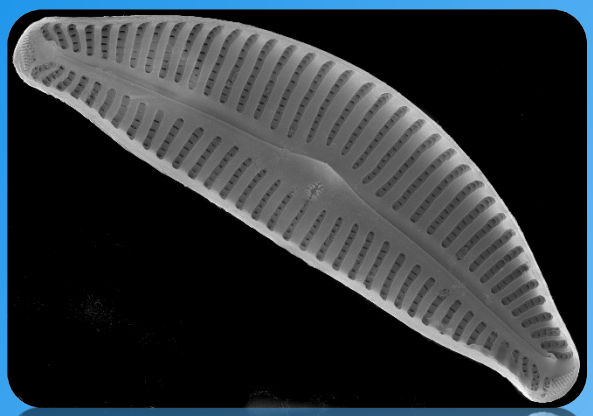

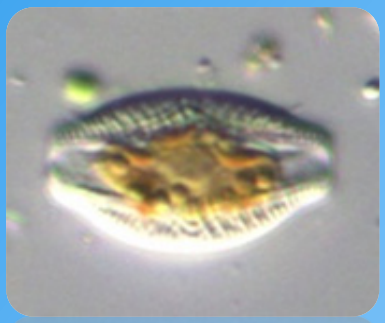

Periphyton Assemblages and Associated Environmental Conditions in the Klamath River 2004-2013

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Photo credits for cover page (clockwise from upper-right):

A. Trinity River at Weitchpec 7/17/2013 (Yurok Tribe Environmental Program); B. *Calothrix* sp. (National Institute of Environmental Science, Japan); C. Collecting periphyton sample at Klamath River at Weitchpec 7/17/2013 (Yurok Tribe Environmental Program); D. *Epithemia sorex* (Paula Furey); E. C*ymbella_affinis* (Potapova, M. 2011. *Cymbella affinis*. In Diatoms of the United States. http://westerndiatoms.colorado.edu/taxa/species/cymbella_affinis).

EXECUTIVE SUMMARY

This report is the second phase of a long-term analysis of periphyton data collected in the years 2004-2013 on the lower and middle Klamath River (i.e., between Iron Gate Dam and Turwar, just upstream of the Klamath Estuary) in California, as well as the lower Trinity River by the Yurok, Hoopa, and Karuk Tribes, and other entities. In the first report, we examined the longitudinal, seasonal, and inter-annual patterns in periphyton species composition and biomass. In this report, we evaluate hypotheses regarding the importance of various environmental factors controlling the temporal and longitudinal dynamics of periphyton communities.

Periphyton, also known as benthic algae, are algae growing attached to river substrates such as cobbles, sand, and aquatic plants. Periphyton are valuable indicators of ecosystem status due to their ecological and biogeochemical importance, sensitivity to human-induced changes in water quality, and ubiquitous distribution. Photosynthesis and respiration by periphyton and aquatic plants can degrade dissolved oxygen and pH, resulting in water quality conditions that are chronically stressful to fish.

Periphyton samples from river cobbles were collected approximately monthly from June through October at 11 long-term monitoring sites. Using a microscope, the algal species in each sample were identified, enumerated, and biomass was calculated (technically 'biovolume' but to facilitate understanding of this report by a general audience, we primarily use the term 'biomass' rather than 'biovolume'). Periphytic chlorophyll *a* concentrations were also quantified. A comprehensive database is included as an electronic appendix.

Although primarily the focus of the Phase I report, the addition of 2013 data necessitated reanalysis of longitudinal, seasonal, and inter-annual patterns in periphyton species composition and biomass. Results were similar with a total of 150 species identified in the 398 samples collected at the long-term monitoring sites. Periphyton assemblages in the Klamath River were dominated by diatoms, which on average comprised 92.5% of relative biomass (as estimated from biovolume measurements), followed by cyanobacteria (6.0%) and green algae (1.5%).

Cluster analysis of periphyton assemblages identified three statistically different periphyton groups (denoted Groups 1 through 3), each occupying distinct reaches and months. Group 3 occurred primarily in the upstream reach (river miles 190 to 160: Iron Gate Dam, Interstate-5, and Quigley's) for June through October. All groups were dominated by attached diatom species, but relative to other groups, Group 3 had a higher percentage of sestonic (i.e., free-floating, not attached) species, including the cyanobacteria *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*, consistent with the presence of Iron Gate and Copco reservoirs upstream. Sites in the middle reach (river miles 129 to 100: Seiad Valley and Happy Camp) fell either into Group 3 or Group 2 depending on season (Group 3 more prevalent in July-October). Group 2 had the highest relative biomass of diatoms and lowest relative biomass of cyanobacteria. Sites in the lower reach of the Klamath River (river miles 60 to 6) and the Trinity River typically fell into Group 2 in May-June and transitioning into Group 1 for July through October. Group 1 was dominated by nitrogen-fixing species, including three diatoms (*Epithemia sorex*, *Epithemia turgida*, and *Rhopalodia gibba*) with cyanobacterial endosymbionts (i.e., cyanobacteria living inside the diatom) as well as the cyanobacterium *Calothrix* sp.

To examine longitudinal, seasonal, and inter-annual patterns in periphyton community composition, we grouped species into functional groups (e.g., nitrogen-fixers, etc.) and used multivariate statistical techniques such as Non-metric Multidimensional Scaling (NMDS) and cluster analysis. Species composition and biomass were then related to environmental conditions (e.g., streamflow, nutrients, water temperature, and air temperature, compiled from a variety of data sources) using graphical comparisons, "envfit" analysis, classification and regression trees (CART), bivariate regressions, and mixed-effects models.

Periphyton assemblages in the Klamath River were strongly associated with temporal variations in flow conditions (e.g., decreasing flow from spring to fall) and spatial gradients in nutrient concentrations (e.g., decreasing from upstream to downstream). In the NMDS "envfit" analysis, the difference between the upstream (Group 3) and downstream periphyton assemblage structures (Groups 1 and 2) was associated with low nutrient concentrations (r^2>0.6 for TN, SRP, and TP, and r^2 = 0.47 for nitrate-nitrite), while site-normalized flow largely separated the two downstream groups (downstream sites sampled in spring and early summer [Group 2] vs. downstream sites sampled in summer and fall [Group 1], r^2=0.56). The classification tree model further illustrated the interactive effects of nutrients and flow on periphyton assemblages showing that periphyton assemblages in downstream sites sampled in summer and fall (Group 1) were associated with more stable hydrological conditions (<54% median flow). Groups 2 and 3 were characterized by higher site-normalized flows (> 54.5% of annual median flow), and were differentiated from each other by SRP concentrations (Group 2 < 0.035 mg/L and Group 3 > 0.035 mg/L).

In the mixed-effects models, nitrate-nitrite concentrations and site-normalized flow were the best predictors of percent benthic nitrogen-fixing periphyton biomass (adjusted r^2 of 0.44). Results from bivariate regressions support the same conclusions, with nitrate-nitrite concentrations explaining *longitudinal variation* and flow explaining *temporal variation* within sites. For example, nitrate-nitrite was inversely associated with relative benthic nitrogen-fixing biomass between sites for both individual samples (r^2=0.34) and for June-September seasonal means (r^2 = 0.74; p<0.00001 for both). For downstream stations where relative benthic N-fixer biomass was generally higher and flow was less moderated by impoundments (Orleans, Saints Rest Bar, Weitchpec, and Turwar), flow was strongly inversely related to relative benthic N-fixer biomass on an inter-annual basis (r^2 = 0.73; p = 0.003).

Although statistically significant, environmental variables explained less of the variation in *total periphyton biomass* metrics (i.e., algal biovolume or periphytic chlorophyll *a*) than they did for the *composition of periphyton assemblages* (i.e., percent benthic nitrogen-fixing periphyton biomass, or the three groups from the cluster analysis). For example, the regression trees r^2 value was 0.50 for predicting percent benthic nitrogen-fixing periphyton biomass but was 0.25 for periphytic chlorophyll *a* and 0.21 for periphyton biovolume. In the mixed effects models and regression trees, the best predictor of periphytic chlorophyll *a* and periphyton biovolume was site-normalized flow (e.g., higher biomass at lower flows), with other significant predictors including nitrate-nitrite (higher biomass at lower concentrations) and air temperature (higher biomass at lower temperatures).

Despite lower nutrient concentrations at downstream sites overall periphyton biomass (and to a lesser extent, periphytic chlorophyll *a* concentrations) was higher at downstream sites than at

upstream sites. This inverse longitudinal relationship between periphyton biomass and nutrients may be explained by the ability of benthic N-fixers (Group 1) to overcome nitrogen limitation. In addition, because the sampling protocol targets microscopic algae from cobble substrates which may adequately characterize downstream periphyton assemblages but not the extensive amounts of filamentous algae (e.g., *Cladophora* sp.) and macrophytes which are present in upstream reaches, the nutrient effect at upstream stations is underestimated.

Overall results showed a strong inverse relationship between the relative abundance of N-fixers and nitrogen concentrations in the Klamath River, and flow was consistently a significant explanatory variable for periphyton biomass metrics in CART, multiple regression, and mixed-effects models. In addition, when seasonal co-variation in flow and development of periphytic biomass is accounted for, we observed a strong inverse effect of flow on relative N-fixer biomass.

The long-term data described in this report provides valuable insight into seasonal and longitudinal patterns of benthic algal communities in the middle and lower Klamath River system. Our evaluation of the linkages between environmental variables and the composition of periphyton assemblages and biomass can inform river management decisions such as reducing upstream nutrient loads, setting flow regimes, and potential dam removals.

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1 INTRODUCTION

1.1 DESCRIPTION OF STUDY AREA

The Klamath River is one of the major salmon rivers of the western United States. Its uppermost tributaries originate in southern Oregon and drain into Upper Klamath Lake, the Link River and Lake Ewauna, where the Klamath River proper begins. From this point, the river flows through a series of impoundments, including Keno, J.C. Boyle, Copco, and Iron Gate Reservoirs. Below Iron Gate Dam, the river flows 190 miles to the Pacific Ocean, mostly through a confined canyon. The climate is Mediterranean, with cool wet winters and springs featuring rainfall at lower elevations and snow at higher elevations, and hot dry summers that are moderated in downstream reaches by a cooling maritime influence.

This study focuses on the lower and middle mainstem Klamath River (i.e., between Iron Gate Dam and Turwar, just upstream of the Klamath Estuary), as well as the Trinity River which is the largest tributaries to this reach (Figure 1).

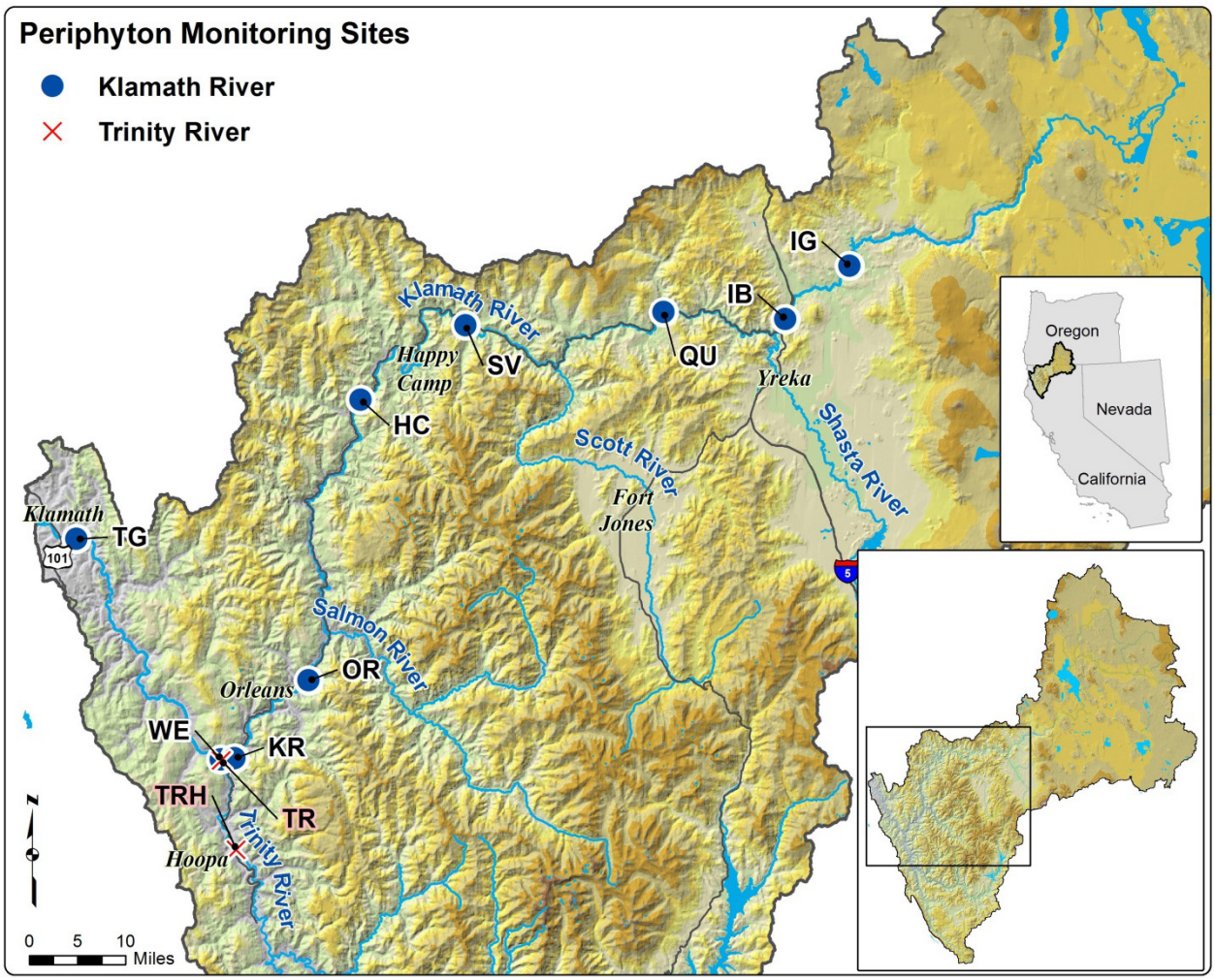

Figure 1. Location of long-term periphyton monitoring sites on the Klamath and Trinity rivers.

1.2 BACKGROUND

The Klamath River and some of its tributaries are designated on the Clean Water Act (CWA) Section 303(d) list as impaired water bodies. The list of impairments varies by state and reaches within states, but includes pH (only in Oregon reservoirs), water temperature, nutrients, organic enrichment/low dissolved oxygen (DO), sedimentation/siltation, ammonia toxicity, microcystin, and chlorophyll *a* (NCRWQCB 2010). Total Maximum Daily Loads (TMDLs) have developed for the river and its tributaries by the U.S. EPA, Oregon Department of Environmental Quality (ODEQ 2010) and the North Coast Regional Water Quality Control Board (NCRWQCB 2010).

Water quality is a concern in the Klamath River because it affects culturally and economically important salmon fisheries as well as public health. During the warm summer months, dissolved oxygen and pH follow a 24-hour cycle in which photosynthesis by aquatic plants and algae attached to the streambed (periphyton) elevates pH and dissolved oxygen concentrations during the day. Respiration at night by those same organisms has the reverse effect, depressing dissolved oxygen and pH (Nimick et al. 2011). The resulting low nighttime DO and high daytime pH can exceed water quality standards and be stressful to fish (NCRWQCB 2010). NCRWQCB (2010) established a periphyton biomass numeric target of 150 mg of chlorophyll *a*/m^2 as a seasonal maximum reach-average for the Klamath River mainstem downstream of the Salmon River.

Periphyton communities are known to be valuable indicators of ecosystem status due to their ecological and biogeochemical importance, their sensitivity to human-induced changes in water quality, and their ubiquitous distribution across ecosystems (e.g., McCormick and Stevenson 1998). Predictable relationships between periphyton abundance, taxonomic composition, nutrient content and water quality have been identified in a variety of systems, including their effect on large diel fluctuations in pH and dissolved oxygen. For example, nutrient enrichment of the South Umpqua River, Oregon was linked to periphyton growth and large diel fluctuations in dissolved oxygen and pH concentrations (Turner et al. 2009). In addition to contributing to large fluctuations in water quality, periphyton assemblages also reflect flow, nutrient, riparian, substrate, and land-use condition (e.g., Hart et al. 2013; Stancheva et al. 2013; Weilhoefer and Pan et al. 2006; Pan et al. 2004; Biggs and Smith 2002). Pan et al. (2006) showed that benthic diatom assemblages were affected by channel morphology, instream habitat, and riparian conditions, and many studies have shown the effect of nutrients such as nitrogen and phosphorus on benthic algal composition (e.g., Wagenhoff et al. 2013; Wu et al. 2009; Dodds et al. 2002).

Thus, an understanding of periphyton community dynamics as well as long-term trends in benthic algae can inform both important aspects of water quality dynamics and potential management actions to improve water quality. Given the established role of periphyton as drivers of water quality (described above), Tribes and other entities (see below) began monitoring periphyton in the Klamath River in 2004. Because these data had not yet been analyzed in a detailed fashion, Klamath River Tribal Water Quality Work Group provided funds for Phase 1 of initial comprehensive analysis of the Klamath River long-term periphyton monitoring dataset (Asarian et al. 2014).

1.3 STUDY GOALS

This study is a follow-up to Asarian et al. (2014), which examined the longitudinal, seasonal, and inter-annual patterns in periphyton species composition and biomass in the Klamath River in 2004-2012. The overall goal of this study was to evaluate hypotheses regarding the importance of various environmental controlling factors on the temporal and longitudinal dynamics of the periphyton communities in the Klamath River for 2004-2013.

2 METHODS

2.1 PERIPHYTON SAMPLING SITES AND SAMPLING METHODS

A full description of sampling methods and lab analyses can be found in Asarian et al. (2014), but are briefly summarized here.

Periphyton samples were collected at eleven long-term monitoring sites, including nine in the Klamath River and two in the Trinity River, in the years 2004 and 2006-2013 (Figure 1 and Table 1). Additional sites were sampled in some years, including special studies using different sampling protocols, and although those results are not discussed in this report, the comprehensive dataset is included as electronic Appendix E. The number of sites sampled per year ranged from three to eleven, with samples generally collected at a monthly frequency. The length of the sampling season varied by year and ranged between May and November. All periphyton samples were collected from 1 to 5 cobbles in each site. Sampling entities included the Yurok Tribe, Hoopa Tribe, Karuk Tribe, Watercourse Engineering Inc., MaxDepth Aquatics, and the North Coast Regional Water Quality Control Board (NCRWQCB).

Table 1. Site characteristics and environmental data sources for long-term periphyton monitoring sites and on the Klamath and Trinity rivers.

	Site Description	Site Code	River Mile	Latitude	Longitude	Drain. Area (km^2)	Elev. (ft)	Periphyton Data Source by Year	Nutrient Data Source by Year	Water Temperature Source by Year	Meteorology Location/ Source
Klamath River Sites	KR below Iron Gate	IG	189.73	41.931083	-122.442200	11,992	2169	Watercourse/NCRWQCB/ MaxDepth 2004; Watercourse 2007; Karuk 2008, 2011-2013	USFWS 2004; Watercourse/ PacifiCorp 2007; Karuk 2007-2008, 2011-2013	USFWS 2004; Karuk 2007-08, 2011-2013; USFWS for gaps	Brazie Ranch/ CalFIRE
	KR at Interstate 5 Bridge	IB	179.00	41.831110	-122.591940	12,553	2028	Watercourse/NCRWQCB/ MaxDepth 2004; Watercourse 2007-2008; Karuk 2009, 2010,2011,2013	USFWS 2004; Watercourse/ PacifiCorp 2007; Karuk 2007-2008, 2011-2013	USFWS 2004; Karuk 2007-08, 2011-2013; USFWS for gaps	Brazie Ranch/ CalFIRE
	KR at Quigley's	QU	160.50	41.837367	-122.864917	15,225	1686	Watercourse/NCRWQCB/ MaxDepth 2004; Watercourse 2007; Karuk 2008, 2011-2013	USFWS 2004; Watercourse/ PacifiCorp 2007; Karuk 2007-2008, 2011-2013	USFWS 2004, 2007-2008, 2011-2013	Oak Knoll/ USFS
	KR at Seiad Valley	SV	128.58	41.842683	-123.218867	17,975	1355	Watercourse/NCRWQCB/ MaxDepth 2004; Watercourse 2007; Karuk 2008, 2011-2013	Karuk 2004, 2007-2008, 2011-2013	Karuk 2004, 2007-2008, 2011-2013 USFWS for gaps	Oak Knoll/ USFS
	KR at Happy Camp	HC	100.66	41.729667	-123.429583	20,846	921	Watercourse/NCRWQCB/ MaxDepth 2004; Karuk 2011-2013	Karuk 2004, 2011-2013	Karuk 2004; USFWS 2011-2013	Somes Bar/ USFS
	KR at Orleans	OR	59.12	41.305600	-123.531583	21,950	358	Watercourse/NCRWQCB/ MaxDepth 2004; Watercourse 2007; Karuk 2008, 2011-2013	Karuk 2004, 2007-2008, 2011-2013	Karuk 2004, 2007-2008, 2011-2013; USFWS for gaps	Somes Bar/ USFS
	KR at Saints Rest Bar	KR	44.90	41.187520	-123.678001	22,617	221	Hoopa 2008-2013	Hoopa 2008-2013	[used WE data]	Notcho/ Yurok Tribe
	KR at Weitchpec (abv Trin. R.)	WE	43.50	41.185833	-123.705556	22,611	194	Yurok/NCRWQCB 2004; Yurok 2006-2013	Yurok/NCRWQCB 2004; Yurok 2006-2013	Yurok 2004, 2006-13 USFWS for gaps	Notcho/ Yurok Tribe
	KR at Turwar	TG	5.79	41.516111	-123.999167	31,339	22	Yurok/NCRWQCB 2004; Yurok 2006-2013	Yurok/NCRWQCB 2004; Yurok 2006-2013	Yurok 2004, 2006-2013; USGS for gaps	Notcho/ Yurok Tribe
Trinity R. Sites	Trinity River at Hoopa	TRH	43.4 +12.4	41.049852	-123.673668	7389	280	Hoopa 2008-2013	Hoopa 2008-2013	USGS 2008-2013	Hoopa/ Hoopa Tribe
	Trinity R. near Weitchpec	TR	43.4 +0.5	41.184444	-123.705278	7685	192	Yurok/NCRWQCB 2004; Yurok 2006-2013	Yurok 2006-2013	Yurok 2006-13; USFWS for gaps	Hoopa/ Hoopa Tribe

2.2 ENVIRONMENTAL DATA SOURCES

2.2.1 HYDROLOGIC DATA

Streamflow data for the Klamath River gages listed in Table 1 were obtained online from the USGS NWIS (http://waterdata.usgs.gov/nwis/). Because not all periphyton sampling sites were located at USGS stream gages, discharge was estimated at some locations using a watershed area accretion method similar to that used by PacifiCorp (2004), Tetra Tech (2009), and Asarian et al. (2009, 2010, 2013). The total watershed area contributing to the ungauged accretions (areas of gaged tributaries were excluded) between each mainstem USGS gage (Iron Gate, Seiad, Orleans, and Turwar) was determined using GIS, and the ratios of individual areas to the total accretion area were calculated. Five-day moving averages of all gages were calculated and accretions for the reaches between the mainstem gages were developed by calculating the difference between the five-day moving averages of the upstream gage, downstream gage, and any gaged tributaries within the reach[1]. The accretion volume was then attributed to the nutrient sampling stations in proportion to their watershed area.

2.2.2 METEOROLOGICAL DATA

Daily mean air temperature and precipitation data for several Remote Automated Weather Stations (RAWS) were obtained from the Western Regional Climate Center's (WRCC) RAWS USA Climate Archive[2]. A meteorological station was assigned to each water quality monitoring station according to proximity (longest distance was 30 miles) and elevation (Table 1). Aside from removing data readily identifiable as erroneous[3], meteorology data were not otherwise adjusted.

2.2.3 WATER TEMPERATURE DATA

The source of water temperature data assigned to each periphyton monitoring site and year is shown in Table 1. Continuous (i.e., hourly or sub-hourly) water temperature data were collected by the Karuk Tribe, Yurok Tribe, and U.S. Fish and Wildlife Service (USFWS) using multi-parameter sensors, with methodology and results described in the following reports: Karuk Tribe (2007, 2008, 2010, 2011, 2012a, 2012b, 2013), Yurok Tribe (2004b, 2004c, 2005, 2010a, 2011a, 2012a, 2013a), Ward and Armstrong (2010). Additional continuous water temperature data was obtained from USFWS Arcata[4]. We calculated daily summary statistics (i.e., mean) when at least 80% of daily measurements were present. For a few stations, daily minimum and maximum

[1] The five-day moving averages were used to avoid the negative calculated accretion values that occasionally resulted from the combination of transit time and rapid changes in flow (i.e., storm events and/or dam releases) at gages.
PacifiCorp (2004) and TetraTech (2009) used seven-day moving averages, but for the May-October period analyzed here, a five-day average was sufficient.
[2] http://www.raws.dri.edu/
[3] For example: Air temperature flatlined at -35.5 °C, or extremely high precipitation values that were not associated with a streamflow increase.
[4] Data request form available at http://www.fws.gov/arcata/fisheries/activities/waterquality/klamathWQ.html

water temperature were also obtained from the U.S. Geological Survey National Water Information System (USGS NWIS)[5], which were averaged to estimate a daily mean[6].

2.2.4 NUTRIENTS AND CHLOROPHYLL DATA

Nutrient samples were generally collected at the same location as the long-term periphyton stations, although in some cases a nearby station was utilized (Table 1). Data were collected by a variety of entities, with methodology and results described in the following reports: Karuk Tribe (2007, 2008, 2010, 2011, 2012a, 2012b, 2013), Yurok Tribe (2004b, 2004c, 2005, 2007, 2008, 2009, 2010b, 2011b, 2012b, 2013b), Armstrong and Ward (2005), ARFO (2005), Raymond (2008, 2009, 2010), Deas (2008), Watercourse Engineering (2011a, 2011b, 2012, 2013, 2014), Kann and Asarian (2007), and Asarian et al. (2009).

Sampling frequency varied by station and year, but was generally monthly in 2004 and bi-weekly for 2006-2013. Parameters analyzed include ammonia (NH3), nitrate-plus-nitrite (NO3+NO2), total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP), and planktonic chlorophyll *a* (CHLA). Some data collection entities did not analyze TN, in which case TN was calculated as Total Kjeldahl Nitrogen (TKN)+ NO3+NO2. Total inorganic nitrogen (TIN) was computed as NH3 plus NO3+NO2. In this report, nutrient concentrations are expressed in units of mg/L as N or mg/L as P.

2.2.5 MATCHING PERIPHYTON SAMPLES TO ENVIRONMENTAL DATA

Because periphyton samples were not collected on the same date as nutrient samples, periphyton samples were assigned environmental data based on the average of environmental values encompassing a 14-day period (the 12 days preceding the periphyton sample, the day of the periphyton sample, and the day following). In most cases this average was composed of a single nutrient sample (although sometimes up to four samples) and 14 days of streamflow, water temperature, and meteorological data. The 14-day period was chosen to correspond with the generally bi-weekly frequency of nutrient samples and is a timescale relevant to periphyton growth.

2.2.6 IMPUTATION OF MISSING ENVIRONMENTAL DATA

Not all environmental parameters were available to match each periphyton sample. The presence of strong longitudinal and seasonal patterns for most environmental parameters allowed us to reasonably impute most missing environmental data; however, if no data were available from a nearby station or date to inform the imputation, then values were left blank/missing. The primary methods for imputation were: 1) using value from nearby date for the same station (5 to 14 days after the periphyton sample was collected, examining flow and temperature to make sure there were no major changes), and 2) estimate by regression (explained below. Following application of those two methods, the 14-day values were then re-calculated. Substantial gaps (~80 samples) still remained for alkalinity, which was only sampled monthly in many years whereas the nutrients were generally collected twice per month. These missing 14-day alkalinity values were

[5] http://waterdata.usgs.gov/usa/nwis

[6] Using a subset of the hourly temperature data, we confirmed that averaging the daily minimum and maximum values results in a value nearly identical to the daily mean.

then filled by substituting 31-day values (the 29 days preceding the periphyton sample, the day of the periphyton sample, and the day following).

Regressions were utilized in two different ways, according to the environmental parameter. For water temperature, linear predictions were developed between daily average water temperature of stations within 30 river miles[7] and daily average water temperature of a particular station needing imputation. The linear equations were then applied to estimate missing data for a particular station. All temperature regressions were highly significant ($p<0.001$) with adjusted r^2 values of >0.995. Longitudinal nutrient dynamics in the Klamath River are well studied, with concentrations decreasing downstream due to dilution and nutrient retention (Asarian and Kann 2006, Asarian et al. 2010); therefore, we utilized a more complex method for estimating nutrients, planktonic chlorophyll *a*, and alkalinity. A mixing equation was used to first provide a preliminary estimate of downstream concentration which takes into account tributary dilution:

$$C_1Q_1 + C_2Q_2 = C_3Q_3$$

Where: C_1 is concentration at upstream station, C_2 is concentration of tributary inputs[8], C_3 is concentration at downstream station, Q_1 is streamflow at upstream station, Q_2 is streamflow of tributary input, Q_3 = streamflow at downstream station. Then, the preliminary estimates of concentrations were linearly regressed against observed values to develop an equation which was then applied to make a more refined prediction of concentration. This regression implicitly accounts for nutrient retention and uses a different equation for each pair of stations and each parameter. Missing nutrient, periphytic chlorophyll *a*, and alkalinity values were only imputed when the regressions were statistically significant ($p<0.05$); in most cases, the r^2 values range from 0.5 to 0.95. Appendix C includes a figure accounting of how many samples for each parameter and site were imputed.

2.3 MULTIVARIATE DATA ANALYSIS

The analysis focused on data collected during the summer (May-August) and fall (September-November) of nine years (2004, 2006-2013) from 11 long-term monitoring sites (n=398).

We used Non-metric Multidimensional Scaling (NMDS) method to characterize both spatial (inter-site) and temporal (seasonal and inter-annual) variation of periphyton community composition using relative biomass. NMDS ordinations were based on Bray-Curtis similarity coefficient (Bray and Curtis 1957), after exclusion of rare species (< 1% biomass) and log-transformation of the data to down-weight the effect of dominant species. The Bray-Curtis coefficient takes into account both species presence and abundance and is commonly used in the analysis of ecological communities (Clarke 1993). The inter-site similarities were used in NMDS ordinations to project their relationships into a low-dimensional space and to best preserve the

[7] TR predicted from TRH (12 miles upstream), SV predicted from HC (28 miles downstream), and QU predicted from a station located just upstream of the confluence of the Klamath River Scott River (18 miles upstream).

[8] Tributary concentrations were assumed to be constant and assigned values based on a mean of 24 samples from various small tributaries to the Klamath River reported in Asarian et al. (2010): TP = 0.012 mg/L, SRP = 0.008 mg/L, TN = 0.081 mg/L, NO3+NO2 = 0.01 mg/L, NH3 = 0.005 mg/L, TIN = 0.015 mg/L. Alkalinity (81 mg$CaCO_3$/L) was not included in the Asarian et al. (2010) report but we calculated it from the same dataset.

ranked distances among them. NMDS does not require any assumptions about the species distribution and allows for user-specified distance measure. To assess how well the inter-site relationships defined by their similarity coefficients were projected onto the NMDS plots, stress values were calculated. The stress value shows how closely the calculated distances (from the NMDS plot) correspond to the actual distances (from the similarity matrix) between the sites, where a lower value indicates a better ordination. A stress value < 0.20 indicates a good ordination (Clarke 1993). The NMDS function was specified to run with 20 random starts in search of optimal solution with the lowest stress value. To explore the environmental variables that explained the patterns in the ordination of periphyton assemblages, a linear fitting function was used ("envfit", Oksanen et al., 2013). This function finds the vector averages of the environmental variables and fits them in the ordination space defined by the species data (NMDS plot). The importance of each vector was assessed using a squared correlation coefficient (r^2). The significance of each vector was tested using 1000 permutations.

Cluster analysis was used to identify groupings in the periphyton assemblages. This analysis uses a hierarchical agglomerative algorithm using the average linkage method to fuse the similar samples into clusters based on similarity between groups of samples. The idea is to identify groups of high within-group community similarity and low between-group similarity. Cluster analysis, based on Bray-Curtis similarity coefficient, was conducted after exclusion of rare species (< 1% relative biomass) and log-transformation of the data to down-weight the effect of dominant species.

Although these techniques were used similarly in the initial Klamath River periphyton analyses contained in Asarian et al. (2014) for the years 2004-2012, inclusion of an additional year of data from 2013 necessitated confirming the original observed patterns.

To determine the environmental conditions under which different periphyton assemblages occurred, classification and regression tree (CART) analysis was utilized (Breiman et al. 1984). This analysis is used for finding important environmental predictors and their interactions in terms of the response variable (e.g., periphyton groups from the cluster analysis, a sample's total biovolume, percent biovolume of benthic nitrogen-fixing species, and periphytic chlorophyll *a*). A CART is constructed after a series of binary decisions are made based on a single predictor variable, which splits the data in two relatively homogeneous subgroups with lowest deviance in a regression tree or the Gini's index values in a classification tree. The advantage of the CART, an alternative to multiple regression, includes that if there are missing values for the splitting variable, the algorithm uses alternative variables (surrogates) with available observations; both categorical and numerical predictors can be used; and the output (a decision tree) is easy to interpret. The resulting full tree may explain the variability in the response variable well but may have limited predictive power due to its complexity (i.e. high number of splits). Therefore, cross-validation is used to trim the tree and produce a less complex final tree. Cross-validation is done by randomly partitioning the original dataset in two subsets: a calibration dataset (90% of samples) and a validation dataset (10% of samples). The size of the final tree model is determined by plotting the mean relative prediction errors from each cross-validation run against the number of splits and selecting the one with the lowest relative prediction error. In regression tree analysis, when the response variable is a continuous variable (e.g., biovolume), the terminal tree nodes provide the predicted mean value for the response variable, the number of samples in the group, and the predictors that define it. The performance of the regression tree model is

assessed by a pseudo-r^2 (measure of goodness-of-fit). In classification tree analysis when the response variable is a categorical variable (e.g., periphyton-based clusters), the terminal tree nodes provide the predicted group, the number of samples correctly and incorrectly classified into the group, and the predictors that define it. The performance of the classification tree model is assessed by classification rate (% of correctly classified samples).

Mixed-effects models are increasingly used in the analysis of ecological data when there might be hierarchical structure in the data (Zuur et al. 2011). These models account for the non-independent nature of observations taken over small spatial or temporal scales. Mixed-effects models have two components, a fixed term (a relationship of interest between a response and predictor variables) and a random term (a categorical variable which contributes noise and might obscure the relationship between the fixed terms). Including a random term in the models improves their explanatory power by accounting for some of the residual variance, which might otherwise remain unexplained. The three most upstream Klamath River sites (IG, IB, and QU) were excluded from the mixed effect model development due to the lack of clear visual relationships between the response variables and predictors at the those sites, perhaps due to their proximity to upstream reservoirs. The two tributary sites (TR and TRH) were also excluded from mixed-effects model development because the relationship between response variables and predictors was different at those sites than at the Klamath River sites. We used a top-down approach for the model selection (Zuur et al. 2011).

First, we fitted multiple regression models using all uncorrelated environmental variables as predictors and the hybrid (stepwise and criterion-based) approach for variable selection. The best model was the one with the lowest Akaike Information Criterion (AIC) value as compared to a null model (without predictors). Second, to find the optimal random structure, we added "Site" or "Season" (Spring, May-July; Summer, August-October) as a random variable and compared the two models with a log-likelihood ratio test. We repeated this last step by adding site autocorrelation as a term (i.e., samples collected over time from the same site will not be independent of each other). The model with the lower AIC was selected as the better one. If there was no significant difference ($p>0.05$) between the models, the simpler one was selected. Next, to find the optimal fixed structure (what variables are significant, $p<0.05$), we compared models with different predictors to find the best model (lowest AIC). All predictor variables were standardized (values subtracted from the mean and divided by the standard deviation) prior to their inclusion in the models in order to account for the variability in their ranges and measurement units. To evaluate whether the model assumptions (normality and homogeneity) were met, we graphically examined plots of residuals and fitted values. All data analyses were performed in R (R Development Core Team 2014).

3 RESULTS

3.1 OVERALL PERIPHYTON ASSEMBLAGE CHARACTERIZATION

Complete periphyton assemblage results are found in Asarian et al. (2014), but are briefly summarized here. Periphyton assemblages in the Klamath River were dominated by diatoms. On average, diatoms comprised 92.5% (range: 25.5-100%) of samples in relative biomass, followed by cyanobacteria (6.0%, range: 0-74.4%). None of the other algal groups (e.g., cryptophytes, dinoflagellates) contributed to more than 1% of relative biomass in a given sample, except for green algae, which averaged 1.5% (range: 0-45.8%). There were a total of 150 taxa found in the samples (Appendix A). The mean taxa richness was 18 (range: 6-30). On average, Shannon diversity index was 1.68 (range: 0.20-2.73) and Simpson diversity index was 0.68 (range: 0.07-0.91) (Table 2).

All ten most frequently observed species were diatoms, including *Nitzschia frustulum* (Kützing) Grunow (94% of samples), *Cocconeis placentula* Ehrenberg (90%), *Achnanthidium minutissimum* (Kützing) Czarnecki (77%), *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot (75%), and *Navicula veneta* Kützing (74%) (Table 3). The ten taxa with the highest mean biomass included nine diatoms such as *Epithemia sorex* Kützing (110.4 x 10^6 $\mu m^3/cm^2$), *Cymbella affinis* Kützing (48.3 x 10^6 $\mu m^3/cm^2$), and *Rhopalodia gibba* (Ehrenberg) Müller (32.2 x 10^6 $\mu m^3/cm^2$) and one cyanobacterium *Calothrix* sp. (7.8 x 10^6 $\mu m^3/cm^2$) (Table 4). Two diatom taxa (*E. sorex, R. gibba*) with cyanobacterial endosymbionts, and heterocystous *Calothrix* sp., all possess the ability to fix nitrogen. The most frequently observed species (*Nitzschia frustulum*) had the least absolute biomass (7.4 x 10^6 $\mu m^3/cm^2$) and very low relative biomass (3.0%) compared to the other nine species (Table 3). Based on relative biomass, the highest mean percentages were attributed to diatoms such as *E. sorex* (22.4%, range: 0-92.8%), *C. placentula* (12.2%, range: 0-80.4%), and *C. affinis* (11.9%, range: 0-96.5%) (Table 4). *Calothrix* sp. ranked tenth for mean relative biomass (2.3%, range: 0-59.8%) and was the only cyanobacteria in the top ten species (Table 4). Nitrogen-fixers included six taxa of cyanobacteria (the benthic *Calothrix* sp. and *Rivularia* sp., and the sestonic [i.e., free-floating] *Anabaena flos-aquae* [Linnaeus] Brébisson, *Anabaena sp*., *Aphanizomenon flos-aquae* [Linnaeus] Ralfs, and *Gloeotrichia echinulata* [Smith] Richter), and three species of diatoms with cyanobacterial endosymbionts (*E. sorex, E. turgida* (Ehrenberg) Kützing, and *R. gibba*) (Appendix A).

Table 2. Summary statistics of species richness and diversity indices calculated from the Klamath River periphyton samples.

Diversity	Mean	Median	Minimum	Maximum
Richness	18	18	6	30
Shannon evenness	0.33	0.31	0.09	0.75
Simpson evenness	0.23	0.21	0.06	0.66
Pielou evenness	0.58	0.60	0.09	0.90
Shannon diversity	1.68	1.72	0.20	2.73
Simpson diversity	0.68	0.72	0.07	0.91

Table 3. Frequency, biomass, and % biomass (i.e., relative biomass) for the ten most frequently observed periphyton species in the Klamath River samples, sorted by frequency (freq.). Minimum biomass for each species was zero. N = number of samples, Med. = median, S.D. = standard deviation, Max = maximum.

			Biomass (% of total biovolume)				Biomass (10^6 x µm^3/cm^2)			
Species	**n**	**% freq.**	**Mean**	**Med.**	**S.D.**	**Max**	**Mean**	**Med.**	**S.D.**	**Max**
Nitzschia frustulum (Kützing) Grunow	376	94	2.95	1.42	4.09	28.88	7.43	2.32	24.30	379.50
Cocconeis placentula Ehrenberg	360	90	12.24	3.68	17.38	80.4	12.81	7.30	18.45	193.63
Achnanthidium minutissimum (Kützing) Czarnecki	308	77	1.16	0.18	3.17	30.38	2.02	0.21	5.40	47.70
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	300	75	0.94	0.27	2	22.01	1.22	0.39	2.90	32.81
Navicula veneta Kützing	296	74	0.98	0.24	2.16	24.96	0.90	0.38	1.40	7.45
Epithemia sorex Kützing	268	67	22.39	13.31	25.45	92.78	110.41	12.86	198.94	1205.89
Cymbella affinis Kützing	260	65	11.94	4.03	18.06	96.54	48.28	4.49	134.00	994.04
Gomphonema angustatum (Kützing) Rabenhorst	251	63	1.24	0.28	3.22	35.09	1.12	0.22	2.27	21.51
Ulnaria ulna (Nitzsch) Compère	240	60	4.22	2.3	5.57	32.26	16.24	3.08	35.51	299.16
Diatoma tenuis Agardh	225	57	3.67	0.31	7.94	71.14	8.20	0.23	21.93	215.81

Table 4. Frequency, biomass, and % biomass (i.e., relative biomass) for the ten species in the Klamath River periphyton samples with the highest mean biomass and percent biomass (top 10 species were the same for both metrics, though their order is somewhat different), sorted by percent biomass. Minimum biomass for each species was zero. The abbreviations are same as in Table 3.

			Biomass (% of total biovolume)				Biomass (10^6 x µm^3/cm^2)			
Species	**n**	**% freq.**	**Mean**	**Med.**	**S.D.**	**Max**	**Mean**	**Med.**	**S.D.**	**Max**
Epithemia sorex Kützing	268	67	22.39	13.31	25.45	92.78	110.41	12.86	198.94	1205.89
Cocconeis placentula Ehrenberg	360	90	12.24	3.68	17.38	80.4	12.81	7.30	18.45	193.63
Cymbella affinis Kützing	260	65	11.94	4.03	18.06	96.54	48.28	4.49	134.00	994.04
Gomphoneis herculeana (Ehrenberg) Cleve	182	46	7.19	0	12.09	82.19	28.64	0.00	76.22	865.29
Diatoma vulgaris Bory	187	47	4.84	0	11.16	79.65	17.05	0.00	62.61	767.48
Rhopalodia gibba (Ehrenberg) Müller	57	14	4.75	0	13.67	83.73	32.20	0.00	185.44	3155.48
Ulnaria ulna (Nitzsch) Compère	240	60	4.22	2.3	5.57	32.26	16.24	3.08	35.51	299.16
Diatoma tenuis Agardh	225	57	3.67	0.31	7.94	71.14	8.20	0.23	21.93	215.81
Nitzschia frustulum (Kützing) Grunow	376	94	2.95	1.42	4.09	28.88	7.43	2.32	24.30	379.50
Calothrix sp.	69	17	2.32	0	8.52	59.8	7.80	0.00	29.38	279.95

3.2 SPATIAL AND TEMPORAL PATTERNS IN PERIPHYTON ASSEMBLAGES

NMDS and cluster analyses performed on 2004-2013 data confirm the spatial and temporal patterns observed in Asarian et al. (2014) for 2004-2012 data. For example, based on cluster analysis on relative biomass of 95 taxa (after removal of 55 rare taxa (<1%) and log-transformation of the data to down-weight the effect of dominant taxa), we identified three major groups of periphyton assemblages (Figure 2, Table 5). These groups were the same ones identified in Asarian et al. (2014) where each group is described in terms of dominant taxa, indicator species, periphyton metrics (if available for more than 50% biomass), and spatial and temporal patterns. The reader is referred to Asarian et al. (2014) for more detailed information.

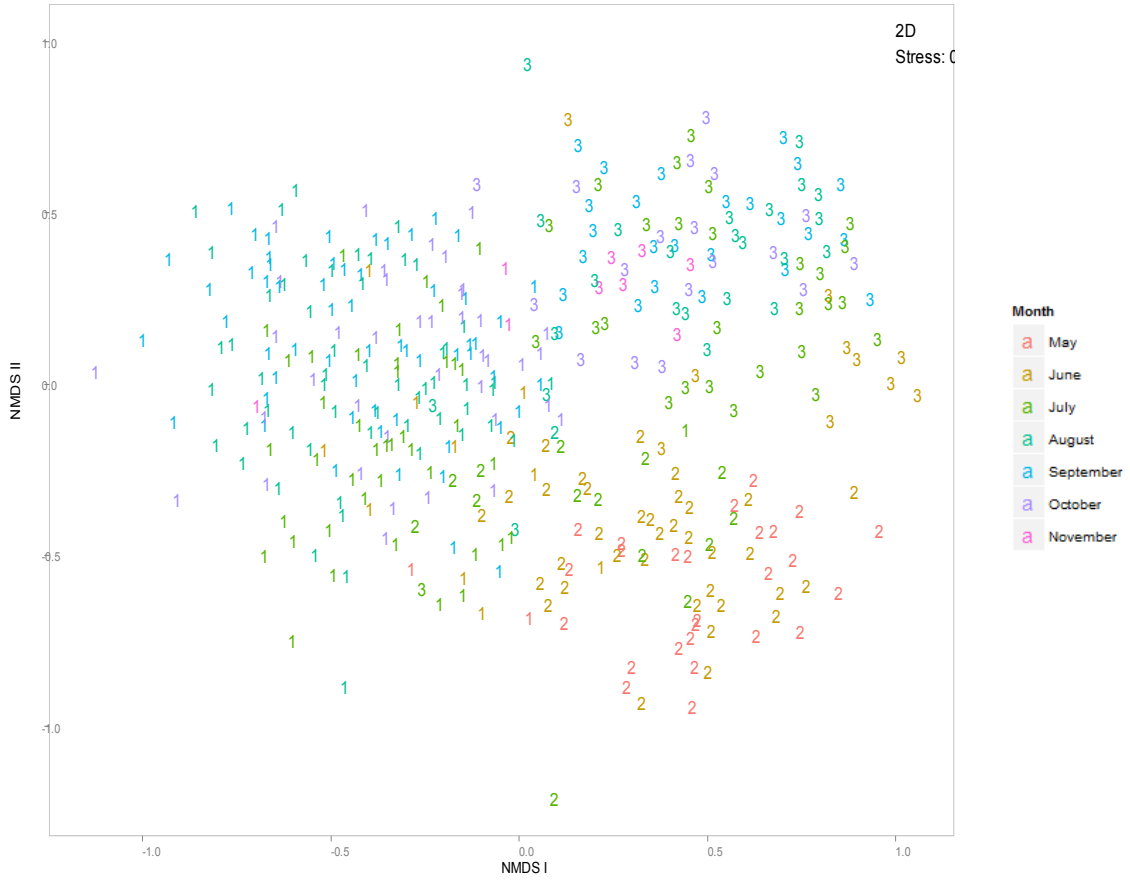

Figure 2. Non-metric Multidimensional Scaling (NMDS) showing the relative similarity of periphyton assemblages for each sample, colored by month and symbolized by the three major groups identified using cluster analysis. The distance between symbols indicates the relative similarity of the samples

The ordination plot based on all the data collected during summer (May-August) and fall (September-November) of nine years (2004, 2006-2013) from 11 sites (n=398) revealed a longitudinal gradient in periphyton species composition from upstream (upper right corner, Figure 2) to downstream sites (left side and bottom, Figure 2). Upstream sites (upper right corner, Figure 2) were more similar to each other than to downstream sites (left side and bottom, Figure 2). However, samples from the same site were more similar to samples from other sites when collected at the same time of the year (i.e., most May and June samples were clustered in the lower right corner of Figure 2). These longitudinal changes illustrated by the NMDS plots correspond to the three major periphyton groups identified by the cluster analysis (Table 5).

There appears to be a seasonal gradient in the periphyton assemblages from late spring-early summer (May-June) assemblages (lower right corner, Figure 2) to late summer-early fall (August-October) assemblages (upper left corner, Figure 2).

Table 5. Number of samples in each cluster group for each site, year, and month. See Table 1for key to site codes.

	Number of Samples			
Site/Year/Month	Cluster 1	Cluster 2	Cluster 3	Total
IG	0	0	24	24
IB	0	0	23	23
QU	0	0	25	25
SV	2	3	19	24
HC	5	2	10	17
OR	18	3	4	25
KR	39	18	1	58
WE	40	12	0	52
TG	35	14	2	51
TR	39	11	1	51
TRH	32	14	2	48
2004	21	1	12	34
2006	8	7	0	15
2007	19	2	13	34
2008	24	9	14	47
2009	24	6	1	31
2010	21	12	0	33
2011	25	21	23	69
2012	32	14	23	69
2013	36	5	25	66
May	2	26	0	28
June	10	36	10	56
July	42	14	28	84
August	56	1	24	81
September	58	0	25	83
October	39	0	18	57
November	3	0	6	9

Some autecological metrics exhibited more or less pronounced inter-annual and longitudinal variation. For instance, relative biomass of benthic N-fixers followed a unimodal pattern of increase from upstream to midstream (sites IG through KR), and then a decrease toward the most downstream sites (WE through TG) in some years (Figure 3). However, with the exception of station TG, many years showed an upstream to downstream increase in dominance by benthic N-fixers, especially in the upper quartile (e.g., 2004, 2008, and 2011-2013).

Seasonally, the highest relative biomass of benthic N-fixers was observed in July-September at downstream sites. Nitrogen-fixers exhibited an interesting upstream migration with the progression of the summer (Figure 4). While nitrogen-fixers were dominant at downstream sites in June, in July-September their biomass increased gradually in the upstream direction as well. This pattern was reversed in October when nitrogen-fixers were again constrained to downstream sites.

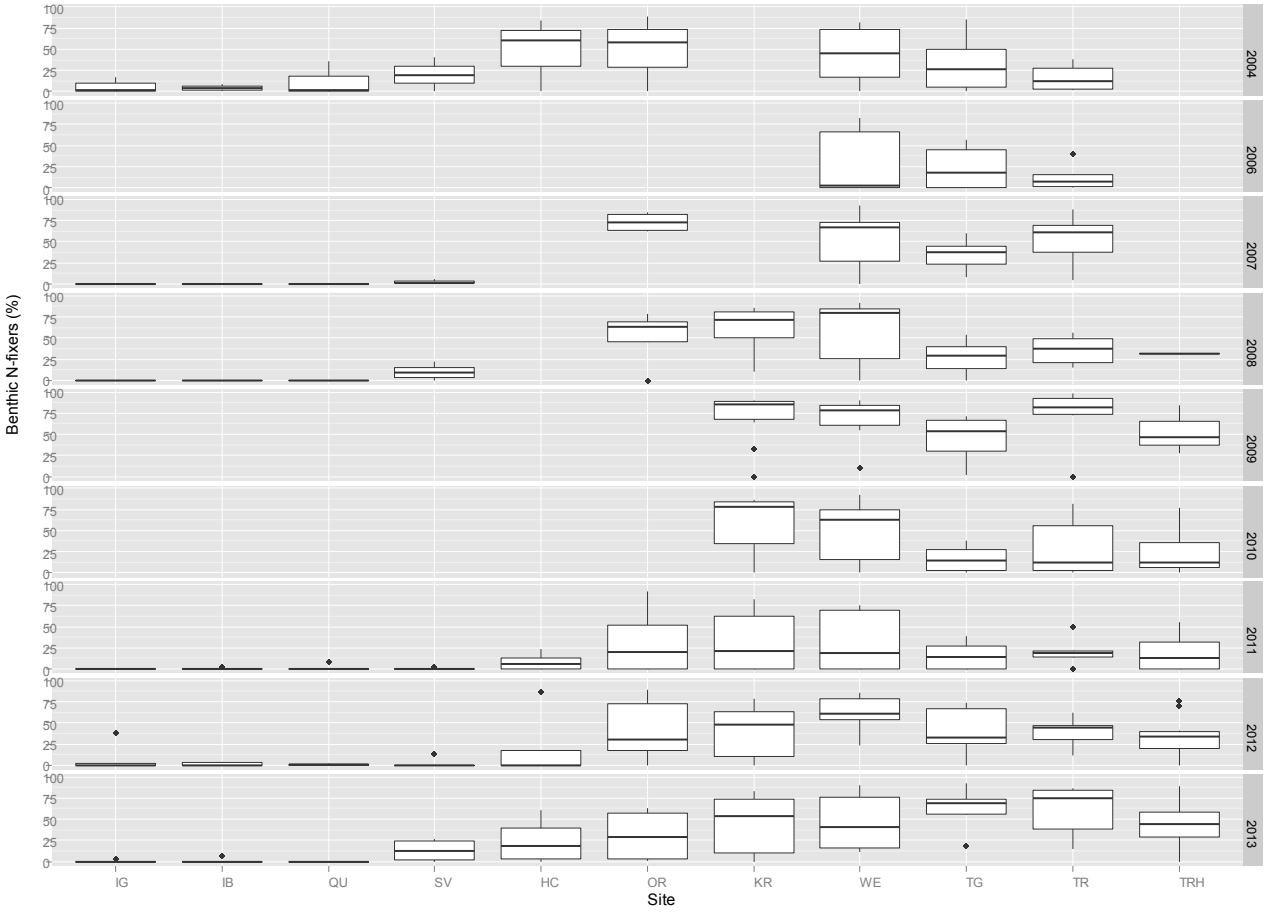

Figure 3. Boxplot of percent biomass of benthic nitrogen fixing periphyton species, by site (columns) and year (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes. The horizontal line inside the box is median, the upper and lower edges of the box are 25th and 75th percentiles, the upper whisker extends to the highest value that is within 1.5 times the interquartile range (75th minus 25th percentile) from the box's edge, and points plotted beyond the whiskers are outliers.

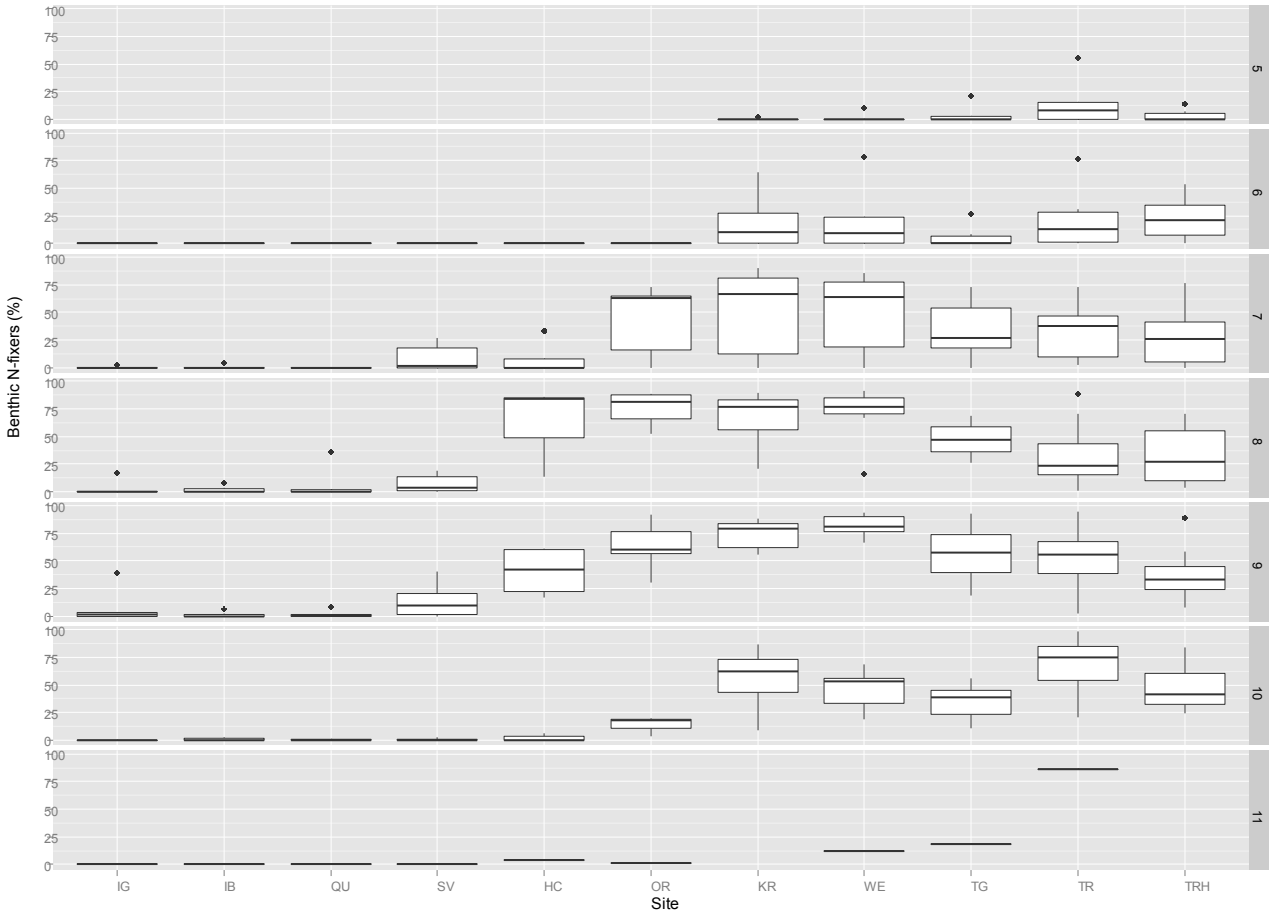

Figure 4. Boxplot of percent biomass of benthic nitrogen fixing periphyton species, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes. The horizontal line inside the box is the median, the upper and lower edges of the box are 25th and 75th percentiles, the upper whisker extends to the highest value that is within 1.5 times the interquartile range (75th minus 25th percentile) from the box's edge, and points plotted beyond the whiskers are outliers.

3.3 SPATIAL AND TEMPORAL PATTERNS OF ENVIRONMENTAL CONDITIONS

Many environmental variables changed longitudinally in the Klamath River. For instance, during the June-October season during which most periphyton samples are collected, flow increased longitudinally (Figure 5) due to tributary inputs while nutrient concentrations (e.g., SRP and NO_3, Figure 5) decreased longitudinally. Site-normalized flow (i.e., flow as a ratio of a site's annual median flow) decreased longitudinally because flows at upstream sites are more constant through the year, in part due to regulation by dams. In contrast because flows at downstream sites are much higher during winter and spring than summer and early fall, site-normalized flow is relatively lower than upstream sties. Water temperature exhibited a more subtle longitudinal pattern with lowest temperatures at the most upstream site (IG), rising to QU and remaining high until OR and then decreasing to TG upstream of the estuary. Nitrate concentrations declined longitudinally until Orleans (site OR), remained low in the downstream sites, and were also very low at Trinity River sites. Unlike nitrate, SRP continuously declined longitudinally.

Seasonal patterns are readily apparent as well (Figure 6, Figure 7). Flow decreased from May through September before rising slightly in October (except at Trinity River sites where it remained low in October). Water temperature peaked in August at all sites. At most sites, NO_3 concentrations were lowest in July and August, with highest values occurring in October.

Seasonal timing in many environmental variables appeared to be strongly associated with flow. For example, visual comparison of the two high-flow years (2010 and 2011) with two low-flow years (2009 and 2013) at site KR indicated than in the high-flow years water temperatures were much cooler in May through July, SRP concentrations were lower, the seasonal decline of NO_3 to non-detect levels was delayed until August (in 2011 only), and the rise in N-fixing periphyton began in August rather than June (Figure 8, figures for other sites are available in appendix B).

As a result of these longitudinal and seasonal patterns, many environmental variables are highly correlated (Figure 8).

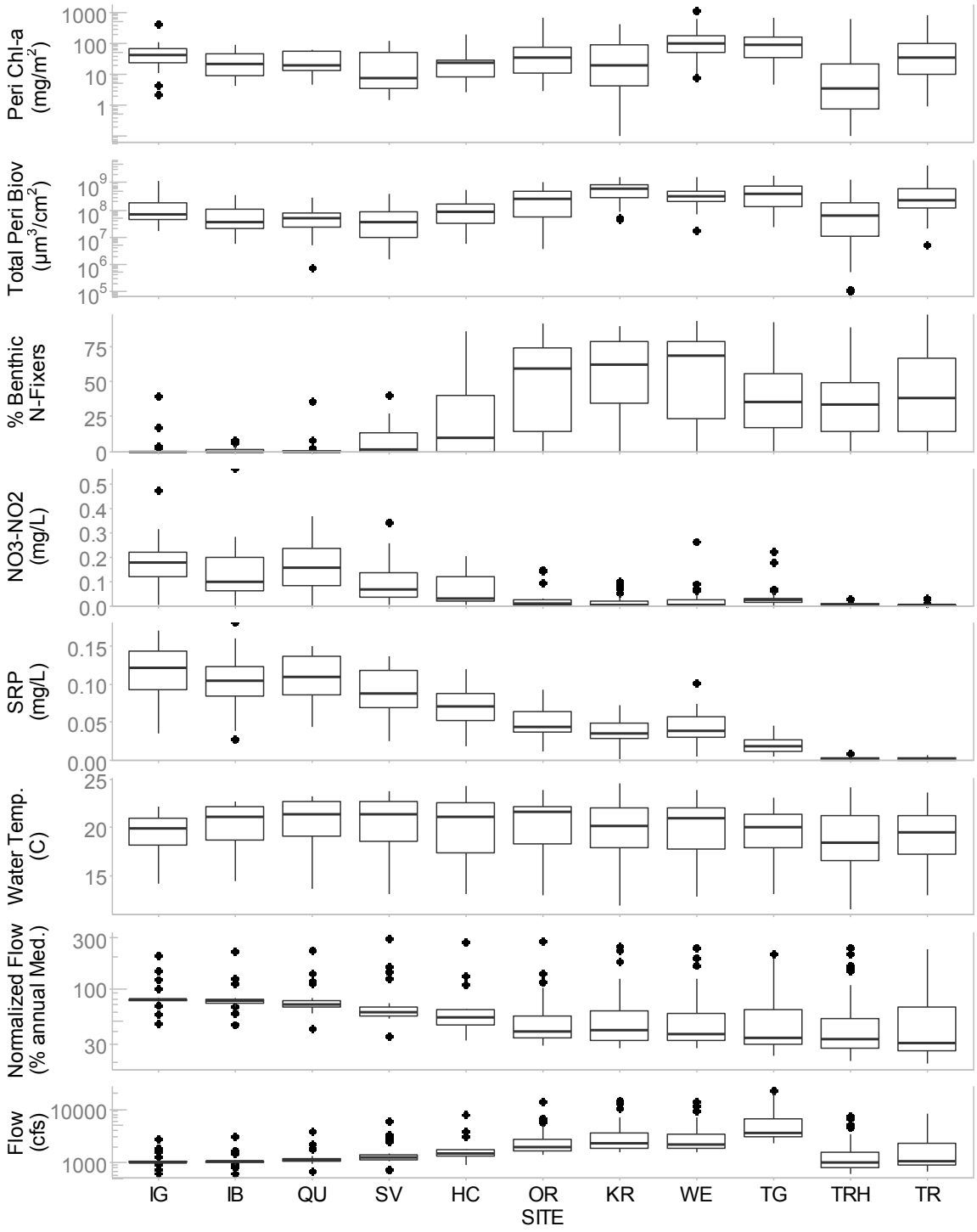

Figure 5. Boxplot of periphyton metrics (top three panels) and environmental parameters (bottom five panels) for individual sites on the Klamath and Trinity rivers for the months of June-October. See Table 1for key to site codes. The horizontal line inside the box is median, the upper and lower edges of the box are 25th and 75th percentiles, the upper whisker extends to the highest value that is within 1.5 times the interquartile range (75th minus 25th percentile) from the box's edge, and points plotted beyond the whiskers are outliers.

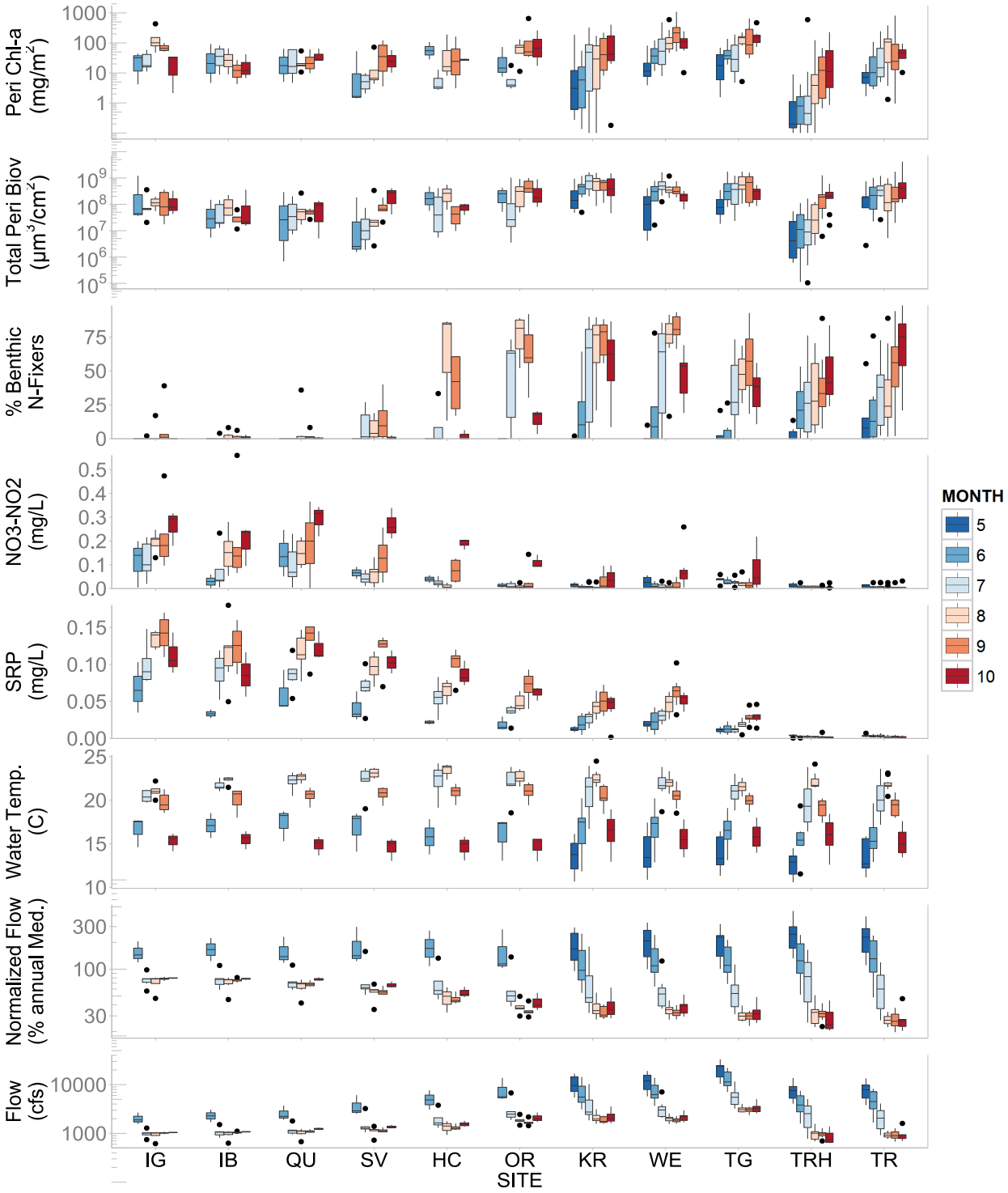

Figure 6. Boxplot of periphyton metrics (top three panels) and environmental parameters (bottom five panels) for individual sites on the Klamath and Trinity rivers, by month for samples collected in May-October. See Table 1for key to site codes. The horizontal line inside the box is median, the upper and lower edges of the box are 25th and 75th percentiles, the upper whisker extends to the highest value that is within 1.5 times the interquartile range (75th minus 25th percentile) from the box's edge, and points plotted beyond the whiskers are outliers.

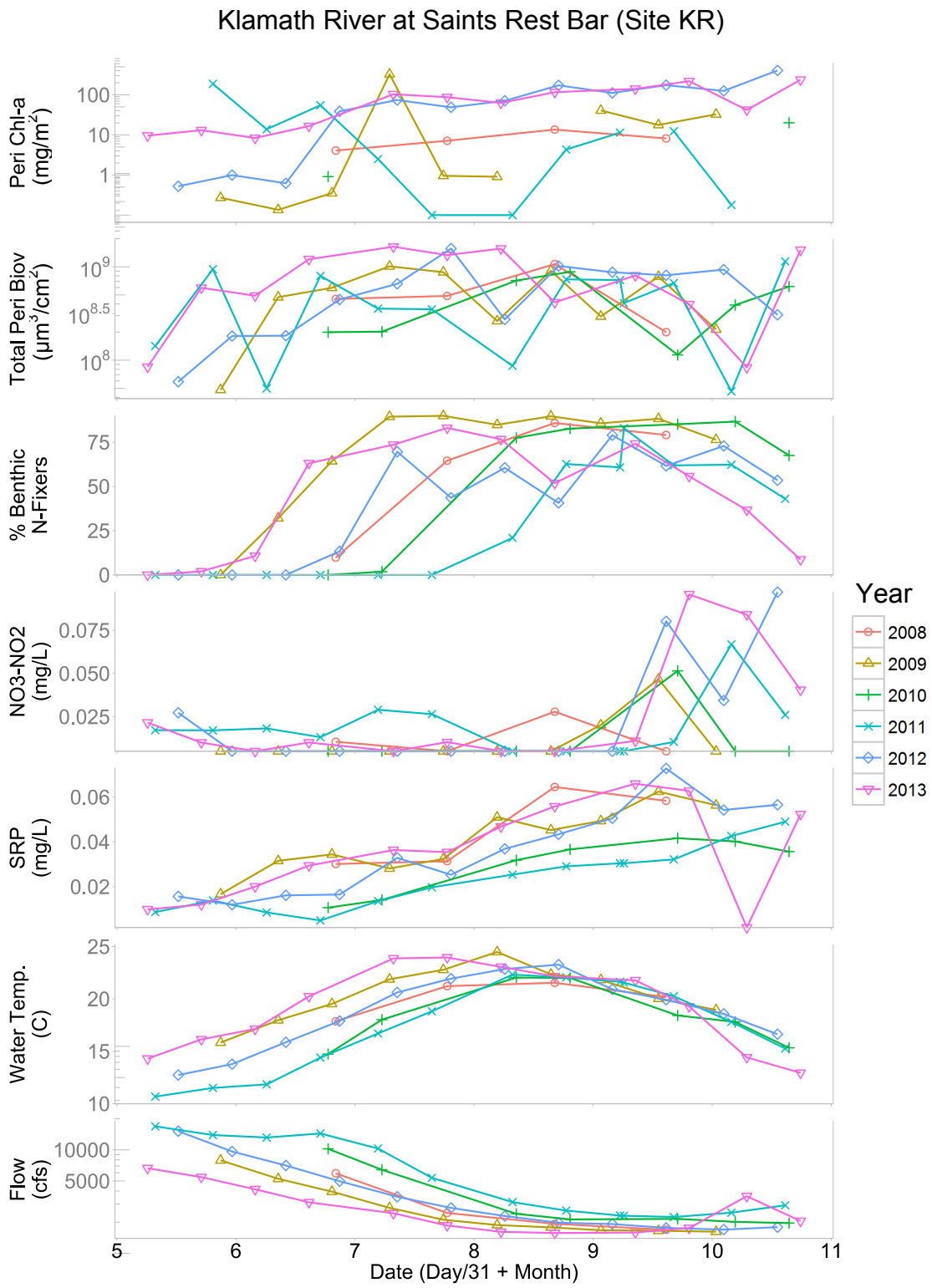

Figure 7. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Saints Rest Bar (KR), for samples collected in May-October. Points on graph represent 14-day averages preceding periphyton sampling dates (see methods above for details). See Table 1 for key to site codes.

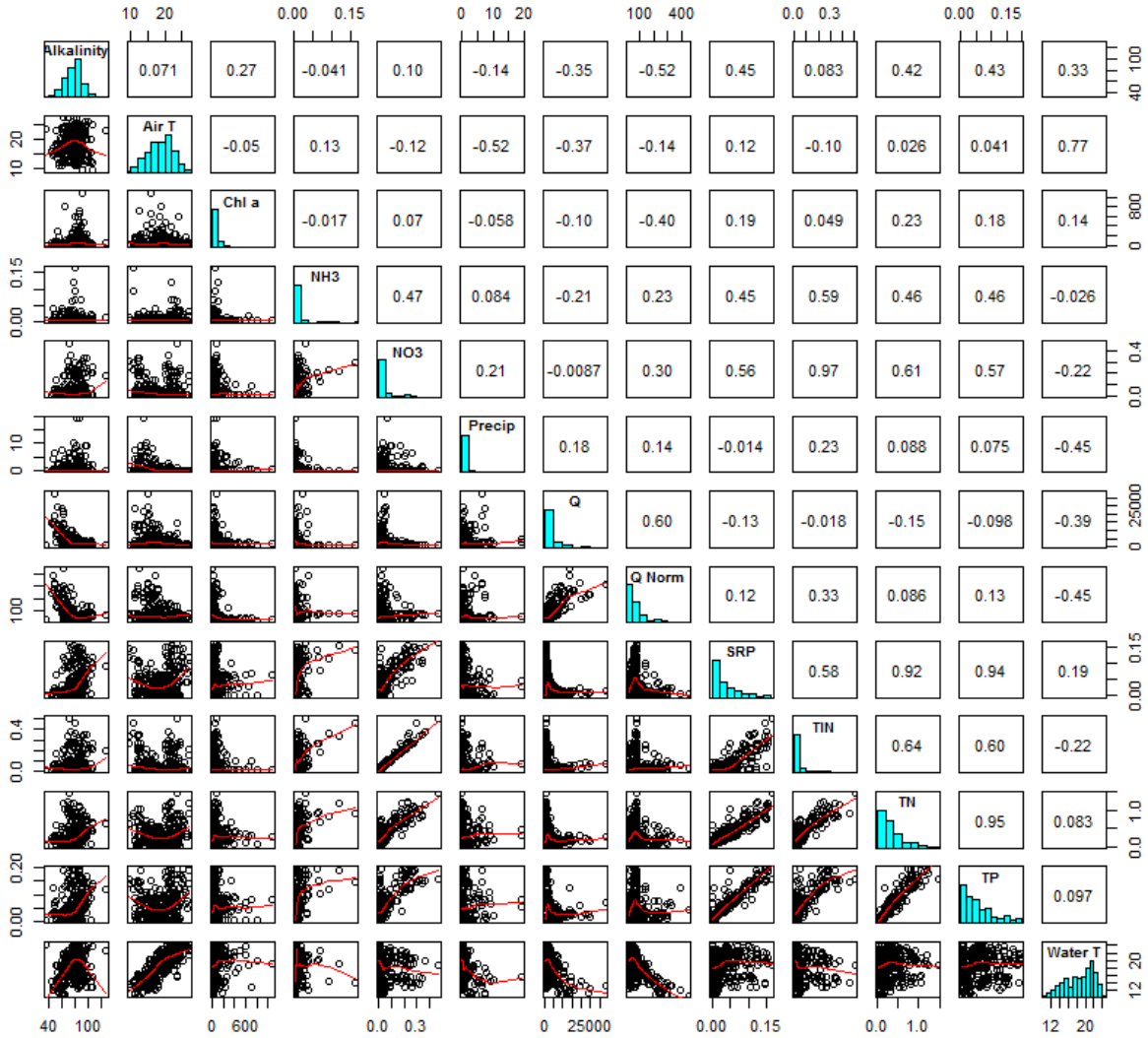

Figure 8. Correlation plot of environmental variables measured in the Klamath River. Numbers in the upper triangle represent Spearman correlation coefficients, scatterplots in the lower triangle show fitted lines (in red) from LOWESS regression. Lower triangle presents a scatterplot with LOWESS trend lines, upper triangle shows the Spearman Rank correlation coefficient, and the diagonal displays variable name and distribution. Abbreviations and units: Alkalinity [mg/L $CaCO_3$]; Air T, air temperature [^oC]; Chl a, periphytic chlorophyll *a* [mg/m^2]; NH_3, ammonia [mg/L]; NO_3, nitrate-nitrite [mg/L]; Precip, precipitation [mm]; Q, flow [cfs]; Q Norm, site-normalized flow[% of median flow]; SRP, soluble reactive phosphorus [mg/L]; TIN, total inorganic nitrogen [mg/L]; TN, total nitrogen [mg/L]; TP, total phosphorus [mg/L]; Water T, water temperature [^oC].

3.4 RELATIONSHIPS BETWEEN PERIPHYTON ASSEMBLAGES AND ENVIRONMENTAL CONDITIONS

3.4.1 COMPARING ENVIRONMENTAL DATA BY PERIPHYTON CLUSTER GROUP

The three periphyton groups identified by the cluster analysis (see Section 3.2 above) analysis details) details were characterized with somewhat different environmental conditions (Table 6). Group 1 (downstream sites sampled in summer and fall) had the highest periphytic chlorophyll *a* concentrations and the lowest site-normalized flow. Group 2 (downstream sites sampled in spring and early summer) had the highest flow and precipitation but the lowest water temperature and planktonic/periphytic chlorophyll *a* concentrations. Group 3 (upstream sites) had the highest nutrient concentrations (e.g., total nitrogen and total phosphorus).

Table 6. Environmental variables for each cluster group (see Section 3.2) and all groups combined. S.D. = Standard deviation. Different superscript letters depict significant differences ($p<0.05$) between groups based on Kruskal-Wallis multiple comparison tests.

	Group 1		Group 2		Group 3		All	
Variable name (units)	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Alkalinity (mg/L $CaCO_3$)	81.81[a]	9.48	63.52[b]	10.51	82.04[a]	14.12	78.32	13.28
Air temperature (^oC)	18.96[a]	3.17	16.94[b]	3.20	19.95[c]	5.06	18.85	3.92
Planktonic chlorophyll *a* (µg/L)	4.3[a]	6.0	2.8[a]	2.2	7.1[b]	8.5	4.8	6.5
Periphytic chlorophyll*a* (mg/m^2)	110.3[a]	158.9	24.6[b]	45.3	42.4[c]	71.8	74.8	128.9
Ammonia (mg/L)	0.006[a]	0.003	0.007[a]	0.007	0.020[b]	0.027	0.010	0.016
Nitrate-nitrite (mg/L)	0.019[a]	0.032	0.019[a]	0.018	0.139[b]	0.107	0.052	0.081
Precipitation (mm)	0.93[a]	2.53	0.90[a]	1.68	0.61[b]	1.66	0.83	2.17
Flow (cfs)	2137[a]	1337	9086[b]	5847	1454[c]	1000	3291	3990
Site-normalized flow (% of median flow)	39.8[a]	22.6	170.8[b]	83.7	76.0[c]	32.6	75.2	65.9
Soluble reactive phosphorus (mg/L)	0.030[a]	0.027	0.012[b]	0.009	0.095[c]	0.042	0.044	0.043
Total inorganic nitrogen (mg/L)	0.025[a]	0.035	0.026[a]	0.020	0.170[b]	0.118	0.063	0.092
Total nitrogen (mg/L)	0.261[a]	0.178	0.162[b]	0.095	0.718[c]	0.314	0.365	0.305
Total phosphorus (mg/L)	0.052[a]	0.048	0.030[b]	0.020	0.127[c]	0.053	0.067	0.058
Water temperature (^oC)	19.9[a]	2.9	15.5[b]	2.7	19.6[a]	3.3	19.0	3.4

3.4.2 NON-METRIC MULTIDIMENSIONAL SCALING (NMDS) ORDINATION

Environmental variables that correlated highly with the ordination space defined by species composition and that could potentially explain their patterns included water temperature and alkalinity (Group 1), flow (Group 2), and nutrients (Group 3) (Figure 9 and Table 7). Water temperature and flow defined a seasonal gradient from samples with low temperature and high flow (i.e., spring and early summer, Group 2) to samples with high temperature and low flow (i.e., late summer and fall, Group 1) as indicated by the opposite direction of their corresponding

vectors (Figure 9). Nutrients (e.g., NO3, SRP, TN, and TP) defined a second gradient, which captured the longitudinal change in periphyton assemblages from upstream nutrient-rich sites (Group 3 in Figure 9) to downstream sites (Group 1 in Figure 9) with lower nutrient concentrations. Planktonic chlorophyll *a* was significantly positively correlated with Group 3 samples (upstream), while periphytic chlorophyll *a* was significantly positively correlated with Group 1 samples (downstream).

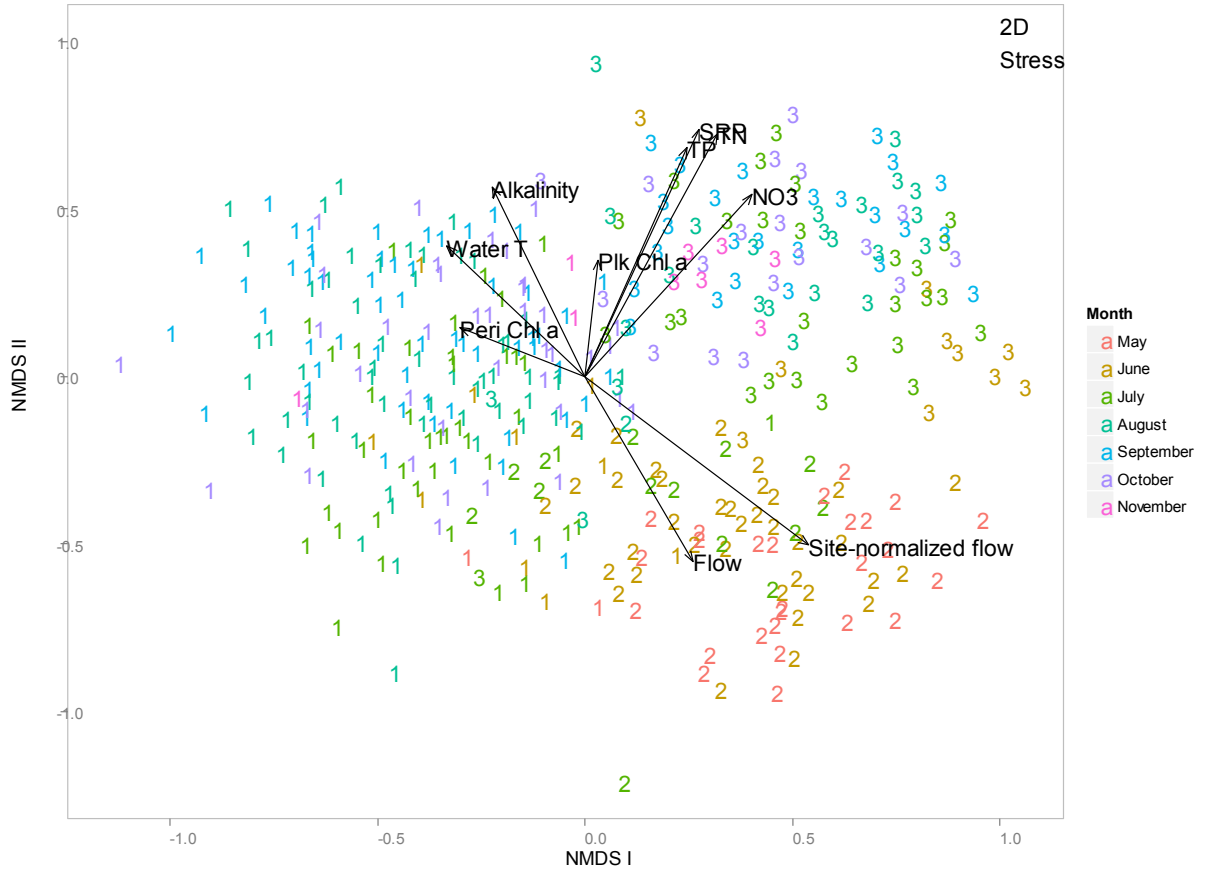

Figure 9. Non-metric Multidimensional Scaling (NMDS) showing the relative similarity of periphyton assemblages for each sample, colored by month and symbolized by the three major groups identified using cluster analysis. The distance between symbols indicates the relative similarity of the samples. Vectors indicate the significant environmental variables ($p<0.05$) and their direction of largest change. Due to high correlation between some variables and ease of representation, only the most significant ones are displayed in the figure. Abbreviations: Water T, water temperature [^{o}C]; NO_3, nitrate-nitrite [mg/L]; Peri Chl a, periphytic chlorophyll *a* [mg/m^2]; Plk Chl a, planktonic chlorophyll *a* [µg/L]; SRP, soluble reactive phosphorus [mg/L]; TN, total nitrogen [mg/L]; TP, total phosphorus [mg/L]. For a complete list of variables see Table 7.

Table 7. Results from the environmental vector fitting in the ordination space of the NMDS plot with variable scores along the two ordination axes (NMDS I– and NMDS II), goodness-of-fit statistic (squared correlation coefficient, r^2) and its significance (p-value). Variable summaries are in Table 6.

Variable name (units)	NMDS I	NMDS II	r^2	p-value
Alkalinity (mg/L $CaCO_3$)	-0.38	0.93	0.38	0.000999 ***
Air temperature (^oC)	-0.38	0.93	0.04	0.001998 **
Planktonic chlorophyll *a* (µg/L)	0.10	1.00	0.14	0.000999 ***
Periphytic chlorophyll *a* (mg/m^2)	-0.96	0.27	0.10	0.000999 ***
Ammonia (mg/L)	0.65	0.76	0.12	0.000999 ***
Nitrate-nitrite (mg/L)	0.60	0.80	0.47	0.000999 ***
Precipitation (mm)	0.32	-0.95	0.00	0.68032
Flow (cfs)	0.44	-0.90	0.38	0.000999 ***
Site-normalized flow (% of median flow)	0.74	-0.67	0.56	0.000999 ***
Soluble reactive phosphorus (mg/L)	0.37	0.93	0.62	0.000999 ***
Total inorganic nitrogen (mg/L)	0.61	0.79	0.48	0.000999 ***
Total nitrogen (mg/L)	0.41	0.91	0.63	0.000999 ***
Total phosphorus (mg/L)	0.38	0.93	0.60	0.000999 ***
Water temperature (^oC)	-0.69	0.72	0.27	0.000999 ***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

p-values based on 1000 permutations

3.4.3 CLASSIFICATION AND REGRESSIONS TREES (CART)

Results from CART analysis revealed that site-normalized flow explained the most variation among the three periphyton groups (Figure 10). Similar to the ordination results, low flows (≤ 54.5% of annual median flow) were associated mainly with periphyton Group 1 (189 samples out of 210, late summer and fall samples from downstream sites). Most of the samples from periphyton Group 2 (76 out of 77 samples) and the majority of samples from Group 3 (96 of 111 samples) were characterized with higher site-normalized flows (> 54.5% of annual median flow). These samples were further structured by SRP concentrations where low SRP concentrations (≤ 0.035 mg/L) were associated with samples from group 2 (spring and early summer samples from downstream sites) while higher SRP concentrations (> 0.035 mg/L) were associated with group 3 (upstream nutrient-rich sites). Overall the CART model's mis-classification rate was 10.6% (42 out of 398 samples).

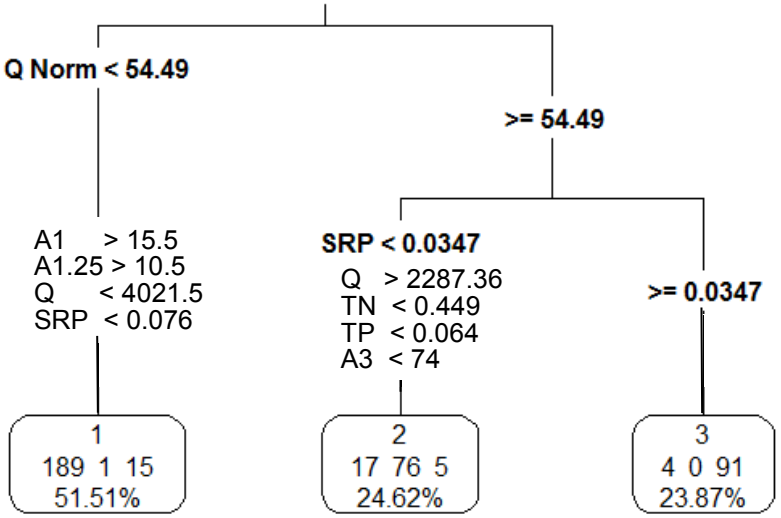

Figure 10. Final classification tree model for the periphyton groups. Names and numbers above each node indicate the predictor variable and its value used for the split. Names and numbers below each node indicate the surrogate variables and their values that can be used as alternatives for the split. Sites that meet the node criteria are split to the next two subgroups. Numbers at the leaves specify predicted periphyton group (1-3), the number of samples for each group (Group 1/2/3), and their proportion of the total sample number (%). Abbreviations: Q Norm, site-normalized flow (% of median flow); SRP, Soluble reactive phosphorus; A1, A1.25, A2, and A3 are days of accrual (consecutive days that flow has remained below 1x, 1.25x, 2x, or 3x the median flow).

The CART analyses predicted increasing periphyton biomass with decreasing site-normalized flow (Figure 11). Samples with lower site-normalized flow (< 50.9% of median flow) were predicted to have the highest biomass (log[19.37] = 266.3 x 10^6 $\mu m^3/cm^2$). These samples were also characterized with lower air temperature (< 21.4°C) and lower TIN (< 0.03 mg/L). Samples with higher site-normalized flow (≥ 50.9% of median flow) were further divided into two subgroups. The first subgroup was associated with lower flow (< 2922.1 cfs) samples, which were predicted to have lower biomass (log[18.6] = 39.8 x 10^6 $\mu m^3/cm^2$). These samples were also characterized with higher air temperature (≥ 18°C), higher TIN (≥ 0.03 mg/L), and higher TP (≥ 0.06 mg/L). The second subgroup consisted of samples with higher flows (≥ 2922.1 cfs) and higher biomass (log[17.49] = 119.6 x 10^6 $\mu m^3/cm^2$). These samples were characterized with lower air temperature (< 18°C), lower TIN (< 0.03 mg/L), and lower TP (<0.06 mg/L). Overall 21% of log-transformed periphyton biovolume variance among sites can be explained by the two predictors in the model.

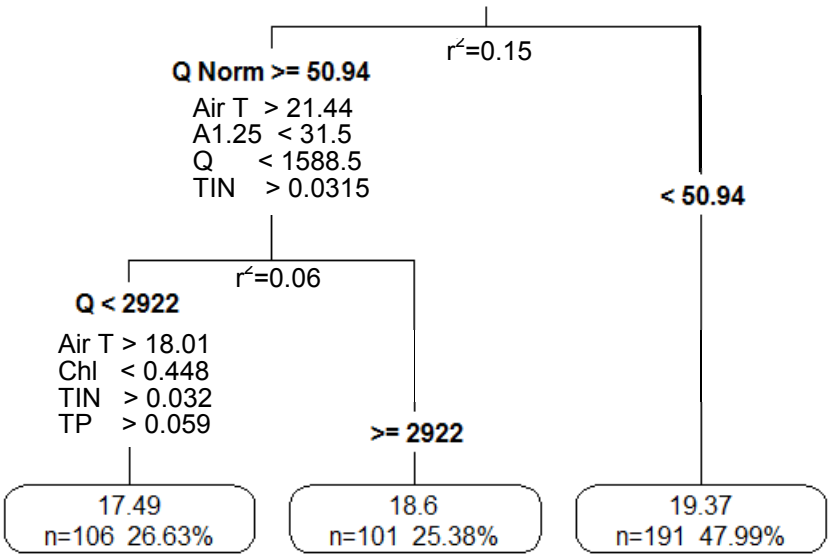

Figure 11. Final regression tree for log-transformed periphyton biomass (r^2 = 0.21). Names and numbers at each node indicate the predictor variable and its value used for the split. Names and numbers below each node indicate the surrogate variables and their values that can be used as alternatives for the split. Sites that meet the node criteria are split to the two sub-groups. Numbers at the leaves specify mean predicted log-transformed values for the response variable (periphyton biomass) , the number of samples (n), and their proportion of the total sample number (%). Abbreviations: Q Norm, site-normalized flow (% of median flow); A1.25, days of accrual (consecutive days that flow has remained below 1.25x the median flow); Air T: air temperature; TIN: total inorganic nitrogen; Chl: chlorophyll; TP: total phosphorus.

The CART analyses predicted increasing periphytic chlorophyll *a* concentrations with decreasing site-normalized flow (Figure 12). Samples with higher site-normalized flow (≥41.36 % of median flow) were further divided into two groups. The first group was associated with TN concentrations < 0.1598 mg/L, which were predicted to have lower chlorophyll *a* concentrations (5.4 mg/m^2). These samples were also characterized with low phosphorus concentrations (TP<0.012 mg/L and SRP<0.0065 mg/L). The second group consisted of samples with higher TN concentrations (≥ 0.1598 mg/L) and higher chlorophyll *a* concentrations (22.1 mg/m^2). Lower site-normalized flows (< 41.36 % of median flow) were predicted to have the highest chlorophyll *a* concentrations (54.9 mg/m^2). Overall 25% of the variance in chlorophyll *a* concentrations among sites can be explained by site-normalized flow and TN concentrations.

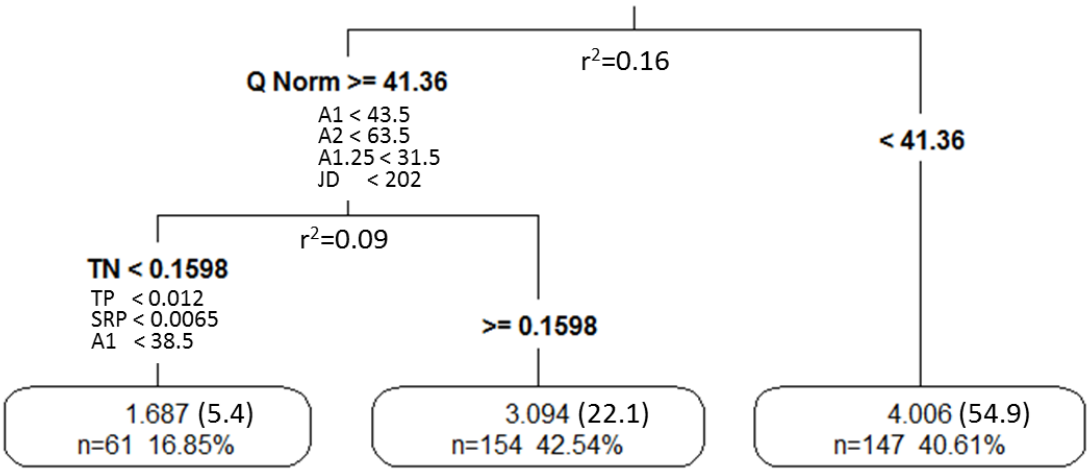

Figure 12. Final regression tree for log-transformed periphytic chlorophyll *a* (r^2 = 0.25). Names and numbers at each node indicate the predictor variable and its value used for the split. Names and numbers below each node indicate the surrogate variables and their values that can be used as alternatives for the split. Sites that meet the node criteria are split to the two sub-groups. Numbers at the leaves specify mean predicted values for the response variable (log-transformed chlorophyll *a*), back-transformed chlorophyll *a* values (parenthetic numbers in mg/m^2), the number of samples (n), and their proportion of the total sample number (%). Abbreviations: TN, Total Nitrogen; Q Norm, site-normalized flow (% of median flow); A1 and A2 are days of accrual (consecutive days that flow has remained below 1x or 2x the median flow); JD, julian day.

Similar to the CART results for total periphyton biomass, the analyses predicted increasing relative abundance of benthic N-fixers (54.49%) with decreasing site-normalized flow (< 49.85% of median flow) (Figure 13). Samples with higher site-normalized flows (≥ 49.85% of median flow) were predicted to have the lowest abundance of benthic N-fixers (9.73%). These samples were also characterized with higher TIN concentrations (> 0.02 mg/L) and low alkalinity (< 73.7 mg/L $CaCO_3$), which could be used as surrogates for site-normalized flow. Overall 50% of the variance in relative abundance of benthic N-fixers among sites can be explained by site-normalized flow.

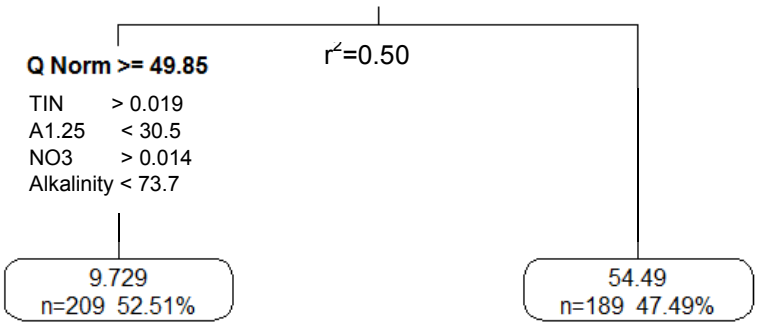

Figure 13. Final regression tree for benthic N-fixers ($r^2 = 0.50$). Names and numbers at each node indicate the predictor variable and its value used for the split. Names and numbers below each node indicate the surrogate variables and their values that can be used as alternatives for the split. Sites that meet the node criteria are split to the two sub-groups. Numbers at the leaves specify mean predicted values for the response variable (chlorophyll *a*), the number of samples (n), and their proportion of the total sample number (%). Abbreviations: Q Norm, site-normalized flow (% of median flow); A1.25, days of accrual (consecutive days that flow has remained below 1.25x the median flow); TIN: total inorganic nitrogen.

3.4.4 BENTHIC N-FIXER REGRESSION ANALYSES

As shown above in sections 2.2 and 2.3, benthic N-fixers, flow, and nitrate exhibit strong longitudinal and seasonal patterns. The apparent association between increasing upstream to downstream relative benthic nitrogen-fixer biomass and decreasing nitrate (Figure 5) is shown in a scatterplot using individual samples collected during June through September (Figure 14). Although significant scatter is evident (most points not fitting the general relationship were September samples) a significant negative relationship was found between the two variables (r^2=0.34, p<0.00001, Figure 14). Because there was greater variation in NO_3 between stations than within a station, the relationship appears to be strongly associated with the longitudinal pattern. For example, the majority of higher nitrate values located to the right of the "Stancheva" threshold[9] were associated with upstream stations (Figure 14).

Due to the seasonal covariation of flow, nitrate, and relative benthic N-fixer biomass[10] the June-September seasonal means were calculated to evaluate inter-annual associations among the parameters. The relationship between the two variables was stronger for the seasonal means (r^2=0.76, p<0.00001, Figure 15) than it was for the individual samples (Figure 14). However, it is clear that the longitudinal gradient is still driving the relationship for the seasonal means, with high nitrate/low relative N-fixer values comprised mostly of upstream stations, and no clear inter-annual pattern was evident for seasonal means within stations (see DWLS smoothers; Figure 16).

[9] Stancheva et al. (2013) determined a benthic N-fixer diatom response threshold of 0.075 mg/L NO3-N. Concentrations below this threshold were associated with increased benthic N-fixing diatoms.

[10] In this case flow and nitrate show a consistent seasonal decrease between June-September while relative benthic N-fixer biomass increases. Thus, the seasonal means provide a way to evaluate specific controlling effects of flow and nitrate independent of seasonal covariation among the parameters.

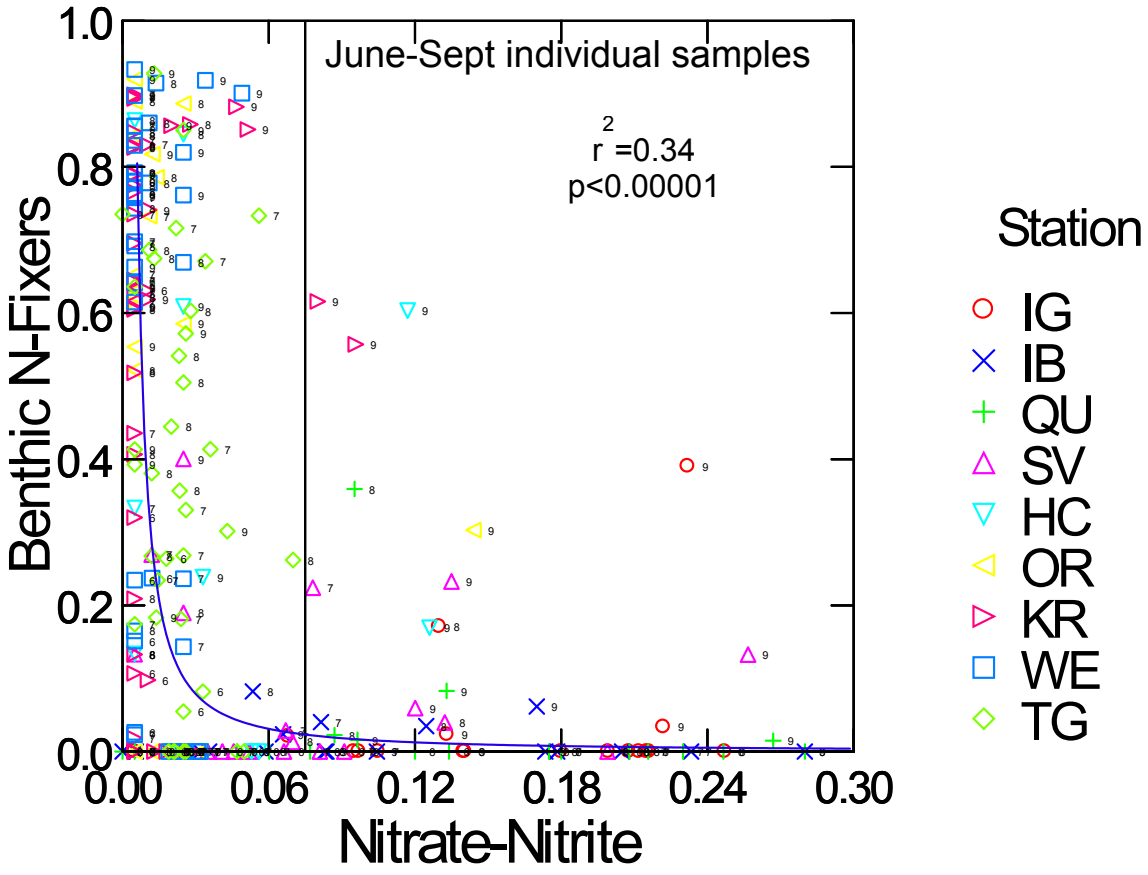

Figure 14. Relative benthic nitrogen-fixer biomass (percent/100) vs. nitrate concentration (mg/L) for individual samples collected at Klamath River in the months of June-September. Linear model was fit to log-transformed nitrate concentration and logit-transformed relative benthic nitrogen-fixers; graph shown here is untransformed. Vertical line at 0.075 mg/L is threshold for periphytic n-fixing diatom abundance (Stancheva et al. 2013).

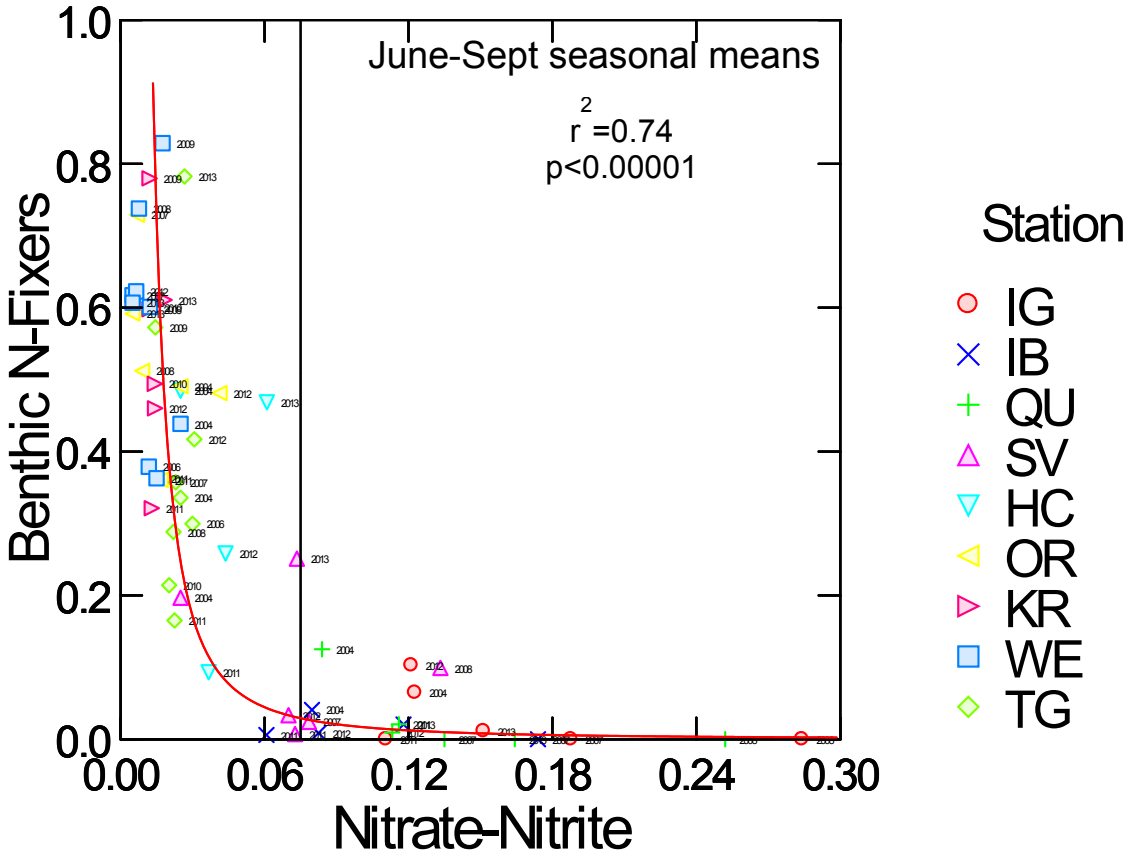

Figure 15. Relative benthic nitrogen-fixer biomass (percent/100) vs. nitrate concentration (mg/L) for June-September seasonal means at Klamath River sites. Linear model was fit to log-transformed nitrate concentration and logit-transformed relative benthic nitrogen-fixers; graph shown here is untransformed. Vertical line at 0.075 mg/L is threshold for periphytic n-fixing diatom abundance (Stancheva et al. 2013).

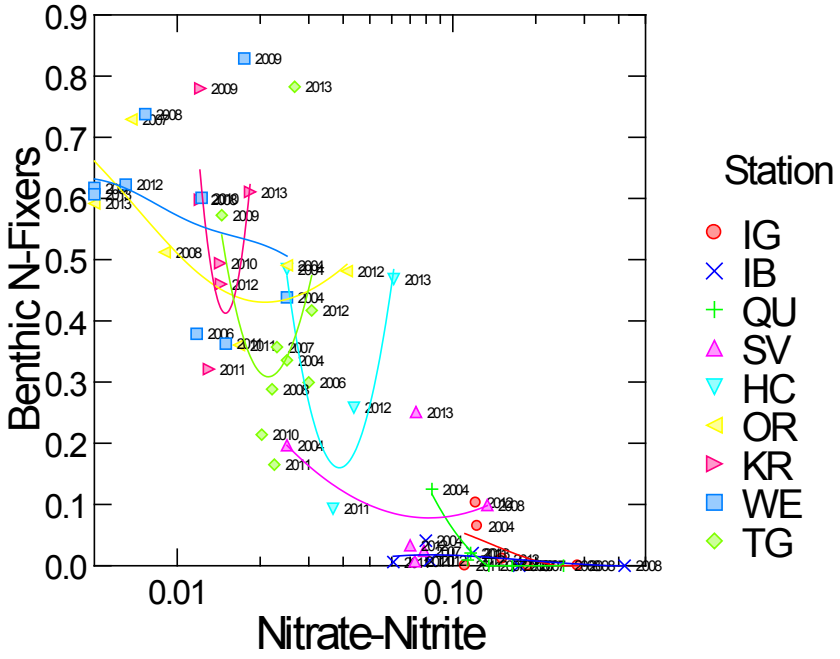

Figure 16. Relative benthic nitrogen-fixer biomass (percent/100) vs. nitrate concentration (mg/L) for June-September seasonal means at Klamath River sites. Nitrate concentration is plotted on a logged scale. A Distance-Weighted Least Squares (DWLS) smoother is fit to each station.

In contrast, the relationship between the June-September seasonal mean relative benthic nitrogen-fixer biomass vs. flow revealed that while no overall linear relationship existed, that flow was negatively associated with benthic nitrogen-fixers within many stations (see DWLS smoother; Figure 17a). Although sample size is relatively small, simple linear regression shows that with the exception of IG, QU, and WE that June-September mean relative benthic N-fixer biomass was negatively related to June-September discharge on a within station basis (Table 8).

Table 8. Regression r^2 and p-values for the relationship between June-September mean relative benthic N-fixer biomass and discharge (cfs).

Station	n	r^2	*p*-value
IG	6	0.156	0.438
IB	6	**0.781**	**0.020****
QU	6	0.12	0.506
SV	6	**0.621**	**0.063***
HC	4	**0.995**	**0.003****
OR	6	**0.623**	**0.062***
KR	6	**0.644**	**0.0558***
WE	9	0.308	0.121**
TG	9	**0.801**	**0.001**

****p*<0.05**

***p*<0.10**

Further evaluation of downstream stations where relative benthic N-fixer biomass was generally higher and flow less moderated by impoundments (OR, KR, WE, and TG) reveals an overall significant negative relationship between the overall lower station mean relative benthic N-fixer biomass and lower station mean discharge (Figure 17b).

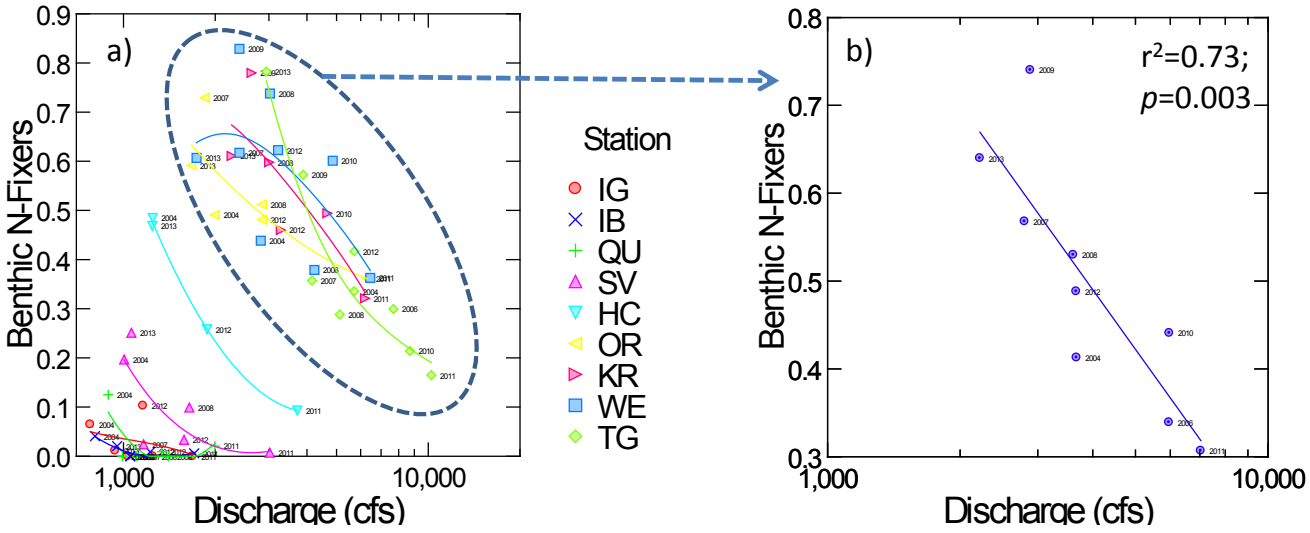

Figure 17. (a) June-September seasonal mean relative benthic nitrogen-fixers (percent/100) vs. June-September seasonal mean discharge (cfs) for all Klamath River sample stations. Relative benthic nitrogen-fixers is logit-transformed and discharge is plotted on a logged scale. A Distance-Weighted Least Squares (DWLS) smoother is fit to each station. (b) June-September overall lower river (OR, KR, WE, and TG) seasonal means of relative N-fixer proportion and discharge, 2004-2013.

3.4.5 MIXED-EFFECTS MODELS AND MULTIPLE REGRESSION MODELS

Amongst all multiple regression models using different measures of periphyton biomass as response variables, the best one (highest r^2=0.44, Table 9) explained the highest amount of variability in benthic N-fixers as a response to NO3 and site-normalized flow. Mixed-effects models revealed the environmental variables most significantly related to periphyton biomass (e.g., biovolume and periphytic chlorophyll *a*) and percentage of benthic nitrogen fixers. The mixed models using Site as a random factor (random intercept) were significantly better ($p \leq 0.05$, lower AIC) than the multiple linear regression models (Table 9). The most important variables explaining the variance in periphyton biovolume included site-normalized flow (Figure 18), air temperature, and nitrate concentrations. Increasing values for predictor variables resulted in decreased periphyton biomass. Air temperature and site-normalized flow were negatively related to periphytic chlorophyll *a*, especially when accounting for temporal autocorrelation at the sites. The two variables most significantly and negatively related to the relative biomass of N-fixers included nitrate concentrations and site-normalized flow (Figure 19 and Figure 20). Site-normalized flow was the most important predictor for both periphyton biomass and percentage of benthic N-fixers. Regression diagnostics revealed that the final models met the assumptions of normality and equal variance.

Table 9. Summary of results from regression models. Mixed-effects models (with random effect) fit the data better (had lower AIC) than fixed models (presented here only for information purposes).Abbreviations: Q Norm, site-normalized flow (% of median flow); ATEMP, average air temperature; and NO3_NO2, Nitrate-nitrite

Response	**Predictors with regression coefficients**	**Random effect**	**Adjusted r^2 ($p \leq 0.05$ in bold)**	**AIC**
log Biovolume	-0.583*ATEMP -0.533*NO3_NO2 -0.507*Q_Norm	None	**0.22**	761.15
log Biovolume	-0.319*Q_Norm	Site		732.98
log Peri Chl	-0.5356*Q_Norm -0.3320*ATEMP	None	**0.11**	773.85
log Peri Chl	-0.45923*Q_Norm -0.3366*ATEMP	Site+ Autocorrelation		732.89
Benthic N-fixers	-9.696*NO3_NO2-19.888*Q_Norm	None	**0.44**	2050.15
Benthic N-fixers	-5.67877*NO3_NO2-19.69734*Q_Norm	Site		2030.53

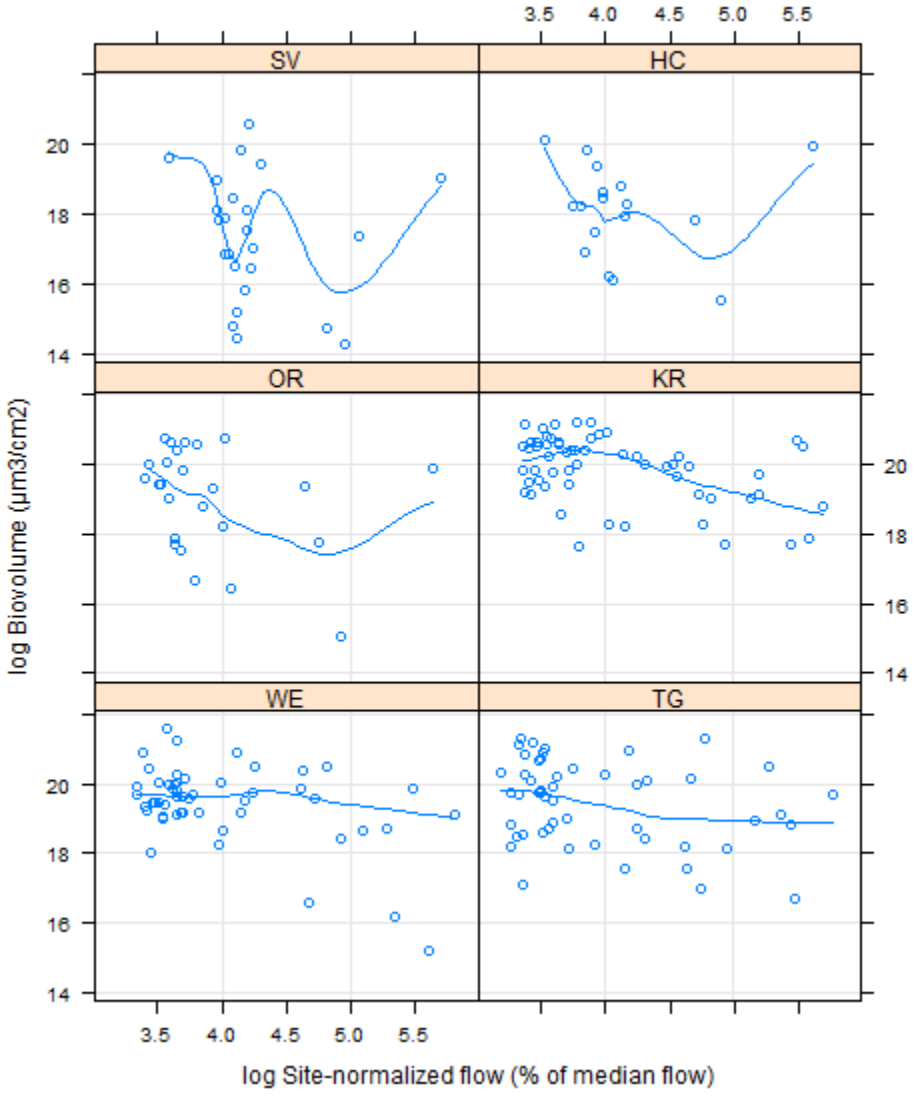

Figure 18. Scatterplots with LOWESS trend lines showing the relationship between periphyton biomass and site-normalized flow for the six sites used in the mixed-effects models

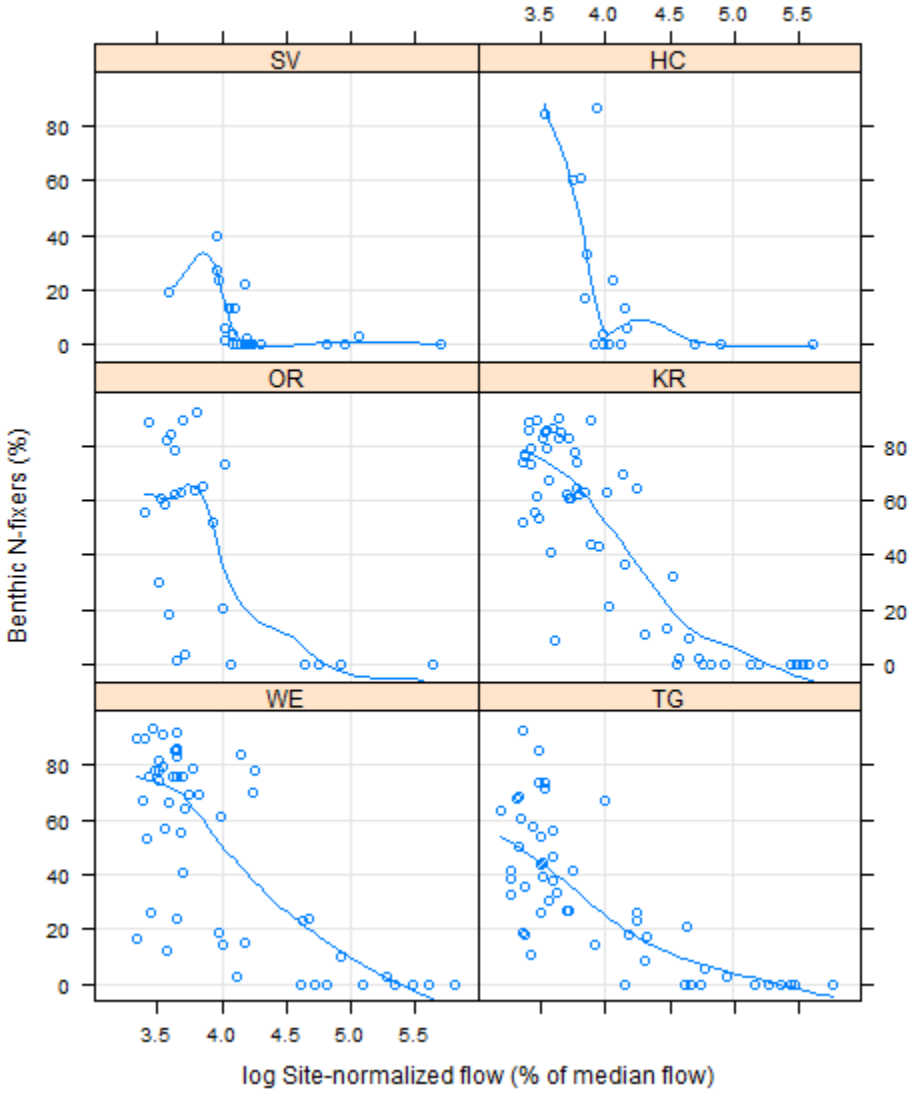

Figure 19. Scatterplots with LOWESS trend lines showing the relationship between benthic nitrogen fixers and site-normalized flow for the six sites used in the mixed-effects models.

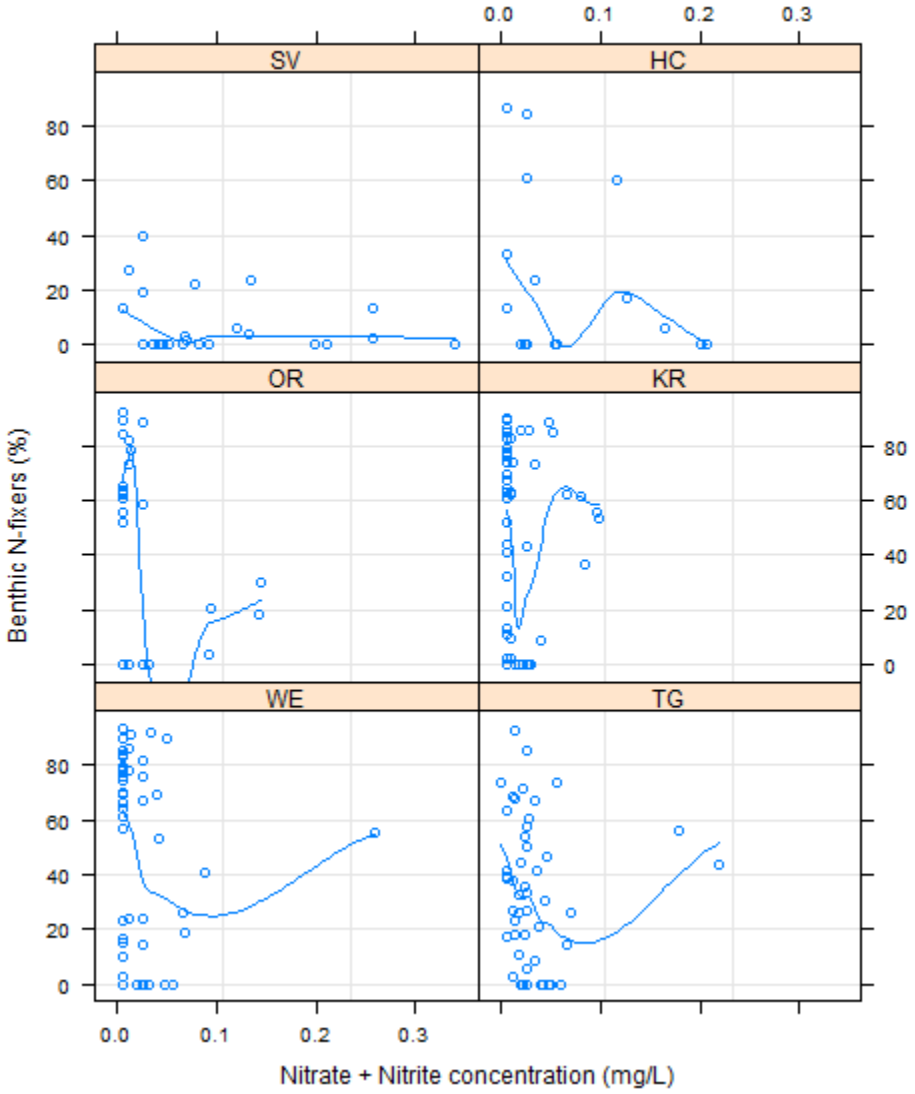

Figure 20. Scatterplots with LOWESS trend lines showing the relationship between benthic nitrogen fixers and nitrate-nitrite concentrations for the six sites used in the mixed-effects models.

4 DISCUSSION

Contrary to the River Continuum Concept (RCC, Vannote et al. 1980), which describes rivers as longitudinal gradients from small mountain streams with limited nutrients and periphyton growth to large rivers with abundance of nutrients and phytoplankton, the Klamath River originates from a hyper-eutrophic lake (Upper Klamath Lake) dominated by planktonic bloom-forming cyanobacteria (e.g., *Aphanizomenon flos-aquae*) (Kann and Smith 1999, Eilers et al. 2004, Eldridge et al. 2013, Oliver et al. 2014). The 'lake and reservoir effects' are clearly reflected by the periphyton assemblages in the upper portion of the river and diminish in downstream direction (for more details see Asarian et al. 2014).

The downstream reaches of the Klamath River are characterized with high periphyton biomass and assemblages dominated by nitrogen (N)-fixing diatoms (e.g., *Epithemia sorex*) and cyanobacteria (*Calothrix* sp.) that reflect overall N-limited conditions (Hill and Knight 1988, Power et al. 1988, Peterson and Grimm 1992). Indeed, our results reveal significantly less nutrients in downstream reaches (Group 1, Table 6, Figure 9) than upstream (Group 3). This inverse relationship between periphyton biomass and nutrients can be explained by the ability of benthic N-fixers to overcome nitrogen limitation. A number of studies have shown inverse relationships between nitrogen concentrations and the relative abundance of N-fixers (Porter et al. 2008, Stancheva et al. 2013, Carpenter et al. 2014), endosymbiont biomass (Stancheva et al. 2013), and the number of endosymbionts within a diatom cell (DeYoe et al. 1992) increased with decreasing nitrogen concentrations. The inverse relationship between periphyton biomass and nutrients may be amplified by sampling design and the sampling protocol used in this study. The protocol targets microscopic diatoms from cobble substrates which may adequately characterize downstream periphyton assemblages; however, the upper stream reaches in the Klamath River, high nutrient concentrations support an extensive amount of filamentous algae (e.g., *Cladophora* sp.) and macrophytes (e.g., *Potamogeton* sp., *Elodea* sp.). To capture filamentous algae and macrophytes as well as periphyton attached to their surface may require a reach-scaled sampling design. For instance, Stevenson and Bahls (1999) proposed a rapid semi-quantitative sampling protocol to capture reach-scale periphyton biomass. Periphyton biomass including filamentous macroalgae can be estimated in multiple sampling locations along multiple-transects in a stream reach by immersing a clear-bottom bucket with 50-dot grid. The protocol used in this study is not designed to adequately characterize filamentous algae or macrophytes and consequently sampled epilithic periphyton biomass in the upper streams may not reflect all primary producers in the segment of the river ecosystem.

Unlike lake phytoplankton (Schindler 1978, Guildford and Hecky 2000), the relationship between nutrients and periphyton biomass in streams is much more dynamic (see reviews by Dodds 2006, 2007). A meta-analysis of 237 nutrient enrichment experiments found lack of biomass response in 42.7% of them and significant increase in 57.8% of them (Francoeur 2001). A national study of 976 streams and rivers (Porter et al. 2008) uncovered a positive correlation (Spearman's $r=0.18$, $p \leq 0.05$) between algal biovolume and nitrate concentrations. Dodds et al. (2002) analyzed published data from 620 sampling stations across the US and established that total phosphorus concentrations above 30 µg/L and total nitrogen concentrations above 40 µg/L result in significantly higher periphytic chlorophyll *a* concentrations. These nutrient concentrations were greatly exceeded for most samples from the Klamath River where total phosphorus and total nitrogen concentrations were 0.067 mg/L and 0.365 mg/L, respectively,

while the mean periphytic chlorophyll *a* concentration was 74.8 mg/m^2. However, mean periphytic chlorophyll *a* concentrations at downstream sites sampled in summer and fall (Group 1, Table 6) exceeded 100 mg/m^2, which is considered high for streams (Welch et al. 1988). Yet, these samples did not have the highest nutrient concentrations (Table 6). This discrepancy can be explained by the abundance of benthic N-fixers and sampling protocol (see previous paragraph). However, the relationship between nutrients and periphyton biomass may not be very strong because individual species in the periphyton assemblage often have different nutrient requirements (Borchardt 1996).

The difference in strength of association between algal biomass and nutrients in lakes and streams is attributed to the effects of light, disturbance, and grazing in lotic ecosystems (Cattaneo 1987). Resources such as light are crucial for benthic diatom growth especially in streams with stable hydrologic regimes (Patrick 1967, Steinman and McIntire 1986, Stevenson et al. 1991, Fanta et al. 2010). Light limitation due to riparian shading can override the effects of nutrient enrichment. Hill and Knight (1988) reported that the nutrient enrichment effects on periphyton biomass was evident in an unshaded northern California stream but not in a shaded stream. In the Klamath River, flow-related disturbance may have significant effects on periphyton assemblages. Frequent physical disturbance associated with substratum instability, high water velocities, and abrasion by sediments (Biggs 1996) or scraping and grazing invertebrates may disturb periphyton assemblages (Steinman 1996) and result in reduced algal biomass (Peterson 1996). NMDS identified three relatively distinct periphyton groups whose distributional patterns ("envfit" analysis) can be largely explained by nutrients and flow (Figure 9 and Table 7). The difference between the upstream (Group 3) and downstream periphyton assemblages (Groups 1 and 2) was associated with nutrients (r^2>0.6 for TN, SRP, and TP, and r^2 = 0.47 for nitrate-nitrite, Figure 9, Table 7), while site-normalized flow largely separated the two downstream groups (downstream sites sampled in spring and early summer vs. downstream sites sampled in summer and fall, r^2=0.56).

Classification tree model further illustrated the interactive effects of nutrients and flow on periphyton assemblages (Figure 10). The model predicted that periphyton assemblages in downstream sites sampled in summer and fall (Group 1) were associated with more stable hydrological conditions (<54% median flow). Groups 2 and 3 were characterized with higher site-normalized flows (> 54.5% of annual median flow). Under the higher site-normalized flow, low SRP concentrations (< 0.035 mg/L) were associated with samples from Group 2 (spring and early summer samples from downstream sites) and higher SRP concentrations (> 0.035 mg/L) were associated with Group 3 (upstream nutrient-rich sites). The consistent relationships between flow and biomass in this study (CART models) suggest that site-normalized flows >50% site median should result in reduced algal biomass. This observation has potential management applications in the control of algal biomass in the river. However, some of the described effects can be modulated by the successional age of periphyton assemblages (Peterson et al. 1990, Steinman and McIntire 1990). Early successional assemblages are dominated by prostrate and/or erect diatom growth forms, which are more resistant to disturbance (Steinman and McIntire 1986, Peterson and Stevenson 1989). These early successional assemblages characteristic of highly disturbed environments correspond to our spring and early summer samples from downstream sites (Group 2) where diatoms comprised 97% of sample's relative biovolume. At later stages of succession and without disturbance, the periphyton assemblage may develop into a complex three-dimensional structure (Hoagland et al. 1982, Korte and Blinn 1983) that includes

an understory of diatoms and a canopy of green algae and cyanobacteria, which may deplete the underlying layers for vital resources resulting in increased senescence (Stevenson et al. 1991, Johnson et al. 1997). Late successional periphyton assemblages are also more susceptible to disturbance (Peterson 1987, Steinman et al. 1987, Peterson and Stevenson 1992, Peterson 1996). Late successional assemblages in stable flow conditions may be typical for downstream sites sampled in summer and fall (Group 1) where diatoms had their lowest relative biovolume (90%) while green algae (1.84%) and cyanobacteria (7.4%) were the highest, compared to Groups 2 and 3. In a review chapter on periphyton biomass in streams, Biggs (1996) summarized that in medium to low disturbance and resource supply, communities are dominated by filamentous cyanobacteria and N-fixers (our Group 1); in medium to high disturbance/ grazing, communities are dominated by tightly-attached to the substrate diatoms (our Group 2).

In addition to the longitudinal nutrient gradient influencing periphyton assemblages in the Klamath River, there was a seasonal hydrological gradient (e.g., flow) acting on downstream stations, which are less impacted by flow regulation from dams and reservoirs. However, downstream stations are subjected to flow variations from seasonal precipitation and tributary inputs. River flow was higher in spring and early summer when periphyton assemblages were dominated by diatoms and lower in late summer and fall when cyanobacteria and N-fixing diatoms were abundant (Figure 9). Low flows can have positive effect on algal growth by supplying nutrients, while high flows can have negative effects by scouring attached algae (Stevenson 1996b). In KR high flows from snow melt and precipitation in spring and early summer result in the lowest periphyton biomass (Group 2, Table 6). After storm events, the periphyton assemblages are reduced to a thin film of scour-resistant diatoms (Steinman and McIntire 1990, Mulholland et al. 1991). There were significant reductions ($p \leq 0.05$) in periphytic chlorophyll *a* before and during the monsoon floods in a New Mexico river network (United States, Tornés et al. 2015). Similar to our findings, a study conducted in the nearby North Umpqua River also found that streamflow was the most important variable structuring periphyton assemblages (Carpenter et al. 2014). However, periphyton assemblage responses to high flows might be species dependent where many diatoms are well suited to withstand such disturbances (Stevenson 1990). The positive effect of low flows in summer and fall result in higher periphyton biomass because of increased nutrient supply (Pan et al. 1996, Stevenson et al. 2008a, Porter et al. 2008).

The mixed-effects and the multiple regression models revealed similarity in response among the three different biomass measurements (biovolume, periphytic chlorophyll *a*, and percent biovolume of benthic N-fixers). All three measures were negatively related to site-normalized flow (Table 9). Other significant predictors included nitrate-nitrite (in biovolume and percent biovolume of benthic N-fixer models) and air temperature (in biovolume and periphytic chlorophyll *a* models). Benthic N-fixers, which included site-normalized flow and nitrate-nitrate as predictor variables, had the strongest relationship of the three multiple regression models (r^2=0.44, Table 9), and the addition of "Site" as a random factor in the mixed effects models provided improved model fit ($p \leq 0.05$, lower AIC). Results from bivariate regressions support the same conclusions, with nitrate-nitrite concentrations explaining *longitudinal variation* and flow explaining *temporal variation* within sites. For example, nitrate-nitrite was inversely associated with relative benthic nitrogen-fixing biomass among sites for both individual samples (Figure 14; r^2=0.34) and for June-September seasonal means (Figure 15; $r^2 = 0.74$; $p<0.00001$ for both). For downstream stations where relative benthic N-fixer biomass was generally higher and flow was

less moderated by impoundments (Orleans, Saints Rest Bar, Weitchpec, and Turwar), flow was strongly inversely related to relative benthic N-fixer biomass (Figure 17b; $p = 0.003$, $r^2 = 0.73$).

These results confirm the commonly observed strong inverse relationship between the relative abundance of N-fixers and nitrogen concentrations in streams (Porter et al. 2008, Stancheva et al. 2013, Carpenter et al. 2014).

High stream flows scour the periphyton assemblages and result in reduced algal biomass (Peterson and Stevenson 1992, Peterson 1996). All three biomass measures exhibited different strengths of association with flow (e.g., different AIC values, Table 9) partially due to the difficulty in characterizing periphyton biomass (Stevenson 1996a). Periphyton biomass in streams, measured as periphytic chlorophyll *a* concentrations, can vary up to four orders of magnitude within a year (Biggs 1996). In addition to temporal factors, periphyton assemblages are structured by a number of environmental factors including resource supply (Steinman and McIntire 1986, Peterson and Stevenson 1989, Stevenson et al. 1991) and disturbance (Steinman et al. 1987, Peterson and Stevenson 1992, Peterson 1996). Also, each measure of algal biomass has its own disadvantages, biovolume estimates must account for error due to cell vacuoles, chlorophyll measurements can be biased if there is nutrient limitation or light limitation, and percent biovolume can have high error variance (Stevenson 1996a). Therefore, it is advisable to use as many measures of algal biomass as possible (Stevenson 1996a), just like in this study. Despite these sources of variability, flow was consistently a significant explanatory variable for periphyton biomass in CART, multiple regression, and mixed-effects models. In addition, the bivariate regression accounting for seasonal co-variation in flow and development of periphytic biomass[11] showed a strong inter-annual effect of flow on relative N-fixer biomass (Figure 17b).

Periphyton metrics, except for % of N-fixers, did not reveal very clear spatial and temporal patterns possibly because species autecological information was compiled from multiple observational studies (Lowe 1974, van Dam et al. 1994, Porter et al. 2008) with different study objectives and designs. However, the successful development of regional or system-specific algal metrics in other areas (Stevenson et al. 2008b, Potapova and Charles 2002, 2007) prompt us to believe that this might be an applicable approach to our study system. This approach is appropriate for well-defined environmental gradients (Munn et al. 2002) such as the Klamath River, which has a strong longitudinal nutrient gradient. The detailed nutrient and water quality information available for the Klamath River (Asarian and Kann 2013, Watercourse Engineering 2013), can be used to develop algal nutrient metrics (i.e., optima and tolerance) for common taxa by using weighted-averaging methods (Stevenson et al. 2008b, Potapova and Charles 2002, 2007).

In summary, this report describes spatial-temporal dynamics of the periphyton assemblages in Klamath River and their relationships with environmental conditions for the period from 2004 through 2013. We used multivariate data analysis to determine these linkages and we found that periphyton assemblages in the Klamath River are strongly associated with both temporal (e.g.,

[11] In North-temperate systems such as the Klamath River, flow declines annually as the algal growing season progresses due to many factors including increased water temperatures and increasing light. Thus, evaluation of seasonal means allows for further evaluation of specific inter-annual effects of flow.

decreasing flow from spring to fall) and inter-annual variations in flow conditions and spatial gradients in nutrient concentrations (e.g., decreasing from upstream to downstream). These results can be used as predictive tools in the management of the river and benefit ongoing efforts to improve its water quality conditions.

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APPENDIX A: PERIPHYTON SPECIES LIST AND TABLE OF AUTECOLOGICAL ATTRIBUTES

Table A1. Frequency and autecological attributes for species detected in 2004-2013 Klamath River samples. LT sites = long-term monitoring sites, excluding special studies. See Table A2 for key to autecological attributes. Species that the lab identified separately but have a single new species name are colored red. Current species name and autecological attributes not shown for the two species detected only in 2013 special studies.

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes													
				LT sites (n=398)	All sites (n=576)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Achnanthes clevei	ACCV	diatom	Karayevia clevei (Grunow) Kingston	6	10	2	2	4	2	2	2	2	4	1	3	5			1
Achnanthes exigua	ACEX	diatom	Achnanthidium exiguum (Grunow) Czarnecki	2	6	2	2	4	2	2	1	2	7	3	3				1
Achnanthes flexella	ACFL	diatom	Eucocconeis flexella (Kützing) Cleve	0	1	2	2	3	1	1	1	1	1	3	3				1
Achnanthes hauckiana	ACHK	diatom	Planothidium hauckianum (Grunow) Round et Bukhtiyarova	2	4	2	2								2				1
Achnanthes lanceolata	ACLC	diatom	Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	95	128	2	2	4	2	2	3	3	5	3	2	2			1
Achnanthes lewisiana	ACLW	diatom	Karayevia suchlandtii (Hustedt) Bukhtiyarova	3	4	2	2	3	1	1	1	1	1	2	3				1
Achnanthes linearis	ACLN	diatom	Rossithidium linearis (Smith) Round et Bukhtiyarova	43	74	2	2	3							3				1
Achnanthes minutissima	ACMN	diatom	Achnanthidium minutissimum (Kützing) Czarnecki	314	446	2	2	6	2	2	1	2	7	3	3	4			1
Achnanthes sp.	ACXX	diatom	Achnanthes sp.	1	1	2	2												1
Amphipleura pellucida	AMPL	diatom	Amphipleura pellucida (Kützing) Kützing	27	54	2	2	4	2	2	2	4	2	2	2	5			1
Amphora coffeiformes	AFCF	diatom	Amphora coffeaeformis (Agardh) Kützing	2	3	2	2	4	2	2	3	3	5	3	1				1
Amphora ovalis	AFOV	diatom	Amphora ovalis (Kützing) Kützing	19	27	2	2	4	2	2	2	2	5	1	3	4			1
Amphora perpusilla	AFPR	diatom	Amphora pediculus (Kützing) Grunow	140	212	2	2	4	2	2	2	2	5	3	3	4			1
Anabaena flos-aquae	ABFA	bluegreen	Anabaena flos-aquae (Linnaeus) Brébisson	3	5	2	1	5									1	1	2
Anabaena sp.	ABXX	bluegreen	Anabaena sp.	2	4	2	1										1	1	2
Ankistrodesmus falcatus	AKFL	green	Ankistrodesmus falcatus (Corda) Ralfs	33	57	2	2											1	2
Anomoeoneis vitrea	AOVT	diatom	Brachysira vitrea (Grunow) Ross	0	1	2	2	5	2	1	2	1	2	2	2				1
Aphanizomenon flos-aquae	APFA	bluegreen	Aphanizomenon flos-aquae (Linnaeus) Ralfs	39	51	2	1	5									1	1	2
Asterionella formosa	ASFO	diatom	Asterionella formosa Hassall	3	8	2	2	4	2	2	2	2	4	1	3				2
Bacillaria paradoxa	BAPA	diatom	Bacillaria paxillifera (O.F.Müller) Marsson	1	1	2	2	6	4	2	4	3	5	3	2				1
Basicladia sp.	BSXX	green	Arnoldiella sp.	1	1														
Caloneis sp.	CAXX	diatom	Caloneis sp.	2	2	1	2												1
Caloneis ventricosa	CAVT	diatom	Caloneis ventricosa (Ehrenberg) Meister	2	4	1	2								2				1
Caloneis ventricosa minuta	CAVM	diatom	Caloneis ventricosa var. minuta (Grunow) Mills	21	41	1	2												1
Calothrix sp.	KXXX	bluegreen	Calothrix sp.	71	104	2	1												1
Chlamydomonas sp.	CHXX	green	Chlamydomonas sp.	5	10	1	2											1	2

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes												
				LT sites (n=398)	All sites (n=576)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Cladophora sp.	CFXX	green	Cladophora sp.	26	40	2	2										2	1	1
Cladophora sp.	CFX9	green	Cladophora sp.	1	1	2	2										2	1	1
Closteriopsis longissima	CBLG	green	Closteriopsis longissima Lemmermann	2	2	2	2											1	2
Cocconeis disculus	CODS	diatom	Cocconeis disculus (Schumann) Cleve	1	1	2	2		3			1							1
Cocconeis klamathensis	COKL	diatom	Cocconeis klamathensis Sovereign	16	16	2	2												1
Cocconeis pediculus	COPD	diatom	Cocconeis pediculus Ehrenberg	7	14	2	2	4	3	2	2	2	5	1	3	4			1
Cocconeis placentula	COPC	diatom	Cocconeis placentula Ehrenberg	370	523	2	2	4	2	2	3	2	5	2	3	4			1
Cosmarium sp.	CSXX	green	Cosmarium sp.	1	2	2	2												
Crucigenia quadrata	CGQD	green	Crucigenia quadrata Morren	0	1	2	2											1	2
Cryptomonas erosa	CXER	cryptophyte	Cryptomonas erosa Ehrenberg	8	9	1	2											1	2
Cyclotella meneghiniana	CCMG	diatom	Cyclotella meneghiniana Kützing	21	40	2	2	4	3	3	5	4	5	2	2				2
Cyclotella ocellata	CCOC	diatom	Cyclotella ocellata Pantocsek	1	1	2	2	4	1	1	1	1	4	1					2
Cyclotella stelligera	CCST	diatom	Discostella stelligera (Cleve et Grunow) Houk et Klee	3	4	2	2	6	2					1	3				2
Cymatopleura solea	CPSL	diatom	Cymatopleura solea (Brébisson) Smith	1	1	1	2	4	2	2	3	2	5	1	2	3			1
Cymbella affinis	CMAF	diatom	Cymbella affinis Kützing	266	373	2	2	4	2	1	1	2	5	2	3	5			1
Cymbella cesatii	CMCS	diatom	Encyonopsis cesatii (Rabenhorst) Krammer	0	3	2	2	3	1	1	1	1	1	3	3				1
Cymbella cistula	CMCL	diatom	Cymbella cistula (Ehrenberg) Kirchner	1	1	2	2	4	2	1	2	2	5	1	3	5			1
Cymbella cymbiformes	CMCM	diatom	Cymbella cymbiformis Agardh	2	2	1	2	3	2	1	1	1	2	2	3				1
Cymbella mexicana	CMMX	diatom	Cymbella mexicana (Ehrenberg) Cleve	10	15	1	2								3				1
Cymbella microcephala	CMMC	diatom	Encyonopsis microcephala (Grunow) Krammer	10	13	2	2	4	2	1	1	1	4	3	2				1
Cymbella minuta	CMMN	diatom	Encyonema minutum (Hilse) Mann	117	181	2	2	3	2						2				1
Cymbella sinuata	CMSN	diatom	Reimeria sinuata (Gregory) Kociolek et Stoermer	173	260	2	2	3	2	2	1	2	3	3	3	5			1
Cymbella sp.	CMXX	diatom	Cymbella sp.	3	5	2	2												1
Cymbella tumida	CMTM	diatom	Cymbella tumida (Brébisson ex Kützing) Van Heurck	11	13	2	2	4	2	1	1	1	4	1	3	5			1
Denticula elegans	DNEL	diatom	Denticula elegans Kützing	5	13	1	2	4	2					5					1
Diatoma hiemale mesodon	DTHM	diatom	Diatoma mesodon (Ehrenberg) Kützing	3	3	2	2	3	1	1	1	1	3	2	3				1
Diatoma tenue	DTTN	diatom	Diatoma tenuis Agardh	225	321	2	2	4	3	2	3	3	5	1	2				1
Diatoma tenue elongatum	DTTE	diatom	Diatoma tenuis Agardh	2	3	2	2	4	3	2	3	3	5	1	2				1
Diatoma vulgare	DTVL	diatom	Diatoma vulgaris Bory	191	274	2	2	5	2	2	2	2	4	1	3	4			1
Didymosphenia geminata	DDGM	diatom	Didymosphenia geminata (Lyngbye) Schmidt	0	1	2	2	6							3				1
Diploneis elliptica	DPEL	diatom	Diploneis elliptica (Kützing) Cleve	2	3	1	2	4	2	1	1	1	3	3	3				1
Diploneis sp.	DPXX	diatom	Diploneis sp.	1	1	1	2												1
Epithemia sorex	EPSX	diatom	Epithemia sorex Kützing	275	395	2	1	5	2	1	2	2	5	2	3				1

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes											
				LT sites (n=398)	All sites (n=576)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Epithemia turgida	EPTR	diatom	Epithemia turgida (Ehrenberg) Kützing	49	68	2	1	5	2	1	2	2	4	3	3				1
Eunotia pectinalis	EUPC	diatom	Eunotia pectinalis (Müller) Rabenhorst	1	2	2	2	2	1	2	1	2	3	3					1
Eunotia sp.	EUXX	diatom	Eunotia sp.	0	1	2	2												1
Fragilaria brevistriata	FRBR	diatom		0	1														
Fragilaria capucina mesolepta	FRCM	diatom	Fragilaria capucina var. mesolepta Rabenhorst	10	30	2	2	4	2						2				1
Fragilaria construens	FRCN	diatom	Staurosira construens (Ehrenberg) Williams et Round	53	79	2	2	4	2	1	1	2	4	1	3	5			1
Fragilaria construens venter	FRCV	diatom	Staurosira construens var. venter (Ehrenberg) Hamilton	104	148	2	2	4	2	2	1	2	4	1	3				1
Fragilaria crotonensis	FRCR	diatom	Fragilaria crotonensis Kitton	7	12	2	2	4	2	2	2	2	3	1	3				2
Fragilaria leptostauron	FRLP	diatom	Staurosirella leptostauron (Ehrenberg) Williams et Round	5	6	2	2	4	2	1	1	1	4	2	3				2
Fragilaria pinnata	FRPN	diatom	Staurosirella pinnata (Ehrenberg) Williams et Round	5	16	2	2	4	2	2	1	2	7	3	3				1
Fragilaria vaucheria	FRVA	diatom	Fragilaria vaucheriae (Kützing) Petersen	82	112	2	2	4	2	2	3	3	5	3	2	2			1
Fragilaria vaucheriae	FRVA	diatom	Fragilaria vaucheriae (Kützing) Petersen	82	112	2	2	4	2	2	3	3	5	3	2	2			1
Frustulia rhomboides	FSRH	diatom	Frustulia rhomboides (Ehrenberg) De Toni	2	2	1	2	2	1	1	1	1	1	2	3				1
Glenodinium sp.	GDXX	dinoflagellate	Glenodinium sp.	1	1	1	2												2
Gloeocystis ampla	GLAM	green		0	1														
Gloeocystis sp.	GLXX	green	Gloeocystis sp.	1	1	2	2												1
Gloeotrichia echinulata	GTEC	bluegreen	Gloeotrichia echinulata (Smith) Richter	2	2	2	1												2
Gomphoneis herculeana	GSHR	diatom	Gomphoneis herculeana (Ehrenberg) Cleve	188	246	2	2								3				1
Gomphonema acuminatum	GFAC	diatom	Gomphonema acuminatum Ehrenberg	1	3	2	2	4	2	1	2	2	5	2					1
Gomphonema angustatum	GFAN	diatom	Gomphonema angustatum (Kützing) Rabenhorst	258	371	2	2	4	2	1	1	1	1		2	5			1
Gomphonema clevei	GFCL	diatom	Gomphonema clevei Fricke	31	40	2	2								3				1
Gomphonema gracile	GFGC	diatom	Gomphonema gracile Ehrenberg emend Van Heurck	0	1	2	2	3	2	1	1	1	3	3	2				1
Gomphonema olivaceum	GFOM	diatom	Gomphoneis olivaceum (Hornemann) Dawson ex Ross and Sims	74	96	2	2	5	2	2	2	2	5	1	3	4			1
Gomphonema parvulum	GFPV	diatom	Gomphonema parvulum (Kützing) Kützing	1	1	2	2	3	2	3	4	4	5	3	1	1			1
Gomphonema sp.	GFXX	diatom	Gomphonema sp.	8	9	2	2												1
Gomphonema subclavatum	GFSB	diatom	Gomphonema subclavatum (Grunow) Grunow	230	325	2	2	3	2	1	1	2	2	3	2				1
Gomphonema tenellum	GFTN	diatom	Gomphonema minutum (Agardh) Agardh	39	61	2	2	3	2			2	5		3				1
Gomphonema truncatum	GFTR	diatom	Gomphonema truncatum Ehrenberg	1	3	2	2	4	2	1	2	2	4	2	3	5			1
Gomphonema ventricosum	GFVT	diatom	Gomphonema ventricosum Gregory	126	172	2	2		1	1	1	1	1						1
Gyrosigma spencerii	GYSP	diatom	Gyrosigma spencerii (Smith) Griffith et Henfrey	16	22	1	2	4							2				1
Hannaea arcus	HNAR	diatom	Hannaea arcus (Ehrenberg) Patrick	18	23	1	2	6							3				1
Lyngbya sp.	LNXX	bluegreen	Lyngbya sp.	8	9	2	2												1
Melosira ambigua	MLAM	diatom	Aulacoseira ambigua (Grunow) Simonsen	1	1	2	2	4	2	2	3	2	5	1	3				2

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes													
				LT sites (n=398)	All sites (n=576)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Melosira granulata	MLGR	diatom	Aulacoseira granulata (Ehrenberg) Simonsen	22	27	2	2	4	2	2	3	2	5	1	3				2
Melosira italica	MLIT	diatom	Aulacoseira italica (Ehrenberg) Simonsen	1	1	2	2	4	2	2	2	2	4	3	3				2
Melosira varians	MLVR	diatom	Melosira varians Agardh	53	82	2	2	4	2	3	3	3	5	2	2	2			1
Meridion circulare	MRCR	diatom	Meridion circulare (Greville) Agardh	2	2	2	2	4	2	2	2	2	7	1	3				2
Microcystis aeruginosa	MSAE	bluegreen	Microcystis aeruginosa Kützing	9	22	2	2	2									1	1	2
Mougeotia sp.	MGXX	green	Mougeotia sp.	2	7	2	2										2		1
Navicula anglica	NVAG	diatom	Placoneis elginensis (Gregory) Cox	3	3	2	2	4	2	2	2	2	5	3	3				1
Navicula capitata	NVCP	diatom	Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	0	1	1	2	4	2	2	3	3	4	3	2	3			1
Navicula cascadensis	NVCS	diatom	Navicula cascadensis Sovereign	3	6														
Navicula cryptocephala	NVCR	diatom	Navicula cryptocephala Kützing	230	332	2	2	4	2	2	3	3	7	2	3				1
Navicula cryptocephala veneta	NVCV	diatom	Navicula veneta Kützing	306	433	1	2	4	3	2	4	4	5	3	1	1			1
Navicula decussis	NVDC	diatom	Geissleria decussis (Hustedt) Lange-Bertalot et Metzeltin	21	38	2	2	4	2	1		1	4	3	3				1
Navicula graciloides	NVGC	diatom	Navicula cari Ehrenberg	31	45	1	2		2				7		2				1
Navicula gregaria	NVGR	diatom	Navicula gregaria Donkin	21	28	1	2	4	3	2	4	3	5	3	2	2			1
Navicula menisculus upsaliensis	NVMU	diatom	Navicula upsaliensis (Grunow) Peragallo	9	9	1	2	4	2			2			2				1
Navicula minima	NVMN	diatom	Eolimna minima (Grunow) Lange-Bertalot et Schiller	6	10	2	2	4	2	3	4	4	5	3	1	1			1
Navicula minuscula	NVML	diatom	Adlafia minuscula (Grunow) Lange-Bertalot	18	26	1	2	4	1			2	1	4	1				1
Navicula mournei	NVMO	diatom	Navicula mournei Patrick	0	1														
Navicula pupula	NVPP	diatom	Sellaphora pupula (Kützing) Meresckowsky	15	21	2	2	3	2	2	3	3	4	2	2	3			1
Navicula radiosa	NVRD	diatom	Navicula radiosa Kützing	1	1	2	2	3	2	2	2	2	4	3	3				1
Navicula reinhartii	NVRN	diatom	Navicula reinhardtii (Grunow) Grunow	2	2	2	2	5	2	2	2	2	5	2					1
Navicula rhynchocephala	NVRH	diatom	Navicula rhynchocephala Kützing	0	1	2	2	4	2	2	4	2	7	2	3	5			1
Navicula sp.	NVXX	diatom	Navicula sp.	46	60	1	2												1
Navicula tripunctata	NVTP	diatom	Navicula tripunctata (Müller) Bory	160	211	2	2	4	2	2	2	2	5	3	3	4			1
Navicula viridula	NVVR	diatom	Navicula viridula (Kützing) Ehrenberg	37	54	2	2	4	2	2	2	3	5	1	2				1
Neidium affine	NDAF	diatom	Neidium affine (Ehrenberg) Pfitzer	0	3	2	2	3	2	1	1	1	4	1					1
Neidium sp.	NDXX	diatom	Neidium sp.	0	1	1	2												1
Nitzschia acicularis	NZAC	diatom	Nitzschia acicularis (Kützing) Smith	19	37	1	2	4	2	4	4	3	5	1	2	3			2
Nitzschia amphibia	NZAM	diatom	Nitzschia amphibia Grunow	99	144	2	2	4	2	3	3	3	5	3	2	2			1
Nitzschia capitellata	NZCP	diatom	Nitzschia capitellata Hustedt	38	71	2	2	4	4			5	6	3	2				1
Nitzschia clausii	NZCL	diatom	Nitzschia clausii Hantzsch	1	1	2	2	4	4	2	2	3	5	3	2	3			1

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes												
				LT sites (n=398)	All sites (n=576)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Nitzschia communis	NZCM	diatom	Nitzschia communis Rabenhorst	136	180	2	2	4	2	4	3	4	5	4	1	1			1
Nitzschia dissipata	NZDS	diatom	Nitzschia dissipata (Kützing) Grunow	197	259	1	2	4	2	2	2	2	4	3	3	4			1
Nitzschia fonticola	NZFT	diatom	Nitzschia fonticola Grunow	3	3	2	2	4	2	2	2	2	4	1	3				1
Nitzschia frustulum	NZFR	diatom	Nitzschia frustulum (Kützing) Grunow	387	528	2	2	4	3	4	3	2	5	3	2	4			1
Nitzschia fruticosa	NZFU	diatom	Nitzschia fruticosa Hustedt	2	3	1	2	3	2		2	3	5	1					1
Nitzschia innominata	NZIN	diatom	Nitzschia innominata Sovereign	56	65														
Nitzschia linearis	NZLN	diatom	Nitzschia linearis (Agardh) Smith	34	42	2	2	4	2	2	2	2	4	3	2	5			1
Nitzschia microcephala	NZMC	diatom	Nitzschia microcephala Grunow	25	34	2	2	4	2	4	3	3	5	1	1	3			1
Nitzschia palea	NZPL	diatom	Nitzschia palea (Kützing) Smith	102	147	2	2	3	2	4	4	5	6	3	1	1			1
Nitzschia paleacea	NZPC	diatom	Nitzschia paleacea Grunow ex Van Heurck	212	295	1	2	4	2	4	3	3	5	2	2	2			1
Nitzschia recta	NZRC	diatom	Nitzschia recta Hantzsch ex Rabenhorst	3	3	1	2	4	2	2	2	2	7	1	3	5			1
Nitzschia sigmoidea	NZSG	diatom	Nitzschia sigmoidea (Nitzsch) Smith	1	1	2	2	4	2	2	3	2	5	2	3	5			1
Nitzschia sp.	NZXX	diatom	Nitzschia sp.	32	47	1	2												1
Nitzschia volcanica	NZVL	diatom	Nitzschia volcanica Sovereign	24	27														
No Algae Present	ZZZZ		No Algae Present	0	2														
Oocystis pusilla	OCPU	green	Oocystis pusilla Hansgirg	1	1	2	2											1	2
Oscillatoria limosa	OSLS	bluegreen	Oscillatoria limosa (Dillwyn) Agardh	0	2	1	2												1
Oscillatoria sp.	OSXX	bluegreen	Oscillatoria sp.	91	122	1	2												1
Pediastrum boryanum	PSBR	green	Pediastrum boryanum (Turpin) Meneghini	4	4	2	2											1	2
Pediastrum tetras	PSTT	green	Pediastrum tetras (Ehrenberg) Ralfs	2	2	2	2											1	2
Pinnularia sp.	PLXX	diatom	Pinnularia sp.	7	12	1	2												1
Rhodomonas minuta	RDMN	cryptophyte	Rhodomonas lacustris var. nannoplanctica (Skuja) Javornicky	8	14														
Rhoicosphenia curvata	RHCU	diatom	Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	308	425	2	2	4	2	2	2	2	5	2	3	4			1
Rhopalodia gibba	RPGB	diatom	Rhopalodia gibba (Ehrenberg) Müller	58	89	1	1	5	2	1	3	2	5	3	2				1
Rhopalodia musculus	RPMS	diatom	Rhopalodia musculus (Kützing) Müller	0	2	1	1												1
Rivularia sp.	RVXX	bluegreen	Rivularia sp.	6	6														
Scenedesmus abundans	SCAB	green	Scenedesmus abundans (Kirchner) Chodat	2	2	2	2											1	2
Scenedesmus acuminatus	SCAC	green	Scenedesmus acuminatus (Lagerheim) Chodat	11	17	2	2											1	2
Scenedesmus bijuga	SCBJ	green	Scenedesmus bijuga (Turpin) Lagerheim	1	1	2	2											1	2
Scenedesmus denticulatus	SCDT	green	Scenedesmus denticulatus Kirchner	1	2	2	2											1	2
Scenedesmus quadricauda	SCQD	green	Scenedesmus quadricauda (Turpin) Brébisson	89	136	2	2											1	2
Schroderia sp.	SHXX	green	Schroderia sp.	1	2														
Selenastrum minutum	SLMN	green	Selenastrum minutum (Nägeli) Collins	10	13	2	2											1	2

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes												
				LT sites (n=398)	All sites (n=576)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Sphaerocystis schroeteri	SFSR	green	Sphaerocystis schroederii Chodat	2	2	2	2											1	2
Spirogyra sp.	SPXX	green	Spirogyra sp.	11	19	2	2										2	1	1
Stephanodiscus astraea minutula	STAM	diatom	Stephanodiscus minutulus (Kützing) Cleve et Möller	1	5	2	2	5	2	2	3	3	6	2	2				2
Stephanodiscus hantzschii	STHN	diatom	Stephanodiscus hantzschii Grunow	3	6	2	2	5	2	3	4	4	6	2	2				2
Stephanodiscus niagarae	STNG	diatom	Stephanodiscus niagarae Ehrenberg	0	1	2	2								3				2
Surirella ovata	SUOV	diatom	Surirella minuta Brébisson	1	2	1	2	4	2		3	3	5	3	2				1
Synedra mazamaensis	SNMZ	diatom	Synedra mazamaensis Sovereign	97	134	2	2	5							3				1
Synedra parasitica	SNPR	diatom	Synedra parasitica (Smith) Hustedt	1	1	2	2								2	3			1
Synedra radians	SNRD	diatom	Fragilaria radians (Kützing) Williams et Round	0	2	2	2	4							2				1
Synedra rumpens	SNRM	diatom	Fragilaria capucina var. rumpens (Kützing) Lange-Bertalot	30	52	2	2	3	2				2						1
Synedra socia	SNSC	diatom	Synedra socia Wallace	4	7	2	2								2				1
Synedra tenera	SNTN	diatom	Fragilaria tenera (W. Smith) Lange-Bertalot	1	4	2	2	2	1	1	1	1	2	2					1
Synedra ulna	SNUL	diatom	Ulnaria ulna (Nitzsch) Compère	246	359	2	2	4	2	2	3	4	7	2	2	1			1
Tabellaria flocculosa	TBFL	diatom	Tabellaria flocculosa (Roth) Kützing	1	1	2	2												2
Tetraedron minimum	TEMN	green	Tetraedron minimum (Braun) Hansgirg	4	6	2	2											1	2
Ulothrix sp.	ULXX	green	Ulothrix sp.	23	46	2	2										2		1
Ulothrix sp.	ULX9	green	Ulothrix sp.	5	5	2	2										2		1
Unidentified flagellate	MXFG	unknown	Unidentified flagellate	1	1														
Vaucheria sp.	VAXX	green	Vaucheria sp.	0	1	2	2												1

Table A2. Key to autecological attributes from Table A1. A list of references is provided below the table.

Attribute Name	Category Code	Category Name	Category Description
Benthic-Sestonic Taxa	1	benthic	primarily or exclusively associated with benthic substrates
	2	sestonic	primarily or exclusively sestonic (planktonic taxa)
Motility	1	motile	taxa with capability of movement in the water column or on submerged surfaces
	2	non-motile	taxa without capability of movement; attached to submerged surfaces
Moisture Requirement	1	in streams	taxa found only in streams, rivers, reservoirs, or lakes
	2	in streams, sometimes wet places	taxa generally found in stream channels; sometimes springs, seeps, or ditches
	3	in streams, often wet places	taxa common in stream channels, springs, seeps, and ditches
	4	wet, moist, or temp. dry places	taxa generally found in springs, seeps, ditches, or soils
	5	exclusively outside water bodies	for example, soil algae
Nitrogen Fixers	1	Nitrogen Fixer	taxon is capable of fixing atmospheric nitrogen
	2	Not Nitrogen Fixer	taxon not capable of fixing atmospheric nitrogen
Nitrogen Uptake Metabolism	1	N autotroph - low org N	taxa generally intolerant to organically-bound nitrogen; some may be 'oligotrophic' or 'mesotrophic species
	2	N autotroph - high org N	taxa tolerant to organically-bound nitrogen; some may be 'eutrophic' taxa
	3	N heterotroph - high org N (facultative)	taxa requiring periodic elevated concentrations of organically-bound nitrogen
	4	N heterotroph - high org N (obligate)	taxa indicative of elevated concentrations of organically-bound nitrogen
Oxygen Requirement	1	always high	nearly 100% DO saturation
	2	fairly high	> 75% DO saturation
	3	moderate	> 50% DO saturation
	4	low	> 30% DO saturation
	5	very low	about 10% DO saturation or less
pH	1	acidobiontic	<7, optimum < 5.5
	2	acidophilous	<7, optimum < 7
	3	circumneutral	around 7
	4	alkaliphilous	>7, occurring ~ 7
	5	alkalibiontic	above 7
	6	indifferent	~ 7
Bahls Diatom Tolerance	1	most tolerant	very tolerant to nutrient and organic enrichment
	2	less tolerant	somewhat tolerant to nutrient and organic enrichment
	3	sensitive	somewhat intolerant to nutrient and organic enrichment; not necessarily 'oligotrophic'
Lange-Bertalot Tolerance	1	very tolerant (1)	polysaprobic: extremely degraded conditions…cf. hypeutrophic
	2	tolerant (2a)	alpha-meso/polysaprobic: highly degraded conditions…eutrophic
	3	tolerant (2b)	alpha-mesosaprobic: degraded (organically-enriched) conditions…eutrophic
	4	less tolerant (3a)	beta-mesosaprobic: somewhat degraded conditions…meso-eutrophic; mesotrophic
	5	less tolerant (3b)	oligosaprobic: low amounts of organic enrichment…mesotrophic; oligo-mesotrophic…i.e. not necessarily pristine
Salinity	1	fresh	< 100 mg/L chloride; < 0.2 ppt salinity
	2	fresh brackish	< 500 mg/L chloride; < 0.9 ppt salinity
	3	brackish fresh	500 - 1000 mg/L chloride; 0.9 - 1.8 ppt salinity
	4	brackish	1000 - 5000 mg/L chloride; 1.8 - 9.0 ppt salinity

Attribute Name	Category Code	Category Name	Category Description
Saprobic	1	oligosaprobic	class: I, I-II; O2 saturation: >85%; BOD5(mg/L): < 2
	2	beta mesosaprobic	class: II; 02 saturation: 70-80%; BOD5(mg/L): 2-4
	3	alpha mesosaprobic	class: II; 02 saturation: 25-70%; BOD5(mg/L): 4-13
	4	alpha meso/polysaprobic	class: II; 02 saturation: 10-25%; BOD5(mg/L): 13-22
	5	polysaprobic	class: II; 02 saturation: <10%; BOD5(mg/L): >22
Trophic Condition	1	oligotrophic	
	2	oligo-meso	
	3	mesotrophic	
	4	meso-eutrophic	
	5	eutrophic	
	6	polytrophic	
	7	eurytrophic	wide range of tolerance to nutrient concentrations; indifferent

References for autoecological attributes:

Bahls, L.L., 1993, Periphyton bioassessment methods for Montana streams: Water Quality Bureau, Department of Health and Environmental Sciences, Helena, MT, 69 p.

Bold, H.C., and Wynne, M.J., 1978, Introduction to the algae. Structure and reproduction: Englewood Cliffs, New Jersey, Prentice-Hall, Inc., 706 p.

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Prescott, G.W., 1962, Algae of the western Great Lakes area, Revised Edition: Dubuque, Iowa, William C. Brown Publishers, 977 p.

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VanLandingham, S.L., 1982, Guide to the identification, environmental requirements and pollution tolerance of freshwater blue-green algae (Cyanophyta): Cincinnati, Ohio, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Office of Research and Development, EPA-600/3-82-073, 341 p.

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APPENDIX B: TIME SERIES OF PERIPHYTON METRICS AND ENVIRONMENTAL PARAMETERS FOR INDIVIDUAL SITES

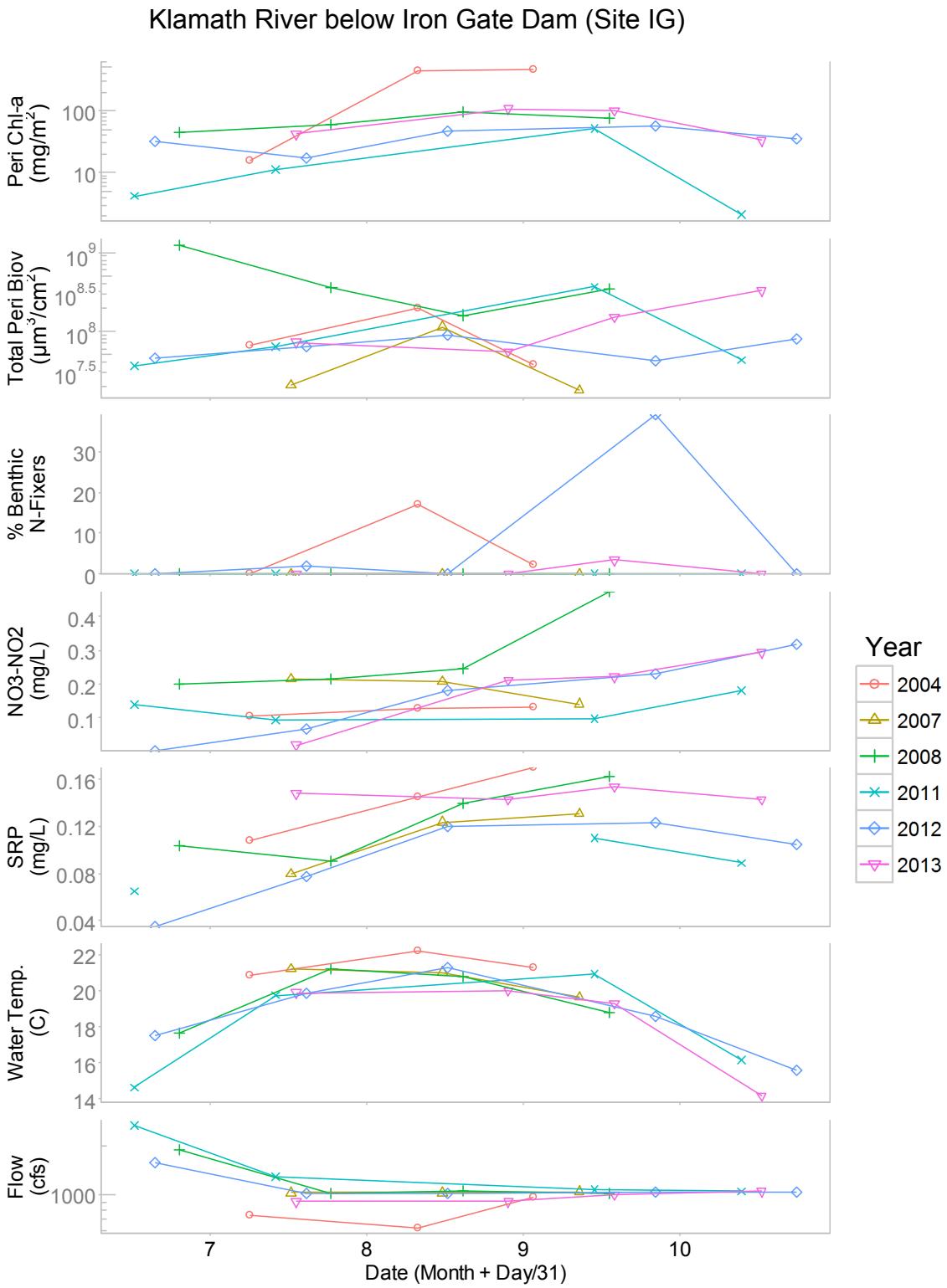

Figure B1. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River below Iron Gate Dam (IG), for samples collected in May-October, for samples collected in May-October.

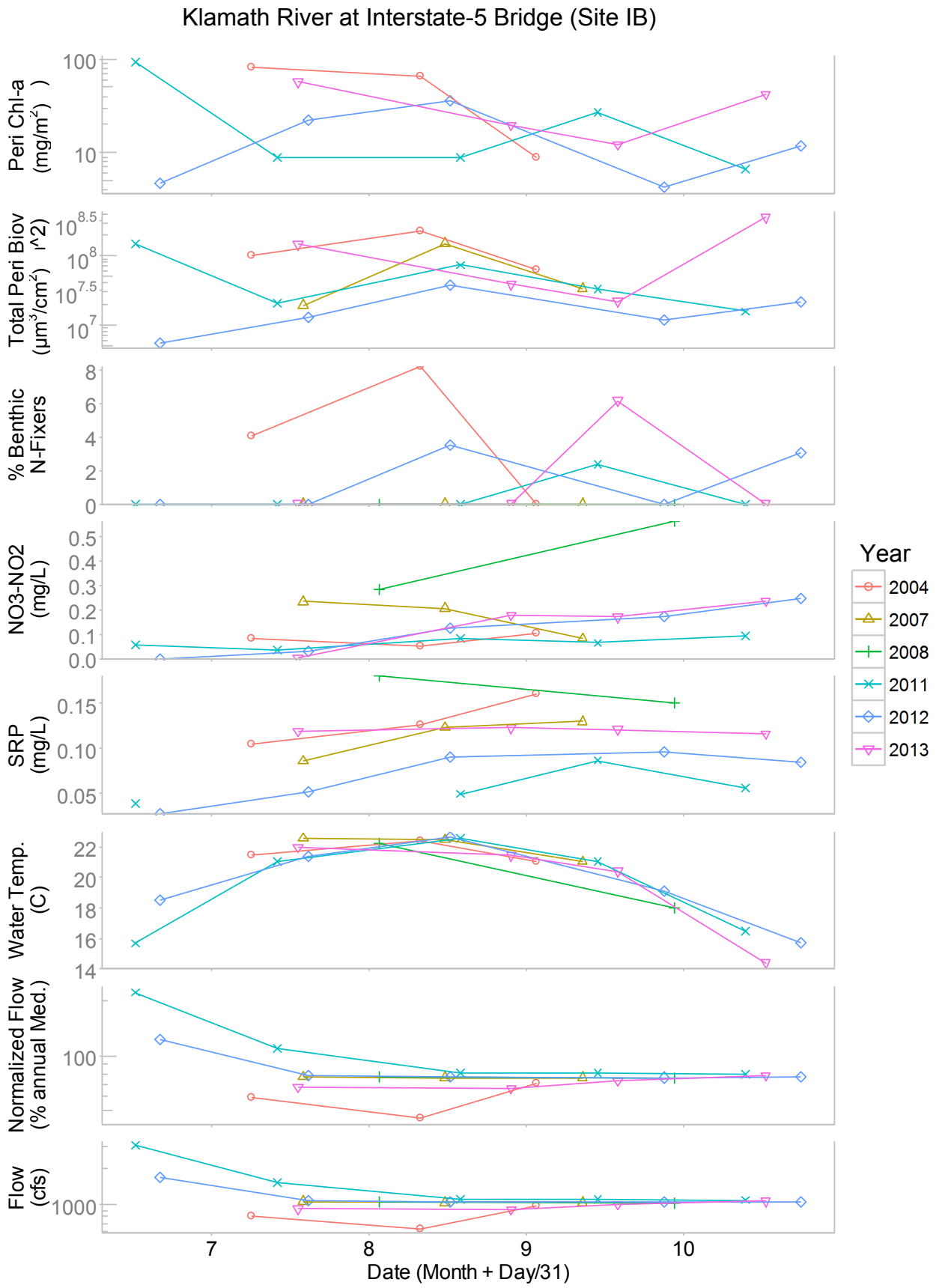

Figure B2. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Interstate-5 Bridge (IB), for samples collected in May-October, for samples collected in May-October.

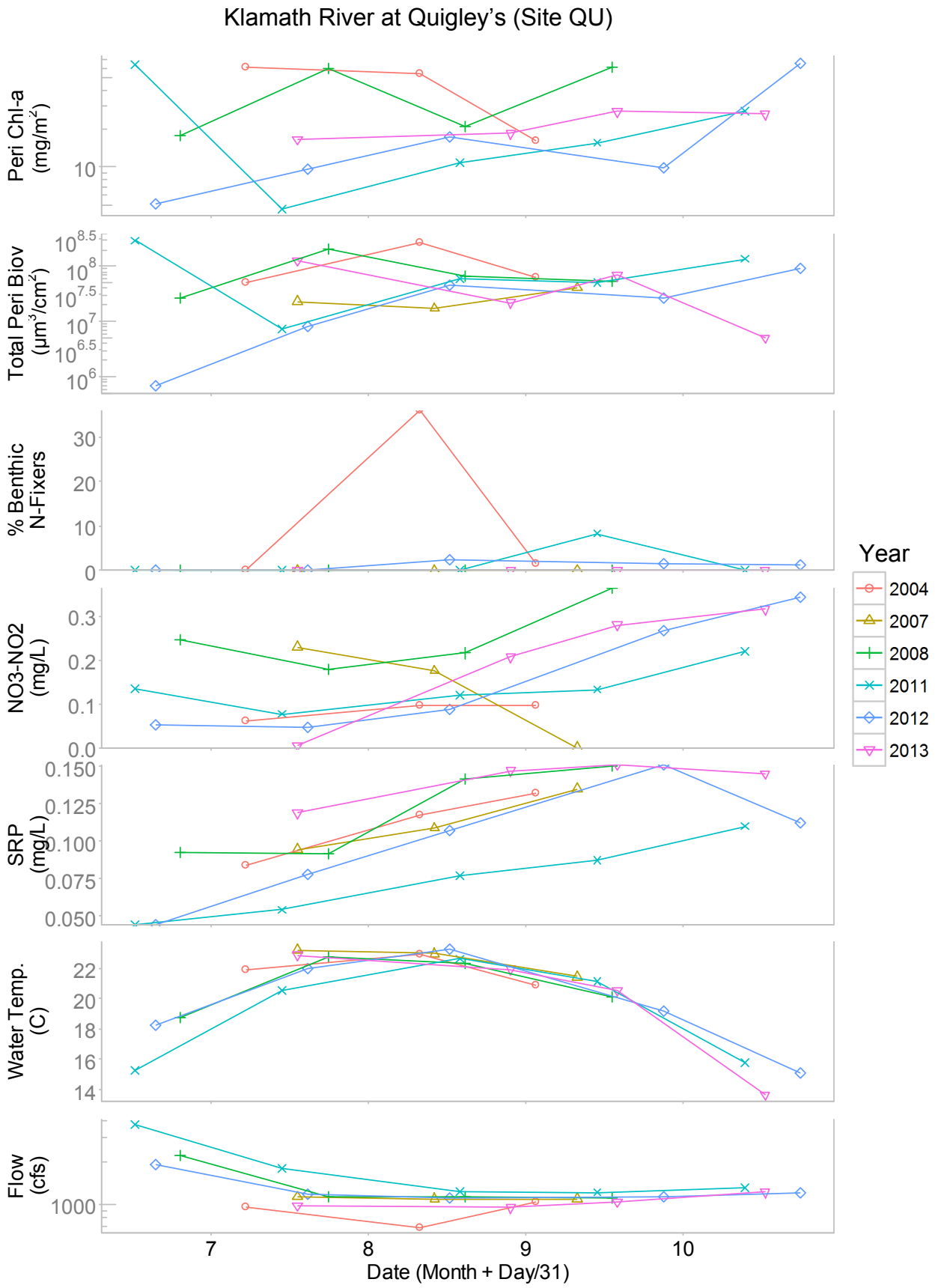

Figure B3. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Quigley's (QU), for samples collected in May-October, for samples collected in May-October.

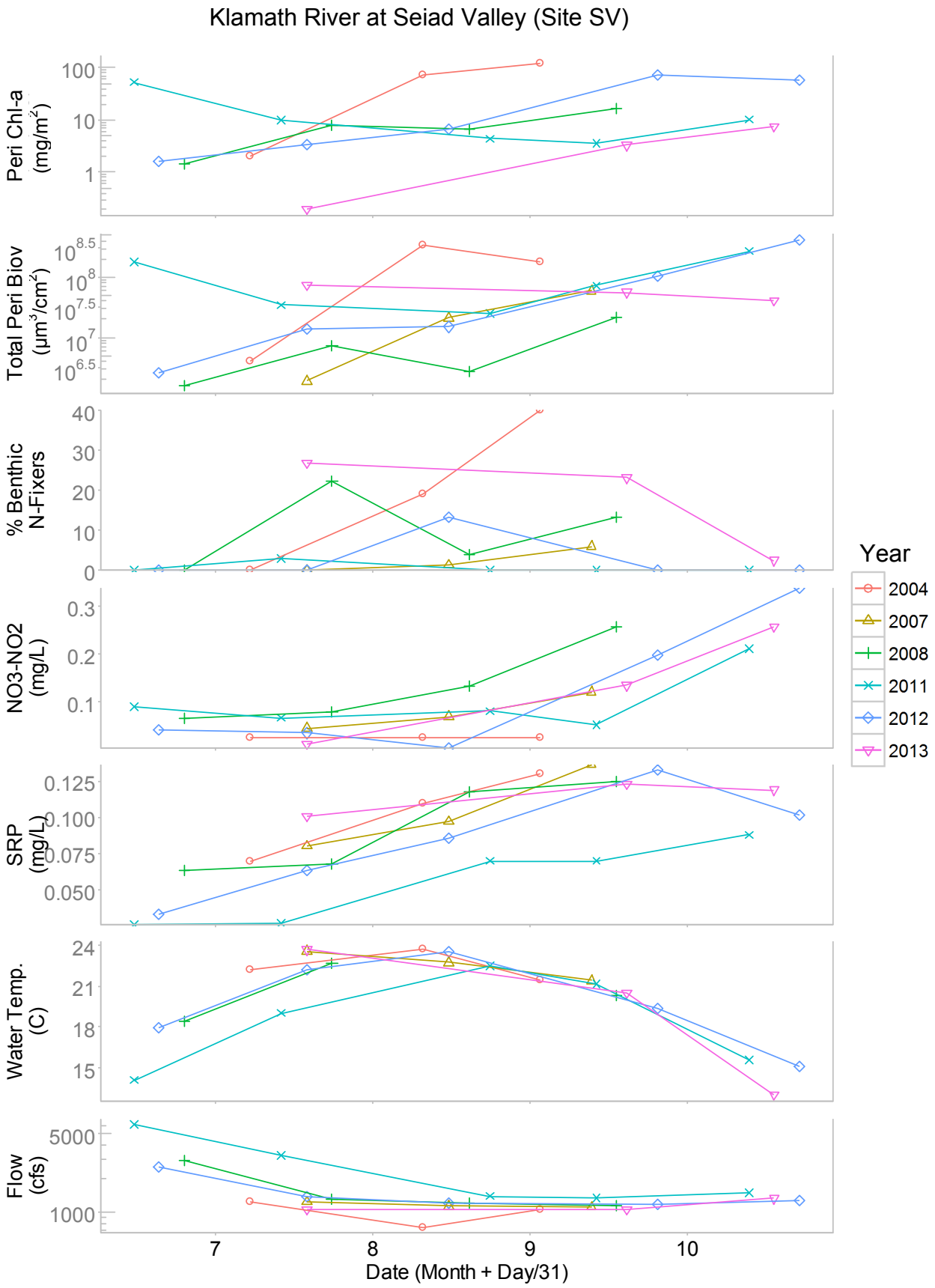

Figure B4. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Seiad Valley (SV), for samples collected in May-October, for samples collected in May-October.

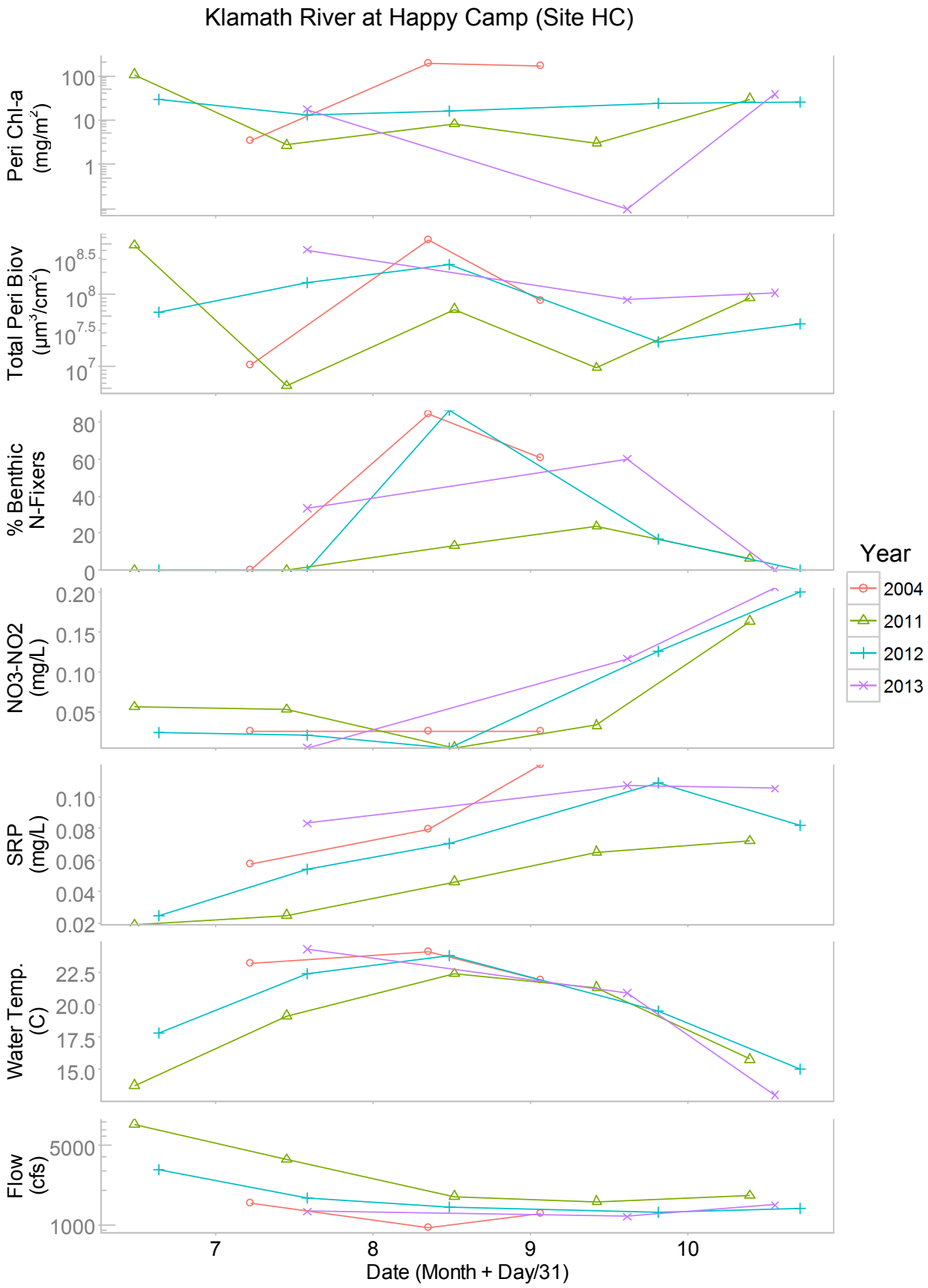

Figure B5. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Happy Camp (HC), for samples collected in May-October, for samples collected in May-October.

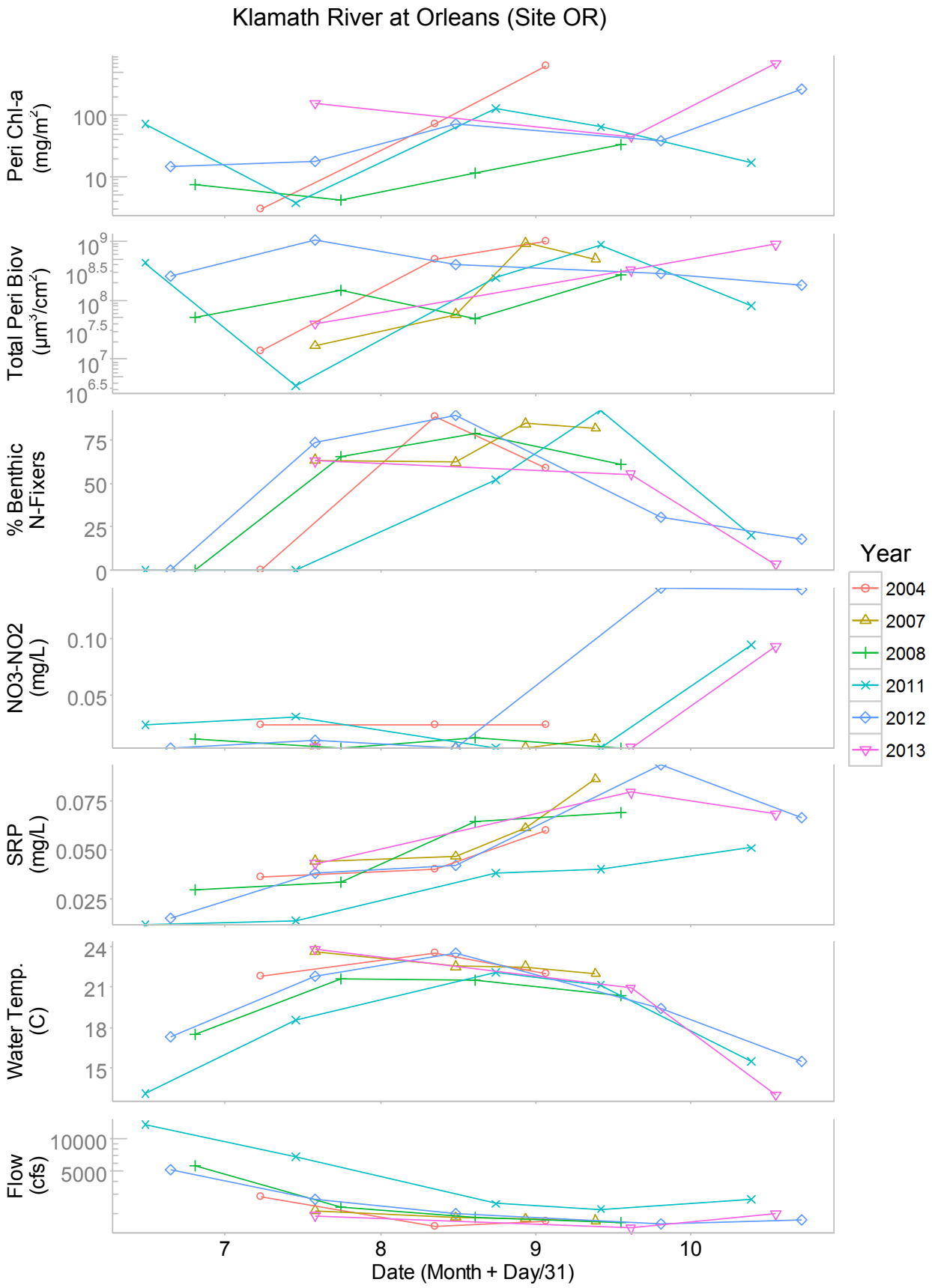

Figure B6. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Orleans (OR), for samples collected in May-October, for samples collected in May-October.

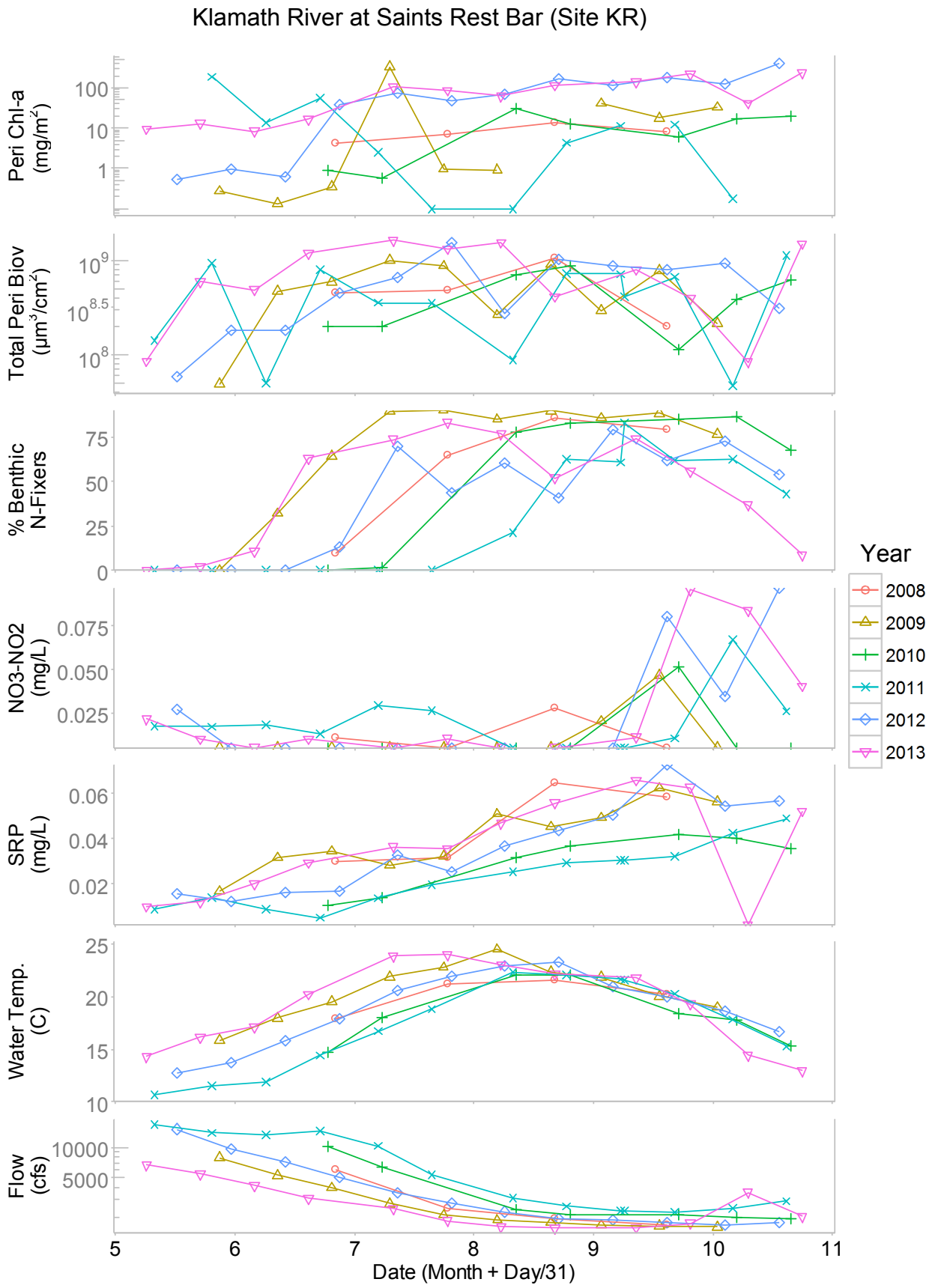

Figure B7. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Saints Rest Bar (KR), for samples collected in May-October, for samples collected in May-October.

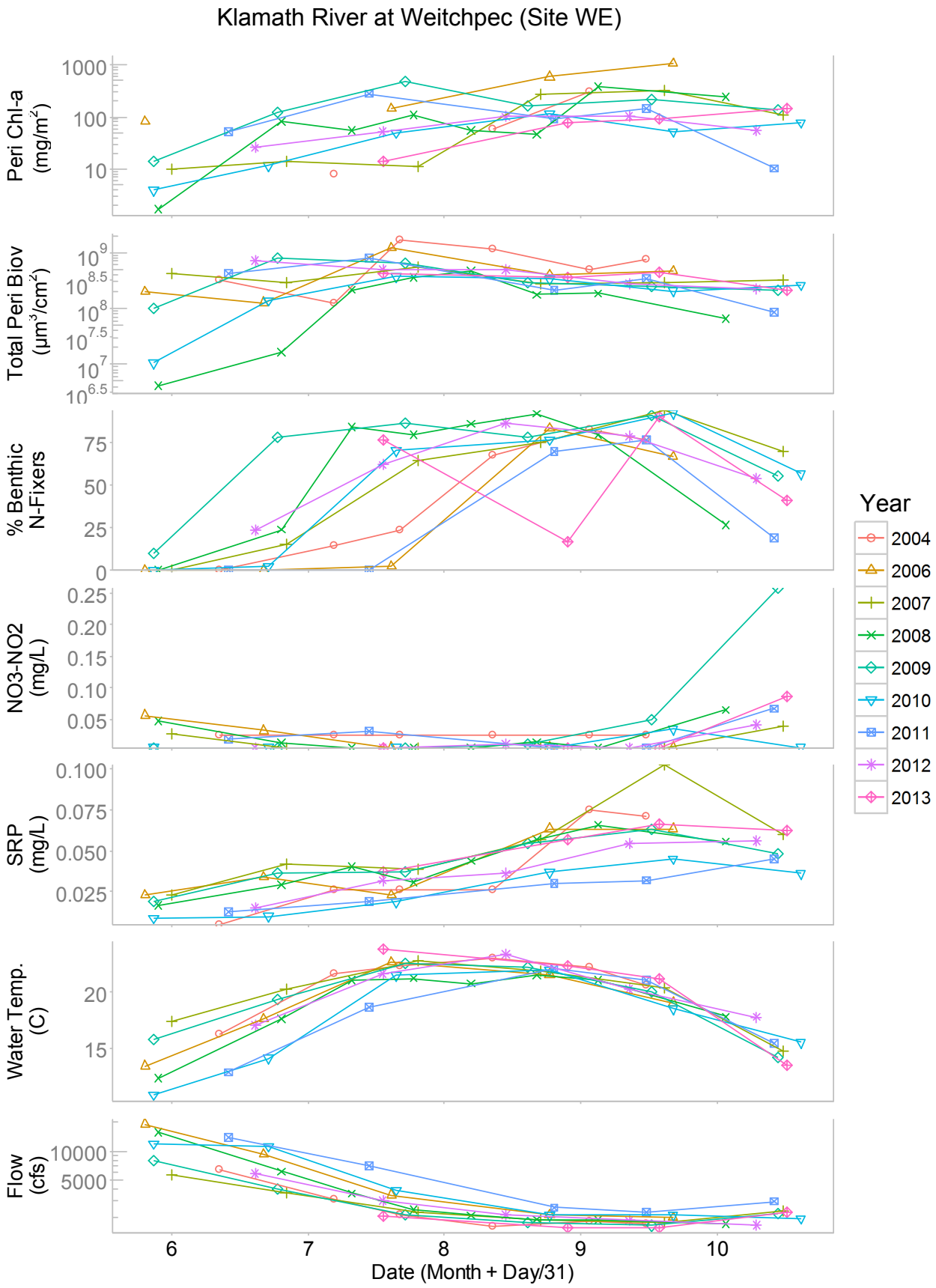

Figure B8. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Weitchpec (WE), for samples collected in May-October, for samples collected in May-October.

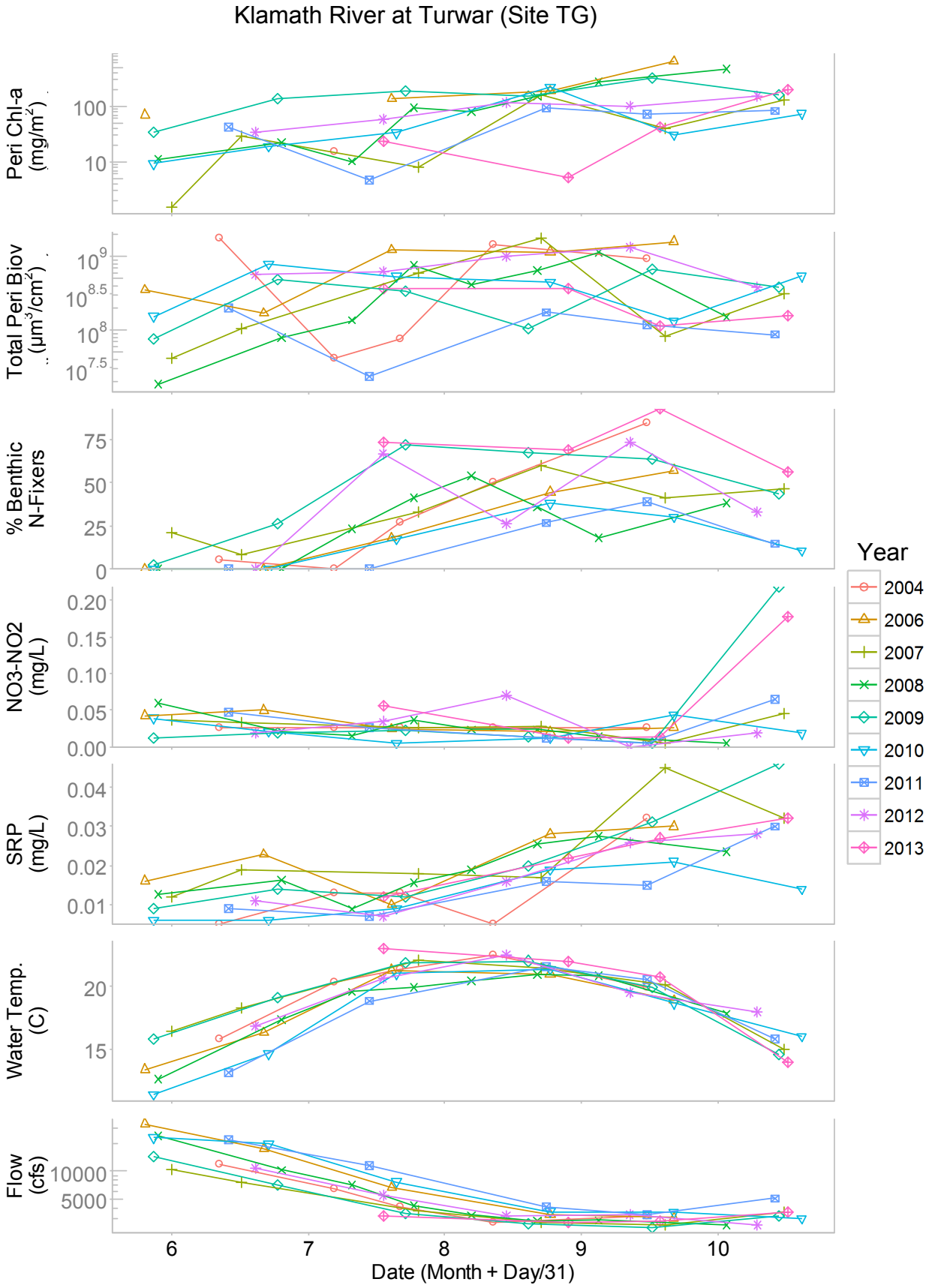

Figure B9. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Klamath River at Turwar (TG), for samples collected in May-October, for samples collected in May-October.

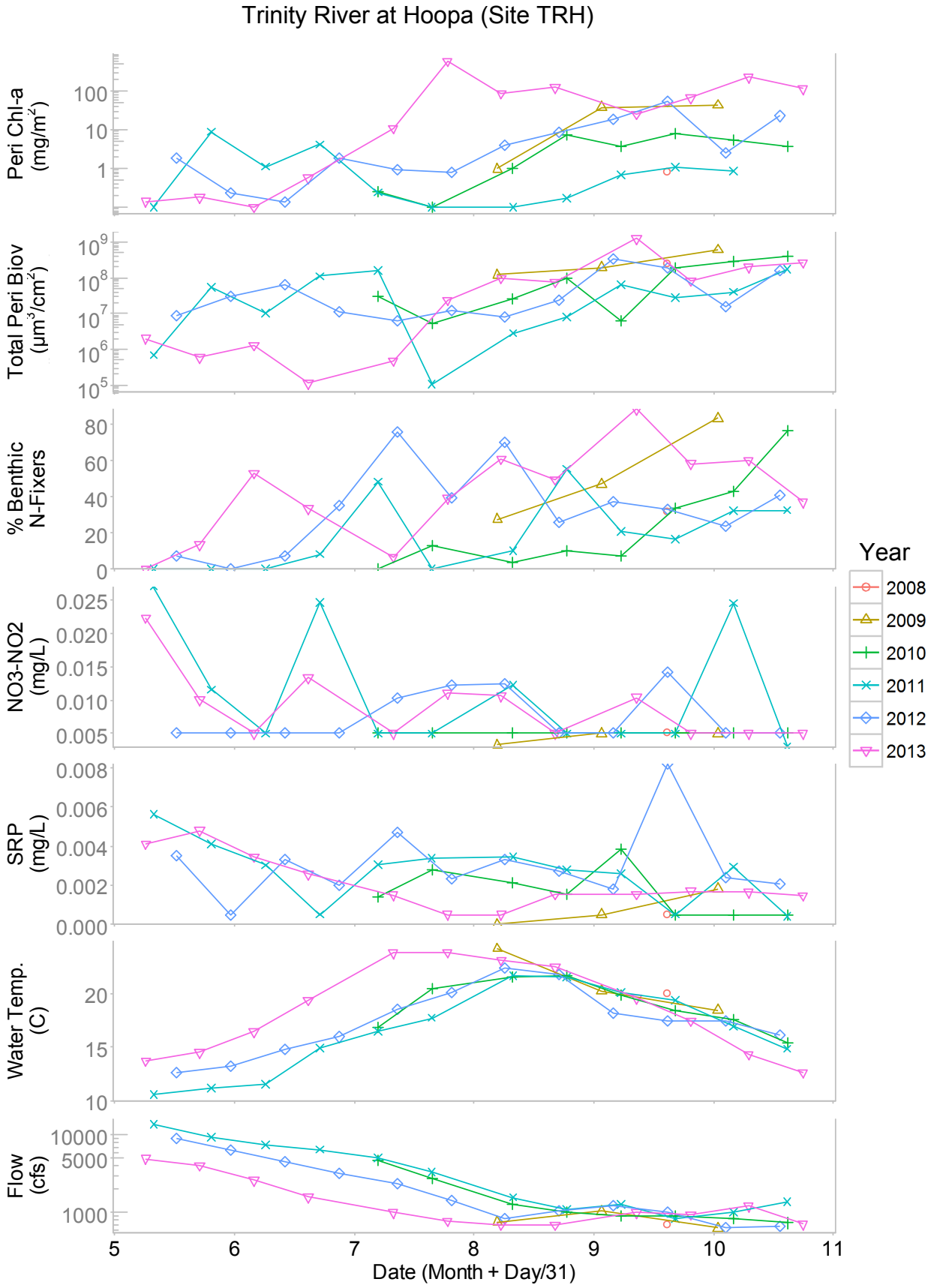

Figure B10. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Trinity River at Hoopa (TRH), for samples collected in May-October, for samples collected in May-October.

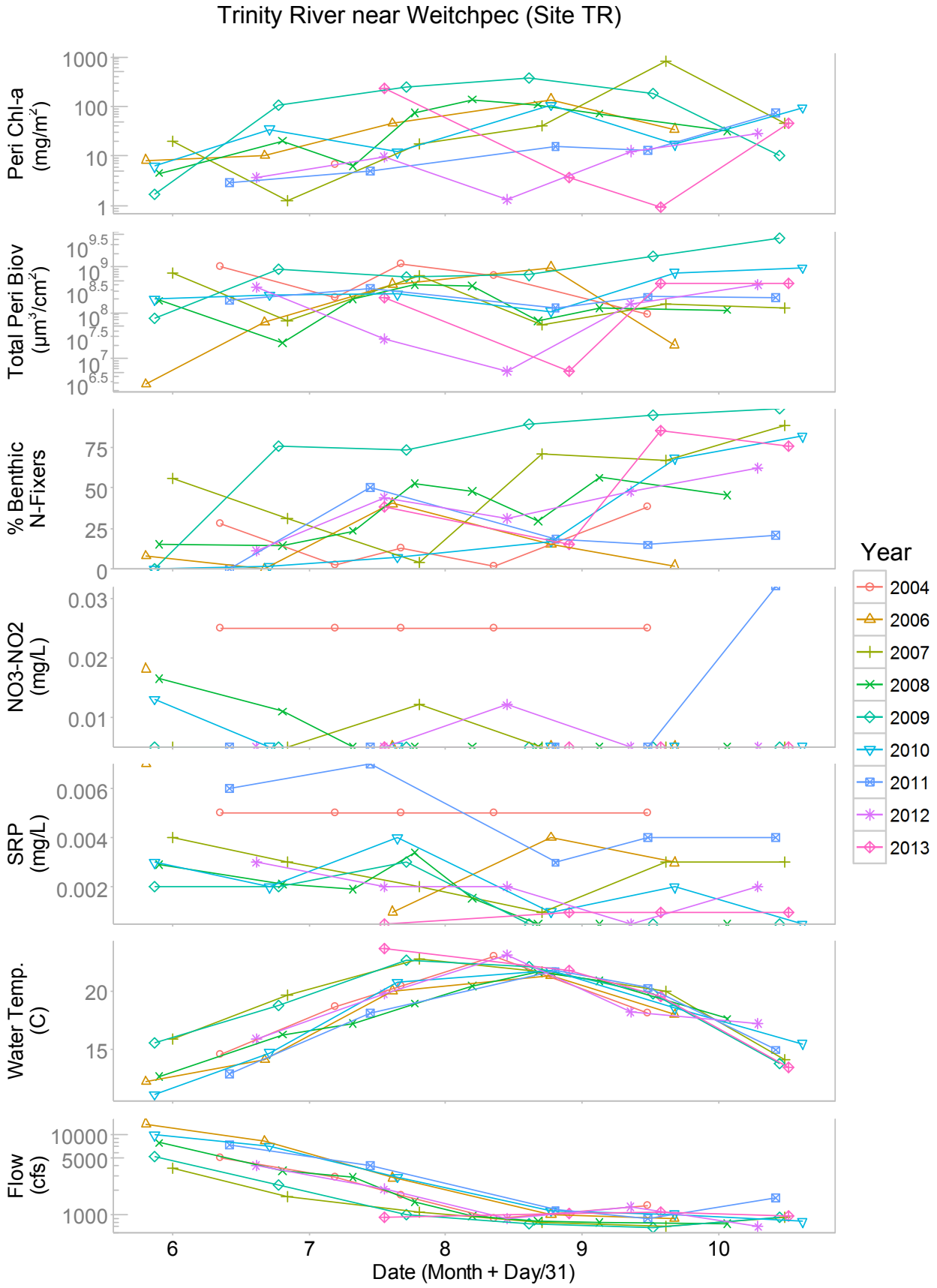

Figure B11. Time series of periphyton metrics (top three panels) and environmental parameters (bottom four panels) for Trinity River near Weitchpec (TR), for samples collected in May-October, for samples collected in May-October.

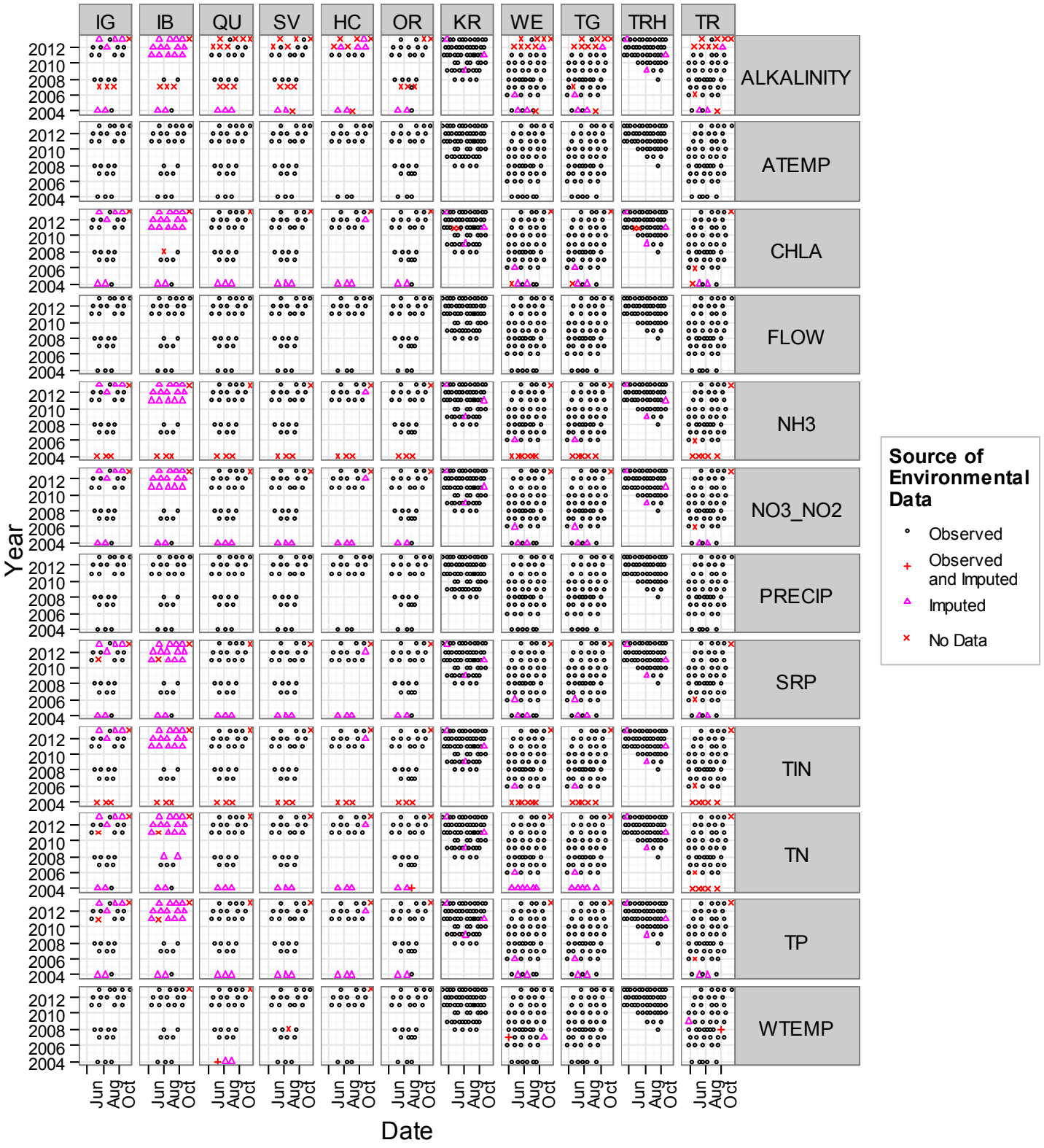

Figure C1. Source of environmental data that were paired with periphyton samples, indicating which data were imputed. Only dates and sites with periphyton samples are shown.