City of Austin
WATERSHED PROTECTION

Phosphorus Concentration Ranges and Periphyton Responses

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Abstract

Biological monitoring represents an effective tool toward determining ecosystem health. Recently it has also been useful for establishing nutrient concentration criteria due to nonlinear responses of biota to nutrient enrichment. Stream benthic diatoms are commonly used to evaluate trophic conditions, and, due to narrow autecological preferences and tolerances, shifts in community composition can be used for establishing nutrient criteria protective of an ecosystem condition. In this study we evaluated the potential of diatoms from streams in the Austin region, spanning a wide trophic gradient, for establishing preliminary nutrient criteria thresholds. We described community stoichiometry and species' shifts relative to phosphorus and nitrogen enrichment with generalized additive models, ordination, and threshold indicator taxon analysis. We found corroborative evidence that eutrophication, notably P-enrichment, impacts periphyton stoichiometry and species composition and abundances. Based on this initial study, establishment of a preliminary numeric nutrient criteria near 9 µg/L TP and 600 µg/L TN would be protective of stream health, above which a stream would be considered in a degraded condition. It was also determined that current TP laboratory minimum detection limits were not sensitive enough to adequately capture ambient phosphorus concentrations near the proposed ecologically relevant threshold.

Introduction

Nutrient concentrations can be measured directly from the water column, but this approach represents a snap-shot in space and time of system water chemistry and may not reflect long-term mean concentrations or excessive storm-driven loadings. Additionally, laboratory techniques need to be sufficiently sensitive to capture concentrations as they approach ecologically-relevant concentrations. An inability to effectively determine if increasing nutrient concentration are approaching a threshold concentration due to laboratory insensitivity could result in a system "regime shift" whereby an ecological threshold is crossed giving rise to a new, less desirable ecosystem condition (Contamin and Ellison 2009; Dodds et al. 2010). System recovery may be costly and difficult to achieve due to legacy effects and inherent resilience and stability of the new ecological condition (Folke et al. 2004). To effectively track system

condition and changes, monitoring programs typically integrate biological indicators that are sensitive to water nutrient concentrations.

Diatom species within periphyton (a cohesive consortium of algae and bacteria) communities have been shown to be effective trophic indicators as they are ubiquitous, integrative of persistent water quality conditions, and species autecology is generally well defined (Stoermer and Smol 1999; Stevenson 2014). Diatom biomass, cellular nutrient contents, and community composition are influenced by the water column absolute (i.e., µg/L) and relative (i.e., nutrient: nutrient stoichiometry) concentrations of nitrogen (N) and phosphorus (P). An overabundance of N and P commonly causes a shift in community composition and an increase in nuisance algal biomass which leads to aesthetic degradation (Wharfe et al. 1984; Biggs and Price 1987; Welch et al. 1988), loss of pollution-sensitive taxa (Quinn and Hickey 1990), clogging of water intake structures (Biggs 1985), and biologically detrimental alterations to the dissolved oxygen concentrations and pH levels in the water column (Quinn and Gilliland 1989).

Excess available nutrients will be reflected in cellular nutrient concentrations as diatoms take up and sequester the abundantly available nutrient, changing the stoichiometric ratios of P, N, and carbon (C) (i.e., C:P, C:N, and N:P; Sterner and Elser 2002; Taylor et al. 2014). Diatom species composition and relative abundances have been more commonly used as indicators of ecosystem condition because of their relatively narrow ecological tolerances (Hill et al. 2001; Stevenson et al. 2008). By coupling water chemistry with diatom species composition, environmental optima and tolerance preferences can be discerned and utilized to score relative ecosystem condition (i.e., indices of biological integrity; Stoermer and Smol 1999). Additionally, periphyton biomass, stoichiometry, and species composition and abundances may have linear or non-linear responses to nutrients (Hillebrand and Kahlert 2001; Stelzer and Lamberti 2001; Frost and Elser 2002; Bowman et al. 2005; Stevenson et al. 2008; Taylor et al. 2014). Identifying stressor-response breakpoints or minimum acceptable changes in community characteristics can form the basis of numeric nutrient criteria thresholds protective of a desired ecosystem condition (e.g., oligotrophic) and services (e.g., contact recreation, aesthetics), or serve as water quality targets achieved through watershed restoration and remediation projects.

As part of a comprehensive regional monitoring and assessment program, the Watershed Protection Department (WPD) has been utilizing periphyton diatom species composition since 1994 as a component to score creek biological integrity. Previous WPD evaluations of the relationship between periphyton stoichiometry and water quality in local streams had limited success due to sampling across a narrow trophic gradient and large variance in the data (Porras 2015, 2016). Austin's creek diatom species have yet to be analyzed comprehensively for cross-site similarities and differences and responses to nutrient concentrations using multivariate and non-linear models. One objective of this study was to evaluate if lower lab detection limits are required to capture ecologically relevant biological responses as elucidated by non-linear responses in periphyton community characteristics. We sampled water chemistry and periphyton from creeks spanning a large nutrient gradient, including sites where P concentrations have been below conventional lab detection limits. Next, we used a suite of non-linear and ordination analyses, including generalized additive models (GAMs), nonmetric multidimensional scaling (NMDS), and threshold indicator taxa analysis (TITAN) to evaluate relationships between periphyton stoichiometry and species composition with ambient stream nutrient concentrations.

These results will help determine if periphyton stoichiometry should be added as a metric to current monitoring programs, and if the larger Environmental Integrity Index (EII) dataset could be utilized to establish local creek nutrient criteria.

Methods

Site Selection and History

The City of Austin has been monitoring water quality in Austin creeks under the EII program since 1994 (Fig. 1, Table 1). Since that time ortho-phosphate has been the typical measure of P in creeks because it is the form most readily utilized by biota; however, TP has been collected occasionally in all sampling reaches over time. Samples were first analyzed for TP at the City of Austin Water and Waste Water Lab in 2001 and again in 2004 where the method detection limit was 20 µg/L. Additional samples were not analyzed for TP until 2011, after which it was routinely determined by the Lower Colorado River Authority Environmental Laboratory Services, which has a method detection limit of 8 µg/L. The TP data collected in 2001 and 2004 was combined with TP data collected from 2011 to 2015 to establish a TP gradient across Austin's creeks (Fig. 2). In some watersheds, a true background level of TP is unknown because samples are typically reported at the method detection limit.

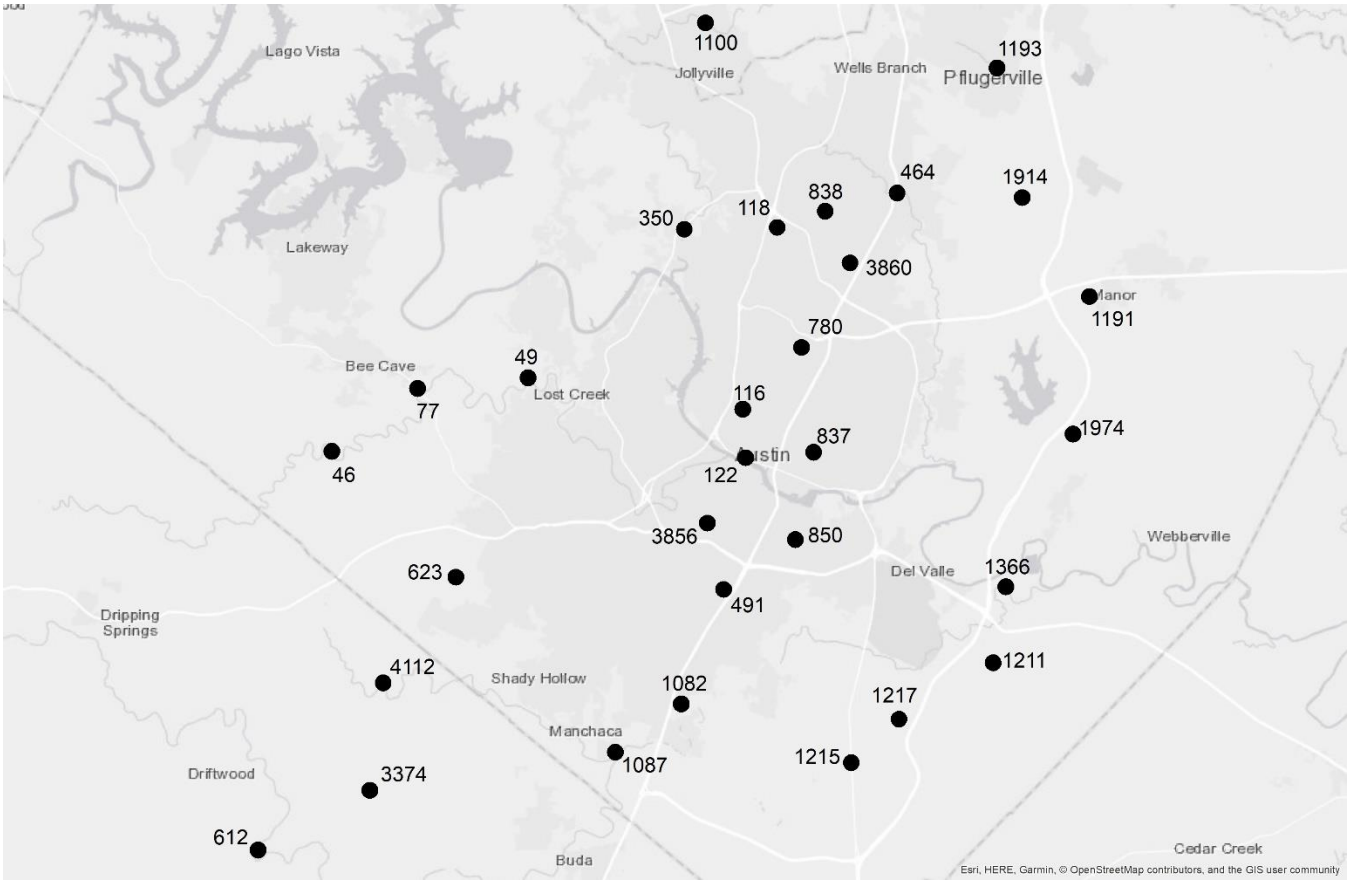

Figure 1. Historic EII site locations and numbers selected for this study.

Table 1: Site name, EII reach, watershed, site number, and location (latitude/longitude; see Fig. 1 for map) of project sites in descending order of average TP concentration (see Fig. 2).

Site Name	EII Reach	Site Number	Watershed	Latitude	Longitude
Lake @ Meadowheath Dr.	LKC3	1100	Lake Creek	30.46489048	-97.7723456
Gilleland @ W. Parsons St.	GIL3	1191	Gilleland Creek	30.34074192	-97.56516542
Waller @ 51st St.	WLR3	780	Waller Creek	30.31634673	-97.72511609
Shoal @ 1st St.	SHL1	122	Shoal Creek	30.26728474	-97.75031308
Dry @ Pearce Rd.	DRE2	1211	Dry Creek East	30.1648278	-97.61619206
North Boggy @ Nile St.	BOG2	837	Boggy Creek	30.268704	-97.714448
North Fork Dry @ FM812	NFD1	1217	North Fork Dry Creek	30.14353474	-97.67064429
Slaughter @ Pine Valley Dr.	SLA1	1082	Slaughter Creek	30.14872059	-97.78555146
Shoal @ Crosscreek Dr.	SHL4	118	Shoal Creek	30.37112504	-97.73660459
Gilleland @ South Railroad Ave.	GIL6	1193	Gilleland Creek	30.44420689	-97.61877736
Decker @ Gilbert Rd.	DKR1	1974	Decker Creek	30.27042222	-97.57981959
Williamson @ IH35	WMS2	491	Williamson Creek	30.2016378	-97.76155148
West Gilleland @ Cameron Rd.	GIL4	1914	Gilleland Creek	30.38348362	-97.60248658
South Fork Dry @ US183	SFD2	1215	South Fork Dry Creek	30.13103891	-97.69556069
West Bouldin @ Cardinal	WBO3	3856	West Bouldin Creek	30.23540476	-97.77190935
Shoal @ 24th St.	SHL2	116	Shoal Creek	30.28838866	-97.75348163
Little Walnut @ Georgian Dr.	LWA3	3860	Little Walnut Creek	30.35397679	-97.69818845
West Country Club @ E. Oltorf St.	CCW2	850	Country Club West	30.2272491	-97.72700909
Onion @ South Austin Regional WWTP	ONI1	1366	Onion Creek	30.20693181	-97.61558726
Little Walnut @ Golden Meadow Rd.	LWA4	838	Little Walnut Creek	30.38070563	-97.70989327
Walnut @ IH35	WLN3	464	Walnut Creek	30.38835955	-97.67208436
Slaughter @ FM 1826	SLA3	623	Slaughter Creek	30.2095626	-97.90356935
Onion near HWY 150	ONI5	612	Onion Creek	30.08543218	-98.01336507
Little Bear @ Ashmun Property	LBR2	3374	Little Bear Creek	30.11231682	-97.95195356
Little Barton @ Barton Creek	LBA1	77	Little Barton Creek	30.29618366	-97.92775105
Bull @ Loop 360 First Crossing	BUL1	350	Bull Creek	30.3716707	-97.78492996
Bear @ Bear Creek Pass	BER3	4112	Bear Creek	30.16088234	-97.94486077
Bear @ Twin Creeks Rd.	BER1	1087	Bear Creek	30.12742048	-97.82190258
Barton @ Shield Ranch Pool	BAR5	46	Barton Creek	30.26978549	-97.97350879
Barton @ Ogletree Pool	BAR3	49	Barton Creek	30.3022731	-97.86854439

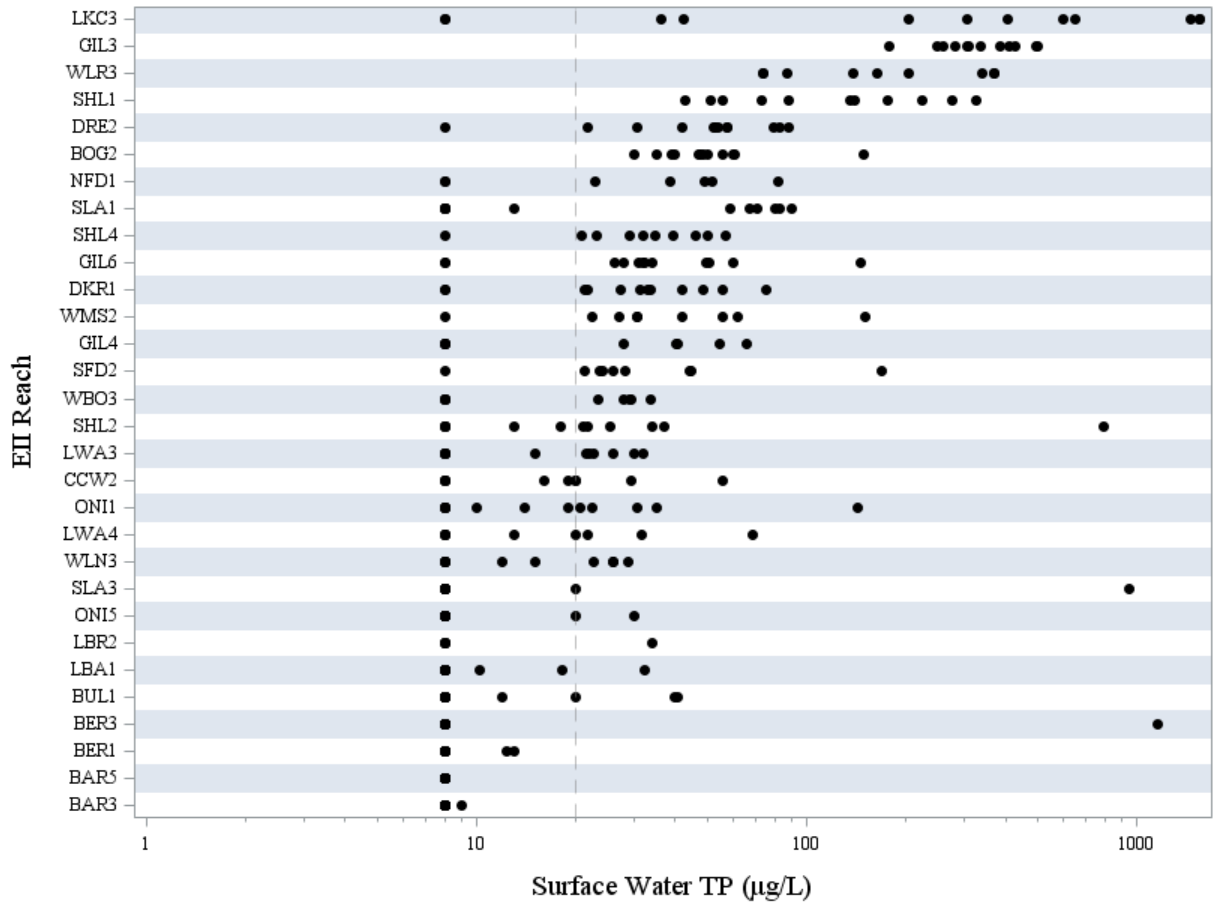

Figure 2: TP concentrations (µg/L) collected in historic (2001, 2004, 2011-2015) EII sampling events from sites selected for this project. Sites are listed in descending order of average TP concentration. Refer to Table 1 for full Creek name and site location. Vertical dashed line at 20 µg/L represents the concentration shown to be a maximum threshold to maintain reference, unimpacted conditions in the Texas Brazos and Trinity River basins by Taylor et al. (2014).

Data

For this study samples were collected during baseflow from 26 April 2016 to 04 May 2016. Sample locations were in open or semi-open canopy (50% or less cover as measured by densitometer) to minimize for the confounding influence of shade on algal growth. The site within EII reach DRE2 could not be sampled because no suitable habitat could be found during the sampling event.

Habitat

At each site staff measured stream velocity, canopy cover, percent cover of visible periphyton, and percent cover of filamentous green algae. Three velocity readings from a Marsh McBirney Flo-mate were collected in the thalwag and averaged for the site. Three canopy cover readings were collected with a densiometer and averaged for the site. Percent cover of visible periphyton and percent cover of filamentous green algae were visually estimated as the percent of the area (not volume) covered by any visible periphyton (including filamentous) and the percent of the area (not volume) covered only by filamentous green algae.

Periphyton
Periphyton was collected from at least fifteen submerged medium-to-large (~20–300 mm) cobble rocks. A 19.6 cm^2 petri dish was used to mark an area to be scraped on each rock. A metal wire brush and ambient site-water rinse was used to remove all attached algae from the marked surface of the rock. Prior to scraping rocks, brushes were rinsed and bristles were scraped with the thumb to minimize cross contamination from the previous site. Scraped material was placed into a single plastic basin where all algae material at a site was aggregated. Total rinsate for all rocks did not exceed 750 mL of ambient stream water. The content of the basin was split into one pre-labeled 500 mL plastic amber bottle and one pre-labeled 250 mL plastic amber bottle. Samples were kept in an ice bath during field collection and then refrigerated until delivery (on ice) to the labs for analysis. The 250 mL sample was preserved with 16 mL of 10% formalin.

The 500 mL bottle was analyzed for periphyton TP, TN, and TC contents (mg/kg), and chlorophyll-*a* concentration and ash free dry weight (AFDW) mass which were normalized to area (mass/m^2) at the Baylor University Aquatic Ecology Laboratory. The 250 mL sample was analyzed for diatom species composition and abundances (org/mL) by Winsborough Inc.

Surface Water
Surface water temperature, specific conductance, dissolved oxygen, and pH were recorded with a Hach Datasonde. Surface water samples were collected upstream of any disturbance from the collection of periphyton rocks pursuant to the following protocol:

- Nitrile gloves were worn by Staff
- Staff prepared one 60 mL syringe, a centrifuge rack, three 40 mL centrifuge tubes (one each for TP, TN, and dissolved N/P [dN/P; i.e., nitrate/nitrite, ammonium, ortho-P]), and one leur lock syringe filter;
- Staff drew and ejected 50–60 mL of site water into the syringe as a rinse;
- Then, staff drew 40 mL of site water into the syringe and discharged the unfiltered sample into the TP-labeled centrifuge tube;
- Next, staff drew 40 mL of site water into the syringe and discharged the unfiltered sample into the TN-labeled centrifuge tube;
- Finally, staff drew 50 mL of site water into the syringe and attached a new luer lock filter. The first 10 mL of the sample was discharged through the filter back into the stream and then the remaining 40 mL was pushed into the dN/P centrifuge tube.

A syringe could be reused throughout the day if there was an initial rinse step at each sample site. A new syringe was used on each new sampling date. New luer lock filters were used at each site. All centrifuge tube samples were placed in a large ziplock bag and kept on ice in the field and kept frozen until delivered to the Baylor University Aquatic Ecology Laboratory for analysis.

Quality Assurance/Quality Control

For each day of sampling, a trip blank and field blank were collected. To collect trip blanks, unfiltered reagent grade water was collected in the WPD lab prior to departure each day into a TN and TP tube, and reagent grade water was pushed through a luer lock syringe into a dN/P centrifuge tube. Reagent grade water was carried to the first site visited each day and used to fill field blank sample bottles in a similar fashion as the trip blank samples. Field duplicates were collected from 10% of the total sites for all parameters except taxonomy.

Data Analysis

The Baylor University Aquatic Ecology Lab asserts to have an analysis method that could detect TP in the surface water at lower concentrations than the LCRA Environmental Laboratory Services method detection limit of 8 µg/L. Total P collected for this project was plotted against TP collected during historical EII events to determine if the analysis method used for this project actually produced values lower than the LCRA method detection limit (Fig. 2). Additionally, the same plot was used by staff to ensure that surface water TP collected for this project fell within the range of historic EII TP concentrations.

WPD Staff followed the methods used by Taylor et al. (2014) to analyze the relationships between the periphyton community structure and surface water nutrients using a combination of generalized additive models (GAMs), non-metric multidimensional scaling (NMDS) ordinations, and threshold indicator taxa analysis (TITAN).

Generalized Additive Model (GAM)

Generalized additive modeling was used to examine the relationships between periphyton nutrient content and surface water nutrients (TP and TN). GAMs were first explored by Hastie and Tibshirani (1986) as an extension to likelihood-based regression models. In likelihood-based regression a response variable Y, is modeled as a linear function $\sum_1^N \beta_i X_i$ of a set of covariates $X_1, X_2, \ldots, X_N$. The β_i of the linear function are estimated by maximum likelihood. GAMs replace the linear function with an additive function $\sum_1^N s_i(X_i)$ where $s_i(X_i)$ is a smoothed function relative to the X_i covariate. The benefits of using a GAM is that the relationship of the covariate and the response variable does not need to be known *a priori*, the interpretation of the analysis is clear, and the model does not need to be linear.

We modeled responses of periphyton C:N, C:P, and N:P ratios to surface water nutrients using GAMs with the mgcv package in R version 3.1.1 (Wood 2006). The gamma distribution was used in all models and cross-validation was used to perform optimal smoothing in each model. Reported R^2 values represent the explained deviance and were calculated as the difference between the null deviance and the residual deviance divided by the null deviance.

Ordination

Non-metric multidimensional scaling (NMDS) is an ordination technique applicable to ecological data with a large number of taxa that may also have a lot of zero counts (McCune and Grace 2002). Rare species (<1% abundance and occurring at fewer than 3 sites) were initially removed from the matrix resulting in 82 species in the final site-species matrix. Species relative abundance data were arcsine transformed to down-weight the influence of abundant species. Preliminary ordinations within a Bray-Curtis distance matrix were used to determine the

optimum number of dimensions that explained the most variance in the site assemblage matrix. Based on 250 iterations and 50 runs with real and randomized data we arrived at a 3-dimensional solution. A Monte Carlo randomization test was run to ensure the final configuration was not simply due to chance. In the final ordination space, sites further apart are more dissimilar in species composition. Species having a significant ($r^2 > 0.2$) influence on site ordination were identified. We overlaid a second matrix comprised of environmental variables (n = 12) on the first (species) matrix to identify significantly related ($r^2 > 0.2$) variables with site separation. Water quality variables were collected on the same day as periphyton; site physical characteristics (i.e., canopy cover, flow permanence) were averages generated from long-term site monitoring.

Threshold Indicator Taxa ANalysis (TITAN)

TITAN splits samples into two groups at a value of a predictor variable that maximizes the association of each taxon with one group (Baker and King 2010). The association is measured by taxon abundances weighted by their occurrence in each group (Dufrêne and Legendre 1997) and standardized to z-scores to allow for cross-taxon comparisons. TITAN distinguishes between increasing (z+) and decreasing (z-) taxa along the predictor variable. In addition, bootstrapping is used to identify reliable taxa for threshold analysis and determine the uncertainty around taxon and community change points. The sum of the z-scores can be used as an indicator of community thresholds by identifying peaks in the sums of all taxa z-scores along the predictor variable associated with the maximum decline from decreasing taxon (z-) or maximum increase from positive taxon (z+).

We analyzed the response of individual diatom taxa to nutrient (TN and TP) enrichment gradients using TITAN2 package in R version 3.1.1 (Baker and King 2010, 2013; Taylor et al. 2014). Taxon were determined to respond negatively or positively to an increase in nutrients if the change in frequency and abundance of the taxon was the same for at least 95% of 750 bootstrapped runs and at least 95% of the 750 runs were significantly different from a random distribution.

Results

Summary Statistics

A summary of all variables collected for this project is provided in Table 2. Algae cover at most sites was dominated by epilithic periphyton rather than dense filamentous mats. In general, canopy cover over the stream reach was below 20%. Baseflow velocities across sites varied between 0.1 and 3.6 ft/s.

The surface water TP concentrations ranged from 3.2 to 184.0 µg/L and ortho-P ranged from 3.7 to 64.7 µg/L. Total N concentrations ranged from 257.0 to 4,104.0 µg/L, with nitrate/nitrite (NO_X) concentrations ranging from 5.1 to 3,010.0 µg/L. Positive correlations were observed between total nutrient concentrations (Pearson correlation, r>0.7) and between inorganic nutrient concentrations with total concentrations (Pearson correlation, r>0.9) (Appendix A). Ammonium (NH_4^+) concentrations were not highly correlated with other forms of N, but were positively correlated with ortho-P (Pearson correlation, r=0.82).

Surface water TP concentrations collected for this project were in the range of historical EII data, but concentrations measured at SLA3, ONI5, LBR2, LBA1, BUL1, BER3, BER1, BAR5, and BAR3 were lower than the LCRA method detection limit of 8 µg/L (Fig. 3). Total P concentration collected at the LKC3 reach, while within the assumed ranged, was on the low end typically observed.

Periphyton AFDW ranged from 11.9 to 65.5 g/m^2, chlorophyll-*a* ranged from 0.1 to 1.7 mg/m^2, TC varied between 48.3 and 112.3 mg/kg, TP ranged from 8.2 to 2,076.0 mg/kg, and TN ranged from 600.0 to 9,000.0 mg/kg (Table 2). Periphyton C:P and N:P ratios were generally over 500 and 12, respectively, and varied by over an order of magnitude; C:N was more constrained between 10 and 50 (Fig. 3). Periphyton AFDW and chlorophyll-*a* were positively correlated (Pearson correlation, r=0.67; Appendix A). It is unclear why chlorophyll-*a* values were much lower in this study than have typically been reported in EII surveys, and for this report we do not discuss further, but it warrants exploration. Periphyton TP and TN were positively correlated (Pearson correlation, r=0.81); periphyton TP was also positively correlated to the surface water concentrations of TP (Pearson correlation, r=0.78) and TN (Pearson correlation, r=0.67) (Appendix 1).

Table 2: Summary statistics for habitat, surface water, and periphyton variables collected from 29 sites.

Variable	Mean (95% CI)	Median	Min	Max
Habitat				
% Algae Cover	45 (33–56)	40	5	95
% Filamentous Algae Cover	12 (6–18)	5	0	55
% Canopy Cover	16 (10–22)	10	0	50
Stream Velocity (ft./s)	1.6 (1.3–2.0)	1.5	0.1	3.6
Surface Water				
Specific Conductivity (µS/cm)	648.0 (607.6–688.4)	656.5	389.9	868.5
Dissolved Oxygen (mg/L)	9.2 (8.4–10.0)	8.8	6.0	15.9
pH	8.0 (7.9–8.1)	8.0	7.4	8.26
Temperature (°C)	22.1 (21.2–23.1)	22.4	17.8	28.7
Ammonium (µg/L)	18.0 (9.8–26.2)	10.7	4.6	115.0
Nitrate/Nitrite (µg/L)	795.2 (502.3–1088.0)	533.0	5.1	3,010.0
TN (µg/L)	1060.7 (716.9–1404.5)	646.0	257.0	4,104.0
DOC (mg/L)	3.6 (2.7–4.4)	3.0	2.1	10.7
Ortho-P (µg/L)	11.3 (5.9–16.6)	6.2	3.7	64.7
TP (µg/L)	36.6 (20.2–53.0)	22.2	3.2	184.0
Periphyton				
AFDW (g/m^2)	31.6 (26.8–36.5)	28.6	11.9	65.5
Chlorophyll-*a* (mg/m^2)	0.7 (0.6–0.9)	0.6	0.1	1.7
TC (g/kg)	80.2 (74.6–85.8)	81.1	48.3	112.3
TN (mg/kg)	5,169.0 (4,358.0–5,979.0)	5,300.0	600.0	9,000.0
TP (mg/kg)	611.7 (425.0–798.4)	522.2	8.2	2076.0

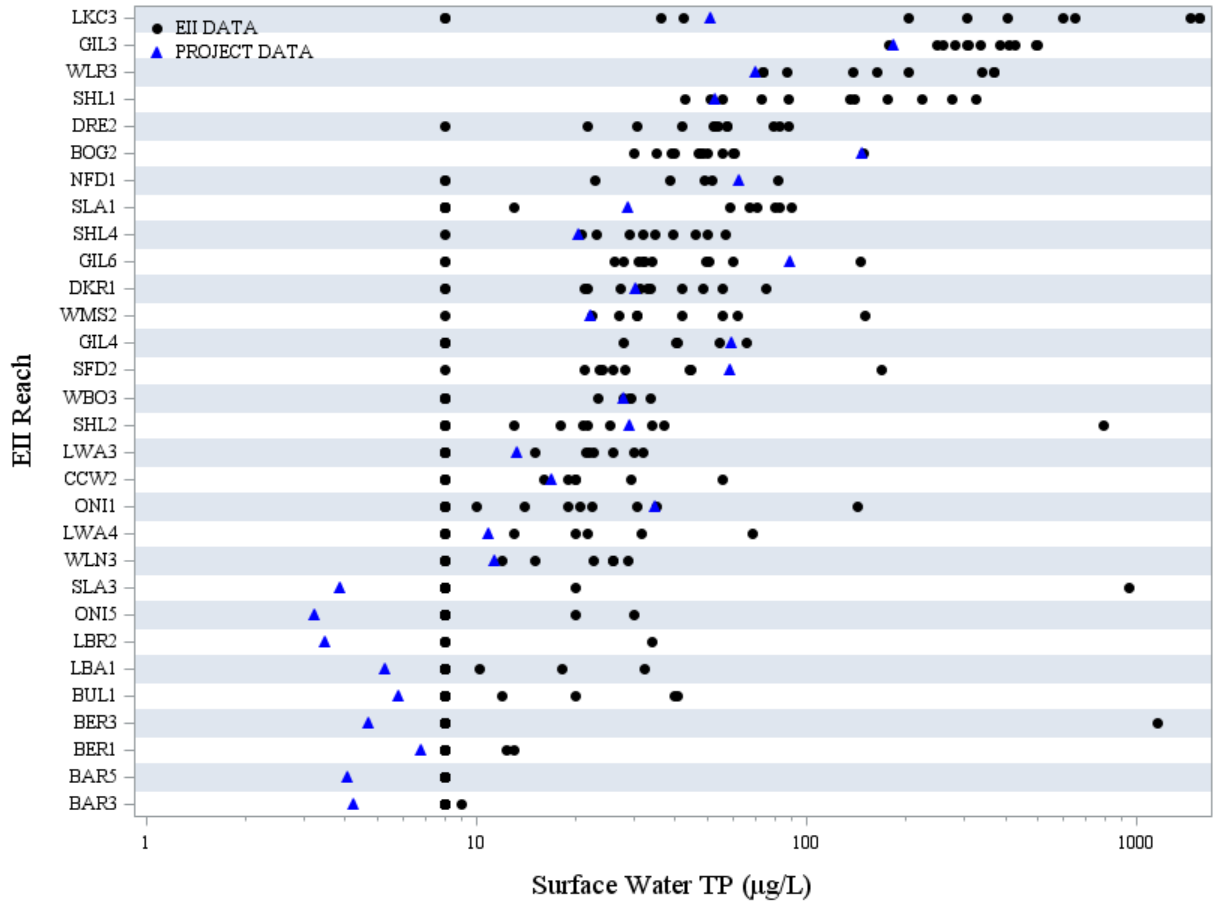

Figure 3: Total P (µg/L) concentrations at the sites sampled in April/May 2016 for this project (triangles) and previously collected (circles) indicating the recent sampling generally fell within the historic range of values. For the most oligotrophic sites need for a lower detection limit is warranted. See Table 1 for full site descriptions. The site within EII reach DRE2 was not sampled during this project because no suitable habitat could be found during the sampling event.

Periphyton Nutrient Content (GAMs)

Periphyton C:P and N:P ratios declined sharply with low levels of nutrient enrichment, especially water column TP enrichment (Fig. 4). At higher levels of surface water nutrients, the confidence interval for the GAMs was increasingly large because there were only a few sites with high concentrations of surface water nutrients. The strongest relationship (i.e., highest r^2) between water TP concentrations was with periphyton C:P, whereas the weakest relationship was with periphyton C:N (Fig. 4 left column). When surface water TP was below 20 µg/L, periphyton C:P ratios spanned a large range (421 to 25,881), but were constrained (100 to 432) when ambient TP was above 20 µg/L. Periphyton N:P also varied to a greater extent (17 to 1,080) when TP was below 20 µg/L, and were more constrained (10-24) when water TP was above 20 µg/L TP.

Significant, but weaker, relationships were observed between water column TN and periphyton stoichiometry (Fig. 4 right column). Periphyton stoichiometric ratios increased sharply when water TN concentration were below 1,000 µg/L.

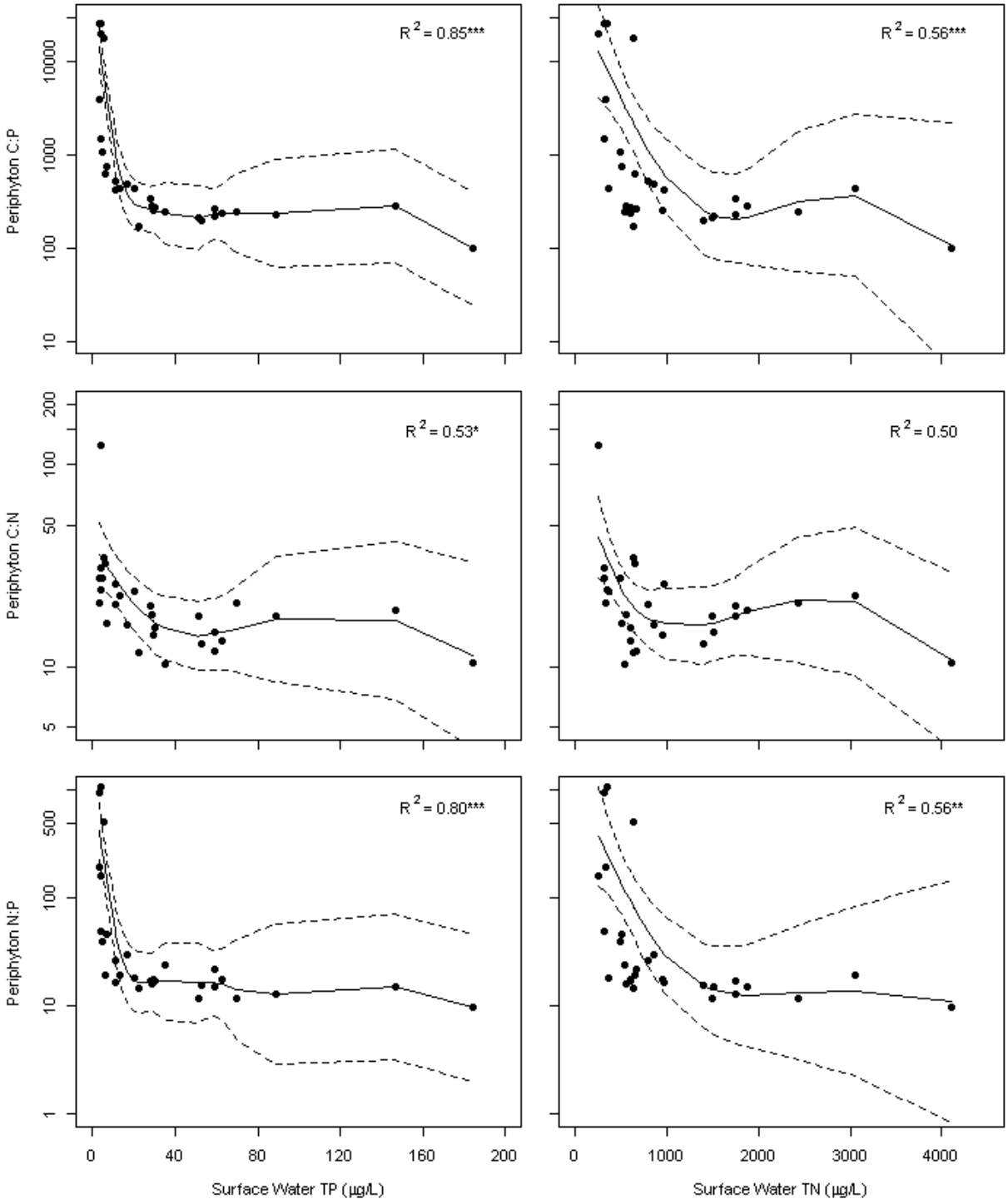

Figure 4: Relationships between periphyton C:P, C:N, and N:P (y-axis; top-to-bottom) against surface water TP (x-axis; left column) and TN (x-axis; right column) with GAM smoothers. Solid lines are predicted values while dashed lines are 95% confidence intervals. R^2 = (null deviance – residual deviance)/null deviance; $p < 0.001$ indicated by ***, $p < 0.01$ indicated by **, and $p < 0.05$ indicated by *.

Community Structure (NMDS ordination)

The final 3-dimensional solution explained over 80% of the variance in species composition across all sites (Fig. 5). In addition, site environmental metrics had significant correlations with site ordinations. We found axis 1 was positively correlated with water velocity (Table 3). Species that were negatively correlated with axis 1 (and thus responded to lower water velocity) were *Caloneis bacillum, C. limosa, C. ventricosa, Encyonema triagulum, Nitzschia amphibia,* and *Rhopalodia gibba* (Table 4). Conversely, *Achnanthidium altergracillimum, A. biasolettianum, E. evergladianum, Encyonopsis microcephala,* and *Sellaphora stroemii* were positively correlated with water velocity. Axis 2 explained the most variance in site diatom species assemblage composition and was correlated with nutrient concentrations (Fig. 5; Table 3). Diatom species positively correlated with axis 2 (and thus responded to lower nutrient concentrations) were *Achnanthidium altergracillimum, A. caledonicum, Brachysira vitrea, Cymbella laevis, Delicata delicata, E. evergladianum, Eucocconeis flexella, Eunotia pectinalis, Gomphonema lateripunctatum,* and *Mastogloia smithii* (Table 4). Only *Gomphonema parvulum* was found to be positively correlated with elevated nutrient conditions. Axis 3 was positively associated with dissolved oxygen (DO; Table 3). Two species, *Reimeria sinuata* and *Rhopalodia gibba,* were found to increase in abundance with decreasing DO concentrations (Table 4), the remainder showed no strong correlations with DO.

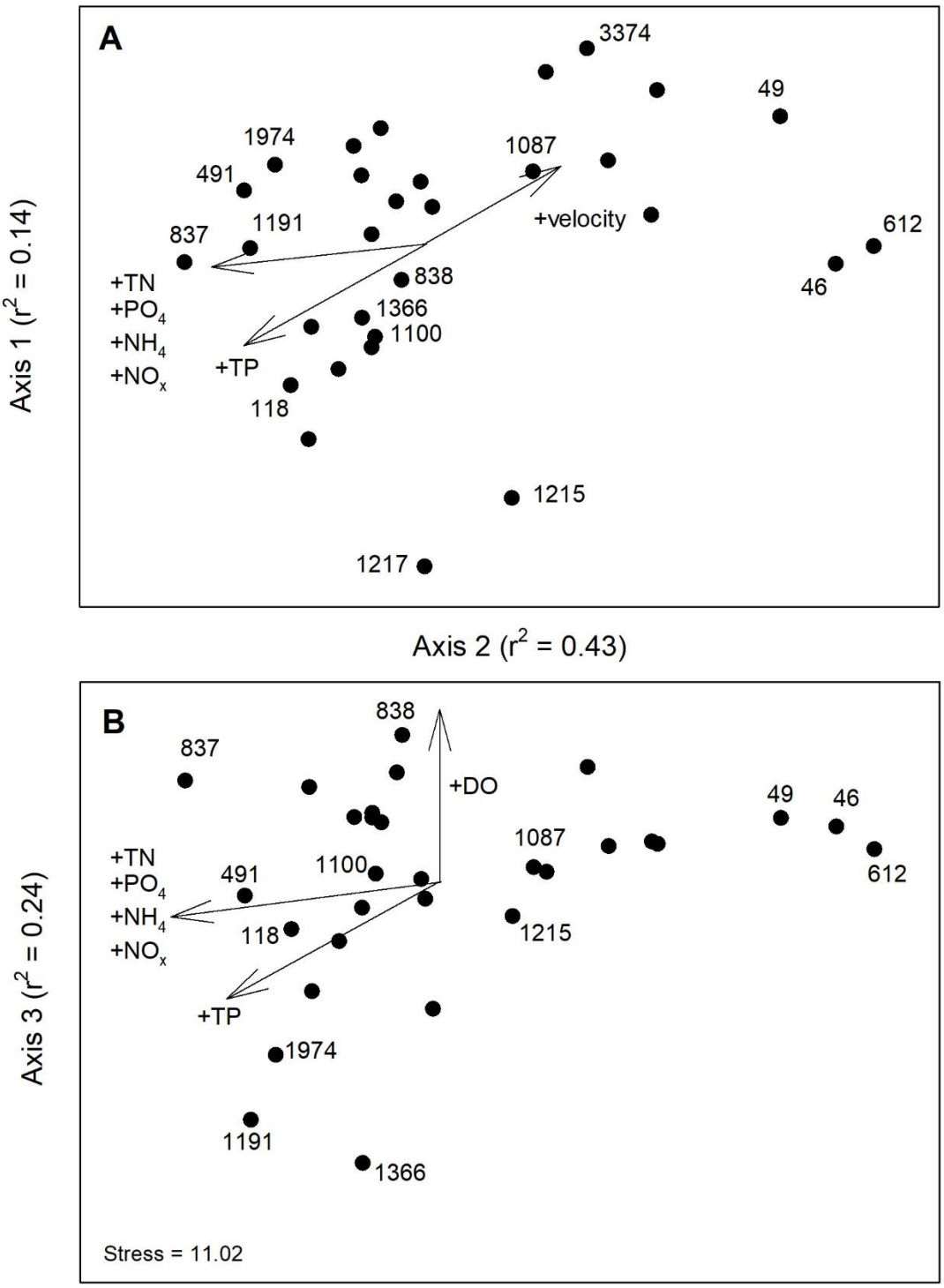

Figure 5. NMDS ordination of sites based on periphyton diatom relative abundances. Refer to Figure 1 for site locations, Table 1 for site names, and Figure 3 for measured TP concentrations corresponding with site numbers. Species strength in relation to each ordination axis can be found in Table 4. Important ($r^2 > 0.2$) water quality metrics associated with axes are overlaid as vector arrows (see Table 3 for coefficients of determination for each parameter with an axis).

Table 3 Environmental and water chemistry coefficients of determination relative to each ordination axis. Correlations (r) > |0.45| shown in bold italics.

Parameter	Axis 1	Axis 2	Axis 3
Dissolved oxygen	0.21	-0.02	***0.46***
Velocity	***0.45***	0.31	-0.18
Total phosphorus	-0.40	***-0.57***	-0.41
Total nitrogen	-0.03	***-0.52***	-0.14
Phosphate	-0.26	***-0.47***	-0.21
Ammonium	-0.29	***-0.52***	-0.11
Nitrate+nitrite	0.07	***-0.47***	-0.02

Table 4 Diatom species coefficients of determination relative to each ordination axis. Correlations (r) > |0.45| shown in bold italics.

Species	Axis 1 (r^2 = 0.14)	Axis 2 (r^2 = 0.43)	Axis 3 (r^2 = 0.24)
Achnanthidium altergracilimum	***0.63***	***0.50***	0.33
Achnanthidium biasolettianum	***0.49***	0.15	0.21
Achnanthidium caledonicum	0.01	***0.73***	0.15
Brachysira vitrea	0.17	***0.59***	0.19
Caloneis bacillum	***-0.64***	-0.04	-0.13
Caloneis limosa	***-0.52***	0.01	-0.02
Caloneis ventricosa	***-0.54***	-0.04	-0.04
Cymbella laevis	0.15	***0.78***	0.16
Delicata delicata	0.13	***0.84***	0.20
Encyonema evergladianum	***0.48***	***0.53***	0.27
Encyonopsis microcephala	***0.49***	0.39	0.12
Encyonema triagulum	***-0.63***	0.03	-0.07
Eucocconeis flexella	0.31	***0.61***	0.28
Eunotia pectinalis	0.40	***0.44***	0.28
Gomphonema lateripunctatum	0.16	***0.72***	0.20
Gomphonema parvulum	-0.09	***-0.49***	0.31
Mastogloia smithii	0.08	***0.57***	0.14
Nitzschia amphibia	***-0.71***	-0.33	0.06
Reimeria sinuata	-0.15	-0.27	***-0.78***
Rhoicosphenia abbreviata	-0.07	-0.30	***-0.52***
Rhopalodia gibba	***-0.50***	-0.02	-0.05
Sellaphora stroemii	***0.49***	0.23	0.21

Taxon Reaction to Nutrients

Threshold indicator taxa analysis (TITAN) identified 19 of the 75 taxa evaluated as pure and reliable responders, with 13 taxa declining and 6 taxa increasing as surface water TP increased (Fig. 6, Appendix B). Assemblage-level analysis showed a threshold of 8.8 µg/L TP (90% CI, 8.1-11.8 µg/L) for all declining species and a threshold of 8.8 µg/L TP (90% CI, 7.6-15.6 µg/L) for all increasing species (Fig. 7).

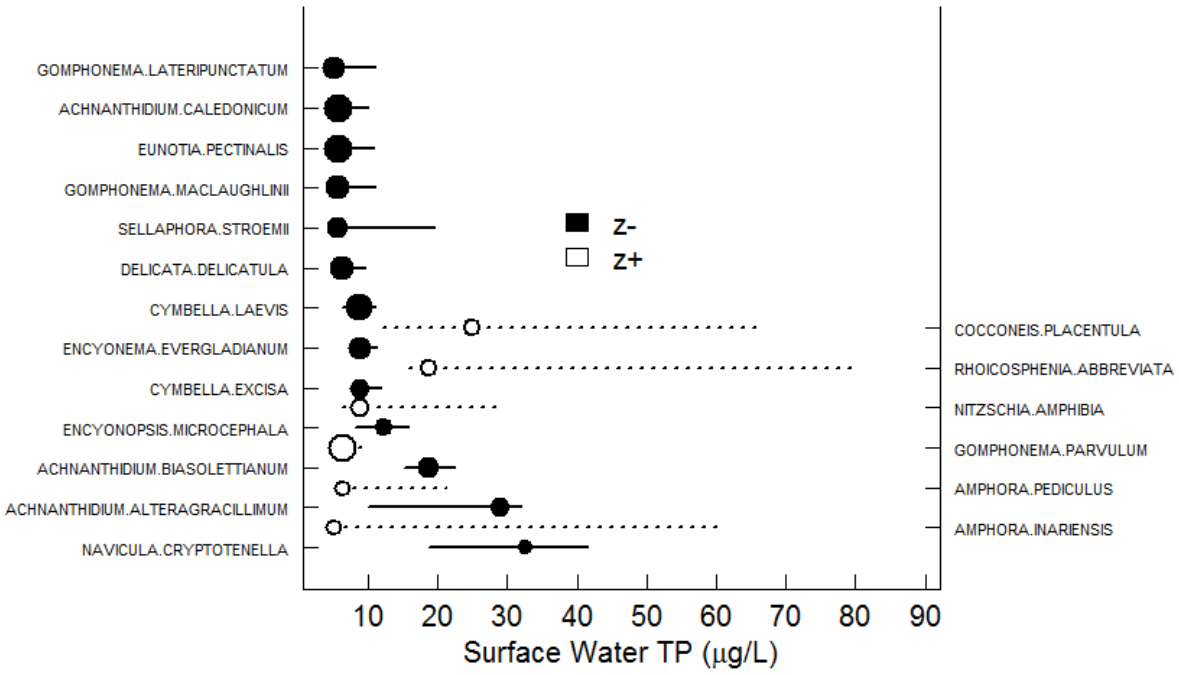

Figure 6: Significant periphyton indicator taxa identified in threshold indicator taxa analysis (TITAN) across surface water TP (µg/L) from 29 sites. Significant indicator taxa are plotted in increasing order with respect to their observed change point. Solid symbols correspond to indicator taxa that declined with increasing TP (z-), while open symbols correspond to those that increased (z+). Horizontal lines overlapping each symbol represent 5th to 95th percentiles among 750 bootstrap replicates.

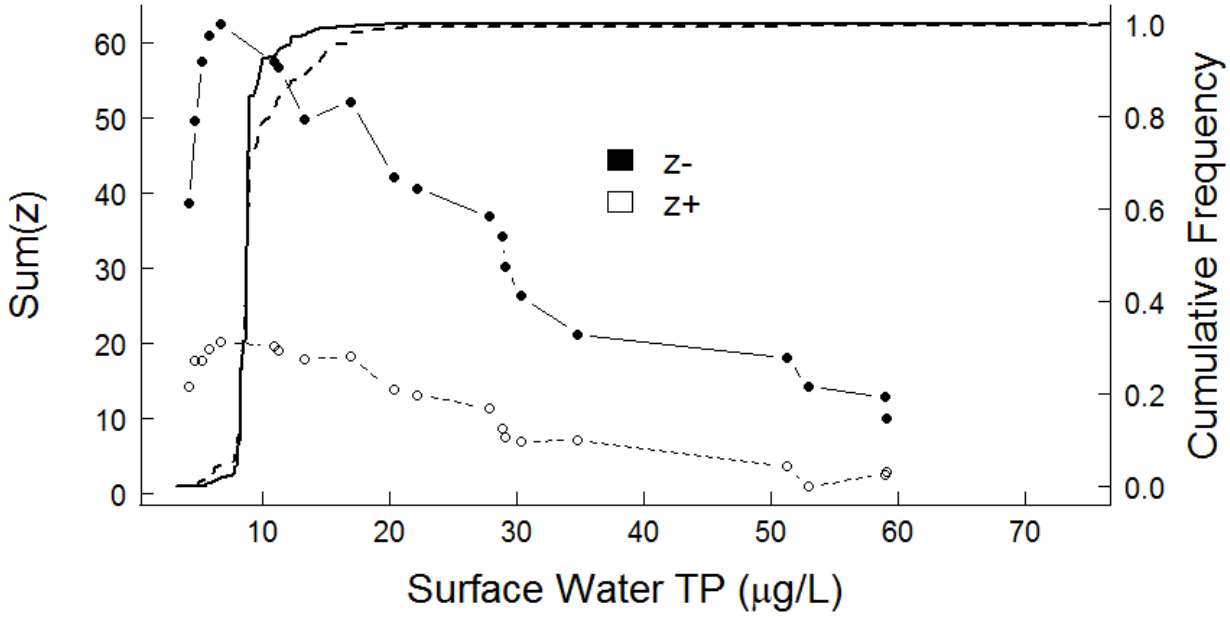

Figure 7: TITAN sum (z-) (aggregate response of negative indicator taxa, black symbols) and sum (z+) (positive indicator taxa, open symbols) are shown in response to an increase in surface water TP concentration. Peak sum (z) scores correspond to the nutrient value resulting in the largest synchronous change among negative and positive indicator taxa, respectively. Solid (negative indictor taxa) and dashed (positive indicator taxa) lines represent the cumulative threshold frequency distribution of the peak sum(z) value obtained among 750 bootstrap replicates for negative and positive indicator taxa, respectively.

In response to increasing TN concentrations, 15 of the 75 taxa evaluated were identified as pure and reliable responders, with 8 taxa declining and 7 taxa increasing (Fig. 8, Appendix C). Assemblage-level analysis showed a threshold of 524.5 µg/L TN (90% CI, 359.5-657.5 µg/L) for all declining species and a threshold of 829.5 µg/L TN (90% CI, 550.0-884.5 µg/L) for all increasing species (Fig. 9).

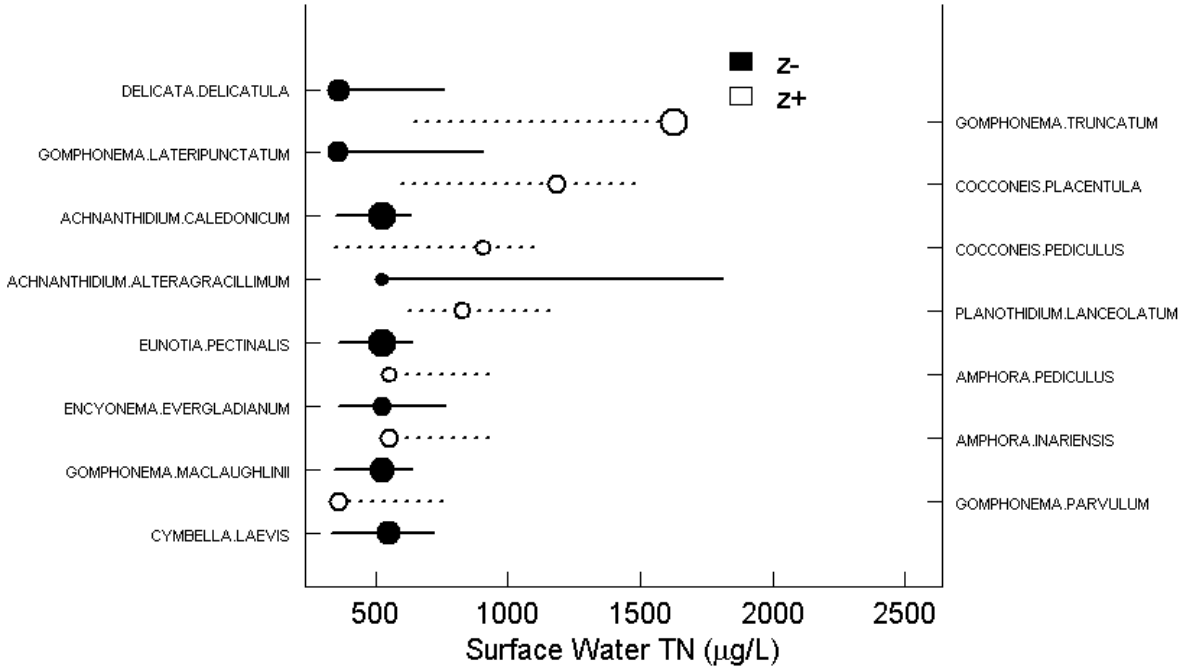

Figure 8: Significant periphyton indicator taxa identified in threshold indicator taxa analysis (TITAN) across surface water TN (µg/L) from 29 sites. Significant indicator taxa are plotted in increasing order with respect to their observed change point. Solid symbols correspond to indicator taxa that declined with increasing TN (z-), while open symbols correspond to those that increased (z+). Horizontal lines overlapping each symbol represent 5th to 95th percentiles among 750 bootstrap replicates.

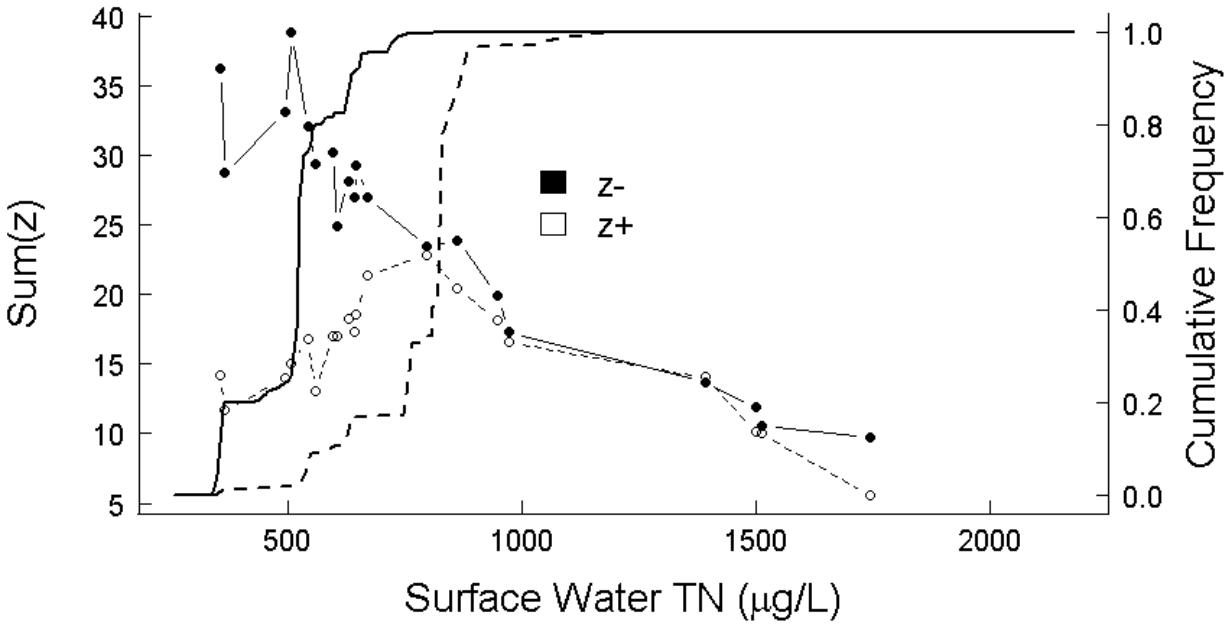

Figure 9: TITAN sum (z-) (aggregate response of negative indicator taxa, black symbols) and sum (z+) (positive indicator taxa, open symbols) are shown in response to an increase in surface water TP concentration. Peak sum (z) scores correspond to the nutrient value resulting in the largest synchronous change among negative and positive indicator taxa, respectively. Solid (negative indictor taxa) and dashed (positive indicator taxa) lines represent the cumulative threshold frequency distribution of the peak sum(z) value obtained among 750 bootstrap replicates for negative and positive indicator taxa, respectively.

Conclusion

Multiple lines of evidence are shown validating the utility of periphyton nutrient concentrations and diatom species composition as sensitive indicators of Austin creek trophic state. We found parsimony between analyses (GAMs, NMDS, TITAN) indicating that phosphorus is the driving nutrient of periphyton ecology, though nitrogen enrichment also impacts diatom species assemblages. While our study was only a single season sampling effort, the corroborative findings suggested TP and TN regulations in the form of a numeric nutrient criteria could be established based on periphyton characteristics. At sites of high ecological integrity (i.e., low nutrient concentrations), changes in periphyton stoichiometry and community composition would provide evidence that ecological integrity is declining irrespective of measured ambient nutrient concentrations that may span a large gradient depending on climatic and hydrologic factors. Conversely, in impacted systems, shifts in periphyton species assemblages could be tracked as evidence of system recovery.

Periphyton stoichiometry suggests P-limitation is common in Austin's creeks. Periphyton from most sites had stoichiometric ratios greater than a suggested balanced C:N:P ratio for algae of 129:22:1; above a C:P ratio of 250 and an N:P ratio of 22, algae are generally considered severely P-limited (Hecky et al. 1993; Sterner 2011). Driven by P-limitation, we observed distinctive break-points in periphyton C:P and N:P stoichiometry along the P gradient. The GAM breakpoint for TP was between 10–20 µg/L TP. Whereas we found relationships between periphyton and TP were stronger, we also observed responses to the nitrogen gradient. The TN change point range was broader based on GAM analysis, with a suggested breakpoint between 500–1200 µg/L. Taylor et al. (2014) similarly found weaker correlations between periphyton stoichiometry with TN concentrations.

Periphyton species composition and abundance responses to the nutrient gradient was evidenced with NMDS and TITAN analyses. Site ordinations provided by NMDS provided a course indication of community similarity among sites and how species composition and relative abundances changed in relation to nutrient concentrations. For example, sites with lower TP and TN concentrations had greater abundances of *Cymbella laevis, Bracysira vitrea, Delicata delicata, Encyonema evergaldianum, E. microcephala, Eucocconeis flexella, Eunotia pectinalis,* and *Mastogloia smithii*. Conversely, *Gomphonema parvulum, Cocconeis placentula, Rhoicosheenia abbreviata, Nitzschia amphibia,* and *Amphora pediculus* were indicators of nutrient enrichment. These species have shown similar nutrient fidelity in periphyton mats sampled from the Great Lakes (Sgro et al. 2007) and rivers across the conterminous United States (Potapova and Charles 2003, 2007). The above taxa and additional species were also found to be sensitive indicators of nutrient enrichment in the TITAN analysis. Species responses to nutrients suggest threshold responses at approximately 9 µg/L TP and 525–830 µg/L TN. Both

thresholds are lower and more constrained than was suggested in the GAM analysis (Taylor et al. 2014). The threshold TP ranges suggested by GAM and TITAN analyses are similar to ecological change point concentrations based on biological communities of the Everglades (10–20 µg/L; McCormick et al. 1996; Hagerthey et al. 2008), mid-Atlantic highland streams (10–12 µg/L; Stevenson et al. 2008), and two north central Texas river basins (20–30 ug/L; Taylor et al. 2014). Unfortunately, the more conservative TP threshold (9 µg/L) exists near what can currently be resolved by standard laboratory reporting limits of detection. The more sensitive laboratory techniques utilized in this study provided TP concentrations at nine sites below the conventional lab detection limits reported by the LCRA Environmental Lab. Nutrient criteria should be protective of the biota that are likely to be lost once exceedance occurs; however, numeric nutrient concentration criteria identified without resolution of concentrations near the critical threshold may not be accurate, defensible, or the most protective.

With biological assessment approaches, it is important to understand potential constraints, limitations, and other driving factors. For example, periphyton nutrient stoichiometry may be influenced by co-nutrient loading or limitations, exposure to shade/irradiance, overflow production of C-rich extracellular products, growth rates, and luxury uptake and storage of a limiting nutrient (Rhee 1973; Droop 1974; Sterner and Elser 2002). To minimize confounding issues, we selected sites with similar flow regimes (e.g., riffles) and that were sun exposed to the greatest extent possible given the existing canopy. Diatom taxonomy is labor and cost intensive, and large datasets of species abundances spanning water quality gradients are typically required to develop effective criteria and autecological inferences. We did not explore relationships between diatom species composition and physical habitat conditions (Pan et al. 2006) or ionic conditions (Hill et al. 2001; Potapova and Charles 2003), which may provide additional thresholds protective of water quality. Recognizing the factors that can influence nutrient criteria establishment, we feel that based on the strength of the relationships observed from this study, the EII dataset going back nearly a decade with determination of stream physicochemical characterization and diatom species composition should be analyzed using similar analytical approaches in this report toward establishment of statistically defensible criteria protective of the biological integrity of Austin's creeks.

Recommendations

- Establish interim water quality objectives for WPD of 8-12 µg/L for TP and 500-600 µg/L for TN in Austin creeks that would be protective of ecological conditions; after further analyses criteria may be developed for different ecoregions across Austin
- Explore statistical methodologies to determine if objectives are being met at the site, reach, or watershed scale based on intra-annual, annual, or multi-year sampling events (cf., Florida Code 62-302.540 http://flrules.elaws.us/fac/62-302.540)
- Analyze broader EII data set for relationships between diatom community composition through time, between ecoregions, and with stream physical and chemical characteristics using multivariate statistics and non-linear models
- Confirm statistically defensible nutrient criteria protective of stream biota based on diatom communities after larger analysis; utilize findings to inform and direct watershed restoration efforts to mitigate eutrophication
- Add periphyton nutrient content and stoichiometry determinations to routine stream monitoring

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Appendix A: Pearson correlation coefficients for periphyton and water column parameters.

Variable	%AC	%CC	%FAC	Vel.	AFDW	Chl-*a*	TC	TN	TP	SW-TC	Cond	DO	NH4	NOx	pH	PO4	Temp	SW-TN	SW-TP
% Algae Cover (%AC)	1.00																		
% Canopy Cover (%CC)	0.19	1.00																	
% Fil. Algae Cover (%FAC)	0.58	-0.01	1.00																
Velocity	-0.26	0.16	-0.24	1.00															
AFDW	0.35	-0.10	0.31	-0.25	1.00														
Chlorphyll-*a*	0.65	0.03	0.36	-0.32	0.67	1.00													
TC	0.13	0.08	-0.28	0.22	0.19	0.28	1.00												
TN	0.03	0.10	0.00	-0.06	-0.25	0.14	0.52	1.00											
TP	0.24	0.24	0.09	0.04	-0.25	0.21	0.37	0.81	1.00										
SW-Carbon	0.02	-0.05	0.41	-0.50	-0.25	-0.17	-0.49	0.09	0.13	1.00									
Conductivity	0.06	0.11	-0.04	0.15	0.10	0.09	-0.03	0.05	0.28	-0.27	1.00								
DO	0.38	-0.19	0.12	-0.02	0.52	0.47	0.14	-0.31	-0.11	-0.41	0.27	1.00							
NH4	0.36	0.21	0.08	-0.04	-0.01	0.31	0.44	0.41	0.45	0.06	0.07	0.01	1.00						
NOx	0.42	0.29	0.25	0.24	0.22	0.51	0.29	0.33	0.58	-0.28	0.54	0.48	0.26	1.00					
pH	-0.02	-0.14	0.05	0.44	-0.18	-0.08	0.10	-0.08	0.13	-0.10	0.15	0.42	0.11	0.38	1.00				
PO4	0.14	0.36	-0.02	0.09	-0.14	0.14	0.33	0.44	0.62	0.12	0.30	-0.04	0.82	0.48	0.24	1.00			
Water Temp.	0.17	-0.12	0.23	0.19	-0.01	0.02	0.13	0.07	0.26	-0.06	0.23	0.39	0.13	0.25	0.34	0.17	1.00		
SW-TN	0.40	0.33	0.32	0.21	0.13	0.41	0.21	0.37	0.67	-0.07	0.53	0.36	0.32	0.97	0.41	0.59	0.27	1.00	
SW-TP	0.22	0.39	0.13	0.07	-0.23	0.10	0.22	0.54	0.78	0.24	0.32	-0.07	0.67	0.58	0.32	0.91	0.16	0.73	1.00

Appendix B: Frequency, z group, z score, changepoint, purity and reliability of species in the TITAN procedure for TP. Purity measures if the change in frequency and abundance of a species is in the same direction for each of the 750 runs. A purity of 0.95 or above was used to define pure species. Reliability measures if the distribution is significantly different from a random distribution for each of the 750 runs. A reliability of 0.95 or above was used to define reliable species.

Species	Freq	Grp	zscore	TP (µg/L)			Purity	Reliability
				Median	5%	95%		
ACHNANTHIDIUM.CALEDONICUM	9	z-	7.67	5.53	5.02	10.03	1.00	1.00
AMPHORA.INARIENSIS	18	z+	3.38	8.83	4.75	58.9	0.98	0.96
AMPHORA.PEDICULUS	19	z+	3.64	9.03	6.28	20.55	0.99	0.98
CALONEIS.BACILLUM	14	z+	2.55	16.8	8.83	60.8	0.94	0.83
COCCONEIS.PEDICULUS	19	z+	2.01	17.75	4.155	59	0.88	0.73
COCCONEIS.PLACENTULA	19	z+	3.6	25	5.735	58.9	1.00	0.99
CYCLOTELLA.MENEGHINIANA	12	z-	1.77	31.25	5.8675	55.05	0.73	0.61
ENCYONEMA.SILESIACUM	25	z+	2.33	5.53	4.75	66.15	0.79	0.72
DENTICULA.KUETZINGII	20	z+	-0.11	43.375	4.58075	66.15	0.65	0.30
FRAGILARIA.CAPUCINA	9	z-	0.43	12.3	3.965	42.95	0.67	0.37
GOMPHONEMA.AFFINE	20	z+	2.4	5.53	4.475	52.05	0.80	0.79
GOMPHONEMA.CLAVATUM	12	z+	1.53	8.55	4.155	32.5	0.58	0.67
GOMPHONEMA.INTRICATUM.V.VIBRIO	6	z-	2.44	28.125	4.985	31.525	0.97	0.69
GOMPHONEMA.PARVULUM	24	z+	5.77	6.28	5.255	9.03	0.98	1.00
NAVICULA.MINIMA	13	z+	1.96	8.83	4.475	59	0.73	0.75
NAVICULA.RADIOSA	14	z-	0.56	25	5.255	58.9	0.53	0.49
NAVICULA.KOTSCHYI	13	z-	2.13	28.45	4.985	36.2875	0.67	0.79
NITZSCHIA.AMPHIBIA	27	z+	3.92	15.1	6.28	29.55	1.00	1.00
NITZSCHIA.DISSIPATA	5	z+	1.3	18.6	9.03	42.95	0.54	0.32
REIMERIA.SINUATA	18	z+	2.73	8.83	6.28	55.9	0.93	0.88
RHOICOSPHENIA.ABBREVIATA	12	z+	3.73	18.6	16.55	79.35	1.00	0.99
SURIRELLA.ANGUSTA	4	z+	1.5	21.25	14.1	40	0.66	0.29
NITZSCHIA.AMPHIBIOIDES	22	z-	2.33	29.7	5.8945	60.8	0.86	0.78
NITZSCHIA.INCONSPICUA	13	z+	2.49	11.3	6.28	79.35	0.97	0.91
NAVICULA.CRYPTOTENELLA	12	z-	4.19	28.95	18.6	41.2625	0.97	0.98
ACHNANTHIDIUM.MINUTISSIMUM	20	z-	2.88	29.7	4.475	64.45	0.88	0.83
ACHNANTHIDIUM.ALTERAGRACILLIMUM	21	z-	5.21	19.55	9.55	31.9	1.00	1.00
NAVICULA.ANTONII	10	z+	2.78	8.83	8.08	66.15	0.95	0.80
DELICATA.DELICATULA	13	z-	6.17	6.28	5.5	9.4285	1.00	1.00
ULNARIA.ULNA	19	z-	3.02	55.05	4.475	56	0.71	0.96
CYMBELLA.TURGIDULA	11	z-	2.01	18.6	4.475	40.26	0.61	0.74
GOMPHONEMA.KOBAYASII	10	z+	0.85	25.5	6.28	60.8	0.66	0.35
ACHNANTHIDIUM.BIASOLETTIANUM	11	z-	5.62	18.6	15.1	22.35	1.00	1.00
CYMBELLA.LAEVIS	9	z-	7.01	8.83	6.28	10.9	1.00	1.00
ENCYONOPSIS.MICROCEPHALA	9	z-	4.5	10.03	8.35	15.8	0.99	0.97
GOMPHONEMA.GRACILE	7	z+	1.43	12.3	6.01	60.8	0.72	0.37
CYMBELLA.NORVEGICA	5	z-	1.55	8.83	4.475	60.8	0.68	0.46
EUNOTIA.PECTINALIS	7	z-	7.56	8.08	5.255	10.03	1.00	1.00
MELOSIRA.VARIANS	9	z+	1.3	14.1	6.01	40.75	0.73	0.52
ENCYONEMA.EVERGLADIANUM	11	z-	6.29	8.83	8.08	11.1	1.00	1.00

Species	Freq	Grp	zscore	TP (µg/L)			Purity	Reliability
				Median	5%	95%		
GOMPHONEMA.MACLAUGHLINII	8	z-	6.36	5.8	4.155	10.03	1.00	0.99
CYMBELLA.EXCISA	7	z-	5.01	8.83	8.08	11.3275	1.00	0.98
ULNARIA.ACUS	10	z-	0.51	18.175	4.155	59	0.62	0.35
CYMBELLA.CISTULA	4	z-	2.41	8.83	4.985	28.45	0.96	0.58
PLANOTHIDIUM.LANCEOLATUM	8	z+	1.91	15.1	9.03	66.15	0.90	0.70
AMPHORA.OVALIS	9	z+	1.2	25	4.985	64.45	0.84	0.53
TABULARIA.FASCICULATA	7	z+	3.46	28.95	9.8715	72.11	0.99	0.88
GOMPHONEMA.ACUMINATUM	4	z+	2.28	21.25	17.75	48.4275	0.82	0.42
GOMPHONEMA.MINUTUM	5	z+	1.21	19.925	8.35	55.05	0.80	0.29
CYMBELLA.SUBLEPTOCEROS	5	z-	2.42	15.1	4.42825	40.75	0.94	0.62
HALAMPHORA.MONTANA	6	z+	1.14	18.6	8.35	60.8	0.65	0.32
GOMPHONEMA.TRUNCATUM	8	z+	1.23	18.6	5.53	66.15	0.76	0.44
NITZSCHIA.PALEA	4	z+	1.48	21.25	14.1	40.15	0.66	0.30
NAVICULA.RECENS	5	z+	1.09	24.7	4.155	56	0.75	0.36
NAVICULA.TRIVIALIS	11	z+	2.67	8.83	8.08	55.9	0.98	0.84
GOMPHONEMA.OLIVACEUM	5	z+	3.75	52.05	16.8	64.35	0.98	0.77
DIPLONEIS.OBLONGELLA	5	z-	0.78	25	4.475	74	0.55	0.38
TRYBLIONELLA.APICULATA	7	z+	2.1	21.25	9.03	75.7	0.97	0.68
RHOPALODIA.GIBBA	6	z+	2.5	44	4.985	60.8	0.89	0.75
AMPHORA.COPULATA	5	z+	1.42	42.95	6.28	75.7	0.63	0.50
DIPLONEIS.PUELLA	5	z+	2.71	55.15	11.1	56	0.86	0.70
NITZSCHIA.FRUSTULUM	5	z+	1.73	29	9.03	56	0.87	0.47
PINNULARIA.MICROSTAURON	5	z-	0.21	15.6	4.39	59	0.59	0.20
CYMBELLA.KOLBEI	4	z-	0.21	19.55	4.05	59	0.56	0.28
CALONEIS.VENTRICOSA	6	z+	1.91	29.7	8.83	59	0.93	0.57
NAVICULA.MENISCULUS	5	z+	1.42	21.25	9.03	54.0825	0.56	0.33
NAVICULA.ERIFUGA	7	z+	1.43	44	4.985	60.8	0.78	0.47
NAVICULA.VIRIDULA.V.ROSTELLATA	4	z+	1.02	29.1	9.03	79.35	0.77	0.36
GOMPHONEMA.LATERIPUNCTATUM	9	z-	5.74	6.28	4.75	11.1	1.00	0.98
MASTOGLOIA.SMITHII	4	z-	6.36	4.75	4.39	31.0975	0.95	0.79
EUCOCCONEIS.FLEXELLA	4	z-	8.53	4.75	4.39	8.83	0.99	0.93
BRACHYSIRA.VITREA	4	z-	6.98	5.53	4.475	8.83	0.98	0.90
SELLAPHORA.STROEMII	9	z-	6.02	5.8	5.255	19.55	0.99	0.99
SELLAPHORA.PUPULA	6	z-	0.94	28.3	5.53	79.35	0.54	0.39
LUTICOLA.GOEPPERTIANA	4	z+	1.54	18.6	12.1	79.35	0.68	0.23

Appendix C: Frequency, z group, z score, changepoint, purity and reliability of species in the TITAN procedure for TN. Purity measures if the change in frequency and abundance of a species is in the same direction for each of the 750 runs. A purity of 0.95 or above was used to define pure species. Reliability measures if the distribution is significantly different from a random distribution for each of the 750 runs. A reliability of 0.95 or above was used to define reliable species.

Species	Freq	Grp	zscore	TN (µg/L)			Purity	Reliability
				Median	5%	95%		
ACHNANTHIDIUM.CALEDONICUM	9	z-	6.74	524.5	348.5	635	1.00	1.00
AMPHORA.INARIENSIS	18	z+	3.96	599.5	525.5	946	1.00	0.99
AMPHORA.PEDICULUS	19	z+	3.4	635	525.5	959	0.99	0.97
CALONEIS.BACILLUM	14	z+	1.42	622.5	472.925	1815.75	0.56	0.61
COCCONEIS.PEDICULUS	19	z+	3.36	904	344	1126.25	0.98	0.95
COCCONEIS.PLACENTULA	19	z+	4.07	1181.25	597.575	1505.25	1.00	0.99
CYCLOTELLA.MENEGHINIANA	12	z+	1.09	904	428.5	2034.112	0.71	0.41
ENCYONEMA.SILESIACUM	25	z+	2.94	500	431	1505.25	0.69	0.96
DENTICULA.KUETZINGII	20	z+	2.31	829.5	566.55	1748.25	0.93	0.77
FRAGILARIA.CAPUCINA	9	z-	2.6	615.5	355	878.65	0.93	0.77
GOMPHONEMA.AFFINE	20	z+	2.03	569	348.5	1626.75	0.89	0.74
GOMPHONEMA.CLAVATUM	12	z+	0.39	635	344	1815.75	0.62	0.41
GOMPHONEMA.INTRICATUM.V.VIBRIO	6	z-	1	593	428.5	1626.75	0.79	0.45
GOMPHONEMA.PARVULUM	24	z+	3.86	577	348.5	765.5	0.99	0.98
NAVICULA.MINIMA	13	z+	2.7	615.5	428.5	1620	0.98	0.90
NAVICULA.RADIOSA	14	z-	-0.13	721.5	359.5	2153.25	0.61	0.44
NAVICULA.KOTSCHYI	13	z+	1.64	615.5	435.5	1626.75	0.90	0.65
NITZSCHIA.AMPHIBIA	27	z+	2.54	524.5	348.5	1512	0.92	0.78
NITZSCHIA.DISSIPATA	5	z+	1.14	644	550	1502.213	0.74	0.26
REIMERIA.SINUATA	18	z+	1.47	599.5	348.5	1815.75	0.73	0.51
RHOICOSPHENIA.ABBREVIATA	12	z+	1.65	1181.25	428.5	1809	0.89	0.58
SURIRELLA.ANGUSTA	4	z-	1.34	644	550	917	0.66	0.25
NITZSCHIA.AMPHIBIOIDES	22	z+	2.94	733	615.5	1502.213	0.95	0.88
NITZSCHIA.INCONSPICUA	13	z+	2.25	635	500	1745.213	0.94	0.87
NAVICULA.CRYPTOTENELLA	12	z-	1.1	655.5	364	1748.25	0.65	0.55
ACHNANTHIDIUM.MINUTISSIMUM	20	z-	0.79	878	325.5	1809	0.50	0.47
ACHNANTHIDIUM.ALTERAGRACILLIMUM	21	z-	3.02	904	517.5	1809	0.99	0.97
NAVICULA.ANTONII	10	z+	3.41	635	532.5	1474.2	0.99	0.93
DELICATA.DELICATULA	13	z-	5.15	500	348.5	760.325	1.00	0.99
ULNARIA.ULNA	19	z+	2.7	435.5	424	1784.7	0.78	0.71
CYMBELLA.TURGIDULA	11	z+	1.73	635	428.5	1755	0.82	0.59
GOMPHONEMA.KOBAYASII	10	z-	1.4	580.5	364	1876.5	0.74	0.43
ACHNANTHIDIUM.BIASOLETTIANUM	11	z+	0.28	1126.25	428.5	2430	0.51	0.45
CYMBELLA.LAEVIS	9	z-	5.52	550	333	721.5	1.00	1.00
ENCYONOPSIS.MICROCEPHALA	9	z-	2.01	721.5	452.1	1626.75	0.92	0.67
GOMPHONEMA.GRACILE	7	z-	1.72	1168.25	500	1235.25	0.63	0.45
CYMBELLA.NORVEGICA	5	z-	2.75	599.5	359.5	959	0.97	0.66
EUNOTIA.PECTINALIS	7	z-	6.77	524.5	359.5	636.1	1.00	0.99
MELOSIRA.VARIANS	9	z+	1.32	635	500	1626.75	0.67	0.48
ENCYONEMA.EVERGLADIANUM	11	z-	4.54	599.5	359.5	765.5	1.00	0.99

Species	Freq	Grp	zscore	TN (µg/L)			Purity	Reliability
				Median	5%	95%		
GOMPHONEMA.MACLAUGHLINII	8	z-	5.93	524.5	344	637	1.00	1.00
CYMBELLA.EXCISA	7	z-	2.43	657.5	517.5	1087.087	0.97	0.75
ULNARIA.ACUS	10	z-	1.19	719.5	346.025	1505.25	0.73	0.45
CYMBELLA.CISTULA	4	z-	3.04	524.5	355	1620	0.86	0.57
PLANOTHIDIUM.LANCEOLATUM	8	z+	3.73	829.5	622.5	1168.25	1.00	0.97
AMPHORA.OVALIS	9	z+	1.62	619	517.5	1755	0.82	0.55
TABULARIA.FASCICULATA	7	z+	4.46	904	596	1126.25	0.99	0.89
GOMPHONEMA.ACUMINATUM	4	z+	1.97	807.5	601.35	1566	0.91	0.45
GOMPHONEMA.MINUTUM	5	z+	0.17	642	428.5	1755	0.53	0.28
CYMBELLA.SUBLEPTOCEROS	5	z+	-0.04	657.5	325.5	2153.25	0.62	0.33
HALAMPHORA.MONTANA	6	z+	1.49	635	569	1168.25	0.76	0.42
GOMPHONEMA.TRUNCATUM	8	z+	5.8	1620	644	1633.5	1.00	0.99
NITZSCHIA.PALEA	4	z+	1.22	644	580.5	1168.25	0.56	0.23
NAVICULA.RECENS	5	z+	0.33	635	344	1505.25	0.38	0.19
NAVICULA.TRIVIALIS	11	z+	2.19	577	551.5	1505.25	0.92	0.74
GOMPHONEMA.OLIVACEUM	5	z+	1.31	1181.25	428.5	2153.25	0.73	0.52
DIPLONEIS.OBLONGELLA	5	z-	1.19	636	339.625	1748.25	0.79	0.43
TRYBLIONELLA.APICULATA	7	z+	2.55	619	574.5	1626.75	0.97	0.67
RHOPALODIA.GIBBA	6	z-	1.76	599.5	503.15	1633.5	0.82	0.50
AMPHORA.COPULATA	5	z+	1.75	644	612	1626.75	0.91	0.47
DIPLONEIS.PUELLA	5	z+	1.4	655.5	569	1512	0.68	0.27
NITZSCHIA.FRUSTULUM	5	z+	1.39	635	569	1512	0.77	0.28
PINNULARIA.MICROSTAURON	5	z+	0.24	733	333	2125.913	0.61	0.27
CYMBELLA.KOLBEI	4	z+	1.23	829.5	325.5	2125.913	0.75	0.45
CALONEIS.VENTRICOSA	6	z+	1.36	599.5	569	1444.5	0.61	0.34
NAVICULA.MENISCULUS	5	z+	2.42	635	619	1181.25	0.91	0.48
NAVICULA.ERIFUGA	7	z+	1.81	615.5	569	1126.25	0.70	0.59
NAVICULA.VIRIDULA.V.ROSTELLATA	4	z+	2.5	657.5	648.5	1755	0.95	0.45
GOMPHONEMA.LATERIPUNCTATUM	9	z-	4.83	500	325.5	904	1.00	0.97
MASTOGLOIA.SMITHII	4	z-	5.31	431	323	574.975	0.98	0.86
EUCOCCONEIS.FLEXELLA	4	z-	8.41	424	348.5	558	0.99	0.91
BRACHYSIRA.VITREA	4	z-	5.48	424	348.5	637	0.99	0.79
SELLAPHORA.STROEMII	9	z-	2.74	517.5	348.5	1181.25	0.95	0.78
SELLAPHORA.PUPULA	6	z+	3.11	635	619	1626.75	0.93	0.68
LUTICOLA.GOEPPERTIANA	4	z+	3.9	1566	754	1815.75	0.99	0.74