Evaluation of Stream Periphyton as Indicators of Biological Condition for Florida Streams

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Prepared by:

Leska S. Fore
Statistical Design
3518 Fremont Ave N
Seattle, WA 98103
leska.fore@gmail.com

Prepared for:

Russel Frydenborg & Nijole Wellendorf
Florida Department of Environmental Protection
2600 Blair Stone Rd.
Tallahassee, FL 32399-2400

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ABSTRACT

Biological measures derived from periphyton samples collected from Florida streams were tested for their response to an independent gradient of human disturbance. Attributes for diatom and non-diatom taxa were used to calculate measures of taxa richness, tolerance and sensitivity, and preferences for pH extremes, nutrients, oxygen and other environmental conditions. These measures of periphyton condition were tested for correlation with independent measures of water quality, nutrients, habitat condition and land use within a 100 m buffer for 10 km upstream of the sampling site. Many periphyton taxa found in Florida streams were not included in published taxa lists; therefore, genus averages of attributes were calculated and applied to missing taxa. Of the 34 candidate metrics tested for diatoms, 14 were significantly correlated with human disturbance for two data sets (derived from two different sampling occasions). Of the 13 candidate metrics tested for non-diatom taxa, none were consistently correlated with human disturbance.

Five diatom metrics that were not redundant or highly correlated with each other were selected for inclusion in a multimetric index, the stream diatom index (SDI). These metrics were percent sensitive cells, percent tolerant cells, percent cells that prefer high oxygen, percent cells that prefer oligotrophic conditions, and van Dam's weighted index for trophic status (TSI). Although significantly correlated with human disturbance, SDI was more highly correlated with pH. When the data were categorized according to pH, a relationship between SDI and human disturbance was not observed. PH was a much better predictor of SDI than human disturbance.

Concurrently with this analysis, Florida DEP sponsored a workshop of 15 algal ecologists to develop a biological condition gradient for periphyton. Experts had periphyton data from 25 streams sites with low pH (< 6.5) and 25 sites with high pH (≥ 6.5). No information was given on site condition or disturbance, except for the pH category. The experts assigned each of the 50 sites to one of six classes of biological condition. SDI was highly correlated with the class assignments of the experts indicating a strong agreement between empirical testing and the knowledge and experience of the algal ecologists. Unfortunately, the BCG ranking for each site was also more highly correlated with pH than with the human disturbance gradient.

Currently the SDI is not appropriate for use as biocriteria due to its poor correlation with human disturbance and its strong association with pH. High values for both nutrients and pH can be due to natural sources in Florida streams. If natural expectations for pH and nutrients can be defined for Florida stream sites, it may be possible to refine the SDI. Until then, periphyton assessment methods are not ready to be implemented as biocriteria.

INTRODUCTION

The regulatory context for the management of surface waters is derived from the mandates of the Clean Water Act (CWA; EPA, 2005; Davies and Jackson, 2006). A primary goal of the CWA is to "restore and maintain the chemical, physical and biological integrity of the Nation's waters." The regulatory framework to accomplish this goal specifies that states must adopt water quality standards to protect their waters. Water quality standards (WQS) include three parts: 1) designated uses, 2) numeric and narrative criteria that protect the uses, and 3) antidegradation policies to prevent deterioration of high-quality waters (EPA, 2006). WQS are part of state law and define the water quality goals for a water body by designating the use(s) and setting criteria necessary to protect the use(s). Once WQS are in place, states are authorized to implement monitoring programs that allow them to report on the attainment of water quality standards and to identify and prioritize waters not attaining standards (Karr and Yoder, 2001; Keller and Cavallaro, 2007). Thus, under federal law, Florida Department of Environmental Protection (DEP) is responsible for reviewing and setting the state's water quality standards.

Florida currently has five designated use classifications for water bodies, Class I through Class V. Most of Florida's water bodies are Class III, which means the water is expected to support recreation and a healthy, well-balanced fish and wildlife population. The current classification system has been in effect for more than 30 years, and during that time scientific knowledge has advanced. Recently, the Florida Stormwater Association requested that DEP amend the existing classification structure for designated uses. In response, DEP has reviewed and proposed a refinement of the current system to better protect both human health and the aquatic life of rivers, lakes, streams, wetlands, and estuaries.

In 2006, DEP formed a Policy Advisory Committee (PAC) of independent experts to help the agency consider the environmental, scientific, technical, economic, legal, and social factors involved with potential changes. Nominations were requested from a wide range of expert stakeholders, *e.g.*, environmental groups, local governments, other state agencies, business and industry, the United States Environmental Protection Agency (EPA).

To determine whether Florida water bodies support their aquatic life use designations, biological indicators have been developed and tested for macroinvertebrate assemblages in streams (Fore *et al.*, 2007a), for macrophytes in lakes (Fore *et al.*, 2007b), and for macroinvertebrates, plants, and diatoms in wetlands (Lane, 2007; Lane and Brown, 2007). Index and metrics derived for each of these water bodies were highly correlated with independent measures of human disturbance. EPA recommends that more than one biological assemblage be used to assess the biological condition of water bodies; therefore, this document describes analysis and testing of biological indicators for periphyton in streams (Stevenson and Bahls, 1999; Rosen, 1995; EPA, 2006).

Periphyton include algae, bacteria, fungi, and protozoa that live in communities attached to stream benthos, *e.g.*, rocks, woody debris, or vegetation. For biological assessment, only the algae are typically used. The algae are divided into two groups for laboratory processing and taxonomic identification: the diatoms have silica cases and can be easily identified to species while the non-diatom algae (sometimes called "soft" algae) are more difficult to identify and are more diverse and include several taxonomic orders. Most bioassessment protocols for periphyton rely on diatoms (Davis *et al.*, 1996).

Diatoms and other algae are phyotosynthetic, and depend on nutrients for growth and reproduction (Carrick *et al.*, 1988; Chessman *et al.*, 1999; Winter and Duthie, 2000). Diatom responses to nutrients are predictable and have been used to develop nutrient criteria (Stevenson *et al.*, 2008a). Development of periphyton indicators for Florida streams is also relevant to recent efforts and requirements to develop nutrient criteria (EPA-State Nutrient Innovations Task Group, 2009).

LITIGATION AND DEVELOPMENT OF NUTRIENT CRITERIA

In July 2008, environmental groups sued EPA for failure to adopt numeric nutrient criteria for Florida water bodies. Under recent legal decisions and agreements, EPA must propose numeric nutrient criteria for Florida lakes and streams by January, 2010 and finalize those criteria by October, 2010. For coastal waters and estuaries numeric criteria must be proposed by January, 2011 and finalized by October, 2011. Florida DEP is working closely with EPA to develop the technical bases for reasonable and protective nutrient criteria.

Florida currently has a narrative nutrient criterion that states "in no case shall nutrient concentrations of a body of water be altered so as to cause an imbalance in natural population of flora or fauna." Florida does not have numeric criteria for nutrients. A multi-metric periphyton index would provide a quantitative measure of human-caused imbalances in the biological condition of streams due to nutrients and other pollutants. Thus, periphyton assessments may potentially be used to support the definition of thresholds for nutrient criteria for Florida streams. Unfortunately, water quality (*e.g.*, nutrients, pH, specific conductance) is highly variable throughout the state due to natural sources, and this is a potentially confounding issue when attempting to isolate human caused changes in periphyton community structure and function.

This document describes the development and testing of biological indicators for diatoms and non-diatom algae. Candidate metrics for biological assessment were derived from published information and from periphyton samples from Florida streams. Metrics were tested against independent measures of human influence. In a parallel effort, an outside panel of algal ecologists evaluated periphyton data from Florida streams and defined categories of biological condition using only taxonomic information from the stream samples. Results from their evaluation and empirical testing described in this document were compared and diatoms evaluated for their potential as indicators of biological condition.

METHODS

Periphyton data were collected from 2005-2007 and many sites were visited twice. Most of the first periphyton site visits occurred during May - August of 2005 (Q2). Most of the second visits occurred from November 2005 – February 2006 (Q4). Q2 and Q4 data collections were part of a year-long statewide study of 150 stream and river sites. Water quality information was collected for all four quarters of the study (Q1-Q4), so water quality data from Q1 and Q2 (March-August 2005) were averaged and matched with Q2 periphyton data, and water quality data from Q3 and Q4 (August 2005-March 2006) were matched with Q4 periphyton data.. Data from Q2 were used to evaluate diatom community structure and test metric responses to human disturbance (Table 1). Data from Q4 were used to develop the human disturbance gradient and confirm metric response to disturbance observed for Q2. Both Q2 and Q4 data were used to identify sensitive and tolerant taxa. A third data set, the validation data, were composed of repeat site visits to some sites in Q2 and Q4 as well as many new sites.

PERIPHYTON COLLECTION AND IDENTIFICATION

Periphyton were collected from a 100 m stream reach using a multi-habitat collection method that targets snags, roots, leaf packs, vegetation, rock, and algal mat in proportion to their occurrence in the reach, per DEP SOP FS 7220. Ten sample aliquots are collected and combined into a single final sample. Substrates that could be reached at arm's length were selected, *i.e.*, the upper 0.5 m of water or less. Wide mouth jars were used to collect 100 mL of site water. A piece of substrate approximately the size of the surface area of the jar was selected and rubbed into the jar. No sediment was collected. The algal/ water slurry was stirred and a pipette was used to remove 4 mL of the algal slurry and then transfer it into a 50 mL centrifuge tube. The process was repeated for a total of 10 substrate types for a final total volume of 40 mL. The sample was then preserved with 4 mL of full strength buffer formalin.

Wet algae were identified using an inverted microscope to count 300 natural units of algae (including diatoms) with non-diatoms identified to the lowest practical level and diatoms recorded as a count of live frustules. A separate aliquot was used to identify diatoms. Diatoms were counted at 1000 X magnificent using a compound microscope. For diatoms, 500 valves were identified to the lowest practical level, typically species. Algal methods followed DEP Biology Section internal SOPs AB-01 through AB-15.

Table 1. Data set name, number of site visits, and type of analysis for which data were used.

Data set	N	Analysis
Q2 (most recent visits)	126	Metric testing
Q2 (LDI < 2)	68	NMS for community structure
Q4 (most recent visits)	148	Confirm metric response; test correlation of human disturbance measures
Q2 and Q4 (most recent visits)	124 x 2	Test for sensitive and tolerant taxa

QUANTIFYING NUTRIENT ENRICHMENT

Four measures of water chemistry were used to develop the water quality (WQ) index. The WQ index was the sum of scored values for conductivity, total phosphorus (TP), ammonia (NH_4), and nitrite/nitrate (NN). Percentiles from statewide probabilistic stream and river data were used to score TP and NN, resulting in ten categories (Table 2). For NH_4 and conductivity, values were scored using five categories rather than ten because the range of values did not include enough unique values to support this ten categories. Scores for conductivity were defined differently for the three regions (Table 3). For NH_4, any value below the method detection limit, 0.03 mg/L, was given a zero, and detected values were assigned 1-4 based on the data distribution. For conductivity, we calculated the 75^{th} percentile of median site values for all streams with LDI < 2 (minimally disturbed) in the statewide database for each region. We considered conductivity values below the 75^{th} percentile to be near natural and assigned those a score of zero (Table 3). Values greater than this level were scaled 1-4 based on the distribution of the data.

The water quality index was calculated by taking the average of all four scores for water quality variables. NH_4 and conductivity were given half the weight in the water quality index of TP and NN because they were considered to be better indicators of human disturbance. Because the variables were on different scales (0–4 vs. 0–9), scores for NH_4 and conductivity were first multiplied by 2.5 before averaging.

Table 2. Percentiles used as breakpoints to define 10 categories to calculate the water quality index.

%Tile	TP	NN
10^{th}	0.02	0.00
20^{th}	0.03	0.01
30^{th}	0.04	0.01
40^{th}	0.07	0.02
50^{th}	0.09	0.04
60^{th}	0.12	0.08
70^{th}	0.17	0.13
80^{th}	0.32	0.24
90^{th}	0.75	0.49

Table 3. Breakpoints used to calculate the water quality index; conductivity was scored separately for the northeast, panhandle and peninsula regions.

	0	1	2	3	4
NH_4	[0-0.03]	(0.03-0.04]	(0.04-0.06]	(0.06-0.1]	>0.1
Conductivity - NE	<170	170-300	300-400	400-500	>500
Conductivity - PH	<198	198-300	300-400	400-500	>500
Conductivity - PS	<406	400-500	500-600	600-800	>800

QUANTIFYING HUMAN DISTURBANCE

The human disturbance gradient (HDG) used to test periphyton metrics was derived from values of the habitat assessment, the WQ index and the landscape development intensity (LDI) index. To measure habitat condition, biologists routinely calculate a habitat condition score (DEP SOP FT3100) whenever stream periphyton samples are collected. The habitat assessment evaluates substrate condition and availability, water velocity, habitat smothering (*e.g.*, by sand and silt), channelization, bank stability, and the width and condition of the riparian vegetation. Two component measures of the habitat assessment (water velocity and habitat smothering) and the observation of canopy cover were also tested as potential independent disturbance indicators, but the total habitat assessment score had the highest correlations with potential algal metrics.

To summarize the intensity of human land use, we used the LDI index developed by Brown and colleagues (Brown and Vivas, 2004). LDI was calculated as the percentage area within the catchment of particular types of land use multiplied by the coefficient of energy use associated with that land use, summed over all land use types in the watershed.

$$LDI = \sum \left(LDI_i * \%LU_i \right).$$

Where,
LDI_i = the nonrenewable energy land use for land use *i*, and
$\%LU_i$ = the percentage of land area in the catchment with land use *i*.

To define the HDG, we converted the three measures of human disturbance to unit-less scores and summed the scores to create HDG values for each stream site. The measures had scores of 0, 1, or 2 indicating low, moderate or high levels of human influence (Table 4). The same scoring rules were used for the habitat index and LDI as were used to develop the SCI (stream condition index) for stream macroinvertebrates (Fore *et al.*, 2007). For the WQ index, percentiles were used to convert index values to scores. Values for the HDG ranged from 0 (indicating minimal human disturbance) to 6 (indicating extreme disturbance).

Table 4. Scoring rules for measures used to calculate the human disturbance gradient (HDG). HDG is the sum of the scores for each site. Habitat index values ranged from 0-100 and represent the field value assigned divided by the total number of points possible (140).

Measure	0	1	2
WQ Index	<2.2	2.2–3.2	>3.2
Habitat index	>65	50–65	<50
LDI	<2	2-3.5	>3.5

TAXONOMIC ORDINATION

Non-metric multidimensional scaling (NMS) is a nonparametric ordination procedure used to identify structure and pattern associated with taxonomic distributions of diatoms. The analysis used taxonomic names and abundances for diatoms only because they represent a unique taxonomic group and are typically identified to a lower taxonomic level than are the non-diatom

algae. Taxonomic names were a mix of genus, species, and variety level identifications. Taxa that occurred at less than 5% of the sites were eliminated to eliminate the influence of rare taxa on the NMS analysis which left 122 unique taxa for the analysis. Data were summarized using the Sorenson similarity index (Bray-Curtis) to calculate taxonomic similarity between all pairs of sites.

The goal for this analysis was to evaluate the influence of regional differences on the structure of diatom communities; therefore, only sites with minimal human disturbance were included to avoid confusing the results with differences associated with human influence. Of the 138 stream sites visited in summer 2005, 68 sites had an LDI < 2.0 and were selected for the NMS analysis.

Results for sites were plotted as a function of the two NMS axes and labeled according to bioregion and pH. Location on Axis 1 for each diatom taxon was compared using correlation with the pH categories defined by Van Dam *et al.* (1994) for diatoms.

IDENTIFYING CANDIDATE METRICS

DIATOMS

Attributes of the periphyton assemblage related to community structure, tolerance and intolerance, autecological guild, and morphological guild were identified for testing in this study (van Dam *et al.*, 1994; Porter *et al.*, 2008). Metrics were derived from these attribute lists for both diatom and non-diatom algae taxa.

Community structure

For periphyton, the total number of taxa typically increases with disturbance in contrast with fish or macroinvertebrates which tend to decline (Cuffney *et al.*, 1997; Stewart *et al.* 1999; Fore, 2002). Percentage dominance was calculated for the single most abundant taxon in each sample.

Tolerance and intolerance

The TSI used trophic status indicator values from Van Dam *et al.* (1994). The tolerance index was calculated as the number of valves found for each taxon multiplied by the tolerance value for that taxon (Potapova *et al.*, 2004). These numbers were summed for each taxon and that number divided by the total number of valves identified in the sample. This format is typical for many algal indices (Whitton and Kelly, 1995).

Taxa were categorized as sensitive or tolerant according to Bahls (1993), who modified initial assignments by Lange-Bertalot (1979) and Lowe (1974). Bahls defined diatom species as generally tolerant to high nutrients (eutrophic), organics (polysaprobic), temperature (euthermal), salts (euhalobus), toxics, suspended solids, or unstable substrate (Bahls, 1993). Sensitive and tolerant taxa designations were also derived from the current Florida data set (see below).

Autecological guilds

Trophic state relates to the presence of inorganic nutrients such as nitrogen, phosphorus, silica and carbon; in contrast, saprobity refers to the presence of biodegradable organic matter and low oxygen concentrations (Van Dam *et al.*, 1994). Eutrophic and polysaprobic diatoms were expected to increase if inorganic or organic nutrients were present in large amounts (Potapova and Charles, 2007). Fertilizer would be one potential source of inorganic nutrient

enrichment; livestock excrement and wastewater discharges could be sources of organic nutrient enrichment. In contrast, oligotrophic and oligosaprobic diatoms should decline with disturbances that increase inorganic or organic nutrient levels.

Many diatoms are known to specifically prefer acidic (*e.g.*, *Eunotia*) or alkaline conditions (Pan *et al.*, 1999; Leland *et al.*, 2001). Construction or agriculture on alkaline soils can erode soils and may increase alkalinity in nearby streams. Irrigation and fertilization can also increase soil alkalinity.

Diatoms in the genera *Epithemia* and *Rhopalodia* are nitrogen fixers because they harbor endosymbiotic cyanobacteria that allow them to convert atmospheric nitrogen into biologically useful forms such as ammonia (Mulholland, 1996). In contrast, diatoms classified as nitrogen heterotrophs can use amino acids created by other organisms as sources of carbon and nitrogen (Tuchman, 1996). Thus, nitrogen fixers should decline as they are replaced by nitrogen heterotrophs that can take advantage of the organic nitrogen associated with fertilization or other disturbances that increase nitrogen.

Morphological structure guilds

Motile diatoms tolerate silt because they can move across unstable substrates without being buried and these taxa are expected to increase as sediment increases. Many studies have noted an increase in motile diatoms associated with erosion and sedimentation which may be caused by a variety of human activities (Bahls, 1993; Kutka and Richards, 1996; Detenbeck *et al.*, 2000). By moving across the substrate, these diatoms avoid being smothered by shifting sediments.

NON-DIATOMS (SOFT ALGAE)

Community structure for non-diatom algae was evaluated using the total number of unique taxa, and percentages of diatom (Bacillariophyta), euglenoid, Chlorophyta, Cryptophyta, Cyanobacteria, and Pyrrhophyta cells (Porter *et al.*, 2008). Nuisance species were provided by Florida DEP biologists (Table 5). Tolerant taxa were derived from testing with this Florida data set (see below).

Table 5. Taxon names of nuisance species provided by Florida DEP biologists.

Taxon name
Microcystis
Microcystis aeruginosa
Oscillatoria
Oscillatoria acutissima
Oscillatoria formosa
Oscillatoria hamelii
Oscillatoria lacustris
Oscillatoria limnetica
Oscillatoria limosa
Oscillatoria princeps
Oscillatoria rubescens
Oscillatoria sancta
Oscillatoria subbrevis
Oscillatoria tenuis
Phormidium
Phormidium aerugineo-caeruleum
Planktolyngbya
Planktolyngbya brevicellularis
Planktolyngbya circumcreta
Planktolyngbya contorta
Planktolyngbya limnetica
Spirogyra
Vaucheria

TESTING INDIVIDUAL TAXON RESPONSE TO DISTURBANCE

Methods used to identify sensitive or tolerant periphyton taxa for Florida followed the methods used to select sensitive and tolerant macroinvertebrate taxa for the stream condition index (SCI; Fore *et al.*, 2007a). The number of occurrences for each taxon was compared for good and poor sites. Taxa more commonly found in good sites were identified as sensitive and taxa more commonly found in poor sites were identified as tolerant. Data from 124 sites (248 site-visits) were divided into two groups on the basis of their HDG values. 'Good' sites were defined as those with moderate levels of disturbance for no more than two components of the HDG or high disturbance for no more than one component. In other words, 'good' sites had HDG <= 2 (N = 144) and poor sites (N = 104) were those with HDG > 2. Thus, the two groups might be more accurately labeled 'better' and 'worse' rather than 'good' and 'poor.'

A 2 x 2 contingency table analysis was used to test whether the number of taxon occurrences in the good vs. poor sites represented a statistically significant difference (Chi-square test, Yates continuity correction, $p < 0.05$). For the SCI macroinvertebrate taxa, selection of taxa was based on criteria more strict than statistical significance of the Chi-square test. To be identified as sensitive, a taxon had to have >87% of their occurrences in good sites. For tolerant taxa, >50% of a taxon's occurrences had to be in poor sites. Very few diatom or soft algae taxa met these more strict criteria; instead, any taxon with a statistical difference was retained.

Consistent with the approach used in testing macroinvertebrate taxa, a minimum of 10 occurrences of an algal taxon was required for the initial round of statistical testing.

Many cells were identified no further than genus. In several cases, the number of occurrences for a genus differed significantly for good vs. poor sites. For each of these cases, the species within the genus were further evaluated. If most of the cells were identified to genus and the genus was statistically significant, then the species were rolled in as well. If the species below a genus that was identified as sensitive (or tolerant) were not also identified as sensitive (or tolerant) and the species represented a large proportion of the genus, that genus was not selected as sensitive (or tolerant).

In addition to the chi-square testing for individual taxa, every genus was also tested for correlation with HDG. Species were not tested in this way because not enough occurrences were available for testing. Percentages of cells in each sample belonging to a particular genus were tested for correlation with HDG. For this analysis, all lower taxa within a genus were "rolled up" and included. Only reasonably abundant genera (>25 occurrences) were tested.

TESTING PERIPHYTON METRICS

About half the diatom taxa names did not have autecological information (48%) because they were not listed in Van Dam *et al.* (1994). To fill in missing values for these species, autecological information was averaged for the entire genus from Van Dam *et al.*'s original master list and applied to species that were missing information (Lane, 2007; Lane and Brown, 2007). For some genera, no information was available.

Metrics were calculated as taxa richness (number of taxa in target group) or relative abundance (percentage of cells belonging to target group divided by the total number of cells in the sample that had autecological information) for each site-visit. For autecological metrics, the highest and lowest values for each attribute were summarized. For example, Van Dam *et al.* (1994) list five values for oxygen tolerance. A value of 1 indicates a requirement for high oxygen, a value of 2 indicates a preference for high oxygen, and so on. For metric calculation values of 1 and 2 were used to define high oxygen preference and were summarized as taxa counts and relative abundance. Similarly values of 4 and 5 were used to calculate a metric indicating tolerance for low oxygen. For pH sensitivity, values of 1 and 2 were used to define acidophilic taxa and values of 4 and 5 to define alkaliphilic taxa. For nitrogen heterotrophy, taxa with values of 3 or 4 were used. For saprobity, values of 1 and 2 were used to define oligosaprobic taxa and values of 4 and 5 to define polysaprobic taxa. Oligotrophic taxa were defined by values of 1 and 2 and eutrophic taxa by values of 5 and 6.

Taxa richness and relative abundance were calculated for sensitive, tolerant, and motile taxa. Genus metrics were only calculated as relative abundance, not taxa richness.

Correlation was used to test for association between candidate metrics and both the HDG and the water quality index (Spearman's *r*). Because the statistical significance of correlation coefficients increases as a function of sample size, very low r-values can be statistically significant. We defined significance *a priori* as a correlation coefficient, $r > 0.4$ (or < -0.4) and correlation in the predicted direction. Although smaller r-values may be significant, a graphical display of smaller r-values are typically not very convincing.

RESULTS

HUMAN DISTURBANCE

Of the 164 sites used for metric testing, about 90 had only minimal levels of known disturbance, that is, HDG = 0 (Figure 1). Other sites showed a good range of condition for metric testing. Sites were evenly spread throughout the three bioregions (Figure 2). HDG were somewhat higher (indicating greater disturbance) in the peninsula. HDG was highly correlated with all of its component measures (Table 6).

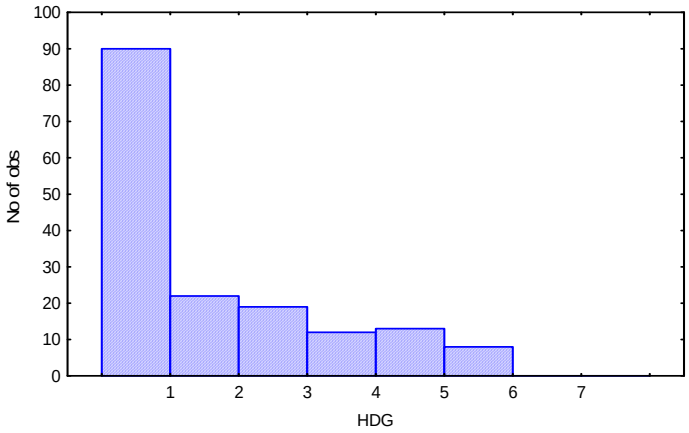


Figure 1. HDG ranged from 0 to 6 for sites used to test metrics.

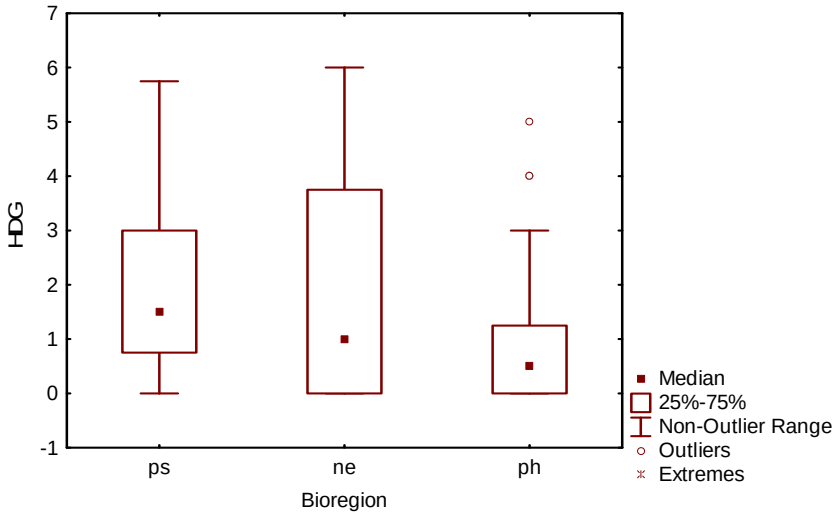


Figure 2. Sites in the peninsula (ps, n=152) tended to have higher HDG values (indicating greater disturbance) than stream sites in the panhandle (ph, n=75) or northeast (ne, n=47).

Table 6. Spearman's correlation values for measures of human disturbance.

	HDG	% Hab index	LDI	WQ index
HDG		-0.71	0.82	0.55
% Hab index	-0.71		-0.60	-0.28
LDI	0.82	-0.60		0.19
WQ index	0.55	-0.28	0.19	

TAXONOMIC ORDINATION

Results from the NMS analysis grouped stream sites from the northeastern section of the state most closely together (Figure 3). The first NMS axis was highly correlated with site values for total P, pH, and conductivity (Table 7). The total number of diatom taxa also tended to increase with the first NMS axis. PH was the most highly correlated with NMS axis 1 (Figure 4).

Individual diatom taxa were also closely associated with the first NMS axis. PH tolerance values from van Dam *et al.* (1994) increased along with the taxon's position along NMS axis 1 (Figure 5). Northeastern sites tend to be more influenced by tannic acid and diatom taxa that prefer more acidic conditions (*e.g.*, *Eunotia*) were associated with these sites. Nonetheless, correlation with pH was high for all sites.

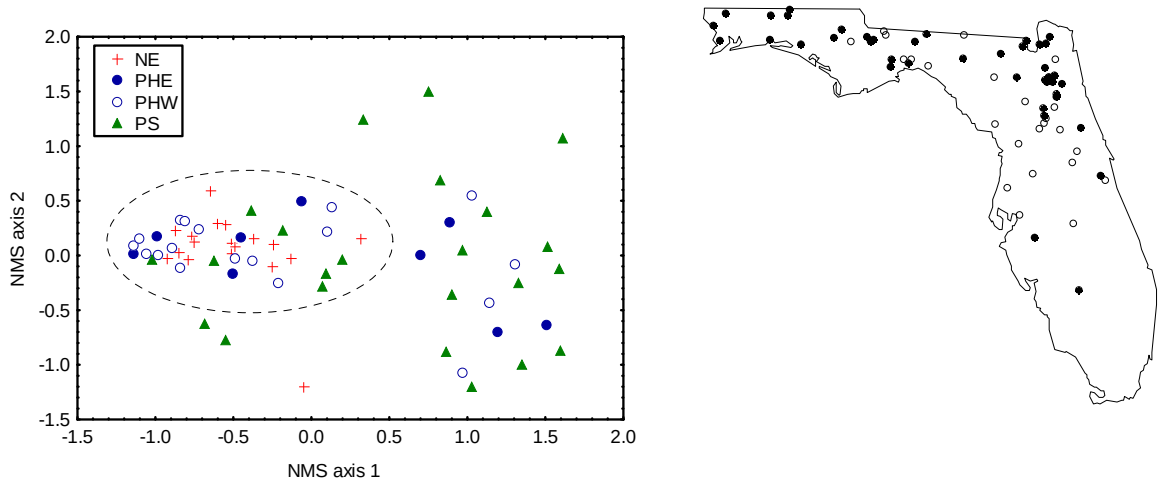


Figure 3. NMS plot: Ordination results show streams sites with LDI < 2 and their relationships based on diatom taxa and their abundances (N = 68 sites). Sites from the northeast (NE) grouped together along with several sites from other regions. Panhandle east (PHE), panhandle west (PHW), and peninsula (PS) sites failed to group together. MAP: Mapped sites show the location of the 68 stream sites and closed circles indicate sites that are included within dashed circle in the NMS plot.

Table 7. Correlation coefficients for measures of site location, site condition and total number of diatom taxa with the first two axes from the NMS analysis (N = 68). Spearman's r values > 0.2 were statistically significant (one-sided test, p < 0.05)

	NMS Axis 1	NMS Axis 2
Latitude	-0.37	0.14
Longitude	0.28	-0.04
Habitat index	0.02	-0.17
LDI	-0.05	0.10
NH4	0.13	0.03
NN	0.47	-0.30
TP	0.53	0.16
Conductivity	0.69	-0.38
PH	0.77	-0.35
Dissolved Oxygen	-0.28	0.08
Velocity	0.19	-0.11
Total number of diatom taxa	0.48	-0.22

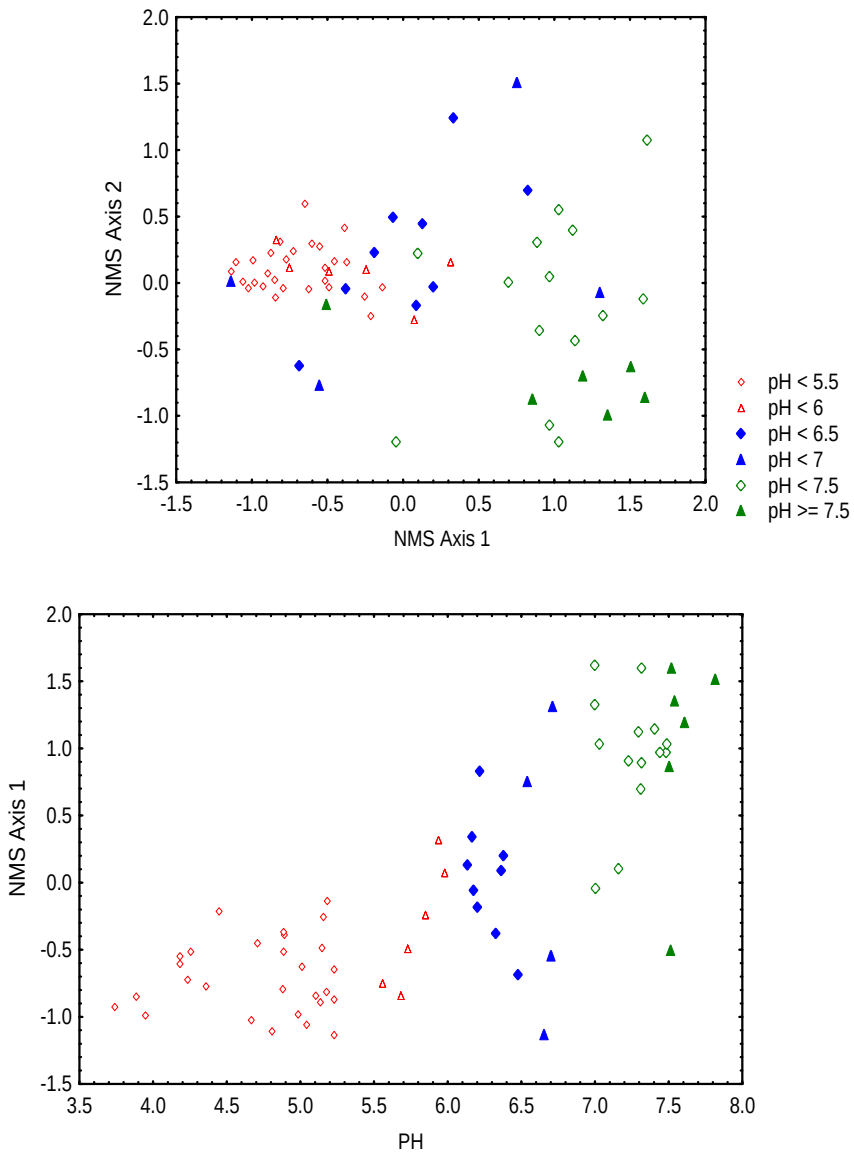


Figure 4. Stream sites (N = 68) plotted in NMS ordination space were associated with pH (upper panel). The strongest correlation was with Axis 1 (lower panel).

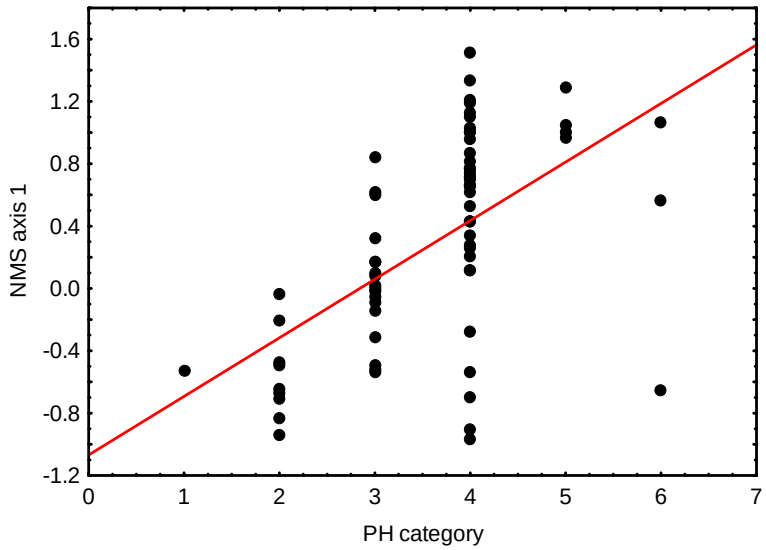


Figure 5. Plot shows the relationship between a diatom taxon's value on the NMS plot (axis 1) and the taxon's values for pH category from van Dam *et al.* (1994). Low values for pH category indicate preference for acidic conditions; high values indicate a preference for alkaline conditions. Note: 71 out of 122 taxa had information on pH tolerance and are shown.

RESPONSE OF INDIVIDUAL PERIPHYTON TAXA TO DISTURBANCE

A total of 563 diatom and soft algae taxa were identified from 248 visits to 124 stream sites (one visit each during Q2 and Q4 sampling periods). Taxa names included cells identified to both genus and species level. All reasonably abundant taxa (> 10 occurrences) were tested for their preference for good or poor sites.

Most of the sensitive and tolerant taxa were identified from the diatoms (Table 8). No non-diatom (soft) algae were identified as sensitive, that is, preferring good sites; six taxa were identified as tolerant. Lack of statistical significance for non-diatom taxa may have been a result of the small numbers of non-diatom algae counted. Most of the taxonomic effort was applied to diatoms which comprised 72% (on average) of the taxonomic units identified from the periphyton samples. Overall, less than 10% of the diatom taxa were identified as sensitive and tolerant. Several diatom genus names showed a statistically significant preference for good (or poor) sites. If the species within the genus followed a similar pattern they were all included as sensitive (or tolerant). If the species within a genus did not show a similar pattern, the genus was eliminated from the list of sensitive (or tolerant) taxa. These corrections meant that the original list of 21 sensitive taxa selected based on chi-square testing increased to 53 taxa when species within the genus were included (See Appendix 1 for a complete list).

Table 8. Results of taxa sensitivity and tolerance testing based on 144 good sites and 104 bad sites.

	# of Taxa names	# of Sensitive taxa (stat. sign.)	# of Tolerant taxa (stat. sign.)	# of Sensitive (taxonomic corrections)	# of Tolerant (taxonomic corrections)
Diatom	307	21	26	53	27
Soft algae	256	0	6	0	7

METRIC CORRELATION WITH HDG

Relatively more metrics were available for testing for diatoms than for soft algae. A total of 14 diatom metrics were consistently correlated with HDG for both data sets while none of the non-diatom (soft) algae metrics were correlated with the HDG (Table 9). Sensitive and tolerant diatoms summarized as either number of taxa or percentage of individuals were significantly correlated with HDG. Metrics derived from van Dam *et al.*'s (1994) attributes for preference of acidic conditions, nitrogen heterotrophy, requirement of high oxygen, preference for oligotrophic condition, preference for eutrophic condition, and trophic state were all significantly correlated with HDG.

Only four algae genera were correlated with HDG for both the Q2 and Q4 data sets (results not shown). Two genera, *Frustulia* and *Eunotia*, both diatoms, were already selected as sensitive taxa and included in the sensitive taxa richness and relative abundance metrics. Two other genera, *Pediastrum* and *Ulothrix*, both soft algae, had too few occurrences to be included on the list of tolerant taxa.

Table 9. Candidate metrics for diatoms and soft algae and their correlation (Spearman's *r*) with HDG during two sampling periods (Q2 and Q4). Only |r|-values > 0.4 are shown ($p < 0.01$). Metrics that satisfied the criterion |r| > 0.4 for both Q2 and Q4 are also indicated.

Metric description	Both?	HDG (Q2)	HDG (Q4)
Diatom metric			
Community structure			
Total number of taxa			
% Dominance (1 taxon)			
Tolerance & sensitivity			
No. of sensitive taxa (Bahls)			
% Sensitive cells (Bahls)			
No. of tolerant taxa (Bahls)			0.44
% Tolerant cells (Bahls)			
No. of sensitive taxa (FL)	yes	-0.46	-0.48
No. of tolerant taxa (FL)	yes	0.53	0.49
% Sensitive cells (FL)	yes	-0.55	-0.50
% Tolerant cells (FL)	yes	0.60	0.53
Autecology			

Metric description	Both?	HDG (Q2)	HDG (Q4)
No. of acidophilic taxa	yes	-0.49	-0.50
% Acidophilic cells	yes	-0.56	-0.50
No. of alkaliphilic taxa			
% Alkaliphilic cells		0.50	
No. of N heterotroph taxa	yes	0.43	0.45
% N heterotroph cells	yes	0.53	0.43
No. of taxa that require high oxygen			
% of cells that require high oxygen	yes	-0.51	-0.48
No. of taxa that tolerate low oxygen			
% of cells that tolerate low oxygen			
No. of oligosaprobic taxa			
% oligosaprobic cells	yes	-0.53	-0.47
No. of polysaprobic taxa			
% polysaprobic cells			
No. of oligotrophic taxa	yes	-0.43	-0.49
% oligotrophic cells	yes	-0.55	-0.52
No. of eutrophic taxa			
% eutrophic cells	yes	0.43	0.43
No. of taxa that require moisture			
% of cells that require moisture			
No. of taxa that tolerate dry conditions			
% of cells that tolerate dry conditions			
Trophic state index (Van Dam)	yes	0.52	0.52
Morphology			
% of motile cells			
Soft algae metric			
Community structure			
Total number of taxa			
% Diatom units			
% Chlorophyta units			
% Cryptophyta units			
% Cyanobacteria units			
% Dominance (1 taxon)			
% Euglenoid units			
% Pyrrhophyta units			
Diatom / (Diatom + Chlorophyta) units			
Nuisance (from FDEP)			
No. of nuisance taxa			
% Nuisance units			
Tolerance			
No. of tolerant taxa (FL)			0.50
% Tolerant units (FL)			0.50

METRIC SELECTION

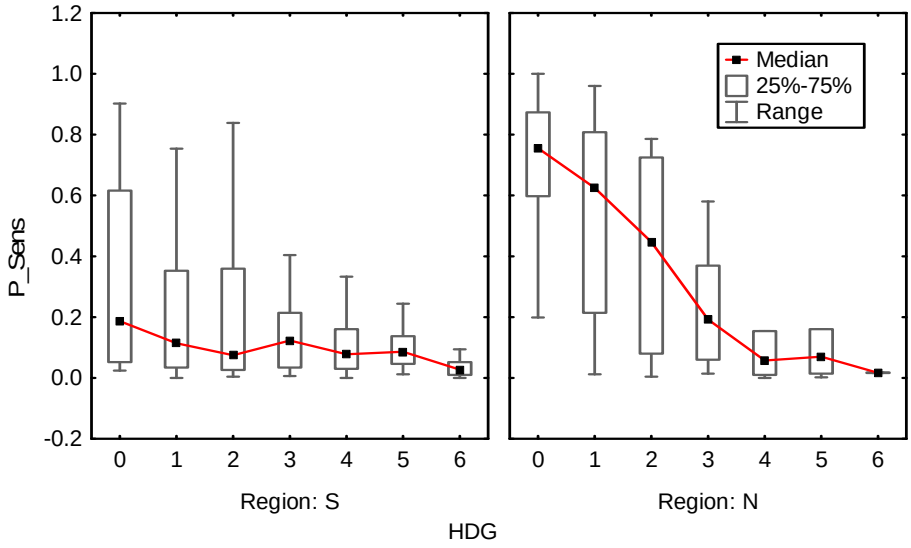
Of the 14 diatom metrics that were correlated with HDG for both data sets, several measured the same attribute of the assemblage and, thus, were conceptually redundant. For example, number of acidophilic taxa and percentage of acidophilic cells summarize the same aspect of the diatom assemblage. When both a taxa richness and a percent abundance version of a metric were significantly correlated with HDG, the percent abundance version was selected because percent abundance metrics tend to reflect a more robust expression of community structure for diatoms.

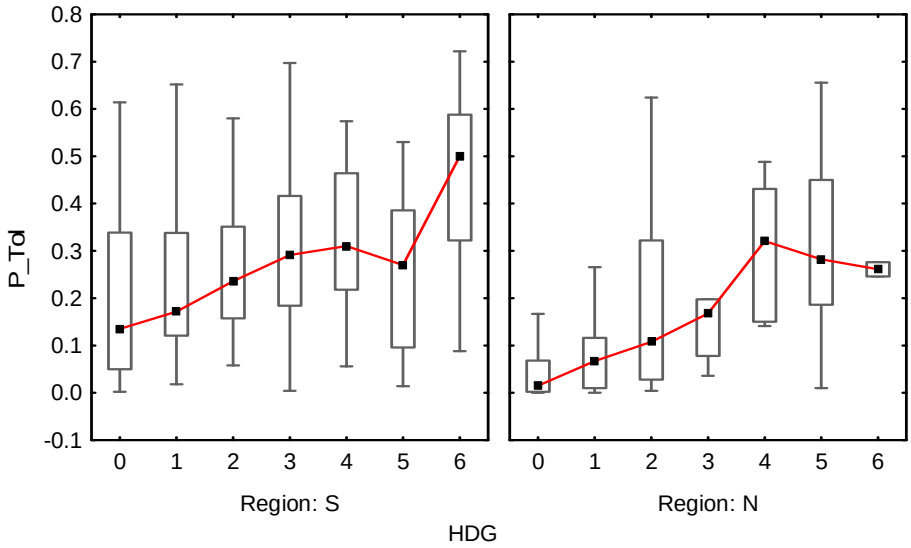
Of the remaining metrics, relative abundance (%) cells indicating acidic and alkaline conditions relate to different types of site condition. In contrast, % cells indicating high oxygen and % cells indicating low oxygen represent opposite ends of the same spectrum. From among the four metrics related to trophic condition, we selected the weighted index (TSI). High correlation of sensitive and tolerant metrics were not surprising because they were calculated for the same data sets used to derive them.

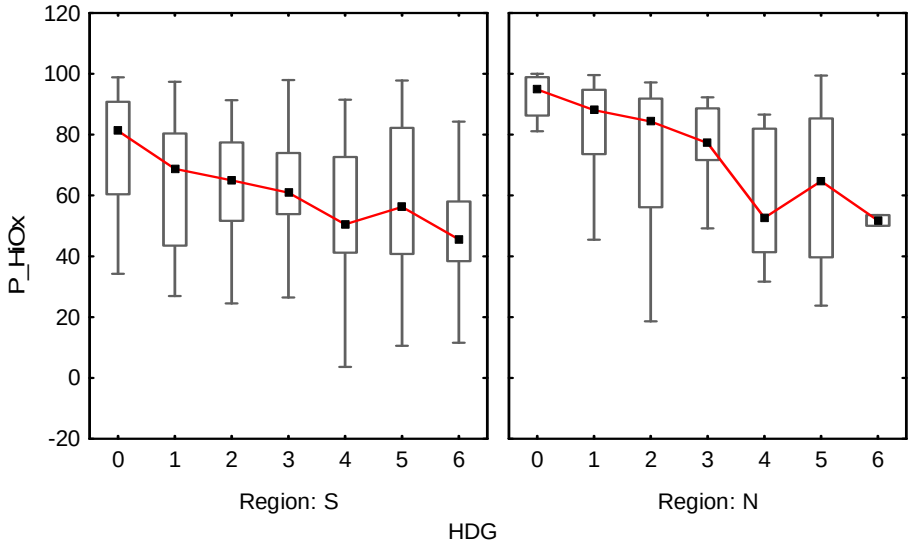
Some autecological attributes derived from van Dam *et al.* (1994) were highly correlated (Table 10) which resulted in several of the derived metrics being highly correlated as well (not shown). To limit redundancy among metrics, five diatom metrics were selected for inclusion in the stream diatom index (SDI): percent sensitive cells, percent tolerant cells, percent cells that prefer high oxygen, percent cells that prefer oligotrophic conditions, and van Dam's weighted index for trophic status (TSI). All five were significantly correlated with HDG in both the northern (northeast plus panhandle regions) and the southern (peninsula region) parts of the state (Figure 6). The NE and PH regions were combined into a single northern region to increase sample sizes and because they were geographically similar.

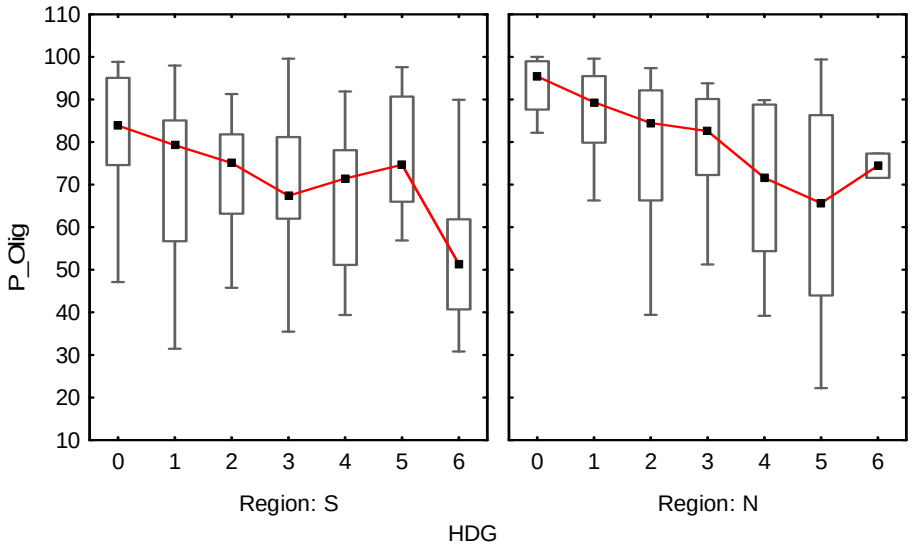
Table 10.Spearman's correlation coefficients for Van Dam *et al.*'s (1994) attribute values for Florida diatom taxa.

	pH	Organic N	Oxygen tolerant	Saprobity	Trophic indicator status
pH		0.29	0.39	0.35	0.62
Organic N	0.29		0.76	0.75	0.65
Oxygen tolerant	0.39	0.76		0.72	0.68
Saprobity	0.35	0.75	0.72		0.68
Trophic indicator status	0.62	0.65	0.68	0.68	


P_Sens
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Median
25%-75%
Range
0 1 2 3 4 5 6
Region: S
0 1 2 3 4 5 6
Region: N
HDG


P_Tol
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0 1 2 3 4 5 6
Region: S
0 1 2 3 4 5 6
Region: N
HDG


P_HOx
120
100
80
60
40
20
0
-20
0 1 2 3 4 5 6
Region: S
0 1 2 3 4 5 6
Region: N
HDG


P_Olig
110
100
90
80
70
60
50
40
30
20
10
0 1 2 3 4 5 6
Region: S
0 1 2 3 4 5 6
Region: N
HDG

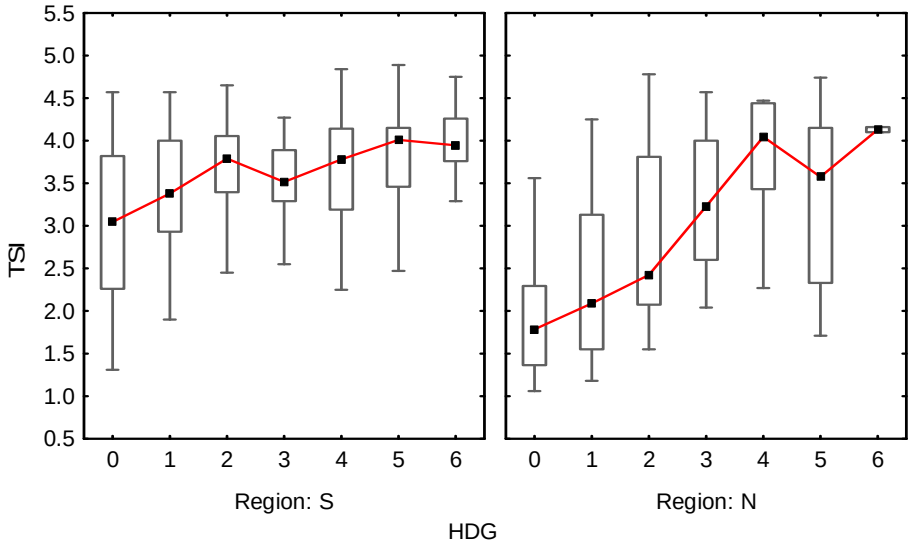


Figure 6. Diatom metrics were correlated with human disturbance similarly in both the northern (N = 122) and southern (N = 152) parts of the state.

DEVELOPMENT AND TESTING OF A STREAM DIATOM INDEX (SDI)

The stream diatom index (SDI) was created by converting the five diatom metrics into unitless scores from 0-10. Percentiles of metric values were used to convert metrics to scores. Summing metric scores provided an SDI ranging from 0-50. Multiplication by 2 converted the SDI into values ranging from 0-100.

Like its component metrics, SDI was highly correlated with HDG and declined as human disturbance increased for both the southern and northern parts of the state (Figure 7; Table 11). Unfortunately, SDI was even more highly correlated with pH and conductivity, which are both influenced by natural gradients as well as human disturbance.

To test whether human disturbance or natural pH gradients were more important predictors of SDI, SDI was regressed against pH, LDI, and the WQ index. When SDI was plotted against pH, the data were categorized according to LDI values and region (northern or southern part of the state). For the resulting eight plots, SDI was strongly associated with pH for all values of LDI and both regions of the state (Figure 8). In sharp contrast, when the data were categorized according to pH and region, the resulting plots of SDI vs. LDI showed no association. Similarly, when data were categorized again for pH and region, plots of SDI vs. WQ index also showed no association. Results suggest that pH is the most important driver of SDI values rather than LDI or the WQ index.

For the periphyton data set, LDI was somewhat correlated with pH, suggesting that human disturbance and pH could be confounded and diatoms may be responding to an underlying aspect of human disturbance that also affects pH. A much larger data set of approximately 49,000 stream samples was used to evaluate the relationship between LDI and pH (Figure 9). For low values of LDI (indicating minimal human disturbance), pH can take on a

broad range of values, but for moderate levels of human disturbance (LDI > 3), pH rarely is observed below 6.0. Furthermore, LDI and pH were not correlated for moderate to high levels of human disturbance.

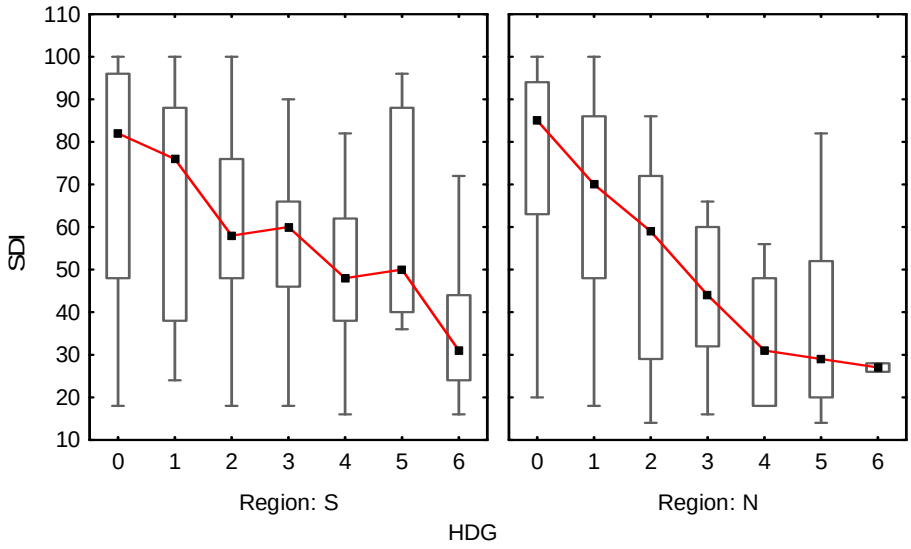


Figure 7. SDI declined with human disturbance in both the northern (N = 122) and southern (N = 152) parts of the state.

Table 11. Correlation of site measures with SDI (Spearman's *r*, ** p < 0.01, N = 274, Q2 and Q4 data sets).

Measure	Correlation with SDI
Latitude	0.17 **
Longitude	0.00
HDG	-0.48 **
WQ index	-0.46 **
Habitat index	0.37 **
LDI	-0.32 **
NH4	-0.06
NN	-0.33 **
TP	-0.38 **
Conductivity	-0.57 **
PH	-0.68 **

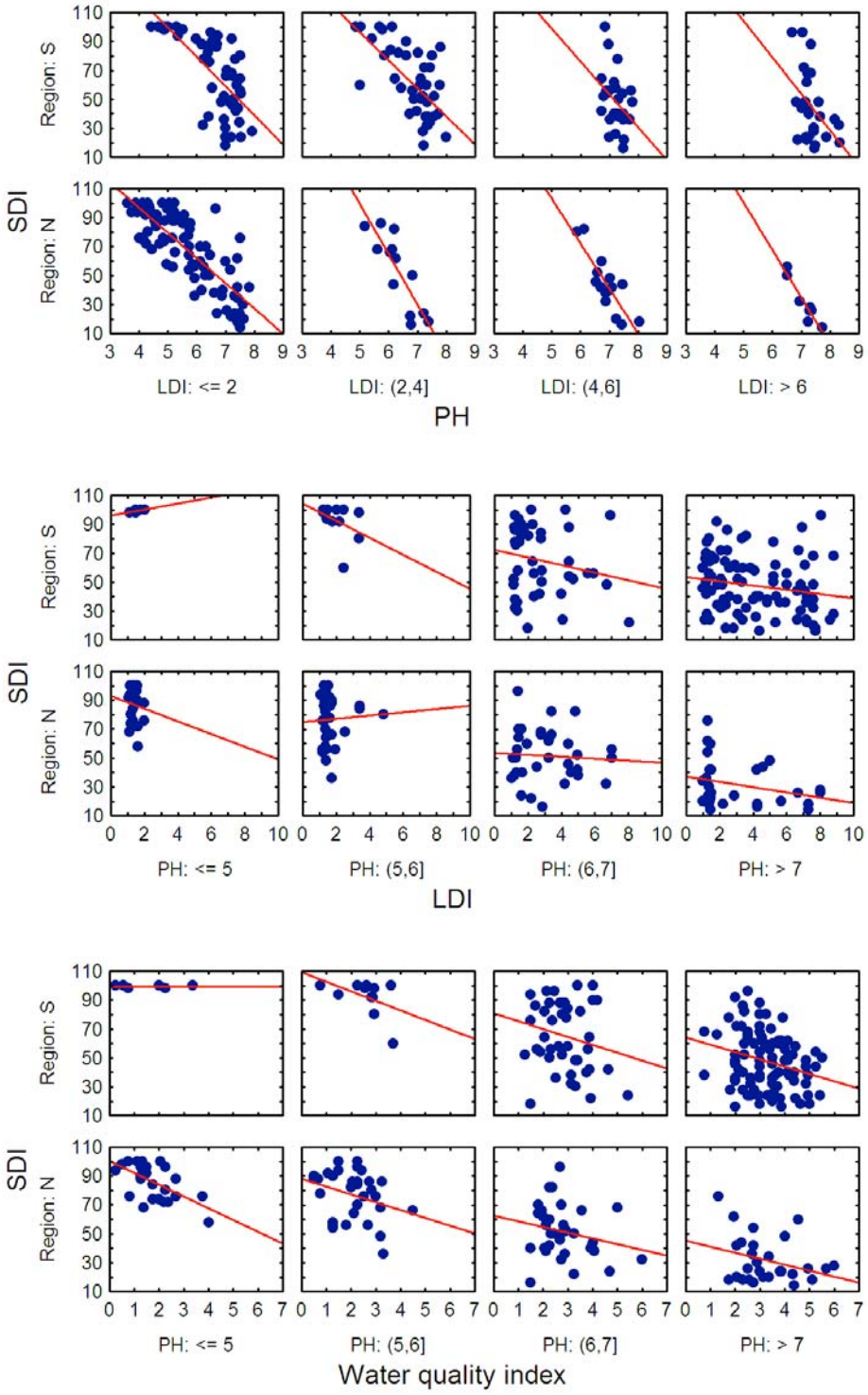


Figure 8. The stream diatom index (SDI) was most highly correlated with pH after isolating for the effects of LDI (top panel). SDI was not strongly associated with either the LDI (middle panel) or the water quality index (bottom panel) even after controlling for pH.

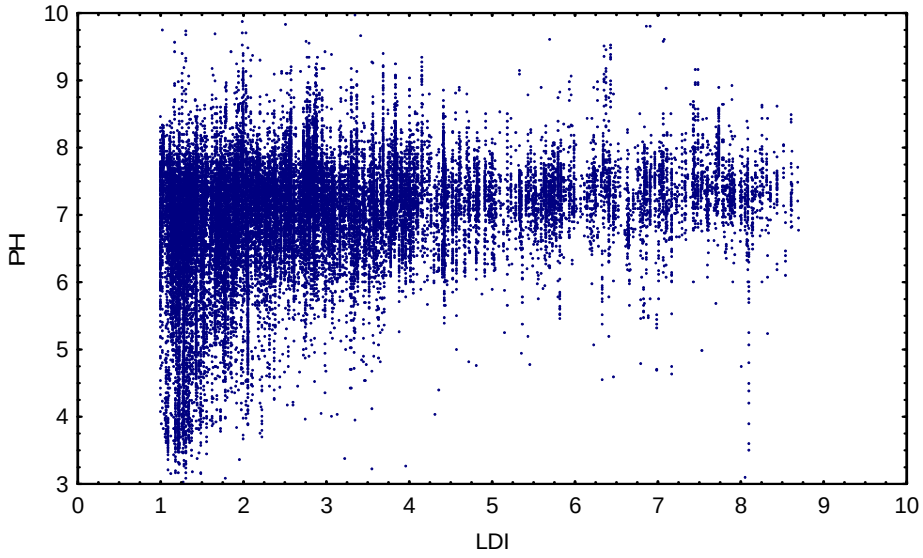


Figure 9. For ~49,000 stream sites (data from FDEP Status and Trends monitoring program), pH has a broad range of values for minimal human influence (LDI < 2). For higher values of human disturbance, pH rarely drops below ~6.

DISCUSSION

Periphyton samples were collected from streams throughout Florida. Stream locations were selected in order to provide a broad range of site conditions that would support development of a human disturbance gradient for metric testing. Metrics for diatoms and non-diatom algae were derived from published lists of diatom attributes (Bahls, 1993; van Dam *et al.*, 1994; Porter *et al.*, 2008). Many of the species found in Florida were not listed on these master taxa lists, even after correcting for taxonomic synonymy.

Other authors addressed this problem for Florida wetlands by using genus-level averages to fill in for species that have no information available for their natural preferences and tolerances (Lane, 2007; Lane and Brown, 2007). When this approach was applied to diatom data, several metrics were highly correlated with human disturbance. To ensure that the results of metric testing were consistent and repeatable, metrics were tested for correlation with HDG using data from two separate sampling occasions. Typically, very few metrics were significantly correlated during one sampling period and not the other; metric response tended to be consistent through time.

To create a summary multimetric index, the stream diatom index (SDI), metric values were converted to unit-less scores and summed. The SDI was highly correlated with HDG and its component measures of human disturbance. Unfortunately, the SDI was even more highly correlated with pH. When the relationship between SDI and the landscape development intensity index (LDI) was tested within discrete categories of pH, there was no consistent or reliable relationship between SDI and human disturbance. Similarly, SDI was not associated with the WQ index when the relationship was tested within categories of pH.

In contrast, when the relationship between SDI and pH was tested within discrete categories of LDI, the association between SDI and pH was even more clear and strong. Other authors have found similar high correlations between diatom metrics and pH, but have either used classification to eliminate this covariate or found the relationship with human disturbance or nutrients to be stronger (Fore, 2002; Lavoie *et al.*, 2006l Stevenson *et al.*, 2008a). Results for Florida streams suggest that pH may be a more important predictor of SDI than measures of human disturbance or water quality.

Four lines of evidence support the idea that pH is an important driver of periphyton response. First, the ordination of diatom taxa for stream sites with minimal human disturbance grouped sites with low pH values. Second, the pH tolerance values for individual taxa were also highly correlated with the first axis of the ordination. Third, the SDI was more highly correlated with pH than with water quality or the LDI, even though metrics were originally selected for their correlation with disturbance. Two of the SDI metrics, percent sensitive and tolerant cells, were specifically defined using measures of human disturbance. This type of correlation with natural gradients as well as human disturbance is common for diatoms metrics (Stevenson *et al.*, 2008b). Fourth, even when data were categorized to control for different levels of pH, SDI still failed to correlate with LDI and the water quality index.

Two conclusions could be derived from this analysis. The first explanation is that diatom assemblages are defined by natural differences in pH for Florida streams. If ambient pH affects Florida diatom assemblages more so than does human disturbance, a diatom index would have limited value as an assessment tool (pH readings would be more practical). A second explanation

would be that human disturbance increases pH (via nutrient and stormwater inputs), and in this case the primary factor influencing the SDI score would be human disturbance, although indirectly through the pH change. Disturbance affects streams in a variety of ways, depending on natural stream features. For example, some streams may be more resistant to an increase in pH. In this case, the magnitude of response in pH may also influence the response of the diatom assemblage.

To test the relationship between pH and human disturbance, data from over 49,000 site visits were used. For sites with minimal disturbance (LDI < 2), sites had a very large range of pH values, that is, little correlation between disturbance and pH. Nonetheless, for sites with greater disturbance, pH values were typically greater than 6.

The relationships between pH, human disturbance and nutrients are complex. The diatom response to this mix of influences can vary as well (Potapova and Charles, 2003). Depending on the natural geologic substrate, type of soil, and presence of spring water, pH conditions can vary dramatically across small distances and between adjacent streams (R. Frydenborg, *personal communication*). Human disturbance, in the absence of adequate Best Management Practices, releases soil and nutrients into streams. Depending on many factors (water transparency, shading, grazers, etc.), these nutrients may increase algal productivity, which in turn (via carbon fixation) increases pH. Another natural source of higher pH is spring water passing through limestone (Stevenson *et al.*, 2004). Higher pH values resulting from human sources include inputs of salt-based fertilizers and cations (calcium, magnesium) washed into streams from human concrete structures. Thus, both natural sources and human actions can cause pH to increase.

During the BCG workshop (see Appendix 2), experts were asked whether periphyton should be used to define biological criteria even though the diatom index was so highly correlated with pH. Most of the algal ecologists believed that periphyton could be used for Florida streams, and only a few had reservations about ability of diatoms to distinguish between the natural variability of nutrients and pH and human-caused changes.

The experts who recommended diatoms be used to develop biocriteria made the following recommendations for improving the stream diatom index (SDI).

- Improve the classification of reference sites for natural gradients, *e.g.*, use geologic, surficial, or soil features to derive natural, regional expectations for pH.
- Evaluate the historic influence of human settlement, *e.g.*, did people settle away from acidic (swamp) sites, to determine if pH changed historically.
- Test additional metrics.
- Improve the level of taxonomy (*i.e.*, more species and variety identifications).
- Collect more data over a longer time period to evaluate diatom variability.

The most promising approach for improving the SDI would be a finer classification of sites for natural features that determine background levels of pH and nutrients. Although the SDI was tested for correlation with human disturbance within pH categories, the pH categories were only derived from field data, they were not based on an underlying examination of geologic features that determine buffering capacity or expectations for pH in the absence of human influence. For this study, additional information about natural expectations for pH were unavailable.

Other recommendations seem less promising. Even if a change in pH could be documented due to human settlement, that would not solve the problem of a lack of correlation with human disturbance when the influence of pH was removed. Additional metrics could be considered; however, many metrics were tested as part of this analysis. Level of taxonomy could potentially be improved, but given the high number of Florida taxa not included on published taxa lists, any improvement would be small. Lack of significant results for the non-diatom taxa could be due to the smaller number of units identified in the periphyton samples compared to diatoms; however, non-diatom taxa are difficult to identify beyond genus and less is known about the natural history of these taxa. Finally, more data could be collected over time, but the problem is not in diatom metric variability, but in the underlying correlation with pH rather than human disturbance.

Whether the current SDI is modified or new indicators are developed for periphyton, they should be carefully evaluated for potential relationships with other natural gradients to ensure that periphyton indicators can reliably detect changes associated with human influence. At this time, FDEP does not consider this version of the SDI to be applicable for use as a bioassessment tool.

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APPENDIX 1. SENSITIVE AND TOLERANT TAXA.

Table A1-1. Taxon name, whether it was selected as sensitive or tolerant, whether it occurred in more than 10 site-visits, preference for good or poor sites (blank cells indicate the taxon did not show a statistically significant preference), total number of occurrences, number of occurrences in good and poor sites, percent of occurrences in good and poor sites, and Chi-square statistic (Yates correction) for comparison of number of occurrences in good vs. poor sites (chi-square stat = 3.841 significant at $p < 0.05$). A red S or T indicates that the species below the genus was selected based on the performance of the genus as a whole.

Cell type	Taxon	Sens Tol	GT 10	Pref	Num occur	Good N=144	Poor N=104	P occur good	P occur bad	Chi sq Yates
diatom	*Aulacoseira*	S	yes	G	115	76	39	0.66	0.34	5.070
diatom	*Aulacoseira distans*	S	no		1	0	1	0.00	1.00	0.027
diatom	*Aulacoseira granulata*	S	no		9	5	4	0.56	0.44	0.036
diatom	*Aulacoseira herzogii*	S	no		8	6	2	0.75	0.25	0.388
diatom	*Aulacoseira nygaardii*	S	no		1	0	1	0.00	1.00	0.027
diatom	*Aulacoseira tenella*	S	no		1	0	1	0.00	1.00	0.027
diatom	*Craticula*	S	yes	G	50	36	14	0.72	0.28	4.304
diatom	*Craticula cuspidata*	S	no		2	0	2	0.00	1.00	0.905
diatom	*Craticula halophila*	S	no		1	1	0	1.00	0.00	0.027
diatom	*Eunotia*	S	yes	G	220	136	84	0.62	0.38	9.951
diatom	*Eunotia bidentula*	S	no		2	2	0	1.00	0.00	0.237
diatom	*Eunotia bilunaris*	S	yes	G	108	78	30	0.72	0.28	14.735
diatom	*Eunotia camelus*	S	no		1	0	1	0.00	1.00	0.027
diatom	*Eunotia carolina*	S	no		3	3	0	1.00	0.00	0.796
diatom	*Eunotia circumborealis*	S	no		1	1	0	1.00	0.00	0.027
diatom	*Eunotia diodon*	S	no		1	1	0	1.00	0.00	0.027
diatom	*Eunotia exigua*	S	no		1	1	0	1.00	0.00	0.027
diatom	*Eunotia femoriformis*	S	no		1	1	0	1.00	0.00	0.027
diatom	*Eunotia flexuosa*	S	yes	G	18	17	1	0.94	0.06	9.000
diatom	*Eunotia formica*	S	yes	G	42	25	17	0.60	0.40	0.002
diatom	*Eunotia implicata*	S	no		3	3	0	1.00	0.00	0.796
diatom	*Eunotia incisa*	S	yes	G	71	54	17	0.76	0.24	12.210
diatom	*Eunotia microcephala*	S	no		1	1	0	1.00	0.00	0.027
diatom	*Eunotia minor*	S	no		8	8	0	1.00	0.00	4.323

Cell type	Taxon	Sens Tol	GT 10	Pref	Num occur	Good N=144	Poor N=104	P occur good	P occur bad	Chi sq Yates
diatom	*Eunotia monodon*	S	yes	G	47	37	10	0.79	0.21	9.144
diatom	*Eunotia naegelii*	S	yes	G	16	12	4	0.75	0.25	1.340
diatom	*Eunotia parallela*	S	no		2	2	0	1.00	0.00	0.237
diatom	*Eunotia pectinalis*	S	yes	G	73	59	14	0.81	0.19	20.699
diatom	*Eunotia pectinalis undulata*	S	no		2	2	0	1.00	0.00	0.237
diatom	*Eunotia pectinalis ventralis*	S	no		2	2	0	1.00	0.00	0.237
diatom	*Eunotia praerupta*	S	no		3	2	1	0.67	0.33	0.081
diatom	*Eunotia praerupta bidens*	S	no		5	4	1	0.80	0.20	0.299
diatom	*Eunotia rhomboidea*	S	yes	G	13	13	0	1.00	0.00	8.174
diatom	*Eunotia serra*	S	no		3	3	0	1.00	0.00	0.796
diatom	*Eunotia transfuga*	S	no		6	5	1	0.83	0.17	0.724
diatom	*Eunotia veneris*	S	no		1	0	1	0.00	1.00	0.027
diatom	*Eunotia zygodon*	S	yes	G	24	23	1	0.96	0.04	13.897
diatom	*Fragilaria javanica*	S	yes	G	15	14	1	0.93	0.07	6.687
diatom	*Frustulia*	S	yes	G	95	69	26	0.73	0.27	12.467
diatom	*Frustulia rhomboides*	S	yes	G	47	39	8	0.83	0.17	13.547
diatom	*Frustulia saxonica*	S	yes	G	103	85	18	0.83	0.17	41.583
diatom	*Frustulia vulgaris*	S	no		2	1	1	0.50	0.50	0.237
diatom	*Luticola*	S	yes	G	37	28	9	0.76	0.24	4.722
diatom	*Luticola mutica*	S	yes	G	52	32	20	0.62	0.38	0.171
diatom	*Navicula cocconeiformis*	S	yes	G	25	20	5	0.80	0.20	4.538
diatom	*Neidium*	S	yes	G	76	52	24	0.68	0.32	4.233
diatom	*Neidium ampliatum*	S	no		2	1	1	0.50	0.50	0.237
diatom	*Neidium densestriatum*	S	yes	G	15	13	2	0.87	0.13	4.187
diatom	*Neidium dubium*	S	no		1	1	0	1.00	0.00	0.027
diatom	*Neidium ladogensis densestriatum*	S	no		8	7	1	0.88	0.13	1.825
diatom	*Pinnularia*	S	yes	G	189	120	69	0.63	0.37	8.697
diatom	*Pinnularia acrosphaeria*	S	no		1	0	1	0.00	1.00	0.027
diatom	*Pinnularia braunii*	S	no		1	0	1	0.00	1.00	0.027
diatom	*Achnanthes exigua*	T	yes	P	156	75	81	0.48	0.52	16.139
diatom	*Achnanthes hungarica*	T	yes	P	70	33	37	0.47	0.53	4.173
diatom	*Achnanthes lanceolata*	T	yes	P	140	70	70	0.50	0.50	7.843
diatom	*Achnanthes lanceolata rostrata*	T	yes	P	91	42	49	0.46	0.54	7.620

Cell type	Taxon	Sens Tol	GT 10	Pref	Num occur	Good N=144	Poor N=104	P occur good	P occur bad	Chi sq Yates
diatom	*Bacillaria paxillifer*	T	yes	P	62	23	39	0.37	0.63	13.800
diatom	*Capartogramma crucicula*	T	yes	P	65	25	40	0.38	0.62	12.832
diatom	*Cocconeis fluviatilis*	T	yes	P	96	44	52	0.46	0.54	8.821
diatom	*Cyclotella*	T	yes	P	59	18	41	0.31	0.69	22.680
diatom	*Cyclotella meneghiniana*	T	yes	P	126	52	74	0.41	0.59	28.284
diatom	*Cyclotella ocellata*	T	no		3	0	3	0.00	1.00	2.137
diatom	*Cyclotella pseudostelligera*	T	yes	P	30	12	18	0.40	0.60	3.769
diatom	*Cyclotella stelligera*	T	no		10	4	6	0.40	0.60	0.730
diatom	*Cyclotella striata*	T	no		6	3	3	0.50	0.50	0.000
diatom	*Diadesmis confervacea*	T	yes	P	168	79	89	0.47	0.53	24.685
diatom	*Gomphonema parvulum*	T	yes	P	201	105	96	0.52	0.48	13.547
diatom	*Hippodonta*	T	yes	P	60	21	39	0.35	0.65	16.065
diatom	*Hippodonta capitata*	T	yes	P	86	35	51	0.41	0.59	15.234
diatom	*Hippodonta hungarica*	T	yes	P	73	31	42	0.42	0.58	9.450
diatom	*Melosira*	T	yes	P	84	41	43	0.49	0.51	3.912
diatom	*Navicula constans*	T	yes	P	23	8	15	0.35	0.65	4.639
diatom	*Navicula cryptocephala*	T	yes	P	149	72	77	0.48	0.52	13.564
diatom	*Navicula gastrum*	T	yes	P	16	3	13	0.19	0.81	9.199
diatom	*Navicula minima*	T	yes	P	170	91	79	0.54	0.46	3.993
diatom	*Nitzschia amphibia*	T	yes	P	114	50	64	0.44	0.56	16.421
diatom	*Sellaphora pupula*	T	yes	P	128	65	63	0.51	0.49	5.161
diatom	*Synedra ulna*	T	yes	P	116	50	66	0.43	0.57	18.896
diatom	*Tryblionella levidensis*	T	yes	P	24	8	16	0.33	0.67	5.597
wet	*Chlamydomonas*	T	yes	P	58	25	33	0.43	0.57	6.180
wet	*Planktolyngbya contorta*	T	yes	P	20	6	14	0.30	0.70	5.839
wet	*Rhabdogloea*	T	yes	P	12	3	9	0.25	0.75	4.325
wet	*Rhabdogloea smithii*	T	no		1	1	0	1.00	0.00	0.027
wet	*Scenedesmus abundans*	T	yes	P	16	4	12	0.25	0.75	6.296
wet	*Scenedesmus bijuga*	T	yes	P	70	26	44	0.37	0.63	16.355
wet	*Scenedesmus quadricauda*	T	yes	P	87	31	56	0.36	0.64	26.294

APPENDIX 2. BIOLOGICAL CONDITION GRADIENT: EXPERTS WORKSHOP

Florida DEP invited 15 experts to a workshop to 1) develop a biological condition gradient (BCG) for stream periphyton data and 2) describe the biological condition associated with "healthy, well-balanced communities of aquatic flora and fauna." The experts used taxonomic data and a small set of calculated metrics to sort data from stream sites along a gradient with six categories. The BCG was highly correlated with the stream diatom index (SDI) but not with an independent measure of human disturbance. In spite of high correlations between the BCG and pH, experts agreed that periphyton should be used for biocriteria. Experts recommended additional statistical analyses, stream classification based on expected pH, and additional ecological studies.

BACKGROUND

In 2001, the National Research Council recommended that states should develop more specific designated use classifications for waterbodies (NRC, 2001). The NRC found the CWA's goals to be too broad to provide the operational definition of designated uses needed to support aquatic life, and recommended greater specificity in defining aquatic life uses. For example, rather than stating that a water body needs to be "fishable," the designated use should specifically describe the expected fish assemblage (***e.g.***, cold water fishery, warm water fishery, or salmon, trout, bass, etc.). *Tiered aquatic life uses* (TALUs) are bioassessment-based statements of expected biological condition in specific water bodies that allow more precise and measurable definitions of *designated aquatic life uses* (EPA, 2005; Davies and Jackson, 2006).

Designated aquatic life uses represent a state's biological goals for their water bodies. Tiered aquatic life uses rely on biological information to more precisely define these goals relative to natural conditions. Bioassessments can then be used to measure attainment of the goals (EPA, 2005). Thus, the TALU approach is designed to support the implementation of biocriteria, and the scientific knowledge of aquatic ecology that they represent, into state and tribal water quality standards. The driving motivation here is to ensure that listings of impaired waters are scientifically defensible and to identify and protect high quality water resources.

The Biological Condition Gradient

The U.S. EPA has outlined a tiered system of aquatic life use designation, along a Biological Condition Gradient (BCG), that illustrates how ecological attributes change in response to increasing levels of human disturbance. The BCG is a conceptual model that assigns the relative health of aquatic communities into one of six categories. The BCG is based on fundamental ecological principles and has been extensively tested and verified by aquatic biologists throughout the U.S. (Davies and Jackson, 2006).

The BCG utilizes ten biological attributes of aquatic systems which respond predictably to increasing pollution and human disturbance. While these ten attributes are measurable, some are not routinely quantified in monitoring programs (***e.g.***, rate measurements such as productivity), but may be inferred via the community composition data (***e.g.***, abundance of taxa indicative of organic enrichment). The attributes are:

I. Historically documented, sensitive, long-lived or regionally endemic taxa
II. Sensitive and rare taxa
III. Sensitive but ubiquitous taxa
IV. Taxa of intermediate tolerance
V. Tolerant taxa
VI. Non-native taxa
VII. Organism condition
VIII. Ecosystem functions
IX. Spatial and temporal extent of detrimental effects
X. Ecosystem connectance.

The gradient represented by the BCG is divided into six levels (tiers) of condition that were defined during a series of national workshops with experienced aquatic biologists from across the U.S. (Davies and Jackson, 2006). The six tiers are described as:

1. Natural or native condition
2. Minimal changes in structure of the biotic community and minimal changes in ecosystem function
3. Evident changes in structure of the biotic community and minimal changes in ecosystem function
4. Moderate changes in structure of the biotic community with minimal changes in ecosystem function
5. Major changes in structure of the biotic community and moderate changes in ecosystem function
6. Severe changes in structure of the biotic community and major loss of ecosystem function.

Davies and Jackson (2006) recommend that regional biological experts adapt the conceptual tiers described by the BCG to local conditions using data from a regional monitoring program. The tiers can then be used in assessment of streams sites and to define thresholds for water quality standards (biocriteria).

FLORIDA WORKSHOP FOR PERIPHYTON – METHODS

To relate periphyton communities in Florida streams to the national BCG model, 15 nationally (and internationally) recognized periphyton ecologists were invited to a three-day workshop (February 27 – March 1, 2009; Table A2-1). Participants were asked to apply and calibrate the general BCG model to stream data collected from Florida streams.

Detailed descriptions of the BCG attributes and tiers were provided to the panel before and during the workshop. Experts discussed the specific attributes and rankings used to create the BCG before turning to the periphyton data to rank Florida stream sites. The panel of experts was provided with taxonomic lists and biological metrics of both diatom and non-diatom taxa for 3 reference stream sites and 50 test samples. The reference streams had minimal human influence and were identified to the panel as reference sites. References sites had LDI values < 2, healthy invertebrate communities, no algal growth that interferes with habitat suitability and were also verified using best professional judgment and field visits.

The panel was NOT given the stream diatom index (SDI) values for any of the sites nor were they given any information about site condition (*e.g.*, LDI, water quality index, or habitat index). The high correlation between diatom metrics and pH was discovered before the BCG workshop and the panel was asked to assign BCG rankings for two sets of data: 25 site-visits with pH < 6.5 and 25 site-visits with pH ≥ 6.5. The experts were asked to adhere closely to the definition for each BCG rank.

A Delphi method was used in which experts rated sites independently, then had a chance to discuss their ratings and make changes based on their colleagues' comments. The experts were also asked to define a threshold for "impaired" conditions based on periphyton data.

Table A2-1. Expert panel member and affiliation.

Expert Workshop Panel Member and Affiliation
Jan Stevenson – Michigan State University
Rex Lowe – Bowling Green State University
Kalina Manoylov – Georgia College and State University
Lithia Metzmier – Texas Commission on Environmental Quality (TCEQ)
Evelyn Gaiser - FL International University
Aga Pinowska – General Atomics
Yangdon Pan – Portland State University
Don Charles – Academy of Natural Sciences
Paul McCormick – South Florida Water Management District (SFWMD)
Michael Stewart – Troy University
Lei Zheng – Tetra Tech Inc.
Jennifer Greenwood – University of TN, Martin
Scott Rollins – Spokane Falls Community College
Lara Panayotoff – KY Department of Environmental Protection (DEP)
Isabelle Lavoie - Universite du Quebec a Trois-Rivieres

RESULTS

The panel assigned the two sets of 25 test samples to BCG Tiers 1 through 6, generating a total of 750 individual site assessments based on periphyton. BCG values averaged for all 15 experts were highly correlated with the SDI for both sets of sites with low and high pH (Table A2-2; Figure A2-1). In contrast, there was no significant correlation between the BCG and the human disturbance gradient (HDG). As noted in the main document (above), the periphyton community appears to respond more strongly to pH and other natural water chemistry gradients than to independent measures of human disturbance.

Table A2-2. Spearman's correlation for pH, the human disturbance gradient (HDG), the stream diatom index (SDI) and the average BCG values for 15 experts.

Low pH (< 6.5)	**pH**	**HDG**	**SDI**	**A_BCG**
pH		0.43 *	-0.67 *	0.68 *
HDG	0.43 *		-0.08	0.23
SDI	-0.67 *	-0.08		-0.76 *
A_BCG	0.68 *	0.23	-0.76 *	
High pH (> 6.5)	**pH**	**HDG**	**SDI**	**A_BCG**
pH		-0.03	-0.38	0.16
HDG	-0.03		-0.23	0.26
SDI	-0.38	-0.23		-0.67 *
A_BCG	0.16	0.26	-0.67 *	

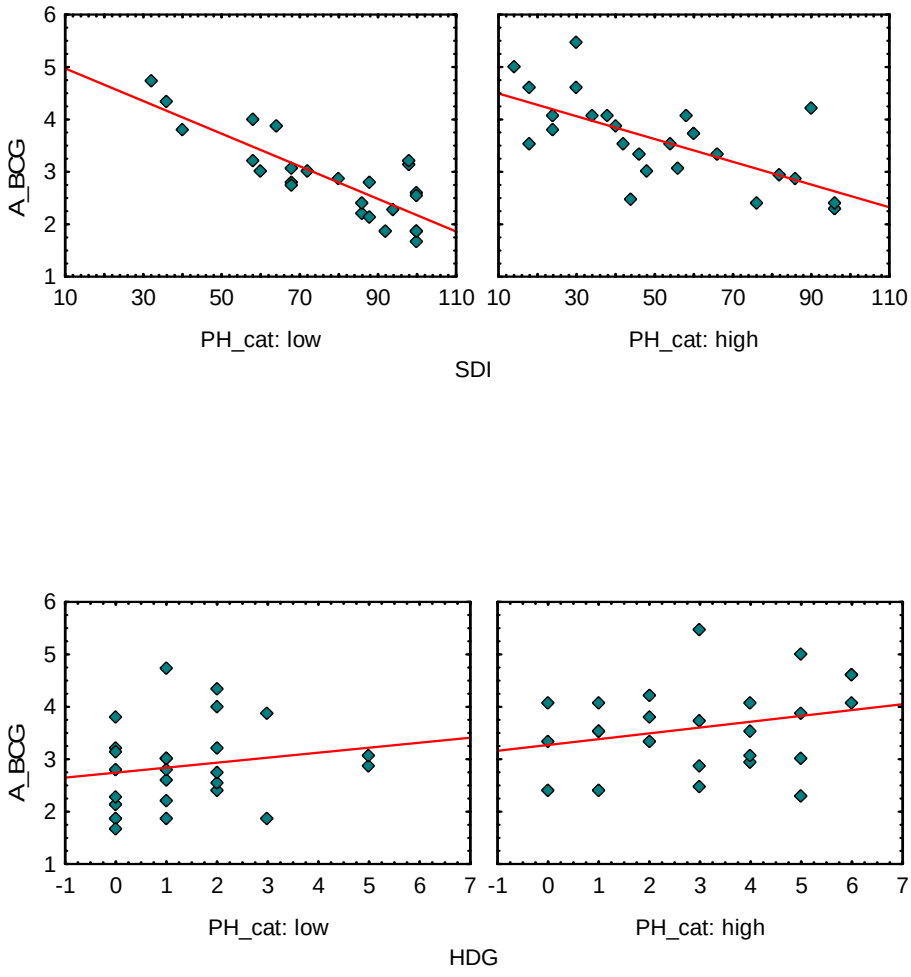


Figure A-1. The average BCG values for all 15 experts were highly correlated with the stream diatom index (SDI; upper panel). BCG values were not significantly correlated with an independent measure of human disturbance (lower panel). Results were similar for sites with low (< 6.5) and high (>6.5) pH.

Defining impairment thresholds

The expert panel was also asked to provide their opinion regarding the impairment threshold for periphyton in streams. They were asked this question:

> *"In your opinion as an aquatic scientist, where specifically along the gradient would you see a point where critical changes lead to a loss of a balanced natural population of flora and fauna?"*

The average initial response to this question was Category 3.5 on the BCG scale from 1 to 6. After discussion, the final average BCG score for this question was 3.1 (Table A2-3).

Table A2-3. Expert responses to the question, "Where specifically along the BCG would you see a point where critical changes lead to a loss of a balanced natural population of flora and fauna?"

BCG Category	Initial Votes	Final Votes
Between 0/1	0	0
Between 1/2	0	0
Between 2/3	6	7
Between 3/4	3	7
Between 4/5	6	1
Between 5/6	0	0

CONCLUSIONS

The BCG category rankings were highly correlated with the stream diatom index (SDI) indicating that the metrics used to summarize diatom condition are founded on ecological theory. Although the results for the experts and the empirical approach agreed, neither the BCG nor the SDI were significantly correlated with an independent measures of human disturbance as summarized by the HDG.

The experts were nearly unanimous in their agreement that periphyton assessments should be used for biocriteria for Florida, even though the diatom indicators were much more highly correlated with pH than human disturbance. Three experts included caveats in their recommendations to use periphyton. One said that periphyton might be better indicators of specific types of disturbance rather than as an indicator of general overall disturbance. Another agreed and said that diatoms are more sensitive to water chemistry and nutrients than to human disturbance. Another was concerned that the data presented represent samples from a system too degraded to identify minimally disturbed sites.

The experts all recommended controlling for the influence of pH and other natural water chemistry gradients before testing for correlation with human disturbance. During the workshop graphs of this type of analysis (see Figure A2-1 above) were provided, but several experts persisted in recommending additional multivariate analysis and modeling to remove the influence of pH. Additional recommendations included:

- Better classification and definition of reference sites, *e.g.*, separating out limestone regions or using geology and surficial deposit data to model pH;
- Test additional metrics;
- Improve the level of taxonomy;
- Evaluate the historic influence of human settlement, *e.g.*, did people settle away from acidic (swamp) sites, to determine if pH changed historically;
- Collect more data over a longer time period.

Florida DEP staff described the complex nature of acidity and alkalinity in Florida streams and the difficulty in predicting the natural pH regime for streams, even within a single stream. Based on these considerations, FDEP does not consider the SDI to be applicable for use as a bioassessment tool.

APPENDIX 3. WATER QUALITY INDEX TESTING

BACKGROUND

We tested diatom metrics against an independent measure of human disturbance, the HDG, which included a summary index of water quality. Before selecting the water quality index (WQI) described above, we evaluated different ways of summarizing measures of water quality. We evaluated three versions of the water quality index that differed based on which measures were included (NH_4, NOx, TP or conductivity) and in the method used to convert raw values into unit-less scores before being summed to obtain the final index value. Only one version (WQ3) was used to calculate the HDG that was used to test metrics above. This appendix describes results of testing for correlation between the interim water quality indexes and diatom metrics.

METHODS

Table A3-1. Measure of water quality, whether it was included in the version of the water quality index, and derivation of the cut-off values used to convert raw values to scores. "X" refers to percentile values from probabilistic sampling throughout Florida and "XX" refers to cut-off values provided by FDEP (see Tables 2 and 3 above).

Measure	WQ2	WQ3	WQ4
NH_4	XX	XX	X
NOx		X	X
TP		X	X
Conductivity	XX	XX	

RESULTS

Table A3-2. Candidate metrics for diatoms and their correlation (Spearman's *r*) with the water quality indexes during two sampling periods (Q2 and Q4). Only |r|-values > 0.3 are shown.

Diatom metric	WQ2	WQ3	WQ4
Q4			
Total number of taxa			
No. of sensitive taxa (Bahls)			
% Sensitive cells (Bahls)		0.32	0.32
No. of very tolerant taxa (Bahls)		0.39	0.36
% very tolerant cells (Bahls)		0.31	
% Dominance (1 taxon)		-0.36	-0.36
No. of acidophilic taxa		-0.40	-0.34
% Acidophilic cells		-0.42	-0.34
No. of alkaliphilic taxa			
% Alkaliphilic cells		0.34	0.30
No. of N heterotroph taxa		0.40	0.42
% N heterotroph cells		0.43	0.42
No. of taxa that require high oxygen			
% of cells that require high oxygen		-0.44	-0.42
No. of taxa that tolerate low oxygen		0.38	0.40
% of cells that tolerate low oxygen		0.41	0.40
No. of oligosaprobic taxa			
% oligosaprobic cells		-0.46	-0.45
No. of polysaprobic taxa		0.33	0.32
% polysaprobic cells		0.32	0.31
No. of oligotrophic taxa		-0.37	-0.34
% oligotrophic cells		-0.46	-0.42
No. of eutrophic taxa		0.31	
% eutrophic cells		0.37	0.33
Trophic state index (Van Dam)		0.46	0.41
No. of sensitive taxa (FL)		-0.37	
No. of tolerant taxa (FL)		0.43	0.43
% Sensitive cells (FL)		-0.41	-0.33
% Tolerant cells (FL)		0.48	0.46
% Sensitive taxa		-0.41	-0.33
% Tolerant taxa		0.50	0.50

Diatom metric	WQ2	WQ3	WQ4
Q2			
Total number of taxa			
No. of sensitive taxa (Bahls)		0.35	0.39
% Sensitive cells (Bahls)		0.31	0.36
#N/A			0.35
#N/A		0.35	0.35
% Dominance (1 taxon)			-0.30
No. of acidophilic taxa		-0.49	-0.42
% Acidophilic cells		-0.54	-0.47
No. of alkaliphilic taxa		0.38	0.36
% Alkaliphilic cells		0.52	0.44
No. of N heterotroph taxa		0.47	0.47
% N heterotroph cells		0.53	0.54
No. of taxa that require high oxygen			
% of cells that require high oxygen		-0.54	-0.51
No. of taxa that tolerate low oxygen		0.37	0.42
% of cells that tolerate low oxygen		0.43	0.47
No. of oligosaprobic taxa			
% oligosaprobic cells		-0.53	-0.52
No. of polysaprobic taxa		0.35	0.39
% polysaprobic cells		0.40	0.41
No. of oligotrophic taxa		-0.41	-0.35
% oligotrophic cells		-0.56	-0.52
No. of eutrophic taxa		0.39	0.38
% eutrophic cells		0.49	0.46
Trophic state index (Van Dam)		0.54	0.50
No. of sensitive taxa (FL)		-0.42	
No. of tolerant taxa (FL)		0.49	0.49
% Sensitive cells (FL)		-0.53	-0.44
% Tolerant cells (FL)		0.61	0.58
% Sensitive taxa		-0.51	-0.39
% Tolerant taxa		0.59	0.54