1988) which may regulate the loading of nutrients, along with riparian zone conditions (Johnson et al., 1997). This has important management implications for setting nutrient criteria. For example, small disturbed estuaries, such as Old Woman Creek in this study, may show more variability over time and space than large disturbed estuaries which may be more modulated due to greater flow. Also, what might be considered a healthy algal assemblage or index score for a large estuary may be different from a healthy algal assemblage or index score for a small estuary.

The metals variables examined in this study were not strong factors in structuring the diatom assemblages based on low eigenvalues of the CCA axes. Metals may, however have other effects on diatom assemblages not examined in this study such as lowering algal productivity. Stevenson & Stoermer (1982) and Stevenson & Lowe (1986) discuss the possible role of toxic loads on species diversity.

Gradients of disturbance based on nutrients and metals in both warm and cool seasons were correlated to LBI scores. The best agreement was

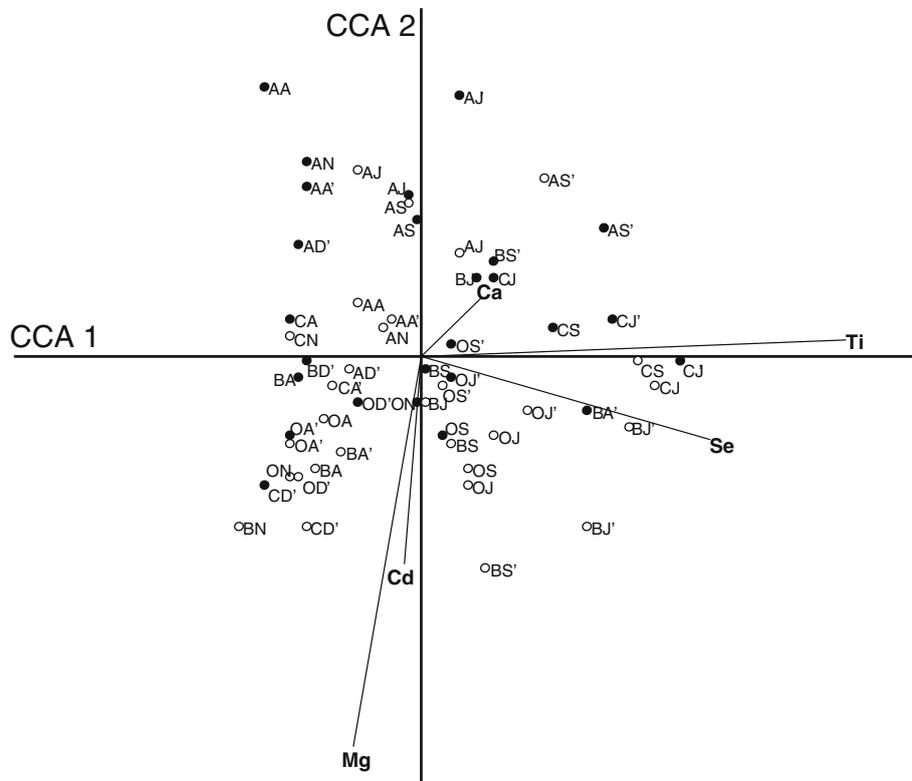


Figure 2. CANOCO of diatom data and selected element concentrations as determined by ICP mass spectrophotometry. In CANOCO, the axes on which the species and sites are simultaneously ordinated are constrained by the environmental variables. Environmental parameters include: calcium, titanium, selenium, cadmium, and magnesium. Sites are represented by circles, solid circles represent downstream sites, hollow circles represent upstream sites. Two-letter site codes represent estuary (A = Ashtabula, B = Black, C = Cuyahoga, O = Old Woman Creek) and month of collection (N = November, D = December, A = April, J = June, S = September). An apostrophe (') means second year of sampling.

between the LBI scores and the gradient of disturbance for nutrient pollution in warm seasons which were moderately well correlated based on the rank correlation test. All sites were dominated by *Navicula lanceolata* in cool seasons which reduced the range in LBI scores. Consequently, the LBI correlated less well with a gradient of disturbance in cool seasons. The implication is that the LBI would be best used in warm seasons to assess these systems. The LBI may be useful in separating 'good' sites from 'bad' sites with regard to nutrient impacts in these estuaries. Based on the LBI, the Ashtabula estuary supported the largest percentage of pollution sensitive diatoms and thus ranked as the least impaired of the estuaries in the study. The Old Woman Creek estuary was somewhat impaired and the Black and Cuyahoga estuaries were most impaired by this measure.

Diatom assemblages in these estuaries may have responded in a threshold manner to these environmental impacts, rather than with a more clearly linear response. The threshold response has been observed for species of other organisms. Effects of urbanization on fish populations (Klein, 1979; Schuler & Gali, 1992) showed a precipitous decrease in Index of Biotic Integrity scores for sites with less than approximately 90% pervious surfaces in their watersheds. Clean water fish taxa are not supported below the 90% threshold. A threshold response by the diatom assemblage to environmental impacts suggests an assimilative capacity by the diatom assemblage. The community may be resilient to slight changes in nutrient concentrations, but greater changes cause a dramatic change in dominant species. LBI scores would tend to remain roughly the same until

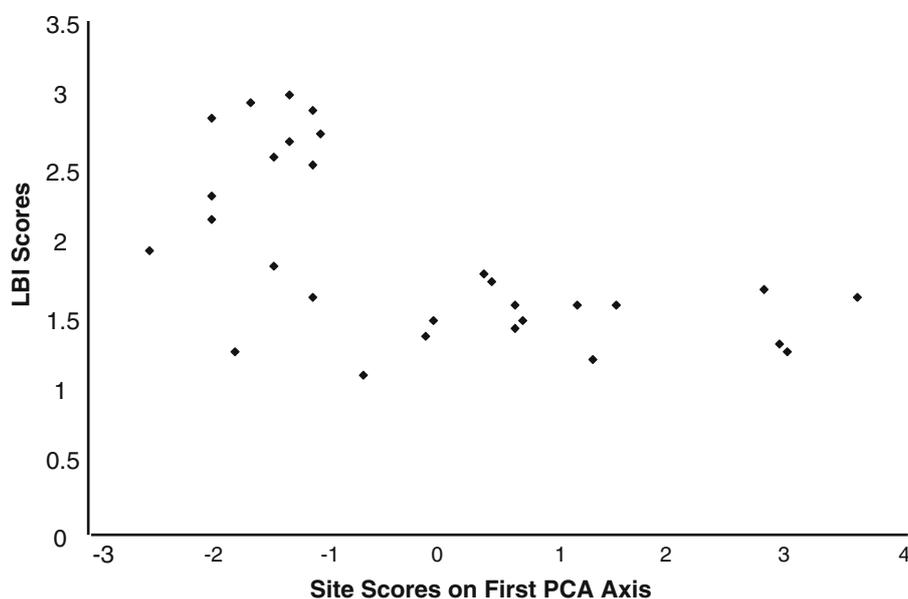


Figure 3. Scatter plot of LBI scores against site scores along the first axis of the warm season, nutrient PCA.

dominant species that are either more pollution tolerant or sensitive shift dramatically within an assemblage. The LBI may lose statistical precision because there is not a clear linear relationship between the disturbance gradient and index scores.

Although the LBI was developed for other ecosystems and specific objectives, it worked remarkably well in Lake Erie estuaries. The values assigned to the species from Lange-Bertalot (1979) are based on empirical observations of effects of organic pollution on diatoms in European rivers, not Lake Erie estuaries. Organic pollution is most related to BOD which was not directly measured in this study. The values were not developed to measure impacts due to metals loadings. Furthermore, it is likely that some species will have different pollution tolerances in Lake Erie estuaries than in European rivers, even though evidence for global similarities in diatom autecology's are indicated in the literature (Lowe, 1974; Beaver, 1981; van Dam et al., 1994).

Many procedures using diatoms have been developed to reconstruct or assess environmental conditions past and present, including multiple linear regression (see Charles & Smol, 1988), weighted average regression and calibration (see Dixit et al., 1993; Dixit & Smol, 1994), weighted average-partial least squares and non-parametric

smoothing techniques (see Potapova et al., 2004). These techniques are more statistically concise than the empirically derived LBI. All of these procedures are designed to infer a particular variable such as chloride or pH from the diatoms. All the researchers above report correlations between diatom inferred and observed variables equal to or greater than the correlations between LBI scores and pollution we report in this study. Yet, the LBI can be used to assess impacts of pollution on the biota in the absence of a more rigorous mathematical model and where assessing the impact of specific nutrients is less desirable than a general pollution indicator. We found the LBI was very effective in differentiating estuaries of varying pollution impacts, and gave a metric that was easier to interpret than CCA and clustering. Future studies should refine metrics like the LBI for application in Great Lakes estuaries by characterizing autecologies of regional populations and their responses to common stressors.

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