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Improved Ecosystem Indicator Tools for Water QualityManagement – Genomic Analysis of Periphyton to IdentifyStressors

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Project Number 2015NH191B

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This report is based on a senior honors thesis prepared by Allison Wood, and presented to the University Honors Program University of New Hampshire as partially fulfillment of her undergraduate degree in Honors Environmental Engineering

Introduction and Problem Statement

Great Bay Estuary is a unique and valuable inland water body located just west of Portsmouth, NH. Water from the Gulf of Maine is driven into the estuary by some of the strongest tidal forces in north america, meeting the discharge of seven freshwater rivers that drain nearly 1000 square miles of watershed area in NH and Maine. Due to the area's geography, Great Bay is one of the most recessed estuaries in the nation, and its tidally-driven ecosystem is a unique environment encompassing a variety of aquatic habitats. The Estuary is home to hundreds of types of birds and fish, including 23 threatened or endangered species (GBNERR, 2011). Since the 1995 establishment of the New Hampshire Estuaries Project, Great Bay has been studied extensively by local and state agencies, as well as EPA. In 2005 the program was centralized at the University of New Hampshire, and re-named PREP: the Piscataqua Region Estuaries Partnership, to include monitoring the parts of the estuary located in Maine. Based on this comprehensive monitoring effort, in 2009 NH Dept of Environmental Services designated the Great Bay as impaired based on its failure to meet various water quality standards for aquatic life, including dissolved oxygen and total nitrogen levels (EPA, 2012). Moving forward, PREP, NH DES and EPA will be looking for economic ways to gather water quality data to prevent further degradation of this unique ecosystem.

Unfortunately, cause–effect relationships of different stressors on an ecosystem are not straightforward, as freshwater and coastal ecosystems respond to nutrient loading in various ways (McQuatters-Gollop, 2009). Therefore, it is useful for scientists to identify a biologic ecosystem component that is reactive to various ecosystem impairments to serve as an indicator of changing ecosystem health. In this study, algae were selected because they are comparable across geographic locations, have been studied extensively, are abundant in aquatic environments, are easy to collect, and their growth is stimulated distinctly by different nutrient conditions. Algae are a particularly useful ecosystem indicator of ecological conditions due to their ability to reflect water quality conditions in a certain aquatic location, based on species type and abundance (Smucker et al, 2013).

In 2009 USGS published a database of algal species which serve as indicators for various water quality conditions, including nutrient enrichment, conductivity, dissolved oxygen, pH, and others (Porter, 2008). This information, in combination with recent success of an attached algae water quality monitoring program by Maine DES, prompted this study to examine attached algae as a potential indicator of water quality in Great Bay.

Objectives

The project had three main objectives. First, determining whether or not algae would work as an indicator of water quality in the great bay ecosystem, an environment where tidal currents are strong and water composition is mixed. This question was explored using multiple riverine inputs from different locations in the estuary. This was accomplished using the USGS list of algal indicator species, using traditional microscopic taxonomic methods. The second goal of the project was to compare traditional microscopic methods of taxonomy with emerging genomic methods, increasing the economic viability of attached algae monitoring. The third project goal, which is still underway, was to generate and use massive amounts of genomic data from the Great Bay ecosystem to see if other organisms might serve as viable indicators of environmental conditions in the bay.

Background / Literature Review

Algae as an Indicator of Water Quality

In 1947 Dr. Ruth Patrick launched a groundbreaking study identifying algae as a potential indicator of water quality in streams (Patrick, 1948). Finding that they are strong indicators of environmental change, she became a proponent of the use of biology to assess the ecological health of streams and rivers in North America. Through her work, the idea that biology could serve as a critical source of information for environmental health was presented and proven, changing the way environmental scientists approach research (Peck, 2014). Today algae, fish, and macroinvertebrates are the most common taxa used as biologic indicators in stream monitoring, however algae have been shown to respond to water quality stressors most distinctly (Magadze et al., 2016).

Algae are an abundant yet diverse group of photosynthetic organisms found in all aquatic habitats. In recent years our knowledge of these organisms has greatly advanced, mainly thanks to new types of data from advancements in electron microscopy and DNA sequencing technologies (Cavalier-Smith, 2007). They are easy to collect, and can be readily identified down to the species level. The species-specific sensitivity of algae to environmental conditions and their high diversity in habitats provide the potential for precise and accurate assessments of physical, chemical, and biological conditions that may be causing problems (Stevenson & Smol, 2003). In addition, algae have short life cycles, meaning they react to any changes in aquatic environments quickly and dramatically, which can be observed via species presence and/or percent abundance, indicating the type and severity of a certain condition (CITATION).

Specifically, attached or benthic algae is a useful indicator of ecological conditions due to its ability to reflect water quality conditions in a certain aquatic location. Attached algae includes diatoms and non-diatoms which attach to surfaces such as rocks and plants. Diatoms are single-celled photosynthetic algae, and are a major type phytoplankton, abundant in fresh and saline waters. Diatoms are effective biological indicators because they respond to various conditions including salinity and various nutrients, including Nitrogen and Phosphorus (Smucker et al.,

2013). Other types of attached algae, such as non-diatom “soft” algae species, are also valuable indicators (Porter, 2008).

Overall, algal bioassessments improve water-quality programs because algae are reliable indicators of water quality (Danielson et al., 2011). Attached algae analysis is a powerful tool for assessment of water quality in streams, and has the potential for application in routine monitoring programs (Mangadze et al., 2016). To date, real applications of such data has been limited due to the lack of available autecological databases from which algal-indicator metrics can be calculated (Porter, 2008). The goal of this research was to explore the use of genomics as a viable alternative method of analysis, to improve monitoring capabilities and lower the cost of biological water quality assessment. Prior to this effort, the use of attached algae for water quality monitoring purposes in the Great Bay Estuary had to be validated using field data, as there is evidence that diatom metrics or indices developed in one geographic area are less successful when applied in other areas (Potapova & Charles, 2007).

Current Applications

USGS

In 2008, USGS published Algal Attributes, a data file containing metrics indicating physiological optima or tolerance to nutrients and other water-quality constituents. The file, created to enhance analysis, interpretation, and understanding of trophic condition in U.S. streams and rivers, includes 37 algal attributes and 101 metric codes which apply to 5,939 algal taxa. Prior to this work, a comprehensive summary of algal autecological attributes for North American streams and rivers did not exist. Use of the database requires taxonomic identification of algal species, currently performed using microscopic techniques to identify algae down to the species level.

Taxa counts converted into % abundance measurements may be matched with taxa in the USGS Algal Attributes file for conversion to algal attributes, which may be manually selected and include salinity, pH, conductivity, and nutrients. Certain attributes contain sub-categories, such as soft algae and diatoms, and regional indicators for nutrient conditions. Each taxa linked to an attribute is given metric codes, which indicate what characteristics of each attribute the taxa represents. For example, taxa that contain the metric label EHTN_1 indicate high TN within the

eastern highlands region, and taxa with the metric label DCOND_HI are diatoms with a high specific conductance optimum. Each taxa in the file is listed alphabetically, and metric labels are indicated for each attribute in columns to the right using numeric metric codes (Porter, 2008).

Maine Department of Environmental Protection

Work by Maine DEP has specifically explored the use benthic algae to assess the quality of Maine's wadeable freshwater streams as it relates to impervious cover. Maine DEP collected samples from 193 sites across the state, encompassing a range of streams from entirely forested watersheds to streams in urban watersheds. Sampling involved using a stiff brush to scrape benthic algae from cobbles or small boulders in riffles or runs of wadeable streams, where water levels were most constant. Algae were counted using traditional microscopy techniques; diatoms were typically identified down to the species level, and some non-diatoms were identified to the genus level. During analysis, enumeration data was converted to % abundance values to reduce the influence of numerically abundant species, similarly to Porter et al, 2008.

Maine DEP developed an empirical method of assigning tolerance values based on local data, rather than using professional judgment or tolerance values from other regions. Algal taxa were categorized as sensitive, intermediate, or tolerant according to Maine stream tolerance values, based upon stressors specific to Maine: Phosphorus, Nitrogen, Conductivity, % Developed watershed, and % Impervious Cover. It was found that metrics based on local tolerance values outperformed metrics that used tolerance values from other parts of the world; it was also found that many metrics used in other algal bioassessments were not useful indicators in Maine, presumably because of regional differences in climate, geology, and predominant anthropogenic stressors. At the end of analysis a novel set of metrics were created; both for algal families associated with streams in disturbed watersheds in Maine and genera associated with minimally disturbed sites in Maine.

In 2012 Maine DEP published a statistical model for analysis of Maine's wadeable streams with the best-performing metrics to evaluate algal community condition relative to the national Biological Condition Gradient (Danielson et al, 2012). The Biological Condition Gradient was published in 2006 in a collaboration between Maine DEP and the Environmental Protection

Agency, and describes how 10 ecological attributes change in response to increasing levels of stressors. The goal of the model is to provide a means to make more consistent, ecologically relevant interpretations and communicate those results to the public (Davies & Jackson, 2006).

From their work to date, Maine DEP has found that sensitivity of bioassessment programs may be enhanced by incorporating stressor-specific metrics when evaluating water-quality. Such metrics serve a critical role in diagnosing sources of impairment. Multimetric indices provide an assessment of overall condition, whereas those implementing water-quality programs can use stressor-specific metrics and autecological indices to prioritize & target actions to restore water quality and monitor improvements of resource condition (Danielson et al, 2011).

Next-Generation Genomic Sequencing

Recent technological developments have caused a major shift in DNA sequencing techniques. Modern methods involve sequencing high numbers of short DNA strands, and have been generally termed "next-generation sequencing", or NGS (Stillman & Armstrong, 2015). These technologies were first introduced to the market in 2005, and have already revolutionized the way scientists process environmental data (Morozova & Marra, 2008). Each organism/bacteria has a unique Ribosomal RNA sequence, which can be identified using a specific primer set for eukaryotes, bacteria, etc. (Smucker et al., 2013). Most NGS studies relating to biodiversity involved sequences that specify only to the family or genus, however diatom assessment typically requires species-level information (Zimmermann et al., 2015).

Data analysis is one of the main challenges of NGS (Smucker et al., 2013). Gathering outputs at the species level of specificity and matching those results to known databases is one of the main challenges in this field currently, and one of the focuses of this study.

Methods

Sampling Methods

Attached algae were chosen for this study because they grow in estuarine & freshwater, and are relatively easy to collect. Previous studies, including work by Maine DEP, have used algae

attached to natural substrate such as rocks. This was considered, however it was determined that for the first study in Great Bay a periphytometer would be more appropriate (Figure 1).

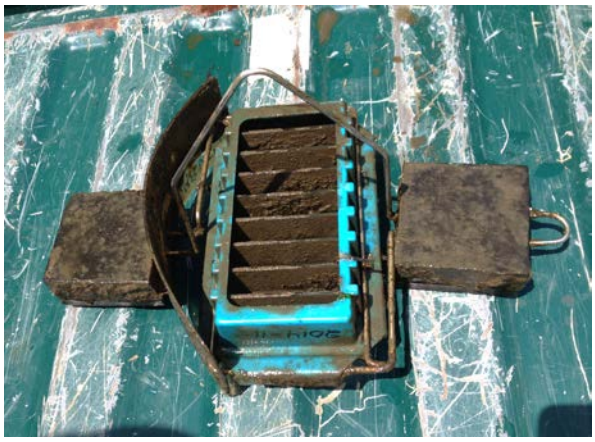


Figure 1: Periphytometer

Controlling for substrate, time, light, flow, depth at each sampling location helped eliminate variability across freshwater, tidal, and estuarine locations. Glass slides were submerged for 2-week intervals, then collected, scraped, and sent to a third party lab for taxa identification.

Sixteen sample sites within the estuary captured the Exeter, Lamprey, and Oyster rivers as well as the bay (Table 1). Approximately six sites were located at inland freshwater portions of the rivers, six sites captured the tidal sections of the rivers, and one site was located in the bay itself (Figures 2 and 3).

Table 1: Site Details

Site	Location	Water Body	Freshwater / Estuarine
001	Haigh Road Brentwood	Exeter River	FW
002	Pickpocket Dam	Exeter River	FW
003	Shaw Hill Road / Rt. 150	Great Brook	FW
004	Chadwick Ln / Gilman St	Little River	FW
005	Gilman St. / Gilman Ln	Exeter River	FW
006	High St. / Rt. 108	Exeter River	FW
007	0.75km below String Bridge	Exeter River	E

008	Exeter Country Club below Parkman Creek Confluence	Wheelwright Creek	FW/E
009	River Road	Squamscott River	E
010	Railroad Bridge, Stratham	Squamscott River Estuary	E
011	Above Wiswall Dam	Lamprey River	FW
012	Packers Falls, upstream of bridge	Lamprey River	FW
013	Downtown Newmarket, below falls	Lamprey River Estuary	E
014	Jackson Landing, Durham	Oyster River Estuary	E
015	Mid Great Bay, buoy	Great Bay	E

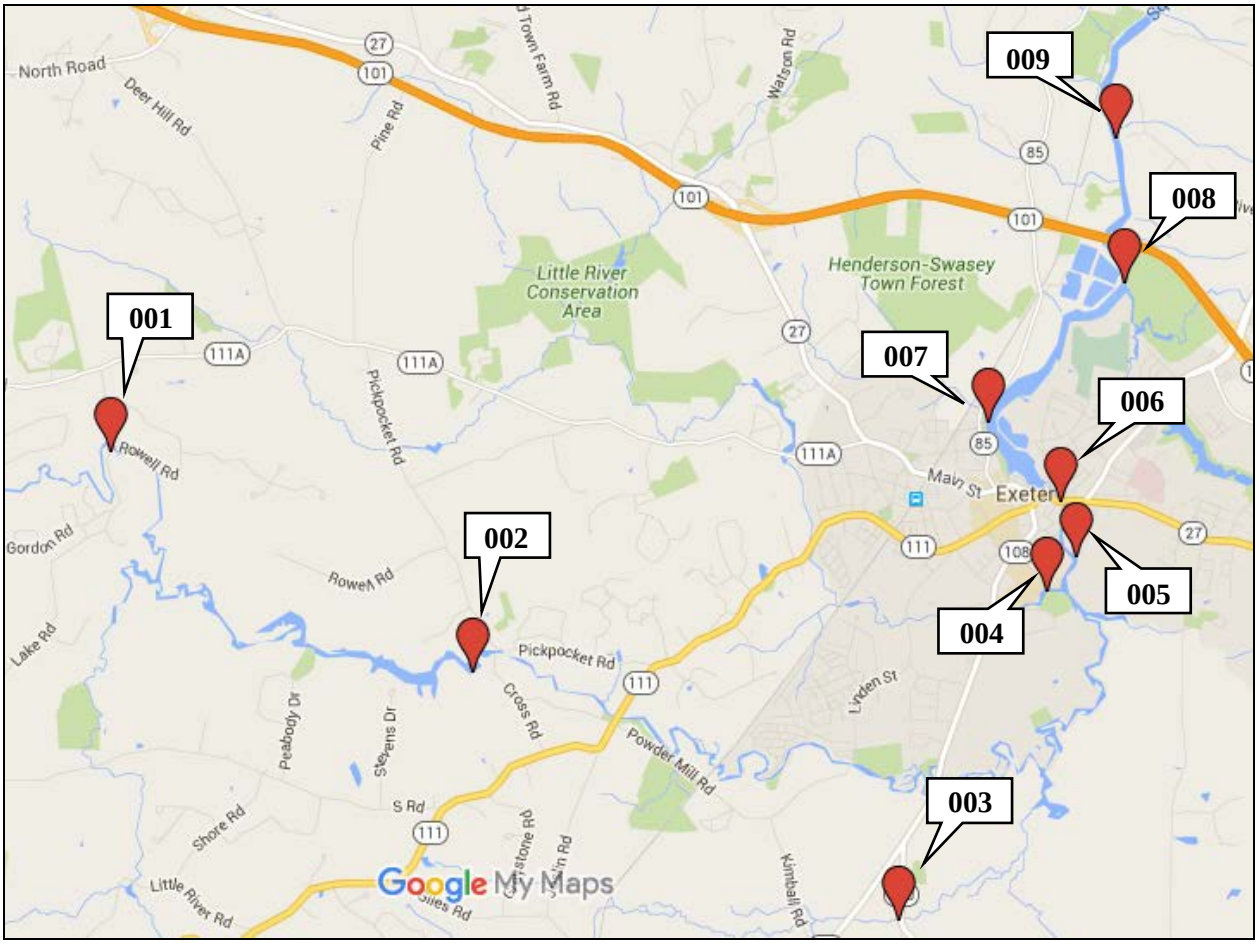


Figure 2: Upriver Site Locations

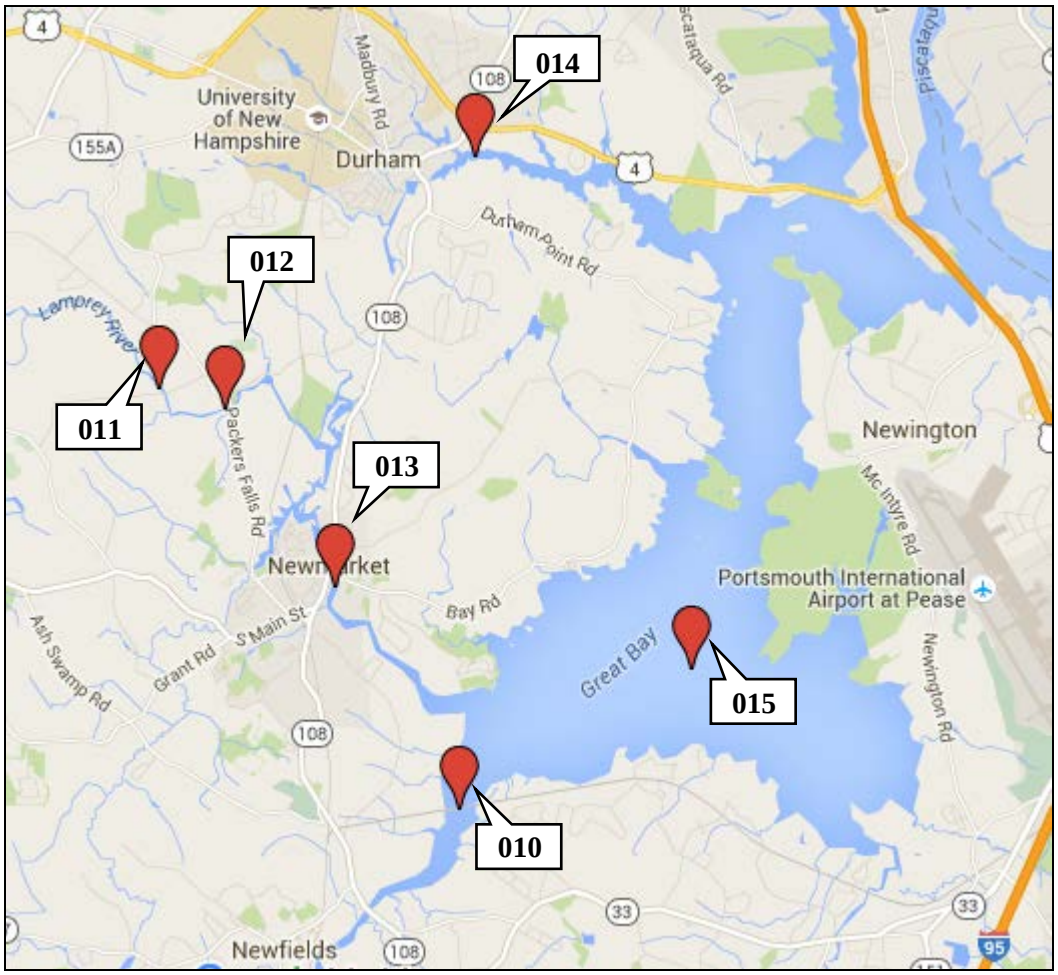


Figure 3: Downriver and Great Bay Site Locations

Traditional Microscopic Analysis Methods

Taxa counts were converted into % abundance for each site, and manually matched with taxa in the USGS Algal Attributes file for association with certain attributes; Salinity, pH, conductivity, and nutrients. Certain attributes contained sub-categories, such as pH indicator taxa for soft algae and diatoms, and regional indicators for nutrient conditions. Each taxa was linked to an attribute and given a metric code, which indicated what characteristics of each attribute the taxa represented. For example, taxa that contained the metric label EHTN_1 indicated high TN within the eastern highlands region, and taxa with the metric label DCOND_HI were diatoms with a high specific conductance optimum. Taxa in the file are listed alphabetically, and metric labels

are indicated for each attribute in columns to the right using numeric metric codes. This analysis was performed manually and yielded basic water quality results from the USGS method, which was then compared to field data using two methods. First data was compared in excel, then data was analyzed in JMP using a principal components analysis. This analysis did not yield any statistically significant results due to the limited size of the data set, however early TP and salinity results yielded unexpectedly distinct patterns, encouraging further study and expansion of the data set.

Genomic Analysis Methods

In partnership with the UNH Genome Center, Illumina sequencing was used to analyze algae and water samples from each sample site. This type of sequencing is the most successful NGS technique to date, and is used worldwide. Illumina machinery can handle complex environmental samples and have an increased input ability compared to previous sequencing technologies. In the Illumina process, a combination of chemical reactions and detection methods are used to sequence large amounts of DNA or RNA strands. Prior to analysis, short pieces of DNA/RNA are washed across a flow cell with selected primers. Those that stick are amplified repeatedly using the polymerase chain reaction, forming clusters. Once colonies have formed, nucleotides tagged with fluorescent indicators are added one at a time, with a unique color identifying each base. As each indicator is added, it is hit with a laser which activates the colors, which are read with a camera. This sequencing produces millions of highly accurate reads, which may then be matched to known sequences in a database to identify what organisms are present in the sample (Illumina, 2016).

Results and Discussion

Chemical Water Quality Data

Water quality data was obtained at each site for Total Dissolved Nitrogen (TDS), Nitrate Nitrogen (NO3-N), Total Suspended Solids (TSS), Ammonia Nitrogen (NH4-N), Phosphate (PO4), Total Nitrogen (TN), and Total Phosphorus (TP). Each site was sampled three times; Trial 1 during June 2014, Trial 2 in September of 2014, and Trial 3 in June of 2015. For each trial, water quality was tested when the periphytometer was deployed and retrieved. This data is

displayed in Table 2, where Sample codes reflect the trial number, deployment or retrieval, site number, and whether the site was freshwater or estuarine.

Table 2: Water quality field measurements

SAMPLE:	TDN (mg/L)	NO3-N (mgL)	TSS (mg/L)	NH4-N (µg/L)	PO4 (µg/L)	TN (mg/L)	TP (µg/L)
T1-D-001-fw	0.434	0.202	27.816	22.518	13.236		
T1-R-001-fw	0.407	0.205	5.135	17.665	9.010		
T2-D-001-fw	0.366	0.116	3.400	9.170	16.209		
T2-R-001-fw	0.398	0.137	0.600	22.831	2.615		
T3-D-001-fw	0.356	0.142	3.200	21.623	5.966	0.644	18.002
T3-R-001-fw	0.412	0.143	1.600	19.748	7.111	0.625	51.480
T1-D-002-fw	0.410	0.179	21.130	28.844	12.431		
T1-R-002-fw	0.368	0.107	2.821	18.996	15.249		
T2-D-002-fw	0.326	0.047	2.000	10.174	15.177		
T2-R-002-fw	0.328	0.033	1.600	19.331	2.615		
T3-D-002-fw	0.339	0.088	3.913	31.112	3.501	0.567	13.377
T3-R-002-fw	0.460	0.166	1.667	25.182	7.680	0.543	21.857
T1-D-003-fw	0.528	0.033	33.890	49.428	48.457		
T1-R-003-fw	0.382	0.003	20.667	40.940	35.777		
T2-D-003-fw	0.461	0.016	1.200	21.663	34.006		
T2-R-003-fw	0.348	0.000	49.167	31.331	32.340		
T3-D-003-fw	0.466	0.040	5.455	33.360	51.425	0.687	169.174
T3-R-003-fw	0.611	0.021	7.500	19.980	77.408	0.731	128.698
T1-R-004-fw	0.311	0.038	3.636	6.814	11.224		
T2-D-004-fw	0.581	0.075	4.412	25.973	20.207		
T2-R-004-fw	0.501	0.112	6.667	35.463	18.554		
T2-R-004-fw-duplicate	0.519	0.074	4.615	35.565	13.600		
T3-D-004-fw	0.540	0.129	3.784	51.589	9.491	0.691	53.258

T3-D-004-fw-duplicate	0.357	0.068	7.826	29.614	9.087	0.552	45.142
T3-R-004-fw	0.492	0.108	5.652	25.299	11.264	0.666	65.346
T1-D-005-fw	0.411	0.154	37.639	28.265	14.645		
T1-R-005-fw	0.482	0.143	6.000	18.084	16.255		
T2-D-005-fw	0.468	0.070	2.833	10.569	15.435		
T2-R-005-fw	0.348	0.009	3.030	8.084	3.907		
T3-D-005-fw	0.383	0.084	11.154	27.366	5.853	0.671	55.345
T3-R-005-fw	0.483	0.126	2.979	96.048	11.538	0.549	27.685
T1-D-006-fw	0.461	0.140	33.478	8.667	45.820		
T1-R-006-fw	0.301	0.007	3.333	8.911	11.022		
T1-R-006-fw-duplicate	0.331	0.010	3.143	10.594	14.846		
T2-D-006-fw	0.361	0.024	0.769	2.747	16.854		
T2-D-006-fw-duplicate	0.338	0.065	3.636	9.540	21.109		
T2-R-006-fw	0.367	0.004	2.286	7.675	5.415		
T3-D-006-fw	0.426	0.095	24.118	21.124	7.154	0.661	44.070
T3-R-006-fw	0.494	0.122	2.581	30.105	11.467	0.513	21.006
T3-R-006-fw-duplicate	0.483	0.128	4.000	24.669	11.676	0.770	40.635
T1-D-011-fw	0.465	0.257	18.754	31.437	17.664		
T1-R-011-fw	0.400	0.161	2.000	18.454	11.022		
T1-D-012-fw	0.476	0.265	9.858	21.692	13.639		
T1-R-012-fw	0.348	0.156	1.961	25.571	29.244		
T1-D-007-e	0.474	0.167	105.325	11.671	15.047		
T1-R-007-e	0.509	0.128	18.667	103.047	9.815		
T2-D-007-e	0.567	0.166	18.571	71.274	26.655		
T2-R-007-e	0.619	0.193	8.286	63.674	14.246		
T3-D-007-e	0.354	0.090	15.455	22.769	12.982	0.866	114.016
T3-R-007-e	0.509	0.195	18.261	14.445	19.955	0.826	105.881
T1-D-009-e	0.287	0.049	127.988	2.380	9.211		

T1-R-009-e	0.496	0.018	58.571	16.132	32.154		
T2-D-009-e	0.974	0.411	45.833	275.746	55.027		
T2-R-009-e	1.077	0.439	70.909	276.843	35.140		
T3-D-009-e	0.447	0.101	147.500	52.838	19.791	1.666	319.659
T3-R-009-e	0.611	0.292	30.588	49.801	39.363	0.990	98.064
T1-D-010-e	0.466	0.112	58.462	159.116	33.966		
T1-D-010-e-duplicate	0.414	0.100	57.500	155.550	33.765		
T1-R-010-e	0.303	0.030	54.286	61.331	30.142		
T2-D-010-e	0.272	0.039	17.027	4.597	33.232		
T2-D-010-e-duplicate	0.258	0.045	12.658	4.411	33.490		
T2-R-010-e	0.212	0.022	25.333	1.271	42.895		
T2-R-010-e-duplicate	0.238	0.020	20.909	6.764	38.156		
T3-D-010-e	0.269	0.050	38.500	31.861	16.528	0.460	76.828
T3-R-010-e	0.391	0.046	26.190	60.935	26.973	0.473	76.151
T1-D-013-e	0.523	0.258	5.909	52.242	17.060		
T1-R-013-e	0.303	0.127	1.333	24.000	14.645		
T2-D-013-e	0.355	0.074	10.476	3.302	31.427		
T2-R-013-e	0.448	0.132	2.857	41.870	17.047		
T3-D-013-e	0.368	0.120	7.000	36.356	7.669	0.654	40.143
T3-R-013-e	0.493	0.145	3.000	35.011	8.063	0.588	18.573
T1-D-014-e	0.413	0.156	3.846	69.151	34.972		
T1-R-014-e	0.336	0.103	10.000	50.276	27.868		
T2-D-014-e	0.397	0.119	0.571	12.185	16.725		
T2-R-014-e	0.325	0.059	38.095	78.612	101.268		
T3-D-014-e	0.437	0.077	13.548	61.578	19.219	0.496	38.006
T3-R-014-e	0.474	0.090	35.625	85.790	36.943	0.540	75.519
T1-D-015-e	0.260	0.054	6.000	56.829	21.287		
T1-R-015-e	0.079	0.007	12.667	10.451	12.230		

T1-R-015-e-duplicate	0.157	0.001	20.625	6.032	13.840		
T2-D-015-e	0.208	0.042	19.815	18.611	29.105		
T2-R-015-e	0.202	0.039	28.000	16.182	36.433		
T3-D-015-e	0.295	0.034	22.800	1.146	6.927	0.307	30.658
T3-R-015-e	0.269	0.042	30.952	45.335	19.049	0.285	26.778
T3-D-016-e	0.233	0.053	32.800	12.633	11.490	0.239	29.834
T3-R-016-e	0.285	0.034	33.333	21.383	21.005	0.376	32.806

Traditional Microscope Data

Taxa identification and counts were obtained from the Academy of Natural Sciences of Drexel University. This data is summarized in Table 3.

Table 3: Microscopic taxa identification results

Taxon ID	Taxon Name	Total Present
1010	Achnanthidium minutissimum (Kützing) Czarnecki	2522
1024	Achnanthidium exiguum (Grunow) Czarnecki	3
1036	Achnanthidium rivulare Potapova et Ponader	78
2122	Achnanthes brevipes Agardh	1
2990	Achnanthes sp. 1 ?	7
6001	Amphipleura pellucida (Kützing) Kützing	9
7010	Amphora inariensis Krammer	1
7043	Amphora pediculus (Kützing) Grunow	1
7073	Amphora subholsatica Krammer	1
7075	Amphora copulata (Kützing) Schoeman et Archibald	3
7161	Amphora sp.	43
10008	Aulacoseira ambigua (Grunow) Simonsen	5
10019	Aulacoseira italica (Ehrenberg) Simonsen	15
16003	Cocconeis placentula var. lineata (Ehrenberg) Van Heurck	139
16004	Cocconeis placentula Ehrenberg	334

16010	Cocconeis fluviatilis Wallace	1
16011	Cocconeis pediculus Ehrenberg	1
16013	Cocconeis scutellum Ehrenberg	197
16035	Cocconeis sp.	1
20001	Cyclotella atomus Hustedt	572
20007	Cyclotella meneghiniana Kützing	109
20011	Cyclotella striata (Kützing) Grunow	3
23048	Cymbella aspera (Ehrenberg) Cleve	1
23068	Cymbella tumida (Brébisson ex Kützing) Van Heurck	111
25004	Denticula subtilis Grunow	1
30004	Diploneis oblongella (Nägeli ex Kützing) Ross	1
30006	Diploneis subovalis Cleve	1
31001	Entomoneis paludosa (Smith) Reimer	1
31003	Entomoneis alata (Ehrenberg) Ehrenberg	1
32003	Epithemia adnata (Kützing) Brébisson	2
33019	Eunotia flexuosa (Brébisson ex Kutzing) Kützing	2
33021	Eunotia formica Ehrenberg	3
33026	Eunotia incisa Smith ex Gregory	25
33036	Eunotia naegelii Migula	1
33059	Eunotia sudetica Müller	12
33066	Eunotia intermedia (Krasske ex Hustedt) Nörpel et Lange-Bertalot	8
33083	Eunotia paludosa Grunow	1
33168	Eunotia implicata Nörpel, Alles et Lange-Bertalot	36
33172	Eunotia faba (Ehrenberg) Grunow	2
33183	Eunotia minor (Kützing) Grunow	139
33185	Eunotia bilunaris (Ehrenberg) Souza	31
33362	Eunotia sp.	5
33395	Eunotia juettnerae Lange-Bertalot	2
33990	Eunotia sp. 1 ?	4
34006	Fragilaria capucina Desmazières	226
34017	Fragilaria crotonensis Kitton	518
34030	Fragilaria vaucheriae (Kützing) Petersen	11

34098	Fragilaria capucina var. gracilis (Østrup) Hustedt	28
34212	Fragilaria sepes Ehrenberg	31
34237	Fragilaria mesolepta Rabenhorst	172
35011	Frustulia vulgaris (Thwaites) De Toni	1
37001	Gomphonema acuminatum Ehrenberg	43
37003	Gomphonema angustatum (Kützing) Rabenhorst	9
37007	Gomphonema gracile Ehrenberg	184
37010	Gomphonema parvulum (Kützing) Kützing	2490
37022	Gomphonema truncatum Ehrenberg	75
37029	Gomphonema subclavatum (Grunow) Grunow	53
37057	Gomphonema turris Ehrenberg	25
37065	Gomphonema olivaceum (Lyngbye) Kützing	1
37071	Gomphonema augur Ehrenberg	39
37080	Gomphonema rhombicum Fricke	25
37084	Gomphonema brebissonii Kützing	5
37118	Gomphonema minusculum Krasske	4
37152	Gomphonema sarcophagus Gregory	2
37168	Gomphonema micropus Kützing	7
37178	Gomphonema minutum (Agardh) Agardh	243
37193	Gomphonema patricki Kociolek et Stoermer	8
37197	Gomphonema kobayasii Kociolek et Kingston	6
37302	Gomphonema drutelingense Reichardt	6
37308	Gomphonema pala Reichardt	6
37310	Gomphonema exilissimum (Grunow) Lange-Bertalot et Reichardt	269
37311	Gomphonema parvulius (Lange-Bertalot et Reichardt) Lange-Bertalot et Reichardt	2
37398	Gomphonema coronatum Ehrenberg	5
37990	Gomphonema sp. 1 ?	250
38004	Gyrosigma spencerii (Smith) Griffith et Henfrey	1
38017	Gyrosigma macrum (Smith) Griffith et Henfrey	3
38030	Gyrosigma sp.	2
44068	Melosira nummuloides (Dillwyn) Agardh	57
44073	Melosira varians Agardh	142

45001	Meridion circulare (Greville) Agardh	3
45002	Meridion circulare var. constrictum (Ralfs) Van Heurck	20
46003	Navicula arvensis Hustedt	2
46014	Navicula cryptocephala Kützing	57
46023	Navicula gregaria Donkin	35
46056	Navicula radiosa Kützing	7
46078	Navicula submuralis Hustedt	1
46104	Navicula tripunctata (Müller) Bory	1
46154	Navicula rhynchocephala Kützing	3
46289	Navicula peregrina (Ehrenberg) Kützing	2
46317	Navicula canalis Patrick	4
46324	Navicula cincta (Ehrenberg) Ralfs	1
46389	Navicula salinarum Grunow	7
46390	Navicula salinicola Hustedt	82
46504	Navicula veneta Kützing	1
46527	Navicula cryptotenella Lange-Bertalot	75
46538	Navicula perminuta Grunow	139
46616	Navicula germainii Wallace	10
46646	Navicula caterva Hohn et Hellerman	2
46648	Navicula erifuga Lange-Bertalot	4
46649	Navicula recens (Lange-Bertalot) Lange-Bertalot	967
46651	Navicula phyllepta Kützing	49
46859	Navicula lanceolata (Agardh) Kützing	3
46896	Navicula rostellata Kützing	1
46990	Navicula sp. 1 ?	50
46991	Navicula sp. 2 ?	685
46992	Navicula sp. 3 ?	17
48004	Nitzschia amphibia Grunow	15
48006	Nitzschia capitellata Hustedt	1
48008	Nitzschia dissipata (Kützing) Grunow	10
48013	Nitzschia frustulum (Kützing) Grunow	15
48015	Nitzschia gracilis Hantzsch	10

48023	Nitzschia linearis (Agardh) Smith	7
48024	Nitzschia microcephala Grunow	2
48025	Nitzschia palea (Kützing) Smith	318
48032	Nitzschia sublinearis Hustedt	6
48122	Nitzschia inconspicua Grunow	222
48123	Nitzschia pusilla Grunow	4
48126	Nitzschia perminuta (Grunow) Peragallo	11
48145	Nitzschia filiformis (Smith) Van Heurck	2
48157	Nitzschia linearis var. tenuis (Smith) Grunow	14
48165	Nitzschia paleacea Grunow	1
48174	Nitzschia reversa Smith	34
48197	Nitzschia brevissima Grunow ex Van Heurck	1
48225	Nitzschia sociabilis Hustedt	3
48229	Nitzschia angustatula Lange-Bertalot	1
48349	Nitzschia tubicola Grunow	3
48351	Nitzschia pellucida Grunow	5
48377	Nitzschia lacuum Lange-Bertalot	28
48381	Nitzschia filiformis var. conferta (Richter) Lange-Bertalot	1
48392	Nitzschia thermaloides Hustedt	3
48417	Nitzschia archibaldii Lange-Bertalot	25
48638	Nitzschia sp.	4
50990	Opephora sp. 1 ?	1
52013	Pinnularia borealis Ehrenberg	1
52045	Pinnularia microstauron (Ehrenberg) Cleve	1
52059	Pinnularia subcapitata Gregory	2
52148	Pinnularia acrosphaeria (Brébisson) Smith	1
52159	Pinnularia gibba (Ehrenberg) Ehrenberg	3
52194	Pinnularia interrupta Smith	1
53012	Surirella sp.	1
54004	Pleurosigma delicatulum Smith	1
57002	Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	5
58001	Rhopalodia gibba (Ehrenberg) Müller	1

62007	Stauroneis smithii Grunow	1
62008	Stauroneis kriegeri Patrick	2
62015	Stauroneis phoenicenteron (Nitzsch) Ehrenberg	1
65064	Surirella brebissonii var. kuetzingii Krammer et Lange-Bertalot	1
65068	Surirella brebissonii Krammer et Lange-Bertalot	5
67004	Tabellaria flocculosa (Roth) Kützing	31
69001	Thalassionema nitzschioides (Grunow) Van Heurck	5
70009	Thalassiosira bramaputrae (Ehrenberg) Håkansson et Locker	1
70029	Thalassiosira proschkinae Makarova	519
70034	Thalassiosira sp.	12
73001	Pseudostaurosira brevistriata (Grunow) Williams et Round	14
73010	Pseudostaurosira parasitica (Smith) Morales	3
76001	Bacillaria paradoxa Gmelin	24
87003	Licmophora sp.	1
89889	Undetermined Pennate	1
89895	Undetermined Centric sp. 1 ?	190
93021	Navicula duerrenbergiana Hustedt	154
93383	Navicula sp.	2
94071	Achnanthes sp.	15
98004	Psammodictyon panduriforme var. continua (Grunow) Snoeijs	4
110004	Encyonema minutum (Hilse) Mann	1
110005	Encyonema silesiacum (Bleisch) Mann	113
110009	Encyonema lunatum (Smith) Van Heurck	1
110063	Encyonema sp.	21
115001	Fallacia pygmaea (Kützing) Stickle et Mann	5
115003	Fallacia cryptolyra (Brockmann) Stickle et Mann	5
115990	Fallacia sp. 1?	6
115016	Fallacia lenzii (Hustedt) Lange-Bertalot	10
115037	Fallacia litoricola (Hustedt) Mann	1
125001	Karayevia clevei (Grunow) Bukhtiyarova	7
125002	Karayevia laterostrata (Hustedt) Bukhtiyarova	2
125011	Karayevia oblongella (Østrup) Aboal	1

130002	Luticola mutica (Kützing) Mann	1
150003	Odontella aurita (Lyngbye) Agardh	1
155003	Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	16
155005	Planothidium peragalli (Brun et Héribaud) Round et Bukhtiyarova	3
155009	Planothidium delicatulum (Kützing) Round et Bukhtiyarova	4
155017	Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	174
155018	Planothidium rostratum (Østrup) Lange-Bertalot	15
155026	Planothidium oestrupii (Cleve-Euler) Edlund	1
170006	Sellaphora pupula (Kützing) Meresckowsky	13
170014	Sellaphora seminulum (Grunow) Mann	43
170033	Sellaphora hustedtii (Krasske) Lange-Bertalot et Werum	3
172001	Staurosira construens Ehrenberg	6
172005	Staurosira construens var. binodis (Ehrenberg) Hamilton	1
172006	Staurosira construens var. venter (Ehrenberg) Hamilton	77
175005	Staurosirella pinnata (Ehrenberg) Williams et Round	18
185006	Tryblionella balatonis (Grunow) Mann	1
185021	Tryblionella calida (Grunow) Mann	2
185023	Tryblionella apiculata Gregory	5
185024	Tryblionella hungarica (Grunow) Frenguelli	2
185025	Tryblionella littoralis (Grunow) Mann	2
185039	Tryblionella compressa (Bailey) Poulin	4
186007	Psammothidium rossii (Hustedt) Bukhtiyarova et Round	1
186008	Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	1
187002	Eucocconeis laevis (Østrup) Lange-Bertalot	2
188001	Lemnicola hungarica (Grunow) Round et Basson	40
189004	Rossithidium anastasiae (Kaczmarska) Potapova	20
190005	Cymbopleura naviculiformis (Auerswald) Krammer	1
192001	Fragilariforma bicapitata (Mayer) Williams et Round	1
192003	Fragilariforma constricta fo. stricta (Cleve-Euler) Poulin	1
193001	Stauroforma exiguiformis (Lange-Bertalot) Flower, Jones et Round	18
194009	Placoneis placentula (Ehrenberg) Mereschkowsky	1
195003	Cavinula pseudoscutiformis (Hustedt) Mann et Stickle	1

197001	Diadesmis confervacea Kützing	31
197002	Diadesmis contenta (Grunow ex Van Heurck) Mann	1
200002	Tabularia fasciculata (Agardh) Williams et Round	35
201001	Ctenophora pulchella (Ralfs ex Kützing) Williams et Round	60
210003	Geissleria decussis (Østrup) Lange-Bertalot et Metzeltin	1
211010	Mayamaea permitis (Hustedt) Bruder et Medlin	1
213001	Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	17
213002	Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin et Witkowski	22
213003	Hippodonta lueneburgensis (Grunow) Lange-Bertalot, Metzeltin et Witkowski	8
218002	Fistulifera saprophila (Lange-Bertalot et Bonik) Lange-Bertalot	5
225002	Berkeleya rutilans (Trentepohl ex Roth) Grunow	43
225990	Berkeleya sp. 1 ?	13
245001	Ulnaria ulna (Nitzsch) Compère	154
245005	Ulnaria acus (Kützing) Aboal	53
2506003	Discostella stelligera (Cleve et Grunow) Houk et Klee	1
2508001	Platessa conspicua (Mayer) Lange-Bertalot	4
8942001	Eolimna minima (Grunow) Lange-Bertalot	25
9049003	Seminavis pusilla (Grunow) Cox et Reid	1
9055990	Gomphonemopsis sp. 1 ?	2
9098003	Halamphora coffeaeformis (Agardh) Levkov	244
9098013	Halamphora veneta (Kützing) Levkov	1
9112001	Grammatophora marina (Lyngbye) Kützing	7

Following the taxa identification, data were processed using Microsoft Excel according to the 2008 USGS Method published by Porter et al. Taxa were quantified in terms of percent abundance at each site (averaged over four trials- June 2014, September 2014, June 2015, September 2015), then taxa were grouped by water quality attributes from the USGS Method. The proportion of diatoms present which indicated the given water quality parameter for each study site is summarized in Table 4.

Table 4: Percent Abundance of various water quality indicators by site ID using USGS method

SITE ID		J-001	J-002	J-003	J-004	J-005	J-006	J-007	J-009	J-011	J-012	J-013	J-014	J-015	S-001	S-002	S-003	S-004	S-005	S-006	S-007	S-010	S-013	S-014	S-015
NF	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TROPHIC	1	0.007	0.005	0.018	0.007	0.007	0.010	0.002	0.000	0.088	0.126	0.002	0.000	0.000	0.072	0.068	0.047	0.066	0.036	0.005	0.000	0.000	0.000	0.000	0.000
	2	0.045	0.003	0.000	0.020	0.020	0.017	0.008	0.000	0.006	0.000	0.014	0.000	0.000	0.000	0.000	0.013	0.005	0.022	0.005	0.000	0.000	0.000	0.000	0.000
	3	0.156	0.038	0.089	0.159	0.100	0.089	0.022	0.005	0.016	0.008	0.129	0.011	0.012	0.036	0.020	0.108	0.202	0.254	0.610	0.000	0.000	0.022	0.004	0.000
	4	0.087	0.000	0.009	0.007	0.025	0.012	0.036	0.008	0.003	0.002	0.012	0.006	0.008	0.152	0.022	0.029	0.012	0.138	0.052	0.020	0.012	0.005	0.031	0.009
	5	0.243	0.316	0.413	0.139	0.396	0.378	0.891	0.907	0.317	0.616	0.709	0.129	0.033	0.231	0.513	0.399	0.404	0.452	0.270	0.761	0.157	0.859	0.308	0.099
	6	0.002	0.000	0.025	0.000	0.000	0.000	0.012	0.070	0.000	0.000	0.072	0.022	0.025	0.003	0.000	0.049	0.003	0.000	0.005	0.154	0.086	0.090	0.132	0.016
	7	0.375	0.616	0.250	0.663	0.446	0.481	0.012	0.000	0.549	0.210	0.026	0.031	0.000	0.403	0.367	0.284	0.287	0.075	0.036	0.006	0.032	0.002	0.015	0.020
EUTROPHIC	1	0.332	0.316	0.447	0.146	0.421	0.390	0.939	0.985	0.320	0.617	0.792	0.157	0.066	0.386	0.534	0.477	0.420	0.590	0.327	0.934	0.256	0.955	0.471	0.124
DIATASTN	1	0.132	0.285	0.401	0.131	0.390	0.366	0.788	0.278	0.292	0.470	0.691	0.073	0.041	0.179	0.481	0.531	0.245	0.260	0.145	0.880	0.128	0.734	0.253	0.049
	2	0.438	0.626	0.074	0.668	0.449	0.482	0.014	0.005	0.567	0.248	0.024	0.017	0.000	0.517	0.376	0.031	0.282	0.069	0.027	0.003	0.005	0.005	0.007	0.004
DIATASTP	1	0.123	0.285	0.204	0.131	0.390	0.366	0.799	0.980	0.291	0.470	0.719	0.126	0.041	0.178	0.481	0.340	0.283	0.256	0.175	0.920	0.150	0.765	0.434	0.067
	2	0.408	0.623	0.044	0.793	0.503	0.536	0.014	0.002	0.552	0.221	0.021	0.017	0.000	0.397	0.372	0.020	0.358	0.231	0.533	0.003	0.005	0.005	0.002	0.002
GNTP	1	0.002	0.000	0.009	0.007	0.005	0.007	0.161	0.205	0.000	0.000	0.515	0.014	0.000	0.000	0.000	0.031	0.050	0.006	0.036	0.692	0.000	0.593	0.035	0.002
	2	0.465	0.621	0.046	0.663	0.447	0.481	0.016	0.000	0.550	0.223	0.014	0.017	0.000	0.483	0.369	0.020	0.264	0.079	0.075	0.003	0.005	0.005	0.002	0.002
GNTN	1	0.021	0.002	0.014	0.007	0.005	0.007	0.175	0.902	0.002	0.000	0.595	0.078	0.000	0.000	0.000	0.031	0.019	0.002	0.002	0.707	0.027	0.797	0.240	0.038
	2	0.347	0.624	0.048	0.677	0.467	0.497	0.014	0.000	0.547	0.223	0.010	0.017	0.000	0.391	0.372	0.052	0.275	0.162	0.086	0.003	0.005	0.005	0.002	0.002
EHTP	1	0.134	0.028	0.199	0.007	0.005	0.005	0.289	0.065	0.024	0.148	0.112	0.048	0.021	0.067	0.028	0.183	0.036	0.026	0.029	0.162	0.140	0.058	0.086	0.038
	2	0.365	0.623	0.046	0.793	0.501	0.536	0.014	0.002	0.550	0.221	0.017	0.017	0.000	0.397	0.372	0.018	0.358	0.205	0.481	0.003	0.005	0.005	0.002	0.002
EHTN	1	0.252	0.313	0.408	0.136	0.415	0.378	0.540	0.012	0.315	0.617	0.072	0.042	0.004	0.245	0.521	0.475	0.257	0.347	0.184	0.057	0.140	0.007	0.031	0.031
	2	0.326	0.608	0.043	0.662	0.446	0.479	0.014	0.005	0.529	0.196	0.010	0.017	0.000	0.362	0.352	0.016	0.271	0.077	0.070	0.003	0.005	0.005	0.002	0.002
SAPROBIC	1	0.163	0.035	0.222	0.035	0.046	0.039	0.030	0.000	0.094	0.128	0.035	0.011	0.012	0.188	0.083	0.160	0.095	0.091	0.050	0.000	0.000	0.000	0.000	0.000
	2	0.589	0.659	0.284	0.828	0.551	0.577	0.154	0.017	0.576	0.334	0.155	0.062	0.012	0.478	0.407	0.228	0.563	0.582	0.714	0.048	0.157	0.031	0.070	0.054
	3	0.054	0.005	0.071	0.005	0.004	0.003	0.329	0.854	0.019	0.029	0.675	0.104	0.029	0.069	0.017	0.155	0.081	0.014	0.059	0.550	0.044	0.854	0.282	0.079
	4	0.125	0.285	0.379	0.129	0.390	0.364	0.457	0.053	0.291	0.470	0.031	0.000	0.000	0.174	0.481	0.408	0.226	0.258	0.138	0.188	0.000	0.002	0.007	0.000
	5	0.002	0.002	0.004	0.000	0.002	0.007	0.012	0.070	0.000	0.002	0.087	0.022	0.025	0.003	0.002	0.013	0.016	0.036	0.029	0.154	0.086	0.092	0.132	0.011
ORG_N	1	0.148	0.035	0.059	0.066	0.093	0.067	0.031	0.000	0.096	0.128	0.035	0.011	0.012	0.174	0.097	0.169	0.111	0.245	0.134	0.000	0.000	0.000	0.000	0.000
	2	0.519	0.646	0.477	0.794	0.506	0.543	0.158	0.713	0.580	0.356	0.089	0.126	0.025	0.533	0.411	0.302	0.420	0.258	0.535	0.068	0.172	0.045	0.280	0.094
	3	0.125	0.287	0.193	0.131	0.392	0.373	0.635	0.053	0.291	0.471	0.182	0.022	0.017	0.174	0.482	0.299	0.280	0.288	0.200	0.185	0.000	0.244	0.055	0.007
	4	0.002	0.000	0.004	0.000	0.000	0.000	0.012	0.070	0.000	0.000	0.068	0.022	0.025	0.003	0.000	0.013	0.000	0.004	0.000	0.154	0.088	0.092	0.143	0.025
POLL_CLASS	1	0.122	0.285	0.195	0.126	0.390	0.364	0.454	0.070	0.291	0.470	0.096	0.042	0.768	0.178	0.481	0.302	0.219	0.247	0.136	0.185	0.091	0.090	0.159	0.103
	2	0.158	0.040	0.323	0.055	0.070	0.057	0.392	0.917	0.023	0.008	0.801	0.179	0.017	0.016	0.008	0.385	0.126	0.178	0.118	0.769	0.093	0.888	0.385	0.076
	3	0.589	0.636	0.245	0.804	0.524	0.548	0.145	0.010	0.570	0.357	0.061	0.042	0.025	0.653	0.431	0.162	0.528	0.477	0.633	0.028	0.125	0.009	0.040	0.025
POLL_TOL	1	0.122	0.285	0.195	0.126	0.390	0.364	0.452	0.070	0.291	0.470	0.096	0.022	0.025	0.178	0.481	0.302	0.219	0.247	0.136	0.182	0.086	0.090	0.132	0.011
	2	0.010	0.000	0.016	0.002	0.000	0.002	0.006	0.014	0.006	0.000	0.054	0.050	0.000	0.000	0.002	0.016	0.017	0.008	0.005	0.011	0.000	0.206	0.093	0.000
	3	0.000	0.000	0.005	0.000	0.000	0.000	0.002	0.005	0.000	0.000	0.005	0.003	0.000	0.000	0.000	0.016	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000
	4	0.411	0.616	0.181	0.658	0.439	0.476	0.087	0.000	0.541	0.300	0.012	0.020	0.000	0.338	0.366	0.023	0.273	0.067	0.034	0.003	0.017	0.004	0.018	0.018
	5	0.203	0.012	0.051	0.012	0.032	0.015	0.006	0.003	0.015	0.005	0.084	0.000	0.000	0.102	0.022	0.036	0.043	0.150	0.057	0.000	0.002	0.025	0.011	0.002
OXYTOL	1	0.476	0.633	0.073	0.711	0.510	0.530	0.044	0.003	0.623	0.310	0.073	0.034	0.021	0.557	0.436	0.151	0.368	0.223	0.141	0.020	0.005	0.002	0.026	0.011
	2	0.030	0.015	0.032	0.144	0.082	0.076	0.016	0.710	0.011	0.002	0.063	0.076	0.000	0.031	0.027	0.052	0.105	0.260	0.488	0.034	0.025	0.016	0.216	0.029
	3	0.163	0.035	0.410	0.007	0.009	0.013	0.307	0.005	0.039	0.174	0.166	0.034	0.033	0.121	0.047	0.230	0.098	0.065	0.098	0.011	0.130	0.255	0.070	0.063
	4	0.123	0.285	0.218	0.126	0.390	0.364	0.454	0.076	0.292	0.470	0.099	0.042	0.025	0.178	0.481	0.345	0.238	0.247	0.143	0.185	0.101	0.107	0.163	0.022
	5	0.002	0.000	0.000	0.003	0.000	0.000	0.017	0.053	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.157	0.000	0.002	0.007	0.000
pH	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.012	0.012	0.225	0.005	0.009	0.008	0.002	0.000	0.006	0.018	0.000	0.000	0.000	0.024	0.000	0.052	0.003	0.024	0.063	0.000	0.000	0.004	0.000	0.000
	3	0.340	0.328	0.277	0.179	0.453	0.410	0.470	0.081	0.406	0.624	0.229	0.039	0.025	0.326	0.569	0.509	0.487	0.517	0.290	0.205	0.086	0.110	0.137	0.011
	4	0.267	0.036	0.470	0.154	0.096	0.094	0.501	0.907	0.041	0.151	0.749	0.171	0.212	0.253	0.068	0.396	0.181	0.373	0.580	0.752	0.403	0.864	0.368	0.330
	5	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.000	0.000	0.002	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	6	0.318	0.611	0.021	0.658	0.440	0.479	0.012	0.000	0.531	0.181	0.010	0.022	0.000	0.314	0.352	0.016	0.309	0.067	0.057	0.003	0.020	0.004	0.004	0.009
SALINITY	1	0.016	0.012	0.225	0.007	0.009	0.008	0.002	0.003	0.093	0.139	0.012	0.000	0.000	0.091	0.068	0.094	0.067	0.053	0.063	0.000	0.000	0.004	0.004	0.000
	2	0.920	0.975	0.768	0.987	0.989	0.983	0.637	0.083	0.891	0.836	0.360	0.095	0.050	0.826	0.922	0.862	0.867	0.921	0.896	0.236	0.241	0.313	0.189	0.056
	3	0.002	0.000	0.000	0.003	0.000	0.000	0.342	0.907	0.000	0.000	0.607	0.098	0.017	0.000	0.000	0.014	0.033	0.004	0.029	0.704	0.015	0.655	0.295	0.049
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.007	0.042	0.050	0.000	0.000	0.004	0.014	0.002	0.002	0.014	0.037	0.011	0.009	0.076
DIATCOND	1	0.000	0.000	0.000	0.000	0.000	0.000	0.159	0.146	0.000	0.000	0.513	0.011	0.332	0.000	0.000	0.005	0.002	0.002	0.000	0.561	0.052	0.604	0.009	0.043
	2	0.184	0.056	0.293	0.033	0.039	0.039	0.005	0.000	0.058	0.076	0.105	0.429	0.021	0.179	0.023	0.113	0.086	0.103	0.118	0.000	0.396	0.025	0.396	0.530
DIAT_CL	1	0.000	0.000	0.000	0.000	0.000	0.000	0.139	0.146	0.000	0.000	0.501	0.000	0.320	0.000	0.000	0.005	0.000	0.002	0.000	0.533	0.017	0.586	0.009	0.043
	2	0.863	0.995	0.752	0.856	0.916	0.914	0.573	0.022	0.904	0.872	0.244	0.521	0.025	0.845	0.902	0.687	0.608	0.464	0.306	0.066	0.548	0.224	0.493	0.566
DIAT_CA	1	0.097	0.015	0.165	0.000	0.002	0.000	0.231	0.151	0.016	0.121	0.510	0.017	0.012	0.024	0.013	0.014	0.012	0.022	0.018	0.561	0.034	0.595	0.000	0.004
	2	0.012	0.021	0.220	0.007	0.012	0.010	0.002	0.000	0.013	0.018	0.000	0.429	0.021	0.022	0.000	0.052	0.014	0.041	0.070	0.000	0.396	0.004	0.396	0.530

Following this analysis, results were run through a principal components analysis using JMP software to observe relationships between the USGS indicator taxa and real field conditions. The first analysis involved the use of TN field data, Land use TN load estimates from a previous study (Geosyntec, 2015) and DIATASTN percent abundance counts. The results, seen in Figure 4, reflect three mildly distinct groups of sites by Nitrogen levels. Green dots represent data from sites 003 and 004, which both had a low anticipated nitrogen load. Blue represent sites 1 and 2, for which a medium nitrogen load was predicted, and pink represents sites 5, 6 and 7 which had a high anticipated load based on land use. Land use nitrogen load estimates were not calculated for sites further downriver.

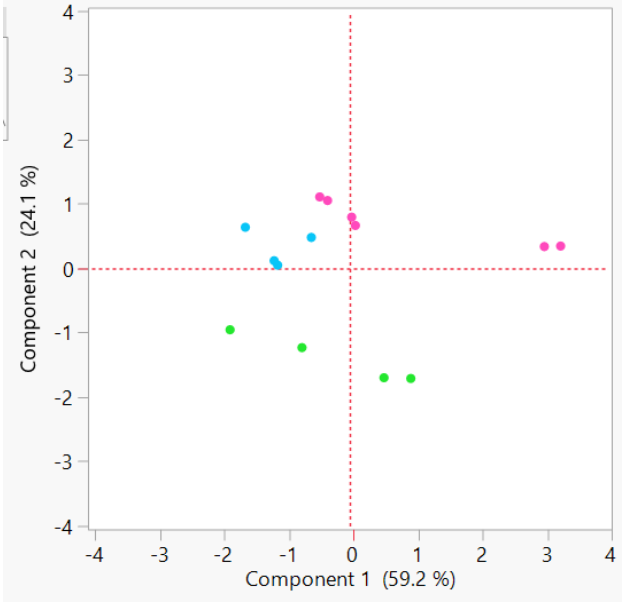


Figure 4: Nitrogen Principal Components Analysis

The next principal components analysis compared TP field measurements with two Phosphorus indicators; Diatom phosphorus, and Eastern Highland Taxa affected by Phosphorus (Figure 5). Orange dots indicate TP conditions below 40ug/L at sites 1,2,6,13, and 15. Blue dots indicate medium TP levels, between 40-75 ug/L and encompassing sites 4,5,14, and 10. Pink dots represent High TP conditions, above 75 ug/L and describe sites 3,7, and 9.

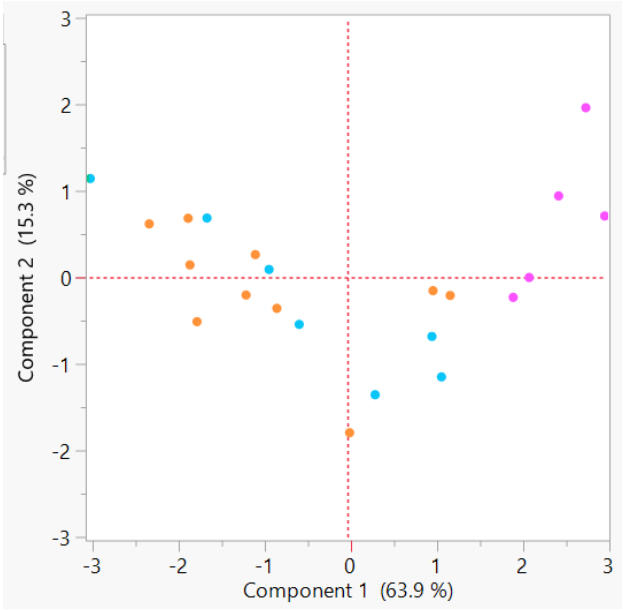


Figure 5: Phosphorus Principal Components Analysis

Diatom phosphorus indicator taxa and eastern highland indicator taxa densities were summed to create total percent abundance measurements for low and high phosphorus conditions. These values were then plotted in excel against TP field data to compare taxa presence to real water quality conditions. Sites 001, 002, 004, 005, 006 and 007 showed promising results (Figure 6). Using the USGS method and taxa database, these sites contained high amounts of taxa which corresponded with water quality field data.

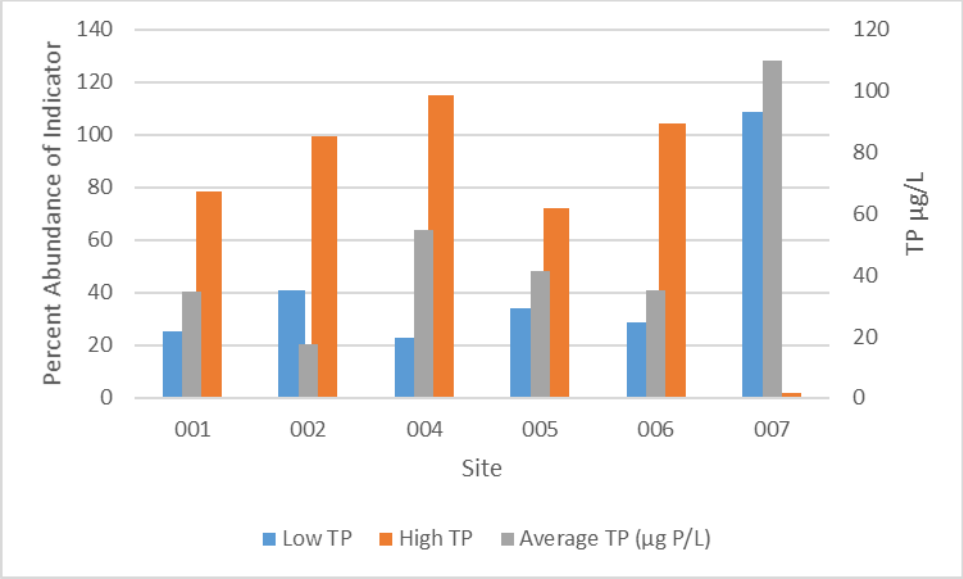


Figure 6: TP Field Data contrasted with Algal Indicator Results

In addition to Nitrogen and Phosphorus, salinity taxa were analyzed as a way of further evaluating the validity of the USGS method for Great Bay. As shown in Figures 2 and 3, site numbers increase as locations move from upriver freshwater rivers downstream into the estuary itself. Looking at Figure 7, sites 1-6 (all freshwater sites) contain primarily taxa indicating low chloride levels, below 500mg/L. Starting at site 7, which is located downstream in the tidal portion of the Exeter River, taxa indicative of chloride levels above 500 start to make up a more substantial portion of the total taxa. Sites 7 and 10, both located in estuarine ecosystems, have barely any taxa indicating Chloride levels below 100 mg/L. Moving further downstream to site 15, located in Great Bay, the largest proportion of taxa indicating Chloride levels above 1000mg/L can be observed.

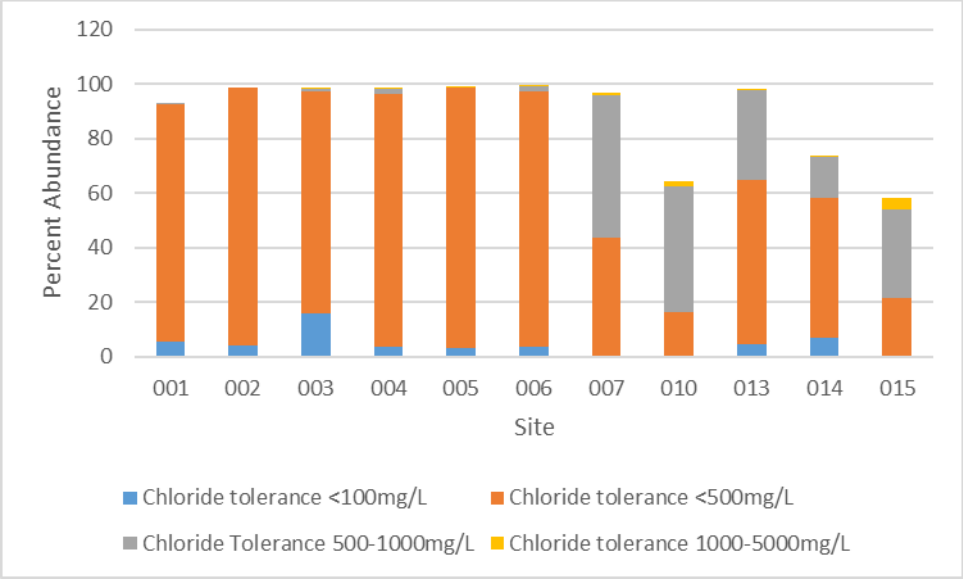
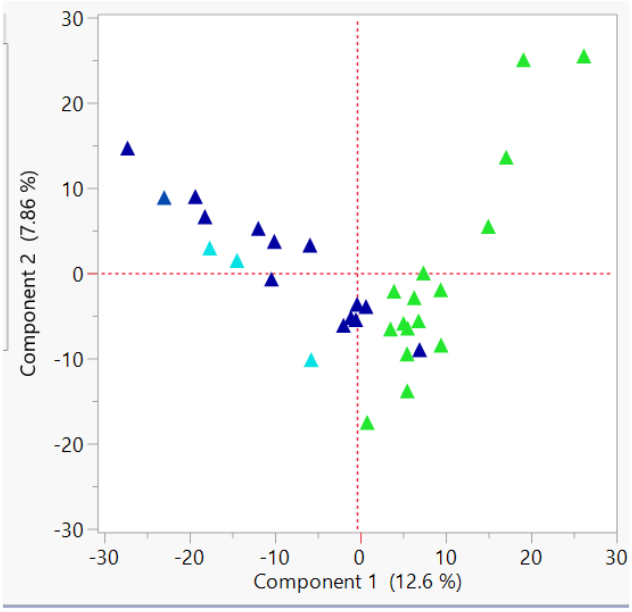


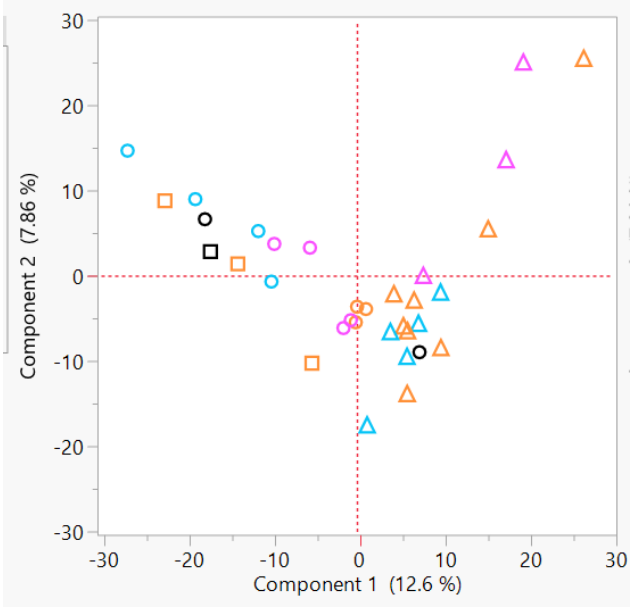
Figure 7: Algal Indicator Results for Salinity

Genomic Data

1. Algae only, bacteria genome data: Light green = upriver, Dark blue = tidal river (7 and beyond), Light blue = Great Bay



2. Algae only, bacteria genome data: Triangle = 1-6, Circle = 7-14, Square = 15-16; Orange = Low TP <40ug/L = 1,2,6,13,15; Blue = Med TP, 40-75 ug/L = 4,5,14,10; Pink = High TP >75 ug/L = 3,7,9



Conclusions

Microscope Results & Great Bay

Analysis of taxonomic results from the traditional microscope taxa identification was limited due to the size of the data set. This in combination with limited field measurements did not yield any statistically viable results, however several patterns were observed which support further investigation regarding the use of attached algae method in the Great Bay region. Specifically, Total Phosphorus and salinity results indicated species of algae (indicators) we would expect to see in certain areas of the estuary based upon field data. Sites known to be high in phosphorus did in fact overall contain more algae species that are high phosphorus indicators, and sites that were closer to the bay contained more species that were indicators of high salinity conditions.

These patterns, based upon the indicator series from USGS, indicate that attached algae may prove to be a viable method for water quality analysis in the unique great bay ecosystem environment.

Barriers to Genomic Analysis

Extraction techniques can have an impact on results, therefore it is important to process samples appropriately. It is unclear whether or not the hard shells of diatoms might affect the success of RNA extraction, and further research is necessary to determine if this is the case. It may be possible that current extraction techniques are not able to obtain a long enough sequence of RNA for the desired level of taxa identification, therefore further exploration of extraction techniques is necessary. Currently, available databases for species identification of algal RNA are limited, therefore further investigation of existing databases must also be included.

Next Steps

Future work will require gathering a larger, more geographically diverse data set to further evaluate algae species which may serve as good indicators for the great bay region. Additional work with genomic analysis will be necessary to determine if algal databases specific enough are available, and to refine current techniques to try to achieve species-level identification. Once this has been accomplished, more work will be possible relating to the identification of new indicators from existing and future genomic data. The University of New Hampshire should continue to work closely with NH-DES and others to identify applicability of any results to state water quality monitoring programs.

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