The use of epilithic and epiphytic diatoms as indicators of organic pollution in the Cheboygan River, Cheboygan County, Michigan

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Abstract: The purpose of this study was to investigate the potential use of diatoms as biological monitors of environmental quality in aquatic systems. Epilithic and epiphytic diatoms were sampled at 14 sites within the Cheboygan River catchment area. These samples were cleaned using standard techniques and mounted on slides, and 300 diatoms were identified to species and counted for each slide. We found that monitoring diatom assemblages provided a more sensitive assessment of environmental quality than traditional water chemistry measurements. We also found more diverse diatom assemblages in wetland areas and locations with more well-lighted surface area.

Introduction

The Cheboygan River is a medium-sized river system flowing into Lake Huron with a catchment area that includes Mullet Lake to the southwest, and the Black River and Black Lake to the southeast. There is a large amount of development and other human-generated disturbance that occurs throughout the watershed, which contributes a great deal of nutrients and organic pollution to begin with. In addition, Cheboygan's wastewater treatment facility, which is located near the river's mouth, contributes a large amount of treated sewage to an already nutrient-rich ecosystem. Certainly this process has long-term effects not only near the source of organic pollution, but on other aquatic systems downstream of it. There are many different methods that could be employed to assess the impact of human activity on the Cheboygan River. However, numerous studies conducted over the past several decades have shown that investigating the species composition of diatom communities is one of the most effective ways to assess the health of aquatic ecosystems when more sensitive monitoring techniques are required.

Diatoms are extremely reliable ecological indicators for a variety of reasons. As living organisms, they reflect the overall health of the ecosystems they inhabit, as opposed to one-dimensional physical and chemical parameters that can change dramatically over a short period of time depending on the time of year, weather conditions, and other allocthanous factors. Diatoms are more suitable for biological monitoring than many other organisms because of their seeming ubiquity, short generation time, sensitivity to changes in nutrient levels, and diverse assemblages (de la Rey, 2004). Species of diatoms are easy to identify relative to other varieties of algae and aquatic invertebrates (Lowe, 1974), and unlike animals such as insects and fish, they are limited in their ability to move between habitats—either they do well in the particular environment in which they are found, or they do not. In addition, it is relatively easy and cost-effective to collect and analyze diatoms, which renders their use

in environmental monitoring very attractive to state-run agencies that are usually operating on a budget.

Perhaps the most important aspect of these studies is the fact that many diatom species are more likely to be found in habitats with very specific physical and chemical parameters. In the mid-1970's, Lowe developed a classification system for diatom genera and species based on their individual habitat preferences or "spectral ranges". We were most interested in the nutrient and saprobien spectra. The saprobien spectrum measures the level of organic pollution that a diatom prefers or, at the very least, is able to tolerate, but is considered to be less important than the nutrient spectrum in determining the location and species composition of diatom populations (Lowe, 1974).

In order to determine how well this concept applies to northern Michigan diatom populations, we decided to sample 14 different locations within the Cheboygan River watershed. The Cheboygan River flows northward from Mullet Lake, merges with the Black River, and then passes through the city of Cheboygan, where a wastewater treatment facility deposits its effluent. We hypothesized that the diatom communities closer to the source of nutrient input would exhibit less diversity, as well as more nutrient-loving and pollution-tolerant species. Another aspect of our study was to investigate the diversity of diatom communities in riverine and coastal wetland habitats dominated by a variety of aquatic and semi-aquatic macrophytes in comparison to some of the more thinned-out locations.

We decided to sample only populations of epilithic and epiphytic diatoms as opposed to plankton because they exhibit the least movement of just about any other organism in the river, and are thus more likely to reflect long-term environmental conditions for the particular area in which they are collected.

Materials & Methods

Over the course of this study, we sampled fourteen different sites within the Cheboygan River watershed. Two were located on western coast of Mullet Lake, two were on the Black River, seven were on the Cheboygan River, one was on the coast of Lake Huron, and two were in Cheboygan Marsh, a coastal wetland area located to the northeast of Cheboygan. At each location, we measured and documented pH, specific conductivity, dissolved oxygen, depth, water temperature, and light (both at the bottom and at the surface of the water). We determined pH using a Fisher Scientific accumet portable AP10 pH/mV monitor, specific conductivity with a YSI 30 Salinity/ Conductivity/ Temperature monitor, dissolved oxygen with a YSI model 55 monitor, and light with a LI-COR Quantum/ Radiometer/ Photometer Model LI-250 light meter.

Water chemistry samples were taken using acid-rinsed Nalgene jars. We avoided handling them as much as possible so as to not contaminate our samples. The samples were placed on ice and returned to the laboratory for analysis. The parameters we measured

included chloride (Cl), total nitrogen (TN), total phosphorus (TP), nitrate (NO_3), and ammonia (NH_3/NH_4^+), as these are often some of the best indicators of human-generated organic pollution.

Both epilithic and epiphytic diatoms were sampled for this project. We scraped several boulders from each site with a stainless steel spoon and collected several specimens of macrophytes that appeared to be adequately encrusted with epiphytes. The samples were contained in labeled whirl packs, placed on ice, and returned to the laboratory for cleaning.

We prepared our algae samples by diluting them with distilled water in a 1000 ml Pyrex beaker, and then combining them with an equal amount 30% hydrogen peroxide solution and 1-2 grams of potassium dichromate crystals. This process was designed to eliminate any organic matter within the sample, leaving the silica diatom valves intact.

We placed the beakers outside of the laboratory until the reaction was over, allowing the solid particles to settle at the bottom over the course of a few hours, and then poured off the remaining fluid and transferred the rest of the contents to a 150 ml Pyrex beaker. Dilutions were performed every 5-6 hours over the course of three days, until the fluid in the beaker was clear and colorless. The cleaned diatoms were then applied to Fisherbrand 22 x 22 mm cover slips with a glass pipette and were subsequently dried using a Fisher slide warmer. We mounted the prepared cover slips on plain Fisherfinest 25 x 75 mm slides with Nafrax and cooked them for approximately 10 minutes on a hot plate set at 215° C. A total of 42 slides were made—28 were used for our analysis, and another 14 were set apart for backup. The remaining diatoms that were not mounted on slides were stored in glass vials for future reference.

Diatom counts were performed using an Olympus BH2 microscope. Under the oil immersion lens (1000 x), we moved randomly throughout the slide, recording the number and species of the diatoms we encountered. Approximately 300 valves were identified and counted on each slide. Although the value of identifying diatoms to genus versus species varies depending on the size and characteristics of environment that is being sampled (Hill, 2001), we chose to identify to species in this study because we encountered a large amount of diversity within a relatively limited number of genera. Consequently, we decided that species-level identification would provide us with the most accurate results for this particular location.

The raw data was entered into a Microsoft Excel spreadsheet, where we calculated the percent abundance of each species at all of our collection sites. Statistically insignificant values where taxon abundance was 1% or less than of the total count, or where taxa was observed only once among sampling sites, were removed prior to multivariate analyses.

We entered this data into Canoco version 4.5, a software program used for multivariate analyses. With this program, we were able to perform a component analysis, which determines the degree to which diatom species abundance at each location was related to the physical and chemical parameters

that we measured. Principal component analysis was used to describe the correlation of most abundant taxa relative to specific environmental factors.

Results

Table 1. A listing of the 14 locations in the Cheboygan River watershed with general descriptions

Site #	Location	General Description	Substrate	Wetland and Aquatic Plants
1	Mullet Lake	Small public beach in residential area. Flanked by private homes.	Silt w/ small boulders	*Chara spp.*
2	Mullet Lake	Littoral area adjacent to road	Silt w/ boulders	*Chara spp.*
3	Thomas Dr, Cheboygan R	Riverine wetland located at private residence	Silt w/ small boulders	*Typha latifolia, Nymphaea odorata, Nuphar variegatum, Potamogeton richardsonii, Chara spp., Carex*
4	M27 & M33, Cheboygan R	Underneath M33 bridge, across from small marina	Boulders	*Myriophyllum spp., filamentous green algae*
5	Lincoln Rd, Cheboygan R	Boat ramp near Lincoln Rd. bridge, fairly disturbed	Silt w/ boulders	*Myriophyllum spp., filamentous green algae*
6	Cheboygan Marina	Marina with sloping, concrete walls, located near river mouth	Silt w/ concrete	*Myriophyllum spicatum,*
7	Cheboygan R Mouth	Rocky littoral area, very disturbed	Silt w/ concrete and larger boulders	*Myriophyllum spp.*
8	Turnaround Point	Disturbed habitat	Silt with concrete and large boulders	*Myriophyllum spicatum, Carex gynandra, Chara spp.*
9	Cheboygan Wastewater Treatment Facility	Concrete pool containing treated sewage that had yet to be de-chlorinated	N/A	*Filamentous green algae*
10	Cheboygan Beach	Public beach	Sand w/ large boulders	*Chara spp., filamentous green algae,*
11	Cheboygan Marsh	Wetland area located near public park	Silt w/ cobble	*Scirpus validus, Scirpus acutus, Eleocharis smallii, Juncus nodosus, Typha latifolia, Scirpus americanus, Chara spp., Saggitaria latifolia, Najas flexilis, Carex spp.*
12	Cheboygan Marsh	Relatively undisturbed wetland habitat, filled with pieces of wood from sawmill	Silt w/ boulders and driftwood	*Cladium mariscoides, Scirpus validus, Scirpus acutus, Eleocharis smallii, Juncus nodosus, Typha latifolia, Scirpus americanus, Chara spp., Saggitaria latifolia, Najas flexilis , Carex spp.*
13	Black River	Residential riverfront property, construction nearby	Silt w/ cobble	*Typha latifolia, Carex hystericina, Chara spp., Myriophyllum spicatum, Carex spp.*
14	Black River	Relatively undisturbed riverfront	Sand w/ boulders	*Scirpus acutus, Thuja occidentalis, Typha latifolia, Chara spp.,Nuphar variegatum, Carex spp.*

Table 2. Physical and chemical measurements taken at each sampling location

Site #	pH	Specific Conductivity (μs)	Dissolved O_2 (mg/l)	Water Temp (°C)
1	8.08	339.2	7.80	23.3
2	8.14	340.1	7.79	25.1
3	7.97	346.2	7.21	25.3
4	8.15	287.6	7.43	27.3
5	8.25	355.3	8.26	28.8
6	8.29	335.6	10.15	28.2
7	8.1	320.0	8.56	28.3
8	8.05	331.2	9.03	26.2
9	7.08	1121.0	3.51	19.8
10	8.35	251.1	8.54	25.2
11	8.72	251.2	11.51	25.4
12	8.45	251.2	10.65	22.7
13	7.69	334.5	6.20	23.1
14	8.25	315.5	9.16	24.2

Table 4. Significant taxa, their spectral ranges, and their maximum relative abundance on rock substrates. Among the total diatom valves counted, 34 genera and 117 species were identified and recorded. The predominant taxa include *Cocconeis placentula* (22.807%), *Cocconeis pediculus* (11.188%), and *Tabellaria fenestrata* (8.236%).

No.	*Abbreviation*	Taxon name	Nutrient Spectrum	Max (%)
1	Achmin	*Achnanthidium minutum*	-	4.43
2	Achsp	*Achnanthidium sp.*	-	1.21
3	Ampped	*Amphora pediculus*	-	0.27
4	Ampova	*Amphora ovalis*	-	0.12
5	Bravit	*Brachysira vitrea*	-	4.30
6	Cocped	*Cocconeis pediculus*	Oligotrophic	11.19
7	Cocpla	*Cocconeis placentula*	Oligotrophic	22.81
8	Cyccom	*Cyclotella comensis*	-	0.49
9	Cycope	*Cyclotella operculata*	-	0.33
10	Cymaff	*Cymbella affinis*	-	1.21
11	Cymgra	*Cymbella gracilis*	-	0.27
12	Cymlun	*Cymbella lunata*	-	0.17
13	Cymmic	*Cymbella microcephala*	-	6.45
14	Cymmin	*Cymbella minuta*	-	0.75
15	Cympro	*Cymbella prostrata*	-	0.82
16	Denkue	*Denticula*	-	0.12
17	Denten	*Denticula kuetzingii*	-	2.20
18	Diavul	*Diatoma vulgaris*	Eutrophic	1.66
19	Encous	*Encyonema cuspidata*	-	0.54
20	Eunrho	*Eunotia rhomboidea*	Oligotrophic - eutrophic	0.36
21	Eunsp	*Eunotia sp.*	-	0.75
22	Fracap	*Fragilaria capucina*	Eutrophic	3.90
23	Fracon	*Fragilaria construens*	Eutrophic	1.26
24	Gomacu	*Gomphonema acumenatum*	-	0.23
25	Gomgra	*Gomphonema gracile*	-	0.31
26	Gomint	*Gomphonema intricatum*	Eutrophic	5.93
27	Gommin	*Gomphonema minutum*	-	7.07
28	Gompar	*Gomphonema parvulum*	Eutrophic	1.07
29	Gomsp	*Gomphonema sp.*	Eutrophic	0.31
30	Gomgrdl	*Gomphonema girdle view*	Eutrophic	1.64
31	Gomtru	*Gomphonema truncatum*	-	0.62
32	Massmi	*Mastogloia smithii*	-	0.66
33	Navgra	*Navicula graciliodes*	Eutrophic	0.21
34	Navrad	*Navicula radiosa*	Eutrophic	2.69
35	Navsp	*Navicula sp.*	Eutrophic	2.80
36	Nitamp	*Nitzschia amphibiodes*	Eutrophic/ pollution tolerant	0.37
37	Nitden	*Nitzschia denticula*	Eutrophic/ pollution tolerant	0.59
38	Nitpal	*Nitzschia palea*	Eutrophic/ pollution tolerant	4.27
39	Nitrec	*Nitzschia recta*	Eutrophic/ pollution tolerant	0.49
40	Nitsp	*Nitzschia sp.*	Eutrophic/ pollution tolerant	0.28
41	Rhicurv	*Rhicosphaenia curvata*	Eutrophic	0.19
42	Rhisp	*Rhicosphaenia sp.*	Eutrophic	0.30
43	Rhogib	*Rhopalodia gibba*	Eutrophic	0.49
44	Synuln	*Synedra ulna*	Eutrophic	6.80
45	Tabfen	*Tabellaria flocculosa*	Eutrophic	8.24

Table 5. Sampling Sites and Maximum Species Occurrences. Notice that *Cocconeis* dominates assemblages at upstream locations (Mullet Lake and the Black River), whereas pollution-tolerant *Nitzschia* is prevalent at locations closer to Lake Huron.

No.	*Abbreviation*	Site	Taxon name	Max (%)
1	Mlake1	Mullet Lake (1)	*Gomphonema minutum*	21.42
			Cocconeis placentula	9.80
2	Mlake2	Mullet Lake (2)	*Cocconeis placentula*	50.31
			Cocconeis pediculus	19.31
3	ThomDr	Thomas Drive	*Gomphonema intricatum*	32.71
			Gomphonema minutum	20.25
4	M2733	M-27 & M-33	*Cocconeis placentula*	42.74
			Cocconeis pediculus	13.86
5	LincRd	Lincoln Road	*Cocconeis pediculus*	29.59
			Achanthidium minutissimum	20.79
6	CRvMar	Cheboygan River Marina	*Nitzschia palea*	21.05
			Synedra ulna	12.69
7	CRvMou	Cheboygan River Mouth	*Cocconeis placentula*	43.3
			Synedra ulna	15.19
8	Turnpt	Turnaround Point	*Cocconeis placentula*	21.79
			Cymbella microcephala	16.41
9	Wtreat	Wastewater Treatment	*Navicula sp.*	58.31
			Nitzschia palea	20.84
10	Beach	Cheboygan Public Beach	*Cymbella microcephala*	23.43
			Brachysira vitrea	12.26
11	CMsh1	Cheboygan Marsh (1)	*Fragilaria capucina*	25.71
			Synedra ulna	10.98
12	CMsh2	Cheboygan Marsh (2)	*Cymbella microcephala*	27.69
			Fragilaria capucina	11.34
13	BlkRv1	Black River (1)	*Cocconeis placentula*	41.07
			Cocconeis pediculus	25.36
14	BlkRv2	Black River (2)	*Cocconeis pediculus*	33.12
			Cocconeis pediculus	20.88

Figure 1. Distribution of diatom species with respect to each site

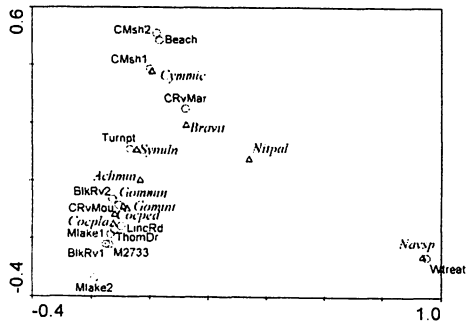


Figure 2. A principal component analysis describing the relationship between abiotic measurements at the sampling location. Notice that with the exception of the wastewater treatment facility, there is very little variation in water chemistry throughout the catchment area.

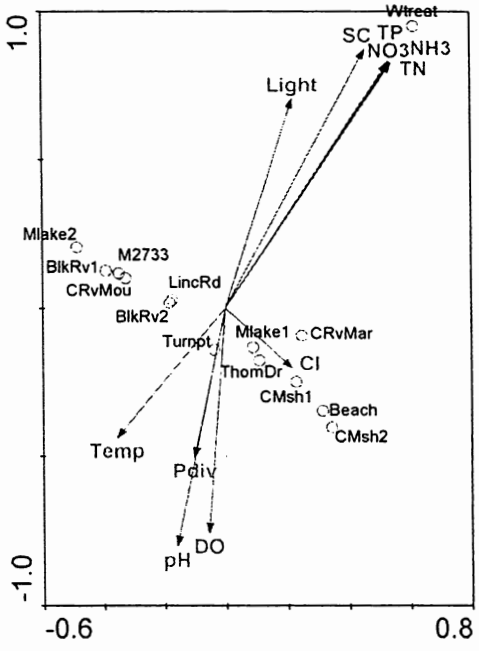


Figure 3. A principal component analysis describing the relationship between locations and different diatom species. There is a clearer correlation of these two factors than in Figure 2.

Figure 4. The concentrations of *Nizschia palea* found at the sampling locations. Notice their prevalence in more nutrient-rich and disturbed habitats.

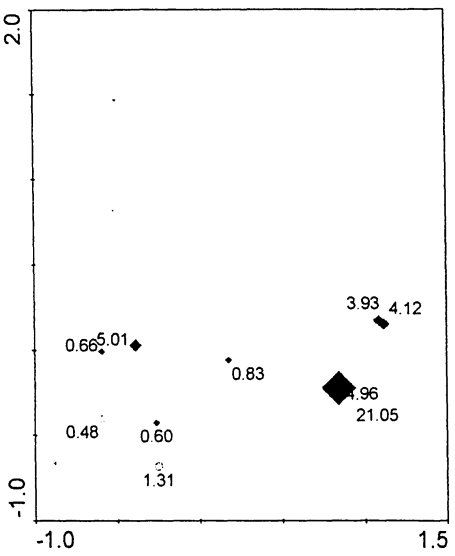
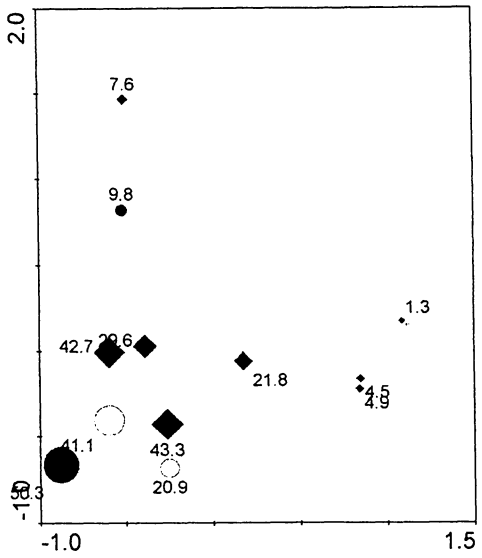


Figure 5. The concentration of *Cocconeis placentula* at our sites. This species is commonly found in more oligotrophic wetlands, as was the case in our study.



Discussion

Based on the data we collected, it is safe to infer that a relationship does exist between diatom assemblages and environmental quality, and that monitoring these communities can provide a more precise indication of water quality than more traditional monitoring approaches. Tables 2 and 3 list all of the water chemistry measurements we took at each of our sampling locations. Notice that with the exception of the wastewater treatment sample, which was anomalous in almost all respects, there is not a large amount of variation in nutrient levels as we progressed downstream. Nevertheless, there is an observable difference in the level of disturbance between more pristine habitats such as Cheboygan Marsh, as opposed to a concrete boat ramp in the middle of downtown Cheboygan. So why are we not seeing a larger difference in the water chemistry?

The answer to this question most likely lies in the fact that these tests are not sensitive enough to detect subtle changes in nutrient levels that would ultimately have a large impact on entire aquatic communities. Figure 3 is a principal component analysis that describes the relationship between abiotic factors and all of the different locations we sampled. Notice that there is not much variation in these respects between the sites, as they are clustered together on the figure. This confirms our observation that there is not a great amount of variation in water chemistry between sites. The diatoms, however, tell a different story. Even as we examined our slides under the microscope, we could see that we were dealing with entirely different

communities from location to location, so obviously there are significant differences between these habitats, even if the water chemistry measurements alone cannot detect them. Figure 3 shows the species composition and distribution across locations. Here you can see that the sites are more scattered across the figure in contrast to the clustering of sites shown in the previous figure. This demonstrates that when diatom assemblages are considered in addition to abiotic measurements, environmental monitoring techniques become a great deal more sensitive.

Diatom assemblages are also extremely useful for monitoring lotic systems because these habitats are inherently ephemeral. There is a constant flow of water through riverine ecosystems coming from other bodies of water, so one-time events upstream of the area being monitored, such as a storm or a leaking septic tank, could drastically change water chemistry measurements. Since epilithic and epiphytic diatoms are for all intents and purposes stuck in whatever habitat they are collected from, they will give a better indication or what conditions are normally like in the sampling area.

We also found evidence that other factors besides water chemistry alone impact diatom assemblages. Although we predicted that species diversity would decrease in the more disturbed habitats located downstream of the wastewater treatment facility, our results showed precisely the opposite trend. In an effort to understand why this occurred, we hypothesized that the increase in nitrogen and phosphorus levels coming from the treated sewage effluent would cause an increase in the growth of aquatic macrophytes at the marina site, which was completely filled with *Myriophyllum* and all sorts of filamentous algae. This location also had sloping concrete walls covered with ridges, which provided a larger well-lighted surface area to which epiphytic and epilithic diatoms could attach, and thus a larger habitat. This situation is consistent with the MacArthur-Wilson Theory of Island Biogeography, which states that larger habitats will contain more diverse populations of organisms. In addition, the fact that boats were constantly coming in and out from the lake might explain the presence of planktonic species.

Species diversity was also higher at all wetland sites, both downstream and upstream of the treated sewage effluent. These habitats were most likely diverse in terms of aquatic and semi-aquatic macrophytes even before the installation of the wastewater treatment facility. The abundance of wetland plants such as *Typha latifolia, Chara spp.*, and *Scirpus spp.*, just to name a few, provides diatoms with a wide variety of substrates (i.e., stems and leaves) to which they can attach. The increased spatial heterogeneity in these environments increases the number of niches in these environments, which results in an increase in diversity of diatom assemblages.

One obvious source of error in our study is the fact that when we performed our counts, we had only been junior phycologists for approximately six weeks, and we might have misidentified a significant number of valves despite our best intentions. Many of the people who use diatoms as bio-indicators spend months or even years becoming

proficient at species level identification, and we simply did not have that luxury given the short duration of our study. Had we had more time and experience with counting, the probability of us misidentifying something would have been drastically lowered.

Another potential source of error might be found in our diatom processing techniques. Because these organisms leave their silica shells behind after they die, one might find valves of a species that established a population at a particular site when environmental conditions were drastically different than those found at the time of collection. To remedy this problem, we might have inspected each of our algae samples prior to cleaning to ensure that the majority of the diatoms were actually alive at the time of collection. This might not have been such a problem with the epiphytic diatoms because many of the macrophytes to which they cling are not long-lived, but it does raise valid concerns regarding the integrity of our rock scrapes.

Since we were prohibited from obtaining samples from the exact source of sewage effluent, we had to settle for only partially treated wastewater that had just been disinfected with Cl_2. This obviously threw off our water chemistry measurements as far as chlorine was concerned, but was probably very similar to what was going into the river in terms of organic pollutants and nutrient levels. Nevertheless, it undoubtedly would have been more accurate had we been able to obtain the pure dechlorinated effluent.

We also ran into an unusual situation in the first Cheboygan Marsh sampling location. Although the conductivity was similar to most of the other sites we had sampled, the chloride readings were extremely high. We knew that the city of Cheboygan stored their road salt somewhere in the vicinity, so the chloride readings made sense, but why was there not a higher specific conductivity? Any number of things could have gone wrong in this situation—the water samples could have been contaminated, or the conductivity meter could have been used incorrectly or malfunctioned in some way. The diatom samples we collected, however, probably gave us a better indication of what was going on in the marsh than did any of our water samples.

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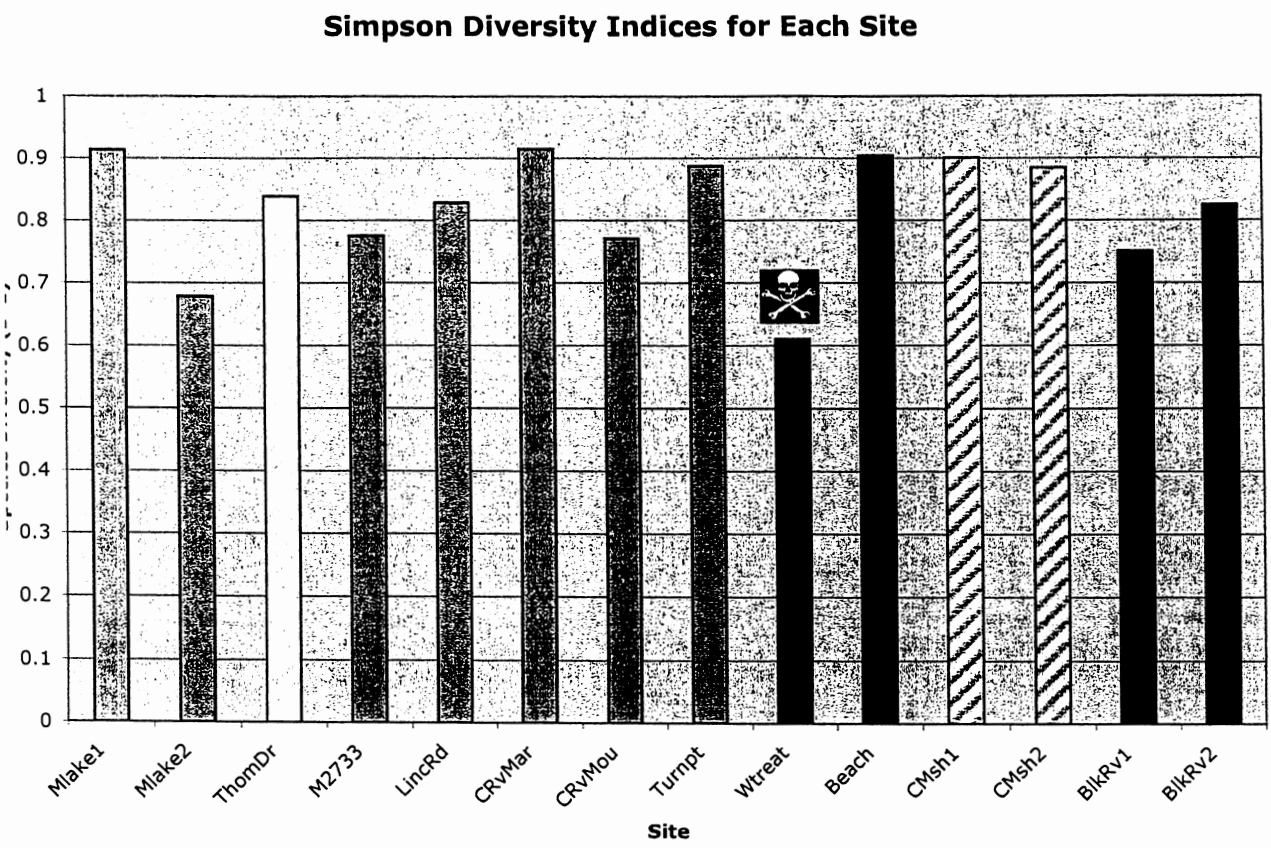

Figure 6. *Simpson Diversity Indices for all 14 sites. Notice that there is a higher D-1 value for wetland sites (striped), and that the lowest is found at the wastewater treatment facility.*

Table 3. Water chemistry measurements taken at each location

	Light (%loss)	DO (mg/L)	Temperature(ϒc)	Conductivity (_s)	Cl (Mg/L)	TN (mgN/L)	NH3	TP	NO3	PlantDiv
7.97	12.471	7.8	29.6	380.6	9.4	0.302	10.1	7.6	19.6	1
8.14	12.927	7.79	25.1	340.1	8.6	0.389	7.7	8.3	27.1	2
7.97	32.8	7.21	25.3	346.2	9	0.398	37.1	12.5	10.7	5
8.15	29.412	7.43	27.3	287.6	8.9	0.625	34	8.5	8.8	3
8.25	15.471	8.26	28.8	355.3	11.4	0.411	9.6	19.8	3.3	3
8.29	31.95	10.15	28.2	335.6	9	0.303	33.8	15.7	15.9	3
8.31	23.83	10.27	27.3	337.6	8.5	0.338	38.9	12	37.9	4
8.05	36.392	9.03	26.2	331.2	8.9	0.319	19.5	13.2	20.1	3
7.08	56.681	3.51	19.8	1121	12.5	7.96	4179.4	1309.36	5688.6	1
8.35	10.161	8.54	25.2	251.1	9.4	0.59	70.8	22	2.2	2
8.72	12.821	11.51	25.4	251.2	188.3	0.52	37.6	10.8	0.3	5
8.45	1.595	10.65	22.7	251.2	8.3	0.358	25.4	10.7	11.9	5
7.69	25.871	6.2	23.1	334.5	5.8	0.45	37.4	17.7	19.2	4
8.25	11.96	9.16	24.2	315.5	6.1	0.523	20.4	20.4	35.4	3