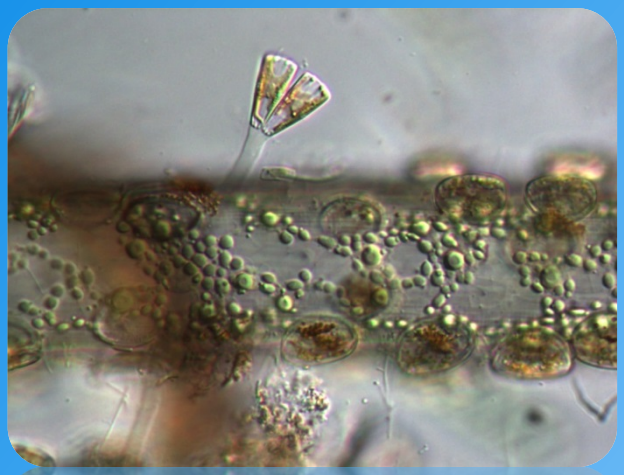

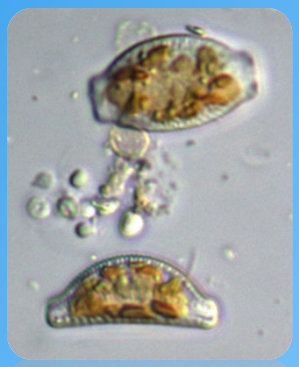

Spatial and Temporal Variation of Periphyton Assemblages in the Klamath River 2004-2012

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Suggested citation:

J.E. Asarian, Y. Pan, N.D. Gillett, and J. Kann. 2014. Spatial and Temporal Variation of Periphyton Assemblages in the Klamath River, 2004-2012. Prepared by Kier Associates, Portland State University, and Aquatic Ecosystem Sciences LLC. for the Klamath Basin Tribal Water Quality Work Group. 50p. + appendices.

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

A. Periphyton in Klamath River above Turwar, 9/27/2012 (Yurok Tribe Environmental Program); B. *Rhopalodia* sp. (Paula Furey); C. Periphyton Klamath River between Happy Camp and Orleans, 8/30/2012 (Eli Asarian); D. *Epithemia Sorex* (Paula Furey); E. *Gomphonema* sp. and *Cocconeis pediculus* growing on *Cladophora* sp.

EXECUTIVE SUMMARY

Periphyton, also known as benthic algae, are algae that grow attached to river substrates such as cobbles, sand, and aquatic plants. Periphyton communities are 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. During the summer in the Klamath River of California, photosynthesis and respiration by periphyton and aquatic plants cause low dissolved oxygen at night and high pH in the day, resulting in water quality conditions that can be chronically stressful to culturally and economically important fish species.

This study analyzes periphyton samples collected at 11 long-term monitoring sites in the years 2004 through 2012 in the lower and middle Klamath River (i.e., between Iron Gate Dam and Turwar, just upstream of the Klamath Estuary), as well as the lower Trinity River which is the largest tributary to the Klamath River. Periphyton samples from river cobbles were collected by the Yurok Tribe, Hoopa Tribe, Karuk Tribe, Watercourse Engineering Inc., MaxDepth Aquatics, and the North Coast Regional Water Quality Control Board. The number of sites sampled per year ranged from three to eleven. Samples were typically collected monthly from June through October, although that varied by year and sampling entity. 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'). Chlorophyll-*a* concentrations were also quantified. Additional sites (i.e., beyond the 11 long-term) were sampled in some years, including special studies using different sampling protocols, and although those results are not discussed in this report, they are included in a comprehensive database as an electronic appendix.

To examine longitudinal, seasonal, and inter-annual patterns in periphyton community composition, we grouped species into functional groups (e.g., heterotrophs, nitrogen-fixers, pollution tolerant, etc.) and used multivariate statistical techniques such as Non-metric Multidimensional Scaling (NMDS) and cluster analysis.

A total of 143 species were identified in the 332 samples collected at the long-term monitoring sites. Periphyton assemblages in the Klamath River were dominated by diatoms, which on average comprised 92.6% of relative biomass (as estimated from biovolume measurements), followed by cyanobacteria (5.9%) and green algae (1.5%).

Periphyton assemblages in the Klamath River show clear longitudinal (i.e., upstream vs. downstream) and seasonal patterns (i.e., May-June vs July-October) in species composition. Cluster analysis based on 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. Although all groups were dominated by attached diatom species, compared to the other two groups, Group 3 had the highest percentage (mean relative biomass of 4.4%) 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 which host large summer blooms of these species. Group 3 also had the highest percent of biomass comprised of taxa tolerant of degraded DO conditions (i.e., >50% DO saturation), nitrogen heterotrophs (i.e., can obtain energy from organic compounds in addition to photosynthesis) including *Nitzschia* spp., and taxa tolerant to organically-bound nitrogen.

Sites in the middle reach (river miles 129 to 100: Seiad Valley and Happy Camp) fell either in Group 3 or Group 2 depending on season. Middle reach stations showed a seasonal progression beginning as Group 2 in June, transitioned to Group 3 in July, and then primarily remained in Group 3 during August through October (although some August and September samples were in Group 1. Group 2 had the highest relative biomass of diatoms (96.1%) and lowest relative biomass of cyanobacteria (2.1%). It also had the largest relative biomass of taxa that are associated with high (near 100% saturation) dissolved oxygen, consistent with the fact that this group occurred early in the season (May and June) when dissolved oxygen levels were high.

Sites in the lower reach fell primarily in Group 1 or Group 2 such that downstream sites (river miles 60 to 6 and Trinity River sites) showed a seasonal progression where the majority of samples fell into Group 2 in May and June, then transitioned to Group 1 in July, and remained in Group 1 during August-October. More than half the mean biomass of Group 1 is composed of 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. The sites and months where nitrogen-fixing species dominate (i.e., downstream reaches in summer) coincide with low nitrogen concentrations measured in water samples, indicating that decreased nitrogen availability may explain the seasonal and longitudinal patterns of dominance of the periphyton community by nitrogen-fixers. In contrast to Group 3, the cyanobacteria in Group 1 were primarily attached rather than sestonic, and Group 1 had the highest relative percent biomass of taxa intolerant to organically-bound nitrogen.

Overall periphyton biomass (and to a lesser extent, chlorophyll-*a* concentrations) were higher at downstream sites than at upstream sites. Total biomass of Group 1was much higher than Group 3 and Group 2, which may explain the higher biomass at downstream sites (Group 1 was found primarily at downstream sites). Periphyton chlorophyll-*a* was generally highest in August or September, whereas differences in total biomass between months were less clear except for low values in May. The higher periphyton biomass downstream relative to upstream does not reflect overall longitudinal patterns in primary productivity because the standardized sampling protocol used in this study does not capture all primary producers in the river ecosystem. The protocol was designed to sample periphyton on cobbles in areas where depth was 1 to 2 feet and velocity was 1 to 2 feet per second, and thus was not designed to adequately characterize deeper areas, filamentous algae (e.g., *Cladophora* sp.), or aquatic macrophytes (i.e., rooted aquatic plants) which can be associated with their own epiphytic (i.e., attached to plants) algal communities.

The long-term data described in this report provide valuable insight into seasonal and longitudinal patterns of benthic algal communities in the middle and lower Klamath River system, and will provide the basis for Phase II of the analysis. The Phase II analysis will incorporate data for various environmental factors such as nutrients, hydrology, water quality, and climate that are available for the same timeframe (2004-2012) as the periphyton samples analyzed in this report. Various multivariate statistical techniques will be used to evaluate linkages between environmental controlling factors and the resulting periphyton assemblages. These evaluations will 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).

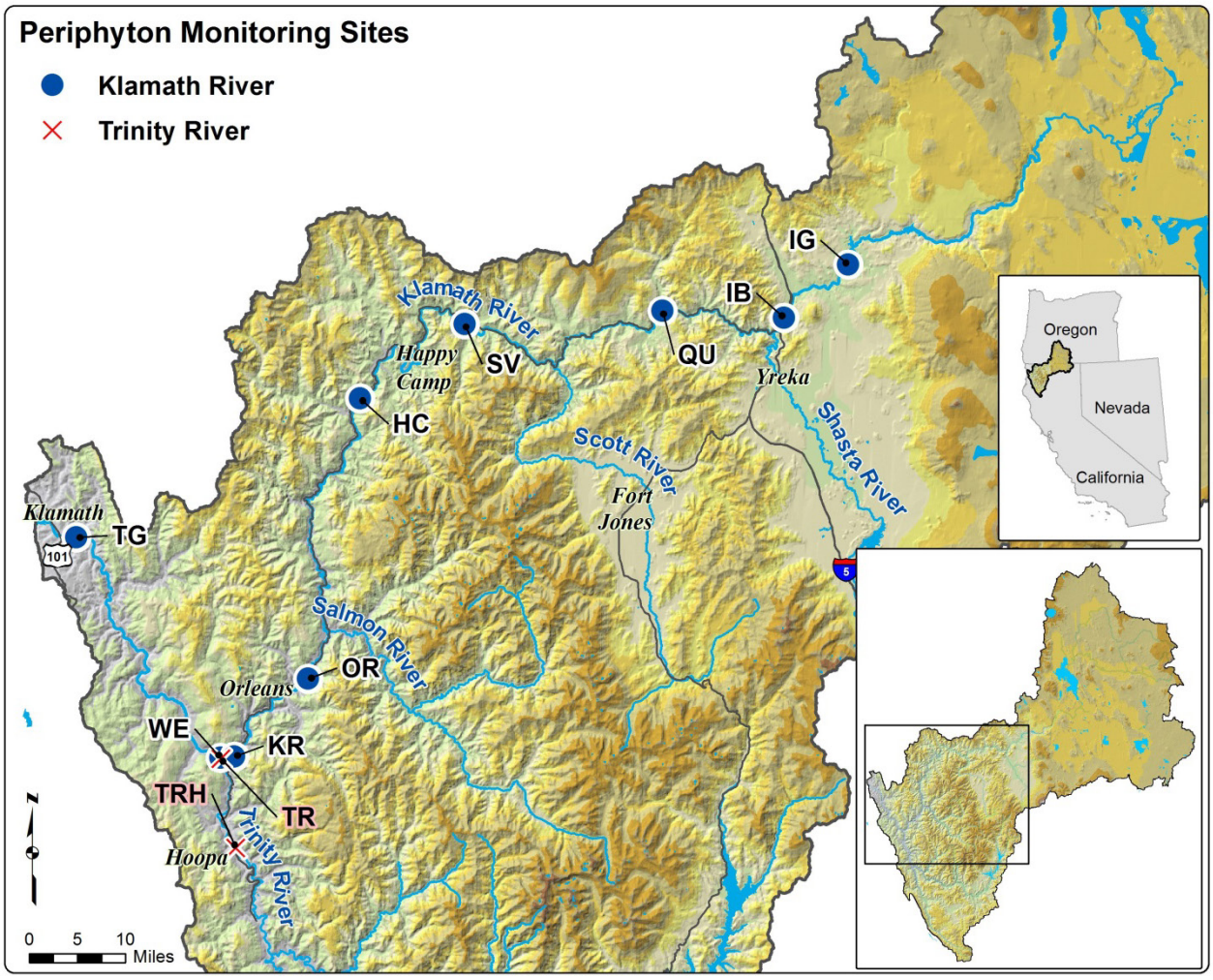

Figure 1. Location of long-term periphyton monitoring sites on the Klamath and Trinity rivers.

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 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 this initial comprehensive analysis of the Klamath River long-term periphyton monitoring dataset.

1.3 STUDY GOALS

The overall goals of this study were to provide the first comprehensive compilation and analysis of periphyton data for the Klamath River and tributaries for the years 2004-2012, including examination of longitudinal, seasonal, and inter-annual patterns in species composition and biomass. Such analyses are intended to provide the basis for the formulation of hypotheses that relate the distribution, biomass, and community structure of the periphyton to physical/chemical dynamics of the Klamath River. These hypotheses will then form the basis for a second phase of this study which will use statistical techniques to determine the importance of various environmental controlling factors on the inter-annual, seasonal, and longitudinal dynamics of the periphyton communities.

2 METHODS

2.1 SAMPLING METHODS

Periphyton samples were collected at eleven long-term monitoring sites, including nine in the Klamath River and two in the Trinity River, from 2004-2012 (Figure 1, 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 (Figure 2). Within the study period, some monitoring sites were relocated short distances due to logistical and access issues. In such cases, the most recent site location and code are used in this report to facilitate comparisons across years. The length of the sampling season varied by year and was as long as May through October. Sampling frequency was generally monthly except the Hoopa Valley Tribe sampled their two sites biweekly for 2009-2012 (Figure 2).

The periphyton sampling protocol was adapted from techniques recommended by U.S. EPA (Peck et al. 2006) and U.S. Geological Survey (Porter et al. 1995). Samples were collected by the Yurok Tribe (YTEP 2004, 2008; Yurok Tribe et al. 2013), Hoopa Tribe (Hoopa TEPA 2013), Karuk Tribe (2011), Watercourse Engineering Inc. (Watercourse Engineering Inc. 2010, as well as additional unpublished data), MaxDepth Aquatics (Eilers 2005), and the North Coast Regional Water Quality Control Board (NCRWQCB 2005). The protocol is briefly summarized here, for additional details refer to the documents cited in the previous sentence.

The sampling locations meet the following criteria: depth of 1 to 2 feet, velocity of 1 to 2 feet per second, and with clear solar path (i.e., no major topographic or riparian shading) (Figure 3). Sampling location selection was not random, but rather was the area most representative of river cross-section (i.e., not the very-near shore assemblage and not the deep water assemblage, which are less extensive). Representative cobbles were selected from the stream bed at each sampling location, avoiding the extremes of algal cover. Selected cobbles were placed in a tub with water and transported to a convenient sample-processing area. For each cobble, a 1 inch by 3 inch microscope slide (96.75 cm^2 area) was held against the cobble so that the remainder of the cobble can be scrubbed off with a brush. Then the slide was removed (Figure 3) and the periphyton was scraped into a sample jar using a razor blade and toothbrush. Two samples (one

for algal speciation and one for chlorophyll), each composed of one or five cobbles, were collected at each sampling location. Sampling began with a 'one-cobble' protocol in 2004 and 2006 but then transitioned to a 'five-cobble' protocol beginning in June 2007 (except that Watercourse Engineering, Inc. samples from the remainder of 2007 utilized the five-cobble protocol).

Algal speciation samples were preserved in Lugol's Iodine.

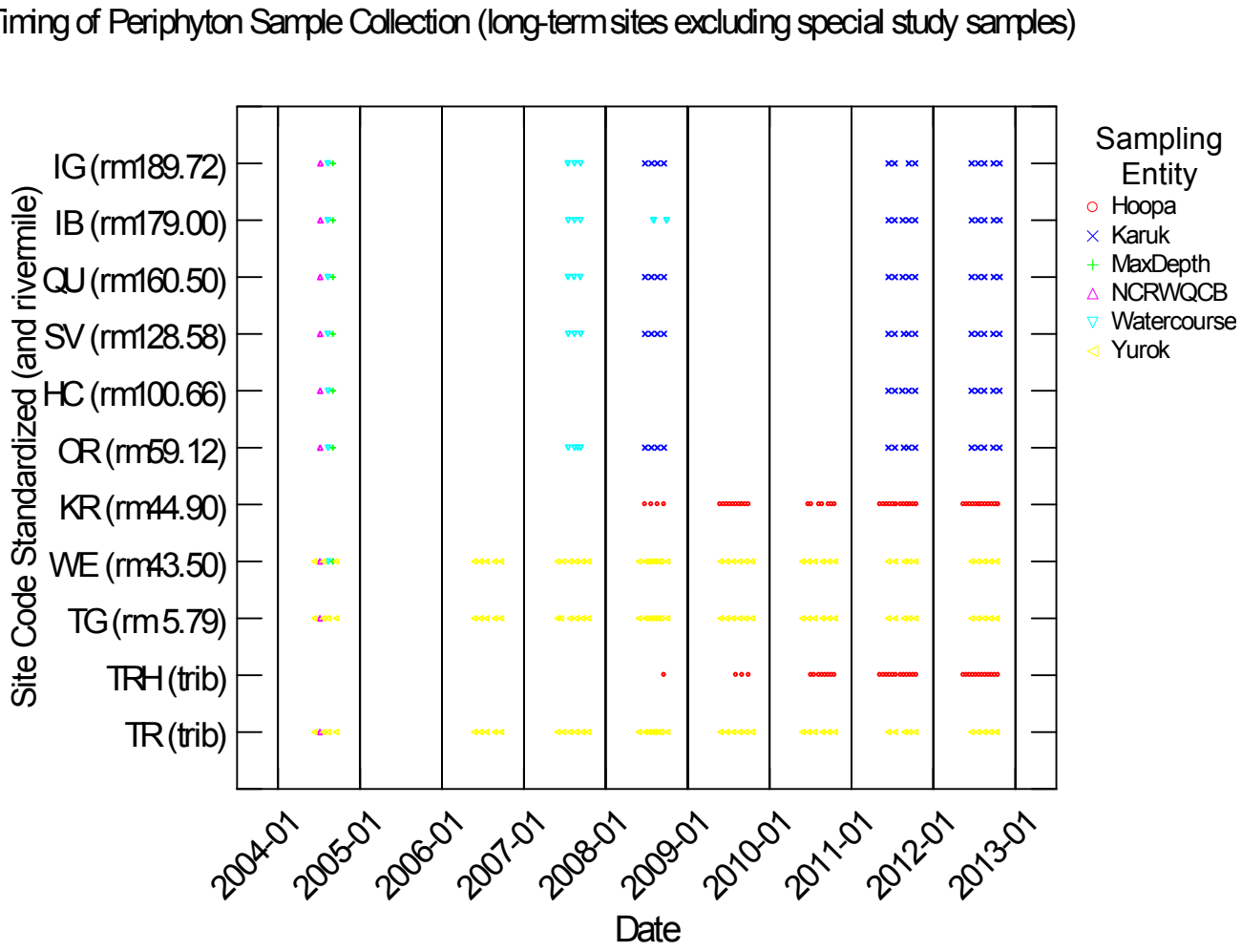

Figure 2. Dates and sites of periphyton sample collection on the Klamath and Trinity Rivers.

Table 1. Site characteristics of long-term periphyton monitoring sites on the Klamath and Trinity rivers.

	Site Description	Site Code	River Mile	Latitude	Longitude	Drainage Area (km^2)	Elevation (ft)	Periphyton Sampling Entity
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, 2011,2012
	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
	KR at Quigley's	QU	160.50	41.837367	-122.864917	15,225	1686	Watercourse/NCRWQCB/MaxDepth 2004; Watercourse 2007; Karuk 2008, 2011,2012
	KR at Seiad Valley	SV	128.58	41.842683	-123.218867	17,975	1355	Watercourse/NCRWQCB/MaxDepth 2004; Watercourse 2007; Karuk 2008, 2011,2012
	KR at Happy Camp	HC	100.66	41.729667	-123.429583	20,846	921	Watercourse/NCRWQCB/MaxDepth 2004; Karuk 2011,2012
	KR at Orleans	OR	59.12	41.305600	-123.531583	21,950	358	Watercourse/NCRWQCB/MaxDepth 2004; Watercourse 2007; Karuk 2008, 2011,2012
	Klamath River at Saints Rest Bar	KR	44.90	41.187520	-123.678001	22,617	221	Hoopa 2008-2012
	KR at Weitchpec (above Trinity R.)	WE	43.50	41.185833	-123.705556	22,611	194	Yurok/NCRWQCB 2004; Yurok 2006-2012
	KR at Turwar	TG	5.79	41.516111	-123.999167	31,339	22	Yurok/NCRWQCB 2004; Yurok 2006-2012
Trinity River Sites	Trinity River at Hoopa	TRH	43.4 +12.4	41.049852	-123.673668	7389	280	Hoopa 2008-2012
	Trinity River near Weitchpec	TR	43.4 +0.5	41.184444	-123.705278	7685	192	Yurok/NCRWQCB 2004; Yurok 2006-2012

Figure 3. Photograph of field crew measuring velocity and light extinction at Klamath River below Happy Camp (HC) periphyton monitoring site, July 18, 2013.

Figure 4. Photographs: (a) collecting a cobble at Trinity River near Weitchpec (TR) periphyton monitoring site, July 17, 2013, (b) a cobble with periphyton removed from everywhere except the 1-inch x 3-inch sampling area (from a different sampling site).

2.2 PERIPHYTON LAB ANALYSIS

Samples for microscopic determination of periphyton density and biovolume were processed by Aquatic Analysts (moved during study period from Milwaukie, OR to White Salmon, WA to Friday Harbor, WA) where enumeration and biovolume measurements were determined according to APHA Standard Methods (1992). Aquatic Analysts' protocol is described in the following sections.

2.2.1 SAMPLE PREPARATION

Permanent microscope slides were prepared from each sample by filtering an appropriate aliquot of the sample through a 0.45 micrometer membrane filter (APHA Standard Methods, 1992, 10200.D.2; McNabb, 1960). A section was cut out and placed on a glass slide with immersion oil added to make the filter transparent, followed by placing a cover slip on top, with nail polish applied to the periphery for permanency. A benefit to this method is that samples can be archived indefinitely.

2.2.2 ENUMERATION

Algal units (defined as discrete particles - either cells, colonies, or filaments) were identified and counted along a measured transect of the microscope slide with a Zeiss standard microscope (1000X, phase contrast). Only those algae that were believed to be alive at the time of collection (i.e., chloroplasts intact) were identified and counted. A minimum of 100 algal units were identified and counted in each sample (Standard Methods, 1992, 10200.F.2.c.).

2.2.3 BIOVOLUME (BIOMASS) ESTIMATES

Average biovolume estimates of each species were obtained from calculations of microscopic measurements of each alga. The number of cells per colony was recorded during sample analysis to arrive at biovolume per unit-alga. Average biovolumes for algae were stored in a computer, and measurements were verified for each sample analyzed. To facilitate understanding by a less technical audience, in this report we primarily use the term 'biomass' rather than 'biovolume', even though the actual units ($\mu m^3/cm^2$) are volume-based not mass-based.

2.2.4 CHLOROPHYLL-A ANALYSIS

The concentration of chlorophyll-*a* in periphyton samples was analyzed by Aquatic Analysts in 2004–2009 (determined fluorometrically) and Aquatic Research Incorporated (Seattle, WA) in 2010–2012 (determined spectrophotometrically using Standard Method 10200H).

2.3 PERIPHYTON TAXONOMY

Taxonomic issues in the periphyton species database were resolved by developing a 'translation' table between obsolete and current species names so that the updated species names could be utilized in analyses (see Appendix A for a list). Sources for the periphyton taxonomy included Diatoms of the United States (http://westerndiatoms.colorado.edu/), Algaebase (http://www.algaebase.org/), and the Academy of Natural Sciences in Philadelphia (http://www.ansp.org/). Algal species information from each sample was summarized in terms of species richness, Simpson and Shannon diversity indices, and evenness. Analyses were based on absolute or relative species biomass.

2.4 PERIPHYTON METRICS

To characterize samples in terms of algal autecology, species were grouped into functional groups (e.g., heterotrophs, nitrogen-fixers, pollution tolerant, etc.). The total relative biomass of each functional group was used to calculate autecological metrics for each sample, so that spatial (i.e. site-to-site), seasonal, and inter-annual patterns could be assessed. Information about the autecology of periphyton species were compiled based on literature and available databases (see Appendix A for a list of metrics, their definitions, and references)

2.5 MULTIVARIATE DATA ANALYSIS

The analysis focused on relatively long-term data collected during the summer (May-August) and fall (September-October) of eight years (2004, 2006-2012) from 11 sites (n=332). All periphyton samples were collected from 1- to 5-cobbles in each site.

We used Non-metric Multidimensional Scaling (NMDS) to characterize spatial (inter-sites) 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 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.

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

To find the species most responsible for the differences among the clusters, indicator species analysis (Dufrene and Legendre 1997) was performed. We calculated relative abundance (RA) of each species for each group. The higher RA of a species in a group, the greater the exclusiveness of the species to the group. We calculated the relative frequency (RF) of each species in each group. The RF value of a species in a group is indicative of its faithfulness to the group. The indicator species value is a product of the relative frequency and relative abundance in each group. Monte Carlo tests with 999 permutations were used to test if the indicator species

value of each species was significantly different from random for each group. Indicator taxa were those that were more abundant and had a higher probability of occurrence in one particular group ($\alpha<0.05$).

To evaluate if periphyton assemblages collected at different sites had significantly different species compositions, samples were analyzed with analysis of similarity (ANOSIM, Clarke 1993). This method tests for significant differences between two or more groups using the rank order of the samples similarity matrix. Similarity values were calculated with the Bray-Curtis similarity coefficient. If two groups had very different species compositions, the dissimilarities between the groups would be larger than those within the groups. Similarity was evaluated by the R statistic, which varies between -1 and 1, with values close to 0 indicating random grouping. The statistical significance ($\alpha=0.05$) of the R statistic was evaluated with 999 permutations. All data analyses were performed in R (R Development Core Team 2012).

3 RESULTS

3.1 OVERALL PERIPHYTON ASSEMBLAGE CHARACTERIZATION

Periphyton assemblages in the Klamath River were dominated by diatoms. On average, diatoms comprised 92.6% (range: 27.5-100%) of samples in relative biomass, followed by cyanobacteria (5.9%, range: 0-72.5%). None of the other algal groups (e.g., cryptophytes, dinoflagellates) contributed to more than 1% of samples relative biomass, except for green algae, which averaged 1.5% (range: 0-45.9%). There were a total of 143 species found in the samples (Appendix A). The mean species 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 (89%), *Achnanthidium minutissimum* (Kützing) Czarnecki (77%), *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot (77%), and *Navicula veneta* Kützing (73%) (Table 3). The ten species with the highest mean biomass included nine diatoms such as *Epithemia sorex* Kützing (111.2×10^6 $\mu m^3/cm^2$), *Cymbella affinis* Kützing (54.1×10^6 $\mu m^3/cm^2$), and *Rhopalodia gibba* (Ehrenberg) Müller (30.8×10^6 $\mu m^3/cm^2$) and one cyanobacterium *Calothrix* sp. (7.2×10^6 $\mu m^3/cm^2$) (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 (5.1×10^6 $\mu m^3/cm^2$) and very low relative biomass (2.7%) compared to the other nine species (Table 3). Based on relative biomass, the highest mean percentages were attributed to diatoms such as *E. sorex* (22.9%, range: 0-91.9%), *C. affinis* (13.3%, range: 0-96.5%), and *C. placentula* (12.1%, range: 0-80.4%) (Table 4). *Calothrix* sp. ranked tenth for mean relative biomass (2.2%, range: 0-59.8%) and was the only cyanobacteria in the top ten species (Table 4).

Relative biomass of species sensitive to nutrient enrichment (Bahls 1993) (69.3%) was higher than less tolerant (20.7%) and more tolerant (1.4%) taxa (Table 5). Nitrogen autotrophs of low organic nitrogen (taxa generally intolerant to organically-bound nitrogen) accounted for a mean

of 45.2% (range: 0-98.3%) and nitrogen autotrophs of high organic nitrogen (taxa tolerant to organically-bound nitrogen) accounted for a mean of 35.2% (range: 0.6-97.3%) (Table 5). Alkaliphilous taxa comprised, on average, the highest sample biomass (47.0%, range: 1.7-98.7%) followed by alkalibiontic taxa (34.2%, range: 0-98.3%) (Table 5). Nitrogen-fixers were 31.0% (range: 0-98.3%) of sample biomass (Table 5). Nitrogen-fixers included five species of cyanobacteria (the benthic *Calothrix* sp. and *Rivularia* sp., and the sestonic [i.e., free-floating] *Anabaena flos-aquae* [Linnaeus] Brébisson, *Anabaena sp*., and *Aphanizomenon flos-aquae* [Linnaeus] Ralfs), and three species of diatoms with cyanobacterial endosymbionts (*Epithemia sorex, E. turgida,* and *Rhopalodia 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	0.59	0.09	0.90
Shannon diversity	0.68	0.72	0.07	0.91
Simpson diversity	1.68	1.70	0.20	2.73

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

			Biomass (% of total)				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	313	94	2.68	1.26	3.73	28.88	5.07	2.06	8.96	84.84
*Cocconeis pla*centula Ehrenberg	295	89	12.10	3.65	17.37	80.40	12.29	6.57	18.14	193.63
Achnanthidium minutissimum (Kützing) Czarnecki	256	77	1.17	0.19	2.95	30.38	2.07	0.23	5.46	47.70
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	256	77	1.01	0.28	2.14	22.01	1.27	0.42	3.08	32.81
Navicula veneta Kützing	243	73	0.96	0.24	2.23	24.96	0.80	0.34	1.27	7.12
Cymbella affinis Kützing	223	67	13.32	5.56	18.88	96.54	54.08	5.66	144.07	994.04
Epithemia sorex Kützing	222	67	22.91	14.40	25.57	91.85	111.24	13.10	188.46	911.78
Gomphonema angustatum (Kützing) Rabenhorst	215	65	1.25	0.30	3.29	35.09	1.18	0.27	2.28	21.51
Diatoma tenuis Agardh	188	57	4.03	0.31	8.51	71.14	8.95	0.31	23.54	215.81
Navicula cryptocephala Kützing	188	57	0.48	0.14	0.94	8.63	0.78	0.13	1.35	7.83

Table 4. Frequency, biomass, and % biomass (i.e., relative biomass) for the ten species in 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)				Biomass (10^6 x µm^3/cm^2)			
Species	**n**	**% freq.**	**Mean**	**Med.**	**S.D.**	**Max**	**Mean**	**Med.**	**S.D.**	**Max**
Epithemia sorex Kützing	222	67	22.91	14.40	25.57	91.85	111.24	13.10	188.46	911.78
Cymbella affinis Kützing	223	67	13.32	5.56	18.88	96.54	54.08	5.66	144.07	994.04
Cocconeis placentula Ehrenberg	295	89	12.10	3.65	17.37	80.40	12.29	6.57	18.14	193.63
Gomphoneis herculeana (Ehrenberg) Cleve	145	44	6.31	0.00	10.68	61.85	23.09	0.00	55.30	489.46
Diatoma vulgaris Bory	157	47	4.96	0.00	11.65	79.65	16.54	0.00	64.52	767.48
Rhopalodia gibba (Ehrenberg) Müller	41	12	4.07	0.00	12.69	83.73	30.75	0.00	194.09	3155.48
Diatoma tenuis Agardh	188	57	4.03	0.31	8.51	71.14	8.95	0.31	23.54	215.81
Ulnaria ulna (Nitzsch) Compère	187	56	3.67	1.50	5.09	26.68	13.86	1.74	30.97	299.16
Nitzschia frustulum (Kützing) Grunow	313	94	2.68	1.26	3.73	28.88	5.07	2.06	8.96	84.84
Calothrix sp.	52	16	2.22	0.00	8.16	59.80	7.21	0.00	27.36	279.95

Table 5. Percent of total biomass for autecological groups for each cluster group (see section 3.2) and all groups combined. Bottom five rows are taxonomic groups, not autecological groups. See Appendix A for key to metrics. NA = the mean percent of biomass for taxa for which no data were available for an autecological metric. S.D. = Standard deviation.

	Percent of Total Biomass							
	Group 1		Group 2		Group 3		All	
Parameter	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Benthic	99.376	1.303	98.177	4.236	95.492	10.980	98.100	6.181
Sestonic	0.590	1.287	1.740	4.246	4.398	11.005	1.835	6.184
Eutrophic.soft	1.568	5.838	2.128	6.109	4.978	11.802	2.573	7.961
Nuisance.1	0.119	0.750	0.733	2.330	3.959	10.919	1.247	5.866
Nuisance.2	1.341	5.871	1.669	6.025	0.873	4.944	1.298	5.677
Poll.tol.1	5.527	5.817	2.838	3.326	6.047	6.083	5.028	5.537
Poll.tol.2	0.403	0.722	1.484	2.025	3.245	6.097	1.385	3.469
Poll.tol.3	0.158	1.186	0.139	0.768	0.059	0.178	0.128	0.928
Poll.tol.4	11.818	12.732	23.202	15.223	58.362	18.146	26.409	24.353
Poll.tol.5	15.398	21.177	25.881	16.797	6.864	8.777	15.676	18.903
Poll.tol.NA	66.695	25.042	46.455	18.846	25.423	16.359	51.373	27.700
Poll.class.1	0.232	0.338	1.056	1.078	3.992	4.570	1.388	2.842
Poll.class.2	17.825	18.421	29.086	14.823	18.868	14.800	20.738	17.337
Poll.class.3	72.416	21.209	63.264	15.175	68.642	20.964	69.300	20.176
Poll.class.NA	9.528	15.723	6.593	10.948	8.498	13.924	8.575	14.281
Moisture.1	6.443	10.659	18.703	14.673	10.542	17.266	10.373	14.410
Moisture.2	65.972	21.210	36.491	21.615	48.065	24.110	54.461	25.274
Moisture.3	12.663	17.478	22.563	15.579	22.578	15.815	17.527	17.309
Moisture.4	0.012	0.044	0.284	0.583	0.145	0.341	0.110	0.349
Moisture.5	0.004	0.055	0.000	0.000	0.039	0.254	0.012	0.135
Moisture.NA	14.904	16.386	21.960	15.356	18.632	15.956	17.517	16.254
Oligotrophic	0.839	1.446	3.089	3.650	3.184	5.850	1.968	3.765
Oligo.mesotrophic	0.739	1.146	2.710	3.358	3.781	4.653	1.981	3.245
Mesotrophic	0.197	0.711	2.004	8.203	0.251	0.804	0.635	4.081
Meso.eutrophic	6.520	10.667	10.319	10.034	11.720	17.955	8.744	12.976
Eutrophic	71.661	18.164	57.066	18.275	60.794	24.344	65.450	20.914
Polytrophic	0.145	0.386	0.172	0.511	1.098	3.297	0.396	1.751
Eurytrophic	5.886	5.878	6.044	5.541	4.031	4.205	5.448	5.465
Trophic.NA	14.013	16.352	18.596	15.319	15.139	15.750	15.378	16.021
Oligosaprobic	0.922	1.477	3.358	3.831	3.354	5.860	2.117	3.837
B.mesosaprobic	77.199	16.875	58.732	17.585	69.982	17.662	71.012	18.735
A.mesosaprobic	2.007	2.548	16.191	12.688	4.943	6.619	6.091	9.206
A.polysaprobic	5.681	5.940	2.815	3.337	5.185	5.218	4.881	5.363
Polysaprobic	0.145	0.386	0.144	0.476	1.098	3.297	0.389	1.749

	Percent of Total Biomass							
	Group 1		Group 2		Group 3		All	
Parameter	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Saprobic.NA	14.045	16.351	18.761	15.334	15.438	15.688	15.510	16.014
DO.high	16.657	21.192	34.149	18.933	11.542	10.099	19.457	20.203
DO.fair.high	47.137	22.669	20.553	13.631	22.896	16.986	34.685	23.220
DO.moderate	21.743	19.188	25.263	14.437	46.008	19.288	28.782	20.837
DO.low	0.283	0.592	1.059	1.542	3.787	4.495	1.363	2.826
DO.very.low	0.030	0.106	0.035	0.179	0.021	0.141	0.029	0.135
DO.NA	14.149	16.339	18.942	15.357	15.744	15.650	15.684	16.007
N.auto.lowN	65.346	20.663	35.301	20.432	14.106	13.114	45.168	28.914
N.auto.highN	18.760	15.873	41.661	18.540	61.758	19.100	35.149	25.087
N.hetero.fac	0.203	0.629	0.361	1.333	1.192	3.067	0.494	1.781
N.hetero.obl	1.549	1.672	3.732	4.273	7.207	8.397	3.510	5.389
Org.N.NA	14.143	16.338	18.945	15.363	15.737	15.656	15.679	16.009
Fresh	0.471	0.924	2.474	8.324	0.533	1.183	0.958	4.197
Fresh.brackish	82.855	16.291	63.897	17.986	76.161	16.472	76.687	18.354
Brackish.fresh	2.676	2.762	18.057	12.662	8.477	7.080	7.775	9.602
Brackish	0.062	0.210	0.052	0.206	0.215	1.098	0.099	0.586
Salinity.NA	13.935	16.366	15.519	15.617	14.615	15.609	14.481	15.967
Acidophilous	0.008	0.076	0.898	7.934	0.457	3.096	0.332	4.149
Circumneutral	0.872	0.961	7.260	7.065	5.294	5.645	3.505	5.278
Alkaliphilous	30.876	23.310	60.563	17.327	66.515	21.984	46.975	27.243
Alkalibiontic	53.907	23.647	11.284	12.032	15.891	20.398	34.160	28.830
Indifferent	0.289	0.499	4.522	5.166	0.545	1.292	1.349	3.142
pH.NA	14.049	16.364	15.472	15.501	11.299	13.532	13.679	15.510
N.fixer	54.823	26.308	7.919	12.495	7.792	14.045	31.762	31.531
Not.N.fixer	45.143	26.442	91.997	12.482	92.099	14.026	68.173	31.505
N.fixer.NA	0.034	0.261	0.083	0.295	0.109	0.219	0.065	0.261
Motile	10.924	17.640	9.887	9.292	7.887	10.992	9.903	14.500
Non.motile	87.599	19.231	90.030	9.326	92.004	11.007	89.298	15.562
Motility.NA	1.478	8.586	0.083	0.295	0.109	0.219	0.800	6.159
bluegreen	7.368	15.143	2.060	4.627	6.411	12.787	5.876	12.943
cryptophyte	0.005	0.049	0.032	0.190	0.015	0.107	0.014	0.113
diatom	91.039	15.816	96.086	7.813	92.557	13.723	92.613	13.895
dinoflagellate	0.004	0.048	0.000	0.000	0.000	0.000	0.002	0.034
green	1.584	5.862	1.823	6.182	1.018	4.989	1.495	5.721

3.2 MAJOR PERIPHYTON ASSEMBLAGE GROUPS IN THE KLAMATH RIVER

Based on cluster analysis on relative biomass of 91 species (after removal of 52 rare species (<1%) and log-transformation of the data to down-weight the effect of dominant species), we identified three major groups of periphyton assemblages (Figure 5). 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 (Table 5, Table 6). ANOSIM results revealed significantly different species assemblages among these three groups (R=0.70, p=0.001). Pairwise comparisons showed that every group had a different species composition ($p \leq 0.001$) compared to the other groups. Groups 1 and 3 were most different (R=0.74, p=0.001).

Group 1 was dominated by diatoms and benthic cyanobacteria (Table 5, Table 6). Among the three periphyton groups, cyanobacteria were most dominant in this group (7.4% biomass) while diatoms were least dominant (91.0% biomass). The most dominant species in this group included a diatom species, with cyanobacterial endosymbionts*: E. sorex* (mean 40.3% biomass) and another diatom *C. affinis* (mean 14.2% biomass). The best indicator species for this group was *E. sorex* (indicator value 0.77). The remaining indicators were all diatoms plus one cyanobacterium *Calothrix* sp. (indicator value 0.20). In addition to *E. sorex* and *Calothrix* sp., other N-fixing species included *R. gibba* (indicator value 0.24) and *Epithemia turgida* (Ehrenberg) Kützing (indicator value 0.10). Species in this group included eutrophic taxa (71.7% biomass), beta-mesosaprobic taxa (77.2% biomass) which live under 70-80% DO saturation and 2-4 mg/L BOD, nitrogen autotrophs at low organic nitrogen (taxa generally intolerant to organically-bound nitrogen, 65.4% biomass), alkalibiontic (53.9% biomass), nitrogen-fixers (54.8% biomass). Spatially, this group included approximately 2/3 of samples collected at downstream (OR, KR, WE, TG) and tributary (TR and TRH) sites (Table 7). Temporally, approximately 2/3 of all August, September and October samples were in this group (Table 7).

Group 2 was dominated by diatoms (mean biomass 96.1%), which reached their highest relative abundance here (compared to the other two groups), while cyanobacteria were least abundant in this group (2.1% biomass) (Table 5, Table 6). The most abundant species in this group were *C. affinis* (mean 22.3% biomass)*, Diatoma tenuis* Agardh (mean 14.2% biomass)*,* and *Gomphoneis herculeana* (Ehrenberg) Cleve (mean 10.2% biomass). The best indicators were *D. tenuis* (indicator value 0.86), *Achnanthidium minutissimum* (Kützing) Czarnecki (indicator value 0.81), *Nitzschia dissipata* (Kützing) Grunow (indicator value 0.72), and *Encyonema minutum* (Hilse) Mann (indicator value 0.71). Species in this group were somewhat tolerant to nutrient and organic enrichment and nearly 100% DO saturation (34.2% biomass). Spatially, this group included 1/3 of samples collected at downstream sites. Temporally, all May (note: May samples were collected only at downstream sites) and the majority of June samples were in this group (Table 7).

Compared to the above two groups, Group 3 had the highest percentage (4.4%) of sestonic species and both diatoms and cyanobacteria had intermediate abundances (Table 5, Table 6). The most abundant species in this group were *Cocconeis placentula* Ehrenberg (mean 33.7% biomass)*, Diatoma vulgaris* Bory (mean 8.9% biomass)*,* and *Gomphoneis herculeana* (Ehrenberg) Cleve (mean 6.4% biomass). The best indicator species for this group included *C. placentula* (indicator value 0.81), *Navicula veneta* Kützing (indicator value 0.74), and *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot (indicator value 0.64). In addition to diatoms, this group included two sestonic species of cyanobacteria *Aphanizomenon flos-aquae*

(Linnaeus) Ralfs (indicator value 0.15) and *Microcystis aeruginosa* Kützing (indicator value 0.09). More than half of species biomass belonged to taxa which live in somewhat degraded conditions. Predominant autecological assemblages in Group 3 were comprised of those tolerant to degraded DO (>50% DO saturation, 46.0% biomass), nitrogen autotrophs at high organic nitrogen (taxa tolerant to organically-bound nitrogen, 61.8% biomass), and alkaliphilous species (66.5% biomass). This group contained some pollution tolerant and potential algal toxin producers. Spatially, almost all upstream sites belonged to this group (Table 7). Temporally, 1/3 of all samples collected in August, September and October were in this group (Table 7).

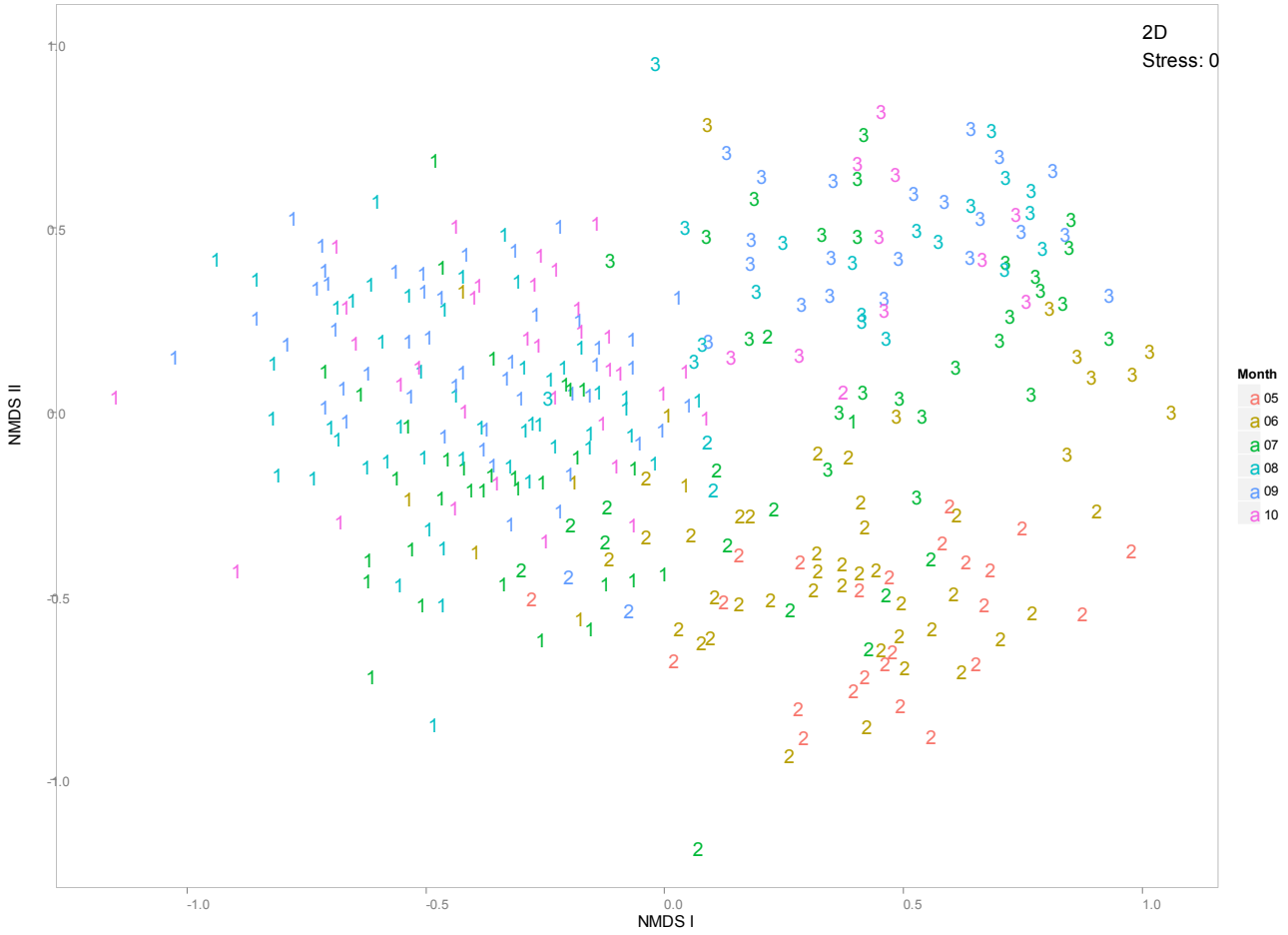

Figure 5. Non-metric Multidimensional Scaling (NMDS) showing the relative similarity of periphyton assemblages for each sample, colored by month and symbolized by the three major groups identified using cluster analysis. The distance between symbols indicates the relative similarity of the samples.

Table 6. Summary of cluster group characteristics, including taxonomic groups, indicator species, autecological groups, sites, and seasonality.

Characteristic		Cluster Group 1	Cluster Group 2	Cluster Group 3
Taxonomic Groups (% biomass)	Diatoms	91% (mostly benthic)	96.1% (mostly benthic)	92.6% (mostly benthic)
	Bluegreen	7.4% (mostly benthic)	2.1% (mostly benthic)	6.4% (mostly sestonic)
Attachment (% biomass)	Benthic	99.4%	98.2%	95.5%
	Sestonic	0.6%	1.7%	4.4%
Species With Highest Mean Biomass (mean %)		Nitrogen-fixing benthic diatom: *Epithemia sorex* (40.3%) Benthic diatom: Cymbella affinis (14.2%)	Benthic diatoms: *Cymbella affinis* (14.2%), *Diatoma tenuis* (14.2%), *Gomphoneis herculeana* (10.2%)	Benthic diatoms: *Cocconeis placentula* (33.7%), *Diatoma vulgaris* (8.9%), *Gomphoneis herculeana* (6.4%)
Indicator Species (indicator value)		Nitrogen-fixing benthic diatoms: *Epithemia sorex (0.77), Rhopalodia gibba (0.24), Epithemia turgida (0.10)* Nitrogen-fixing benthic bluegreens: *Calothrix* sp. *(0.20)*	Benthic diatoms: *Diatoma tenuis (0.86), Nitzschia dissipata (0.72), Achnanthidium minutissimum (0.81), Encyonema minutum (0.71)*	Benthic diatoms: *Cocconeis placentula* (0.81)*, Navicula veneta* (0.74)*, Rhoicosphenia abbreviata* (0.64) Sestonic bluegreens: *Aphanizomenon flos-aquae* (0.15)*, Microcystis aeruginosa* (0.09)
Auto-ecological groups (% biomass)		eutrophic taxa: 71.7%	eutrophic taxa: 57.1%	eutrophic taxa: 60.8%
		Beta-mesosaprobic taxa (under 70-80% DO saturation and 2-4 mg/L BOD): 77.2%	Beta-mesosaprobic taxa (under 70-80% DO saturation and 2-4 mg/L BOD): 58.7% (lowest % among all clusters). Alpha-mesosaprobic (25-70% DO saturation and 4-13 mg/L BOD): 16.2% (highest % among all clusters)	Beta-mesosaprobic taxa (under 70-80% DO saturation and 2-4 mg/L BOD): 70.0%
		Fairly high DO (> 75% DO saturation): 47.1%	High DO (nearly 100% DO saturation): 34.2%	Degraded DO conditions (>50% DO sat.): 46.0%
		Nitrogen autotrophs at low organic nitrogen (taxa generally intolerant to organically-bound nitrogen): 65.4%	Nitrogen autotrophs at low organic nitrogen (taxa generally intolerant to organically-bound nitrogen): 35.3%	Nitrogen autotrophs at high organic nitrogen (taxa tolerant to organically-bound nitrogen): 61.8%
		Alkalibiontic: 53.9%	Alkaliphilous: 60.6%; Alkalibiontic: 11.3%	Alkaliphilous: 66.5%
		Nitrogen-fixers: 54.8%	Nitrogen-fixers: 7.9%	Nitrogen-fixers: 7.8%
Sites and Seasonality	Upstream sites (IG, IB, QU)			Nearly all (98%) June-October [no May data]
	Middle sites (SV, HC)	Some (31%) August-September	Most (80%) June [no May data]	All (100%) July, most (70%) August-October
	Downstream sites (OR, KR, WE, TG)	Most (70%) July, nearly all (99%) August-October	Nearly all (90%) May-June, some (18%) July	
	Trinity River sites (TR, TRH)	Most (62%) July, nearly all (89%) August-October	Nearly all (86%) May-June, some (38%) July	

Table 7. 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	19	19
IB	0	1	17	18
QU	0	0	20	20
SV	2	3	15	20
HC	3	2	8	13
OR	14	3	4	21
KR	31	15	0	46
WE	35	12	0	47
TG	31	14	1	46
TR	31	15	0	46
TRH	22	13	1	36
2004	21	2	11	34
2006	7	8	0	15
2007	17	4	13	34
2008	23	10	14	47
2009	24	6	1	31
2010	20	13	0	33
2011	25	20	24	69
2012	32	15	22	69
May	0	24	0	24
June	7	36	9	52
July	33	13	25	71
August	49	2	20	71
September	47	2	21	70
October	33	1	10	44

3.3 SPATIAL VARIATION OF PERIPHYTON ASSEMBLAGES IN THE KLAMATH RIVER

3.3.1 PERIPHYTON COMMUNITY COMPOSITION

The ordination plot based on all the data collected during summer (May-August) and fall (September-October) of eight years (2004, 2006-2012) from 11 sites (n=332) revealed a longitudinal gradient in periphyton species composition from upstream (upper right corner, Figure 6) to downstream sites (left side and bottom, Figure 6). To discern spatial and temporal variability, a separate ordination analysis based on samples collected within a single year (year 2012, Figure 7) confirmed this longitudinal shift in species composition. Upstream sites (upper right corner, Figure 7) were more similar to each other than to downstream sites (left side and bottom, Figure 7). 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 7). This seasonal pattern was observed in both 2012 and 2011 (years with highest number of samples) and was less pronounced at upstream sites (i.e., smaller distance among upstream sites) as opposed to downstream sites (i.e., larger distance among downstream sites). These longitudinal changes illustrated by the NMDS plots correspond to the three major periphyton groups identified by the cluster analysis (see above section).

Individual autecological metrics superimposed on the NMDS plot revealed that the relative abundance of nitrogen-fixers (Figure 8) and nitrogen autotrophs at low organic nitrogen conditions increased longitudinally (Figure 9), while sestonic species (most common at the two most upstream sites, Figure 10) and very tolerant to nutrient and organic enrichment species decreased longitudinally (Figure 11). ANOSIM results revealed significantly different species assemblages among sites ($R=0.33$, $p=0.001$). Pairwise comparisons showed that some sites had different species compositions ($p \leq 0.001$) compared to other sites. In addition, the similarity among sites decreased as the distances among them increased from upstream to downstream. However, there was no statistically significant difference ($p>0.05$) in species composition among a few pairs of sites in the middle reaches (SV to WE). For example, species composition at site SV was similar to species composition at site HC (downstream from it), site HC was similar to site OR (downstream from it), and site OR was similar to both downstream sites KR and WE.

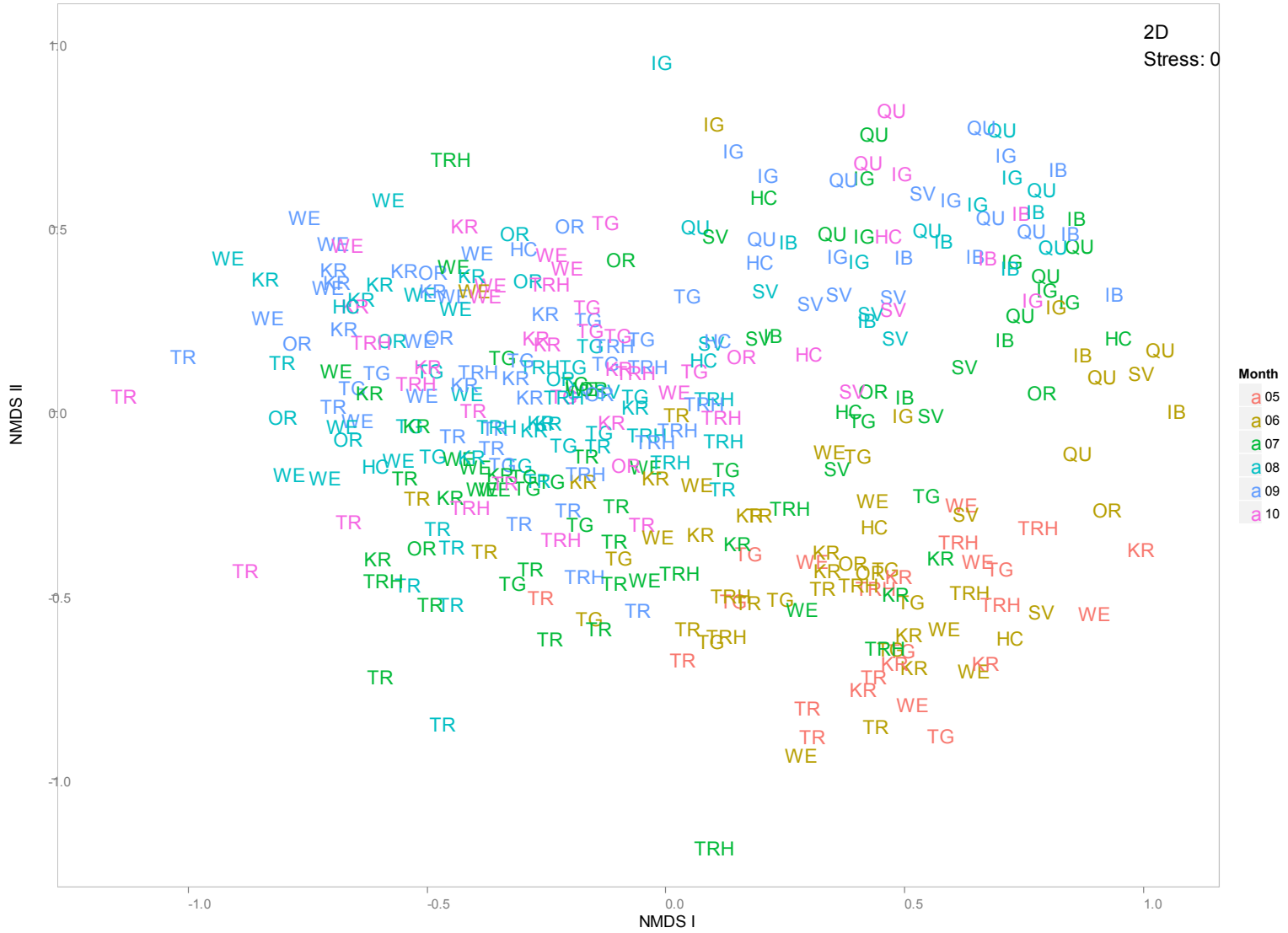

Figure 6. Non-metric Multidimensional Scaling (NMDS) plot showing the relative similarity of periphyton assemblages for each sample, colored by month and symbolized by site (all years of data). The distance between symbols indicates the relative similarity of the samples. See Table 1for key to site codes.

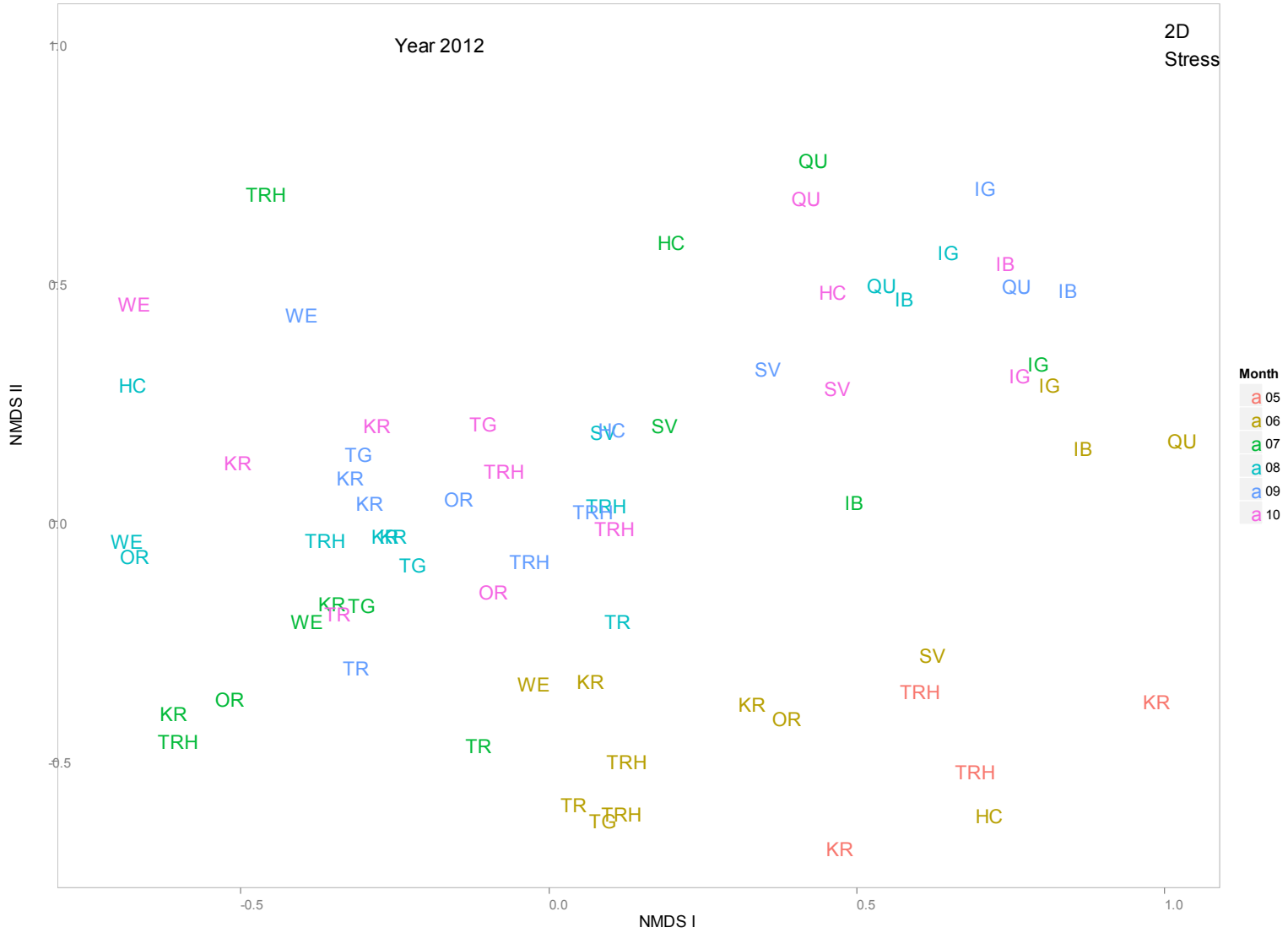

Figure 7. Non-metric Multidimensional Scaling (NMDS) plot showing the relative similarity of periphyton assemblages for each sample collected in the year 2012, colored by month and symbolized by site. The distance between symbols indicates the relative similarity of the samples. See Table 1for key to site codes.

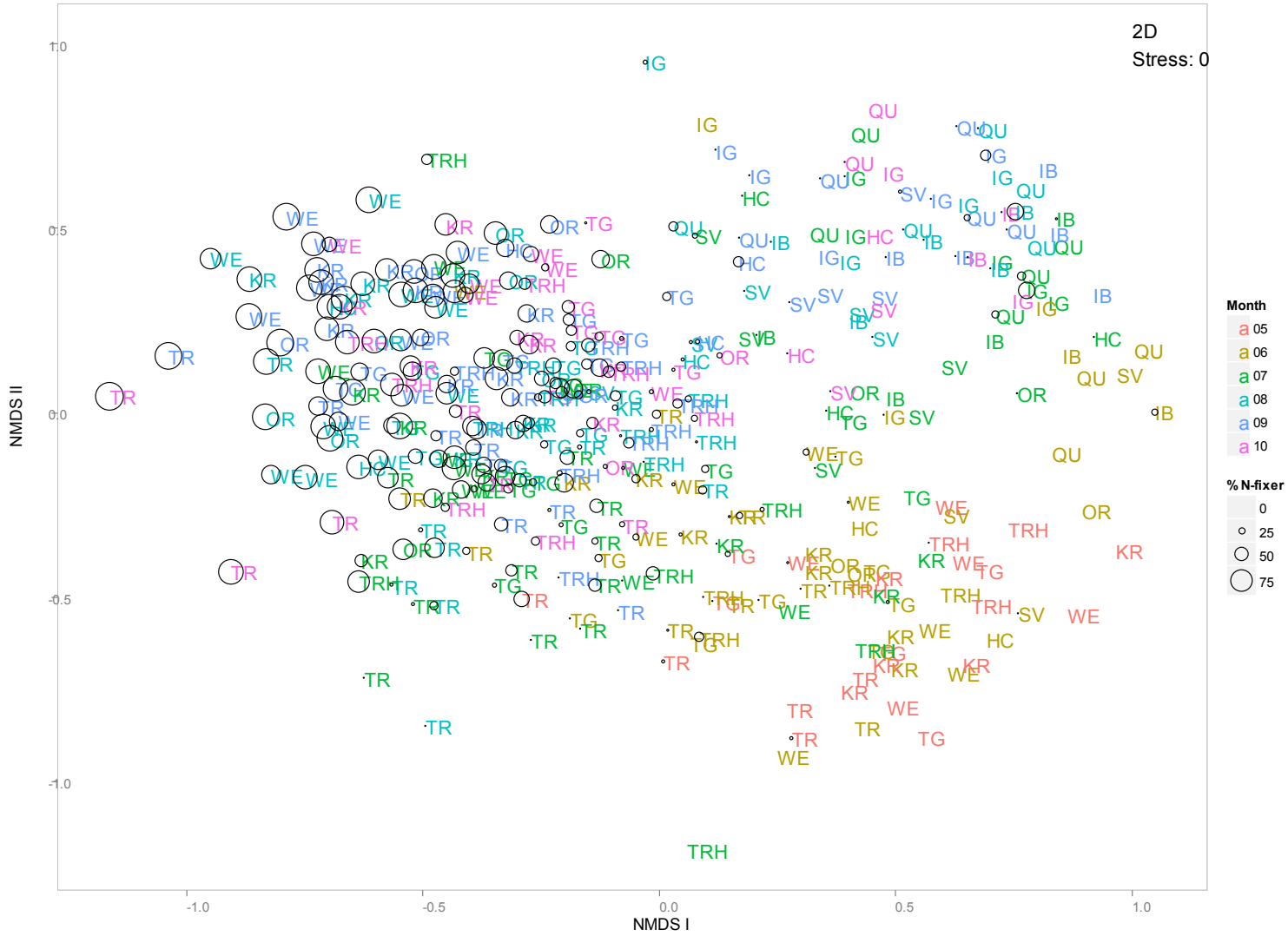

Figure 8. Percent nitrogen-fixer biomass superimposed on Non-metric Multidimensional Scaling (NMDS) plot of the relative similarity of periphyton assemblages for each sample. The distance between symbols indicates the relative similarity of the samples. Individual samples are colored by month and symbolized by site. See Table 1for key to site codes.

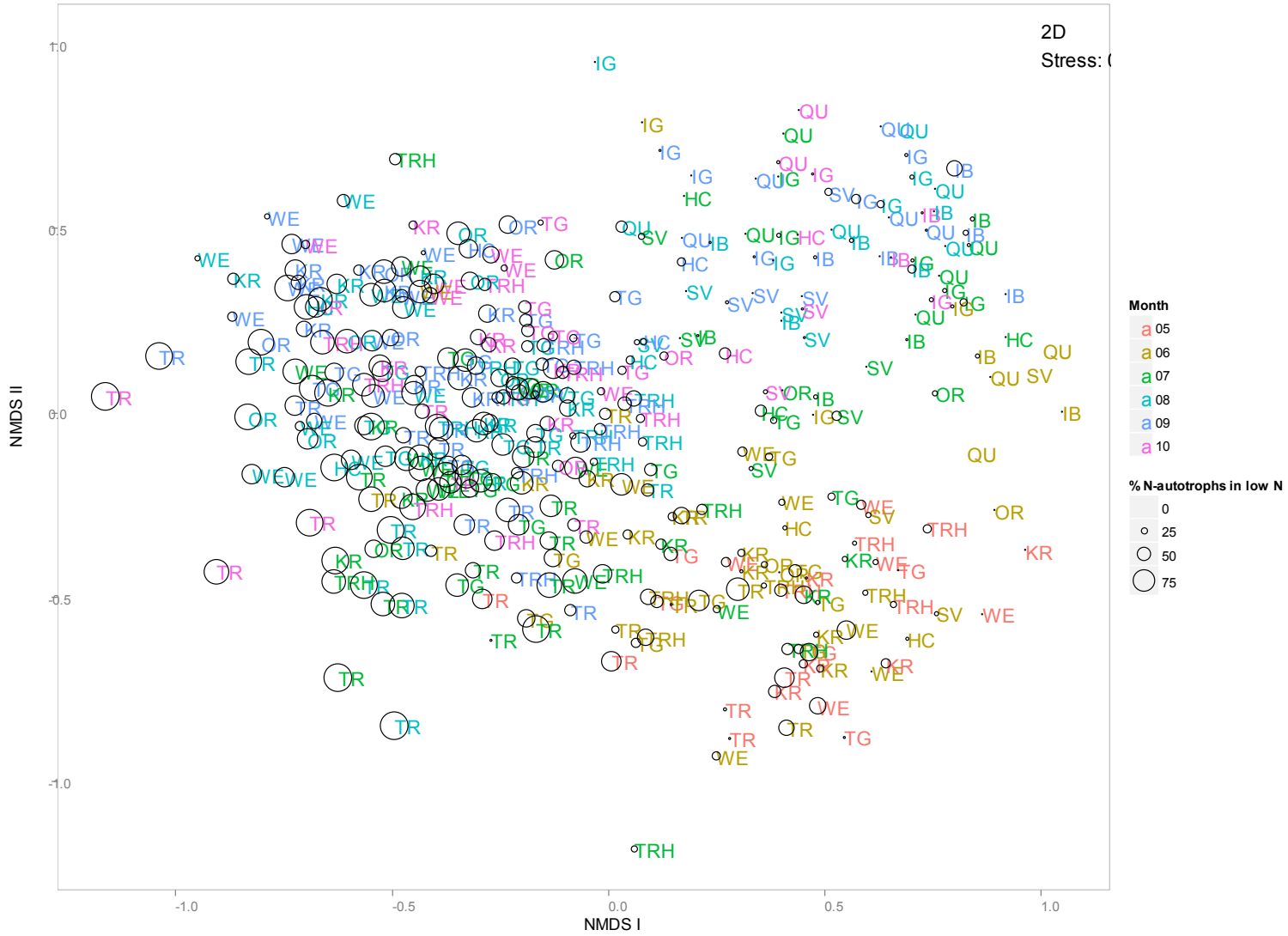

Figure 9. Percent nitrogen-autotroph (in low N conditions) biomass superimposed on Non-metric Multidimensional Scaling (NMDS) plot of the relative similarity of periphyton assemblages for each sample. The distance between symbols indicates the relative similarity of the samples. Individual samples are colored by month and symbolized by site. See Table 1for key to site codes.

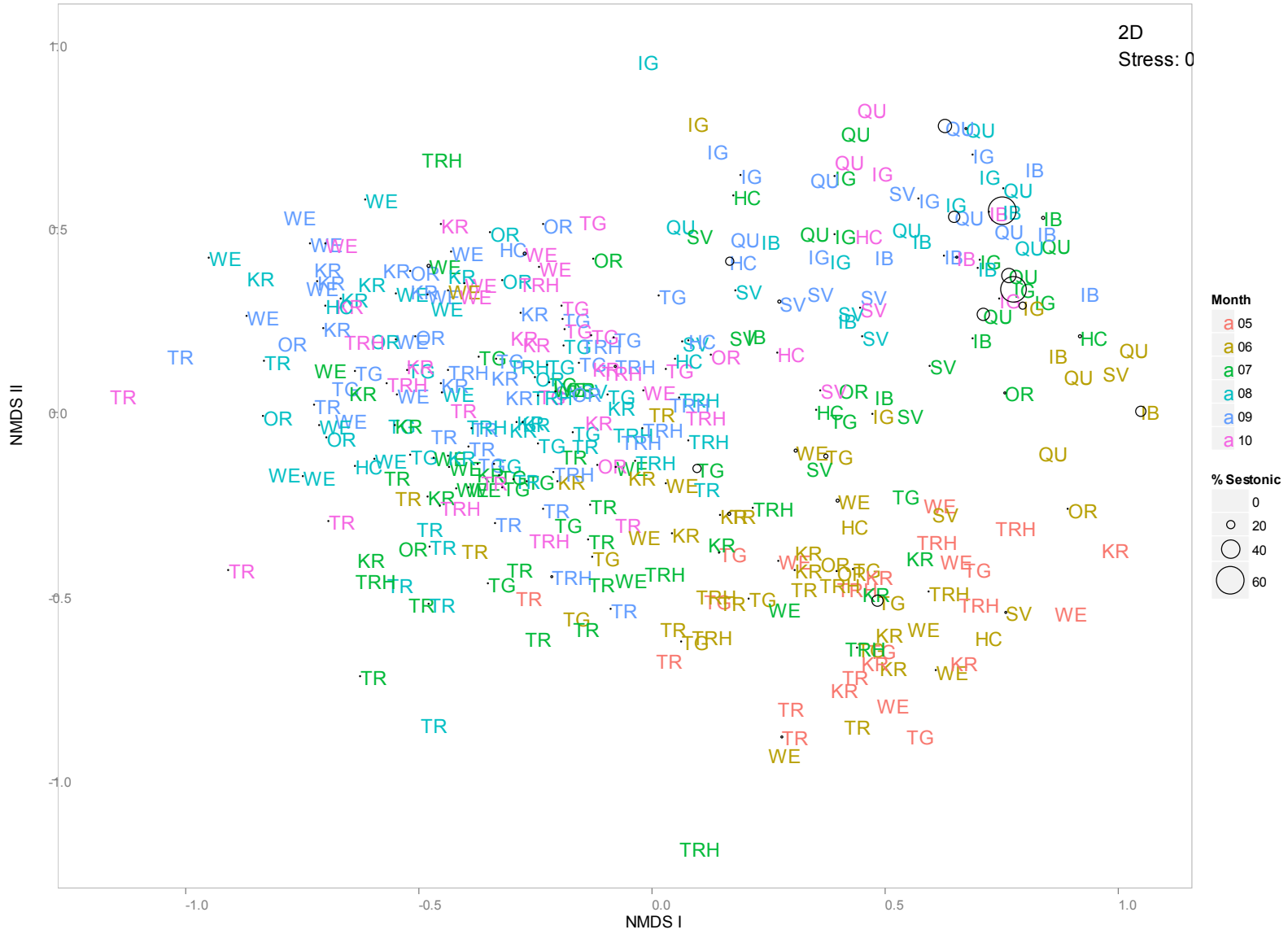

Figure 10. Percent sestonic biomass superimposed on Non-metric Multidimensional Scaling (NMDS) plot of the relative similarity of periphyton assemblages for each sample. The distance between symbols indicates the relative similarity of the samples. Individual samples are colored by month and symbolized by site. See Table 1for key to site codes.

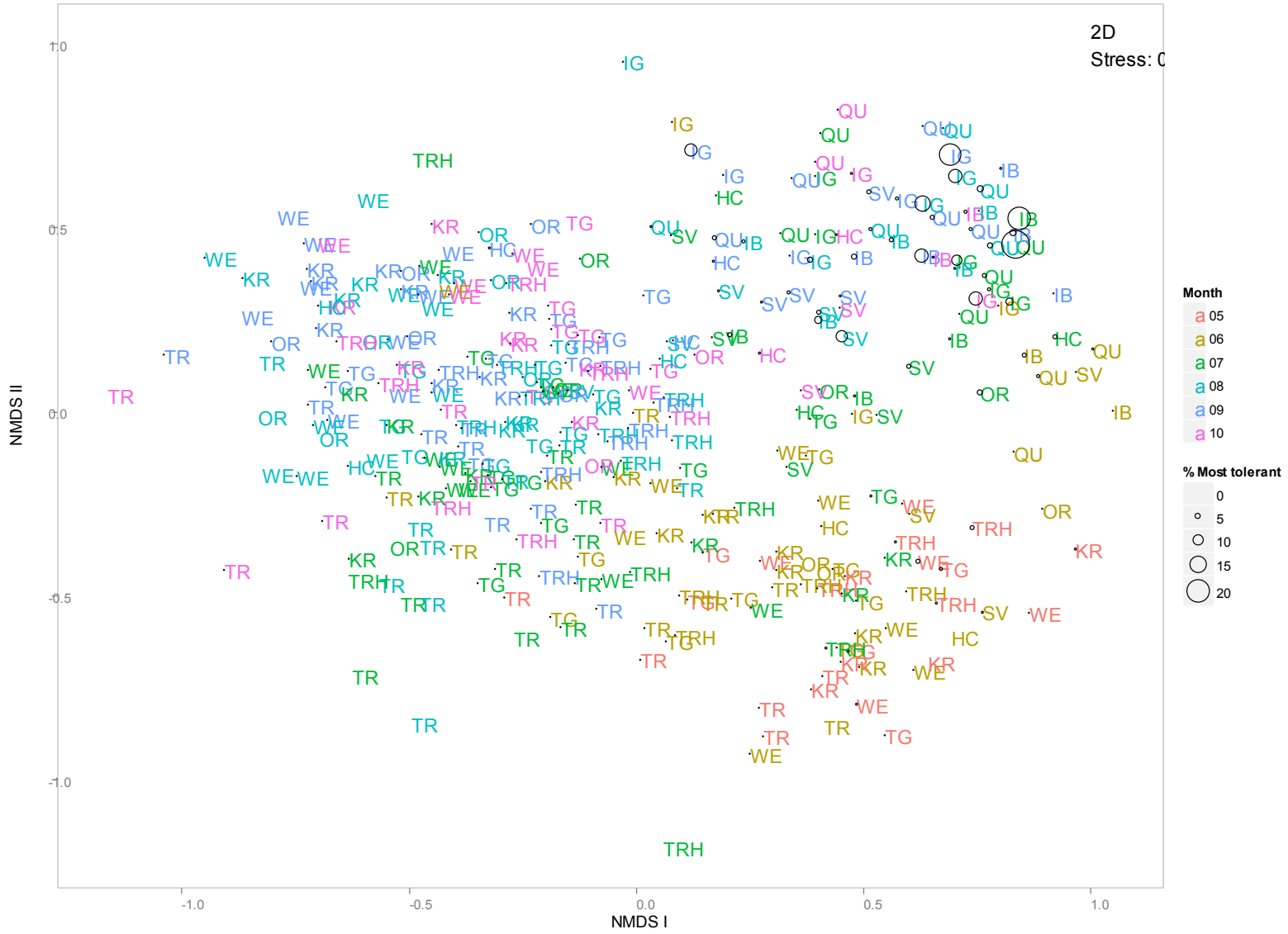

Figure 11. Percent 'most pollution tolerant' biomass superimposed on Non-metric Multidimensional Scaling (NMDS) plot of the relative similarity of periphyton assemblages for each sample. The distance between symbols indicates the relative similarity of the samples. Individual samples are colored by month and symbolized by site. See Table 1for key to site codes.

3.3.2 BIOMASS OF DOMINANT SPECIES, FUNCTIONAL GROUPS, AND ENTIRE ASSEMBLAGE

In general, total biomass increased in a downstream direction (Figure 12a). Longitudinal patterns in chlorophyll-*a* were less clear, although chlorophyll-*a* was highest at the two most downstream sites (WE and TG) (Figure 13a,b,c). Group 1 had the highest biomass, followed by Group 2, and then Group 3 (Figure 12). Chlorophyll-*a* was greater in Group 1 than in Group 2 or Group 3 (Figure 13a,c).

Diatoms were the dominant periphyton group at all sites, although cyanobacteria such as the benthic *Calothrix* sp. sometimes reached high biomass at three downstream sites (OR, KR and WE, Figure 14). The biomass of attached taxa increased in the downstream direction (Figure 14). The biomass of tolerant to pollution taxa decreased downstream while the biomass of sensitive taxa increased (Figure 14). Species characteristic of low oxygen (>30% DO saturation) conditions were more common upstream (sites IG, IB, and QU, Figure 14), compared to downstream sites which had more species preferring high oxygen (>75% DO saturation, Figure 14). Dominant taxa with high nutrient preferences were more common at upstream sites (*C. placentula, G. angustatum, Navicula cryptocephala, N. veneta, Nitzschia frustulum*), compared to others, which were more abundant downstream (*C. affinis, E. sorex*, Figure 15). Nitrogen-fixers at downstream sites were periphytic diatoms (species of *Epithemia and Rhopalodia*) and the only attached nitrogen-fixing cyanobacterium (*Calothrix* sp.). In contrast, for upstream sites the nitrogen-fixers were comprised of planktonic cyanobacteria (*Anabaena flos-aquae*, *Anabaena* sp., *Aphanizomenon flos-aquae*).

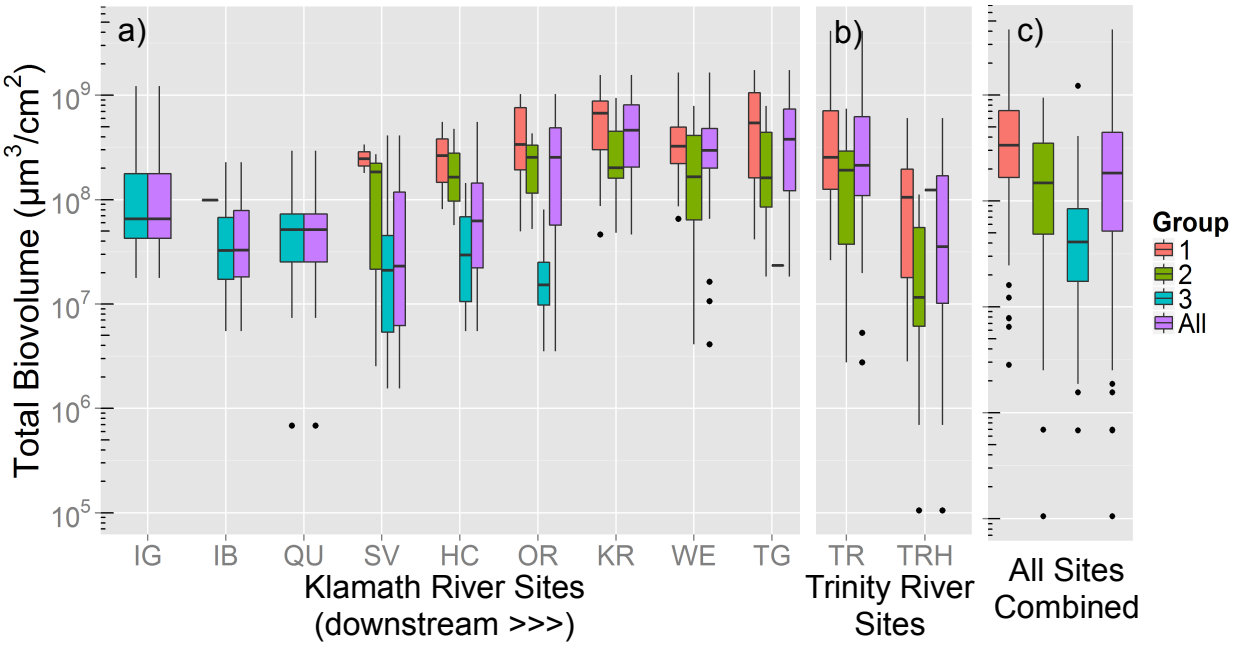

Figure 12. Boxplot of total periphyton biomass, by cluster group, for individual sites on the (a) Klamath and (b) Trinity rivers, and (c) all sites combined. See Table 1for key to site codes. The horizontal line inside the box is median, the upper and lower edges of the box are 25th and 75th percentiles, 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.

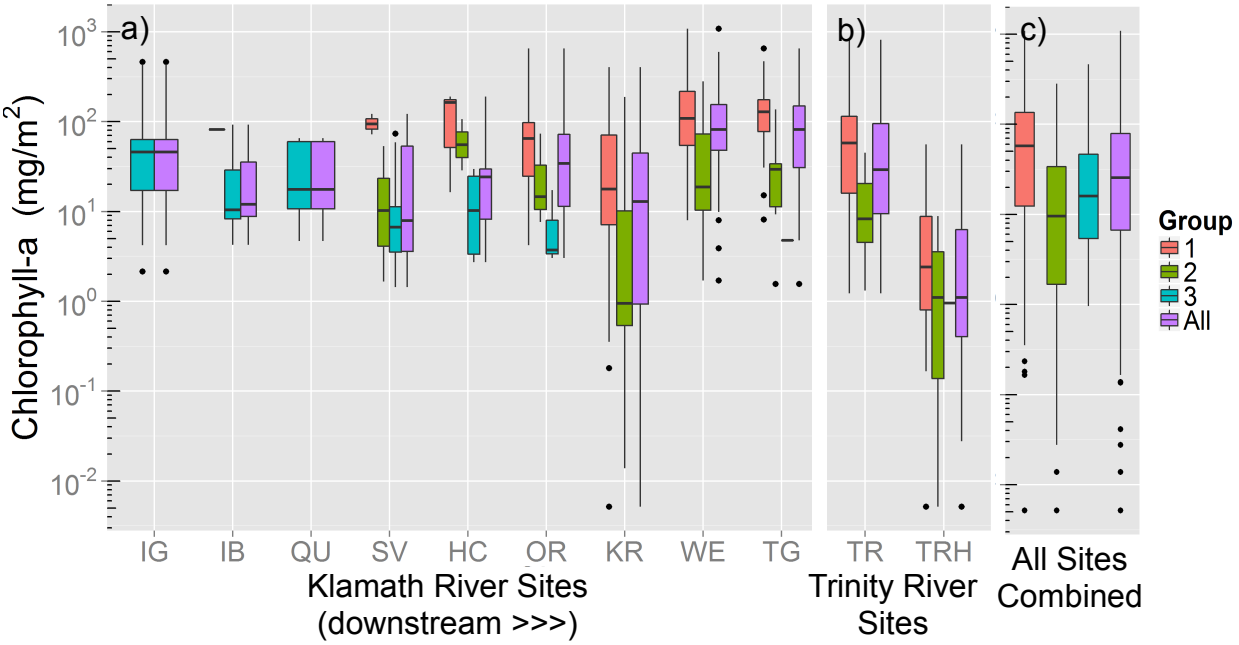

Figure 13. Boxplot of periphyton chlorophyll-*a* density, by cluster group, for individual sites on the (a) Klamath and (b) Trinity rivers, and (c) all sites combined. Note: chlorophyll-*a* data were not available for all samples. See Table 1for key to site codes. The horizontal line inside the box is 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.

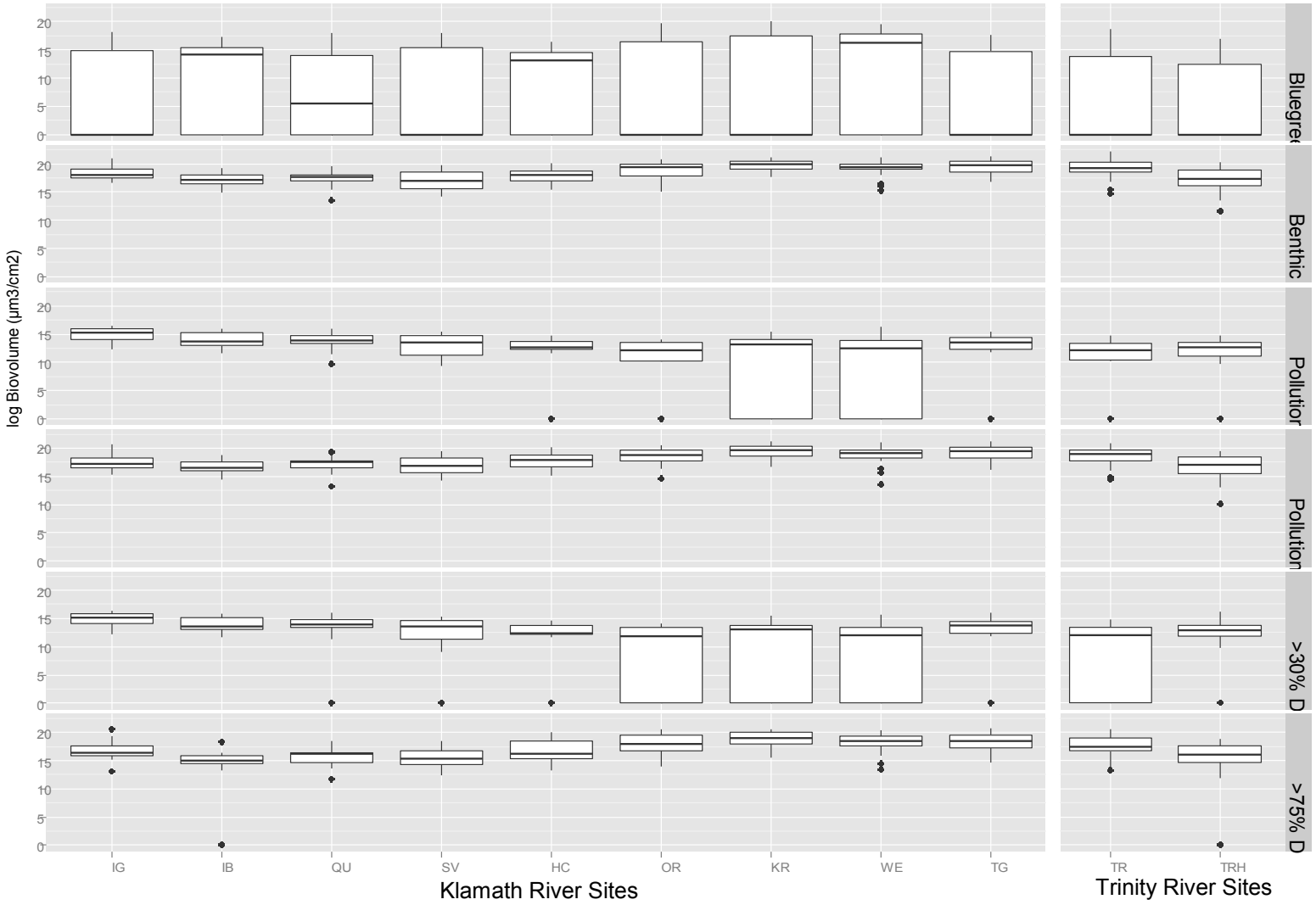

Figure 14. Boxplot showing biomass of taxonomic groups and autecological groups for Klamath River periphyton samples, by site. Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). Y-axis is log-transformed. See Table 1for key to site codes. Pollution class 1 = taxa most tolerant of organic enrichment (Bahls 1993), Pollution class 3 = taxa least tolerant of organic enrichment (Bahls 1993), >30% DO = taxa with low dissolved oxygen requirement, and >75% DO = taxa with fairly high dissolved oxygen requirement.

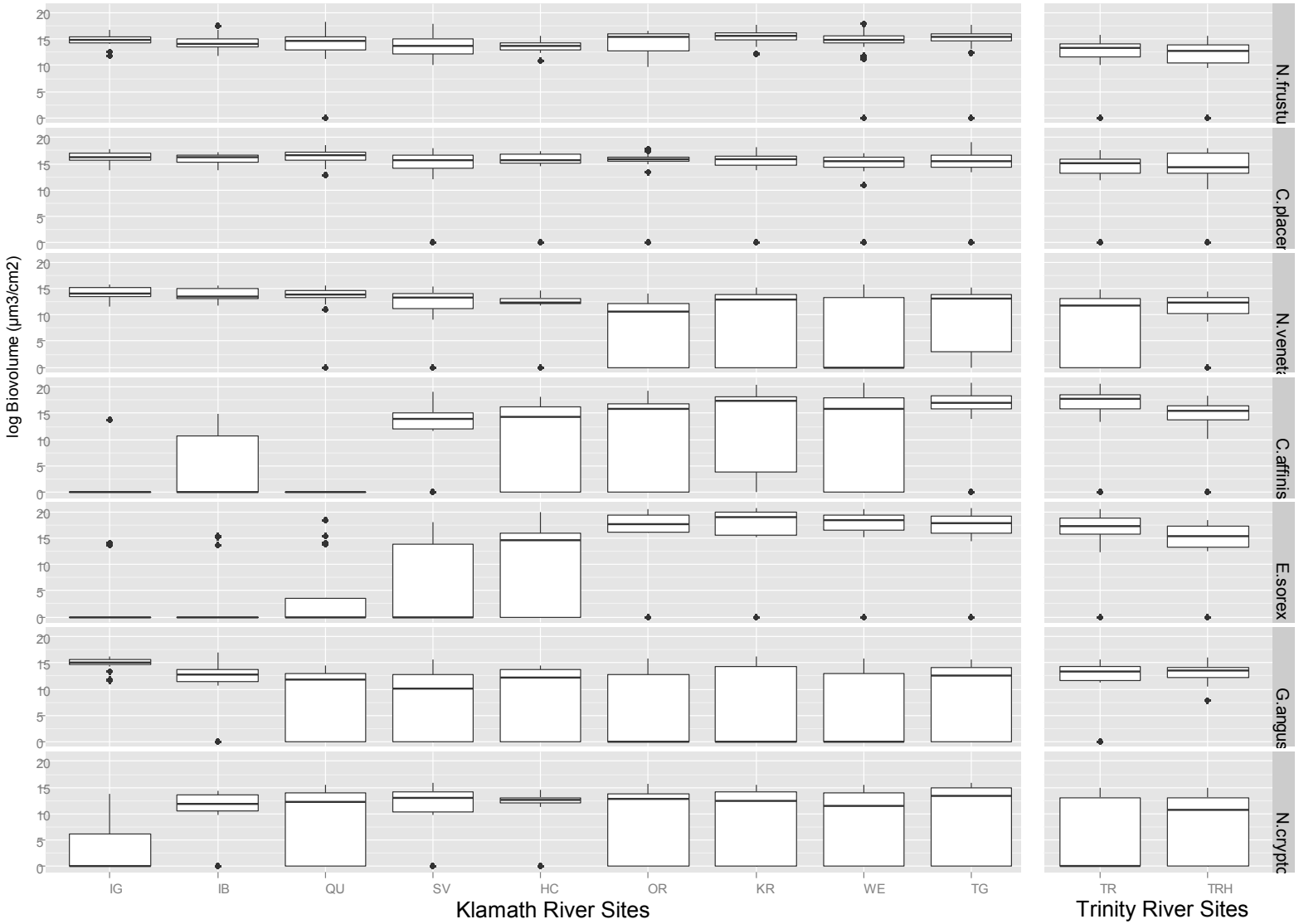

Figure 15. Boxplot showing biomass of common Klamath River periphyton species, by site. Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). Y-axis is log-transformed. See Table 1 for key to site codes.

3.4 TEMPORAL VARIATION OF PERIPHYTON ASSEMBLAGES IN THE KLAMATH RIVER

3.4.1 PERIPHYTON COMMUNITY COMPOSITION

There appears to be a seasonal gradient in the periphyton assemblages from late spring-early summer (May-June) assemblages (lower right corner, Figure 6) to late summer-early fall (August-October) assemblages (upper left corner, Figure 6). Individual ordination plots for two different sites (one upstream, QU and one downstream, KR) confirmed this temporal shift in species composition. For both sites (Figure 16 and Figure 17), samples collected within the same season were more similar to each other than to samples collected within the same year (i.e., smaller distance among spring samples as opposed to larger distances among samples collected in 2012). Total biomass tended to increase in fall (Figure 18), and the relative abundance of nitrogen-fixers (Figure 8) and nitrogen autotrophs at low organic nitrogen conditions (Figure 9) increased toward the end of the summer and early fall when sestonic species (most common at the two most upstream sites, Figure 10) and species 'very tolerant' to nutrient and organic enrichment were also abundant (Figure 11).

ANOSIM results revealed significantly different species assemblages among years ($R=0.26$, $p=0.001$). Pairwise comparisons showed that most years had statistically significant different species compositions ($p \leq 0.001$). However, there was no statistically significant difference ($p>0.05$) in species composition between years 2006 and 2010, 2007 and 2008, and between 2008 and both of 2010 and 2012. The last three years of data (2010-2012) had similar species compositions. In addition to inter-annual species differences, there were also statistically significant differences on a monthly basis ($R=0.21$, $p=0.001$). Confirming the NMDS results above, samples collected in May and June had similar periphyton assemblages ($p>0.05$), as did samples collected in late summer (August-October). Also, the similarity among monthly samples decreased as the time differences between them increased from late spring (May) to fall (October).

Some autecological metrics exhibited more or less pronounced inter-annual variation. For instance, relative biomass of N-fixers followed a unimodal pattern of increase from upstream to midstream (sites IG through HC) and then a decrease toward downstream sites (KR-TG) in most years (Figure 19). This pattern was most clear in years with complete data for all sites (2004, 2011-2012). Similar patterns were observed for nitrogen autotrophs at low organic nitrogen conditions (Figure 20). Nitrogen heterotrophs at high organic nitrogen conditions and pollution tolerant taxa decreased from the most upstream site (IG) toward the next two (IB and QU) in 2004, 2007, and 2012 (Appendix C).

Seasonally, the highest relative biomass of nitrogen-fixers was observed in July-September at downstream sites. Nitrogen-fixers exhibited an interesting upstream migration with the progression of the summer (Figure 21). 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. Sestonic species in June were confined to the two most upstream (IG and IB) and the two downstream (WE and TG) sites (Figure 22). In July and September, site QU experienced an increase in sestonic species. Very tolerant to nutrient and organic enrichment species were abundant at the upstream sites (IG-QU) from July through October (Appendix C).

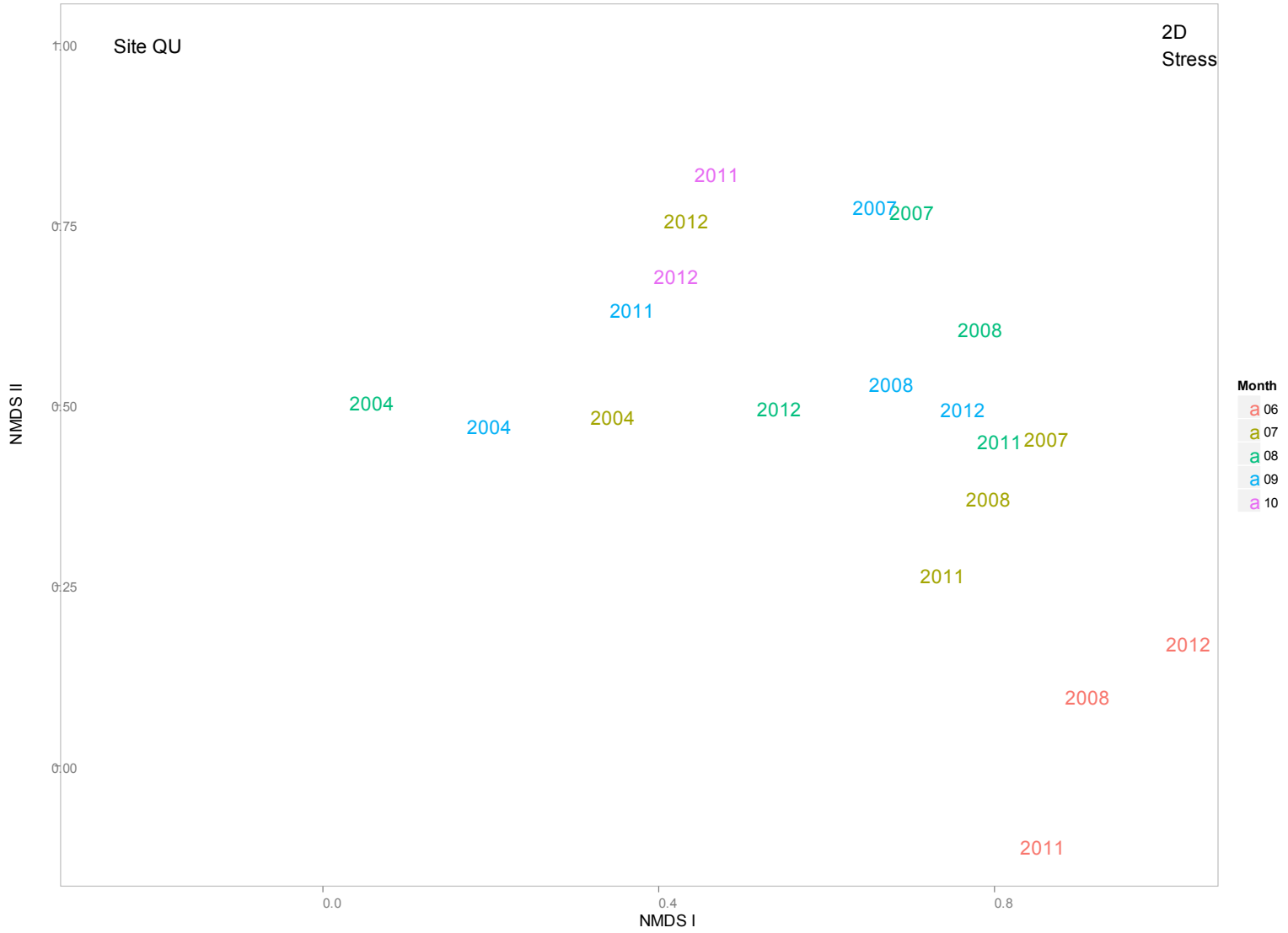

Figure 16. Non-metric Multidimensional Scaling (NMDS) plot showing the relative similarity of periphyton assemblages for each sample collected at site QU, colored by month and symbolized by year.

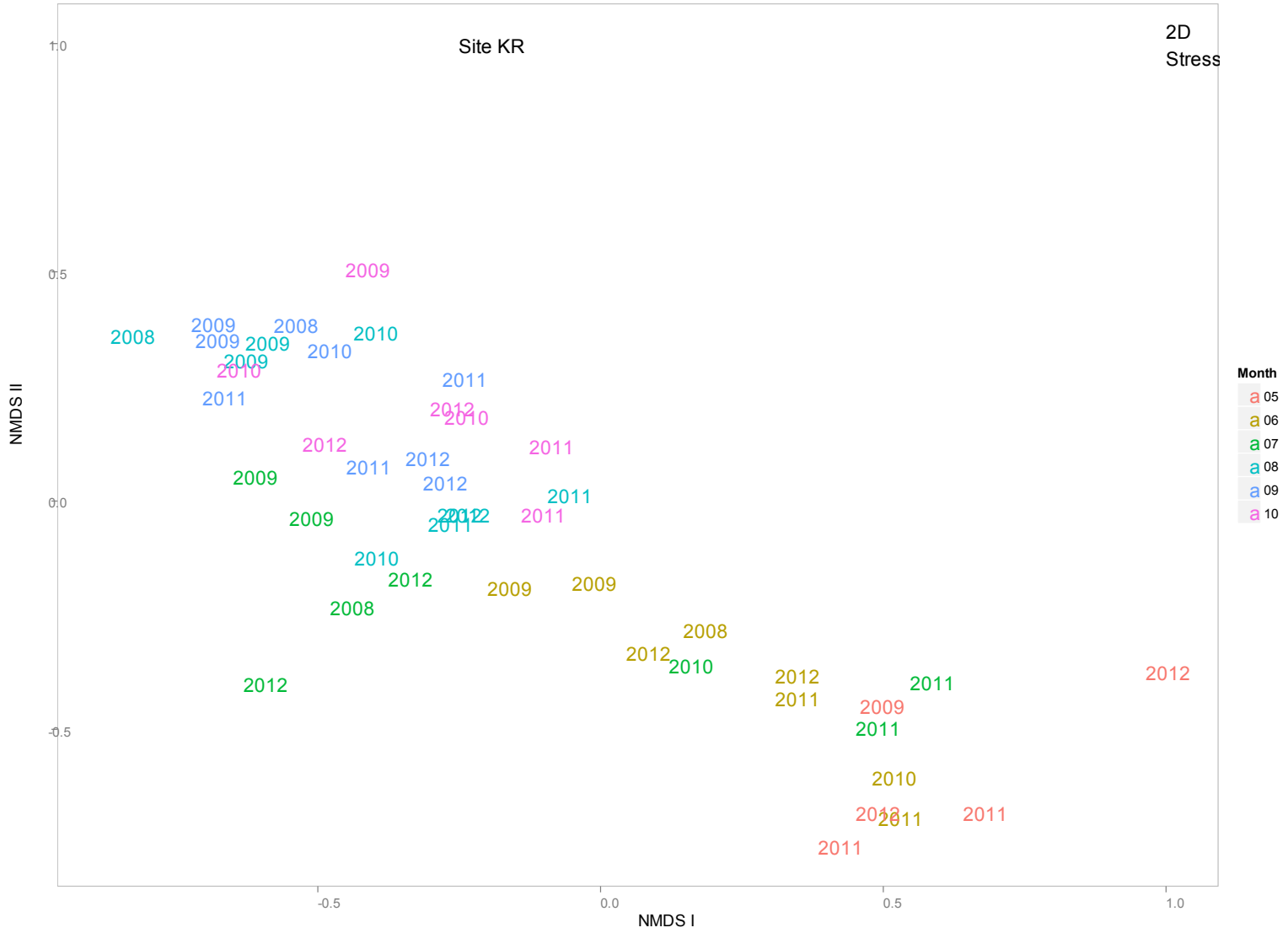

Figure 17. Non-metric Multidimensional Scaling (NMDS) plot showing the relative similarity of periphyton assemblages for each sample collected at site KR colored by month and symbolized by year.

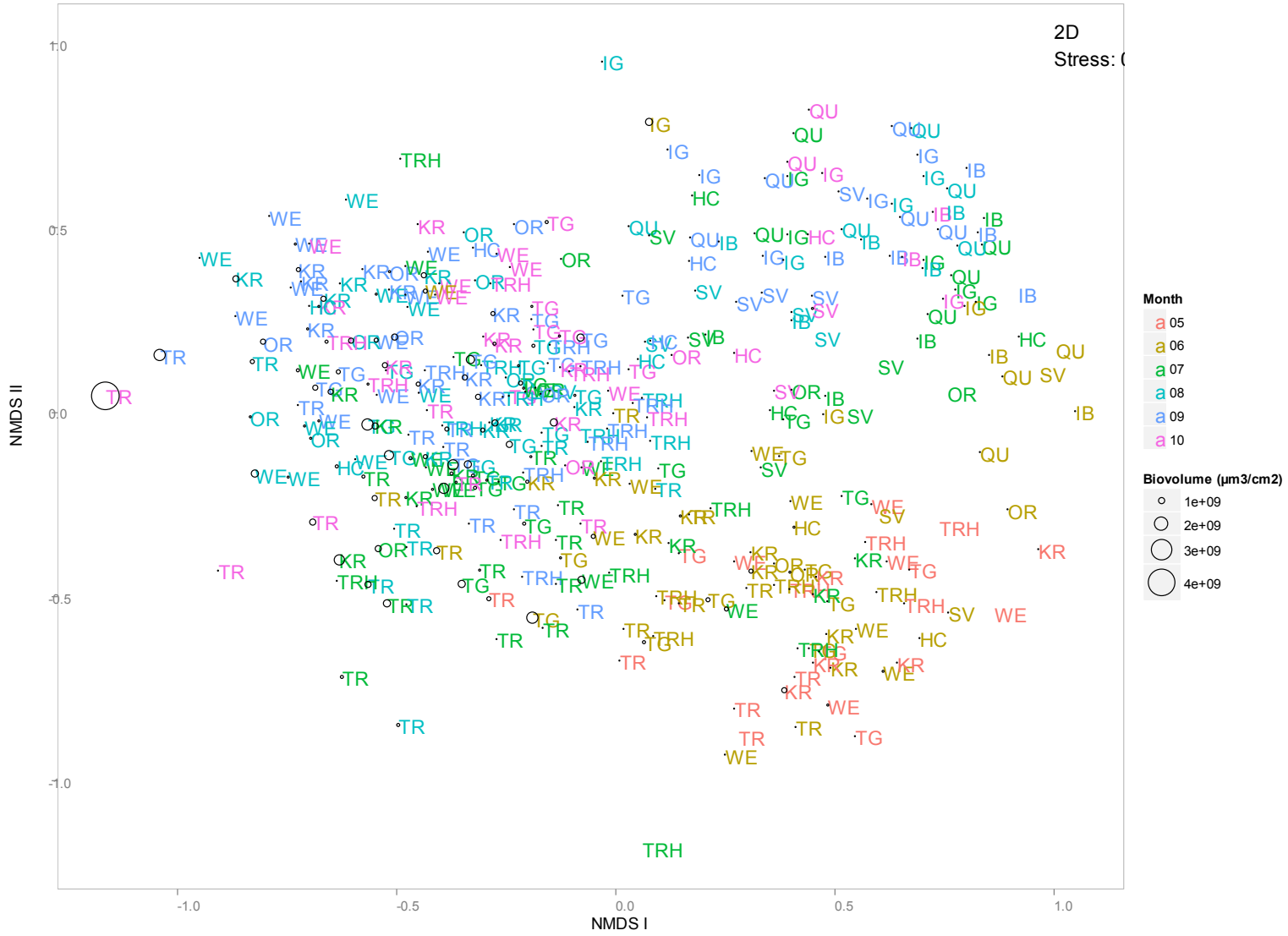

Figure 18. Total biomass overlaid on Non-metric Multidimensional Scaling (NMDS) plot showing the relative similarity of periphyton assemblages for each sample, colored by month and symbolized by year. See Table 1 for key to site codes.

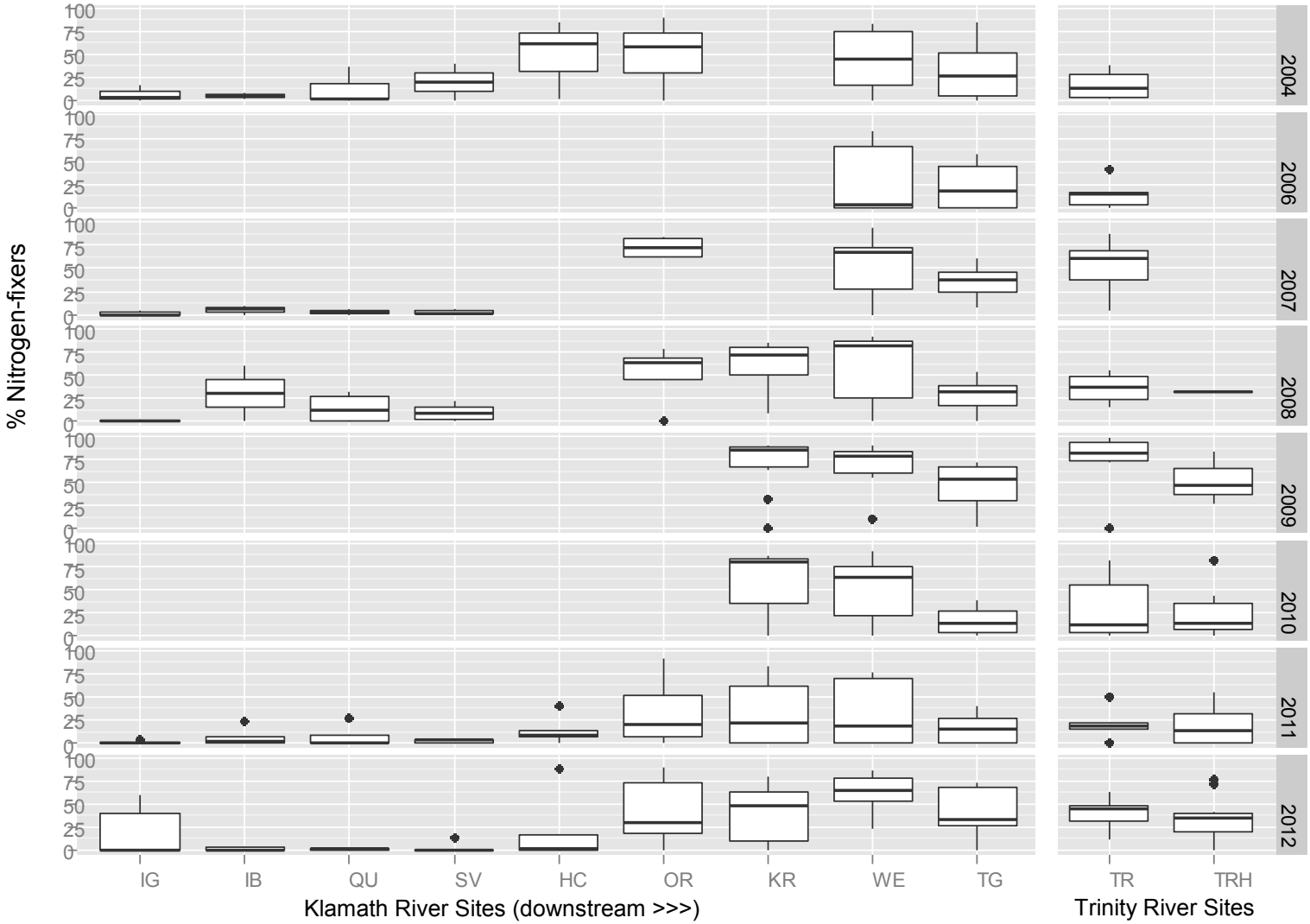

Figure 19. Boxplot of percent biomass of nitrogen fixing periphyton species, by site (columns) and year (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

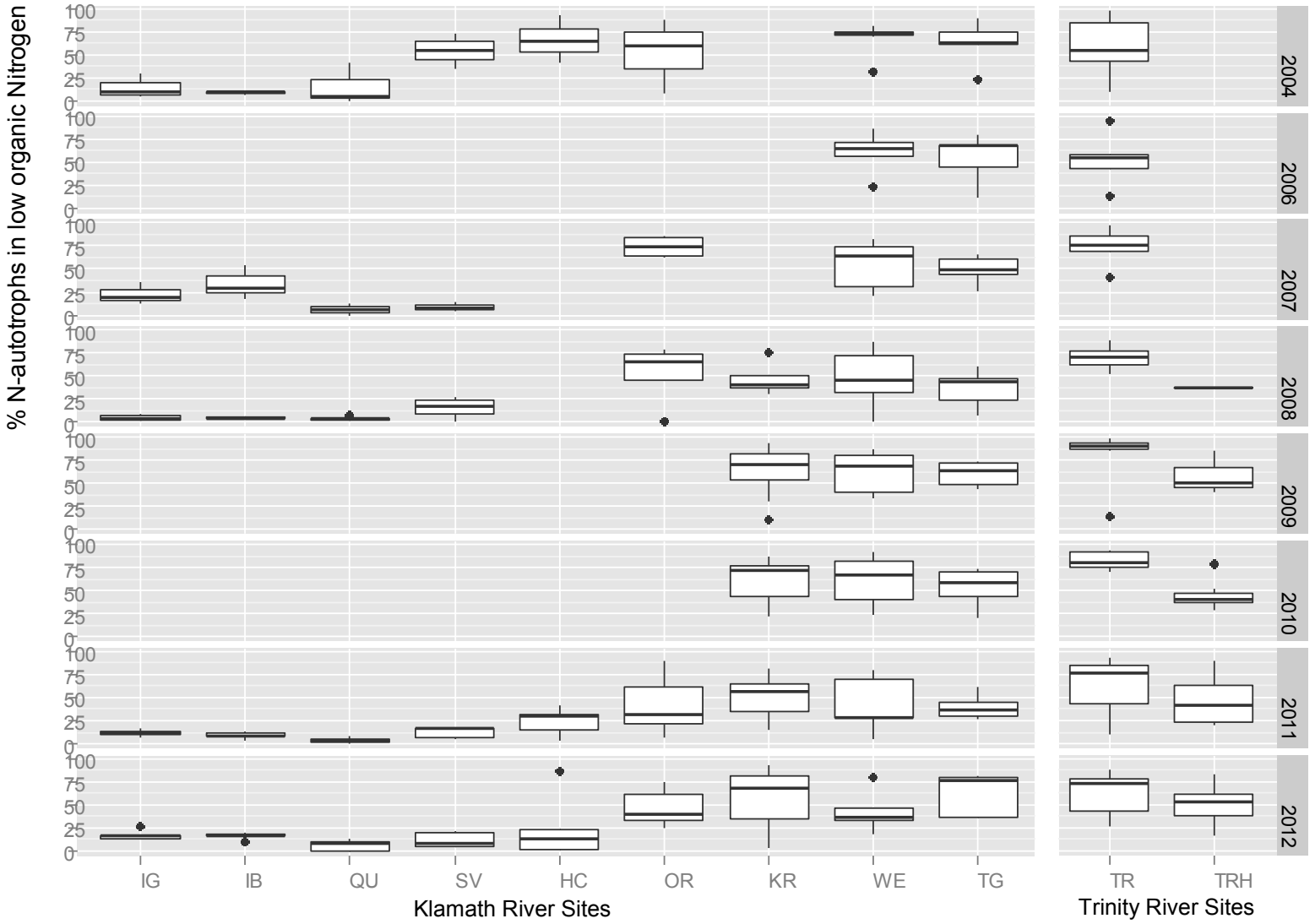

Figure 20. Boxplot of percent biomass of periphyton species that are nitrogen-autotrophs in low organic nitrogen conditions, by site (columns) and year (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

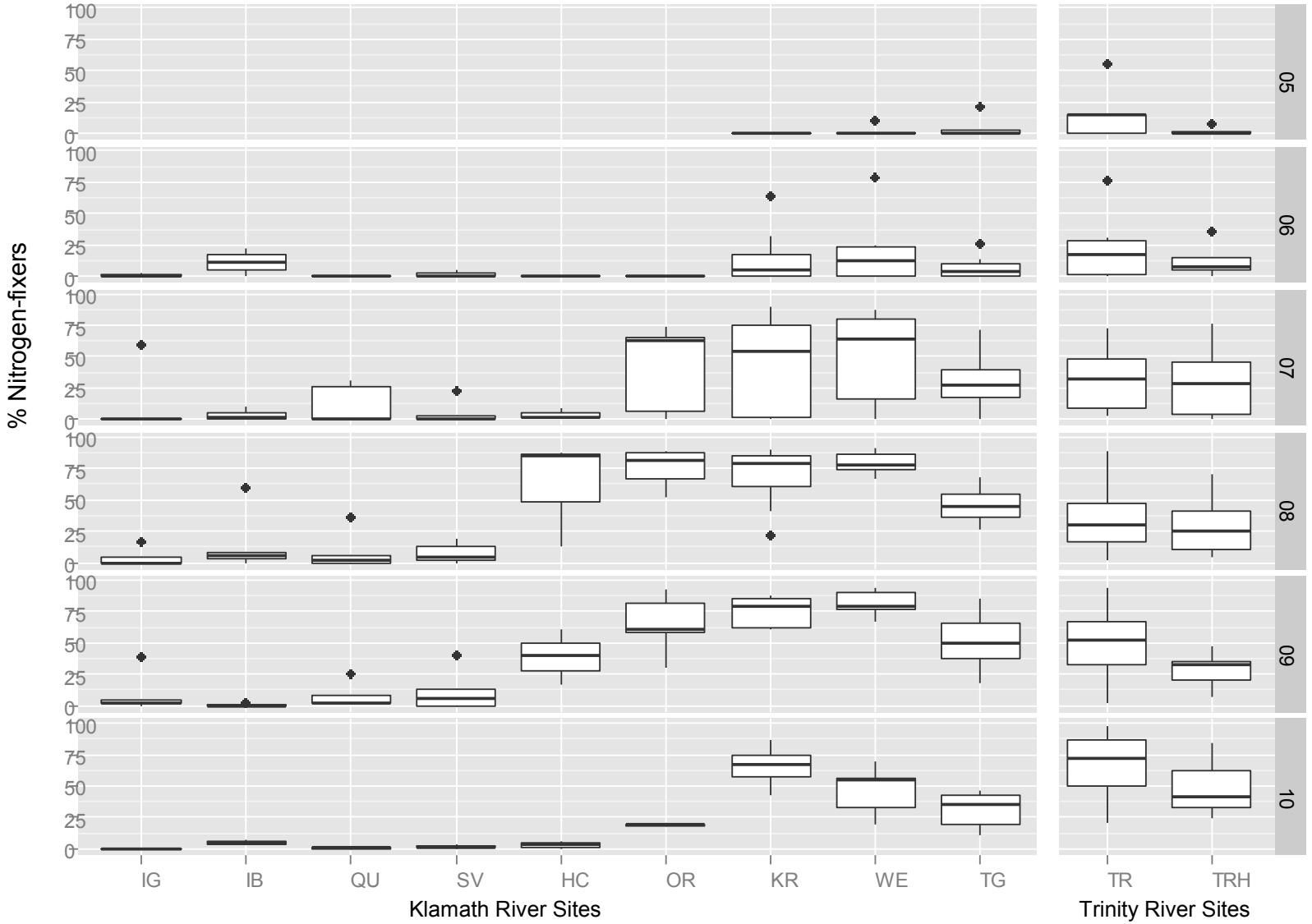

Figure 21. Boxplot of percent biomass of nitrogen fixing periphyton species, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

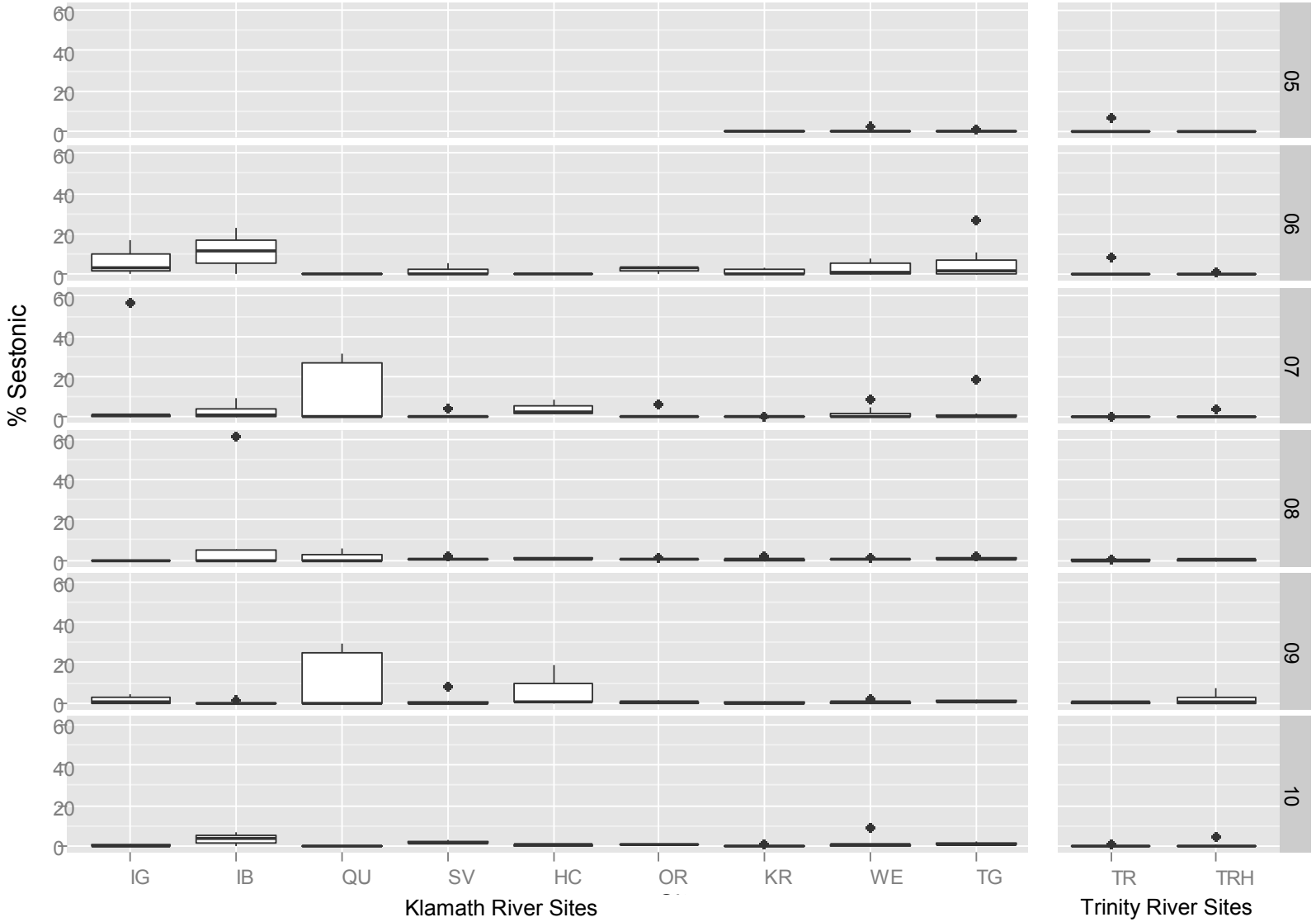

Figure 22. Boxplot of percent biomass of sestonic periphyton species, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

3.4.2 BIOMASS OF DOMINANT SPECIES, FUNCTIONAL GROUPS, AND ENTIRE ASSEMBLAGE

There were no clear inter-annual patterns in the biomass of the two most abundant periphyton groups, diatoms and cyanobacteria. Diatom biomass was higher in July-September (Figure 23), while cyanobacteria were higher in August and September at two sites (KR and WE, Figure 24). Some dominant species (*A. minutissimum, D. tenuis*, Figure 25 and Appendix B) showed highest biomass in May and June, others in July and August (*N. veneta*) or August and September (*N. cryptocephala*), but others peaked in July through October (*E. sorex*).

Total periphyton biomass was lower in May than the other months (Figure 26, right panel, but monthly patterns for June through October were highly variable by site (Figure 26, left panel). Monthly patterns in periphyton chlorophyll-*a* were more pronounced than total biomass, increasing from May to a peak in August or September before decreasing in October (Figure 27).

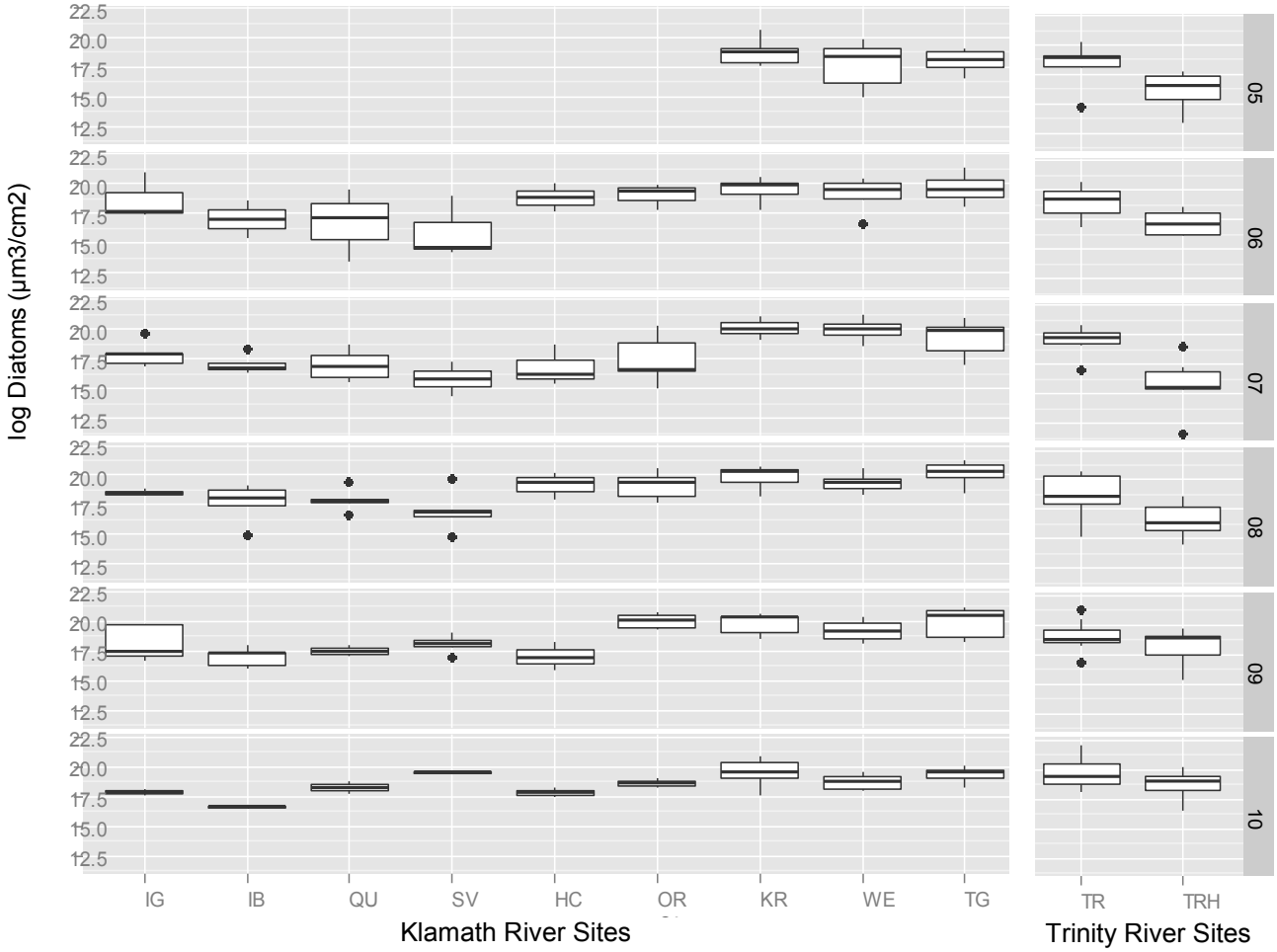

Figure 23. Boxplot of diatom biomass by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). Y-axis is log-transformed. See Table 1 for key to site codes.

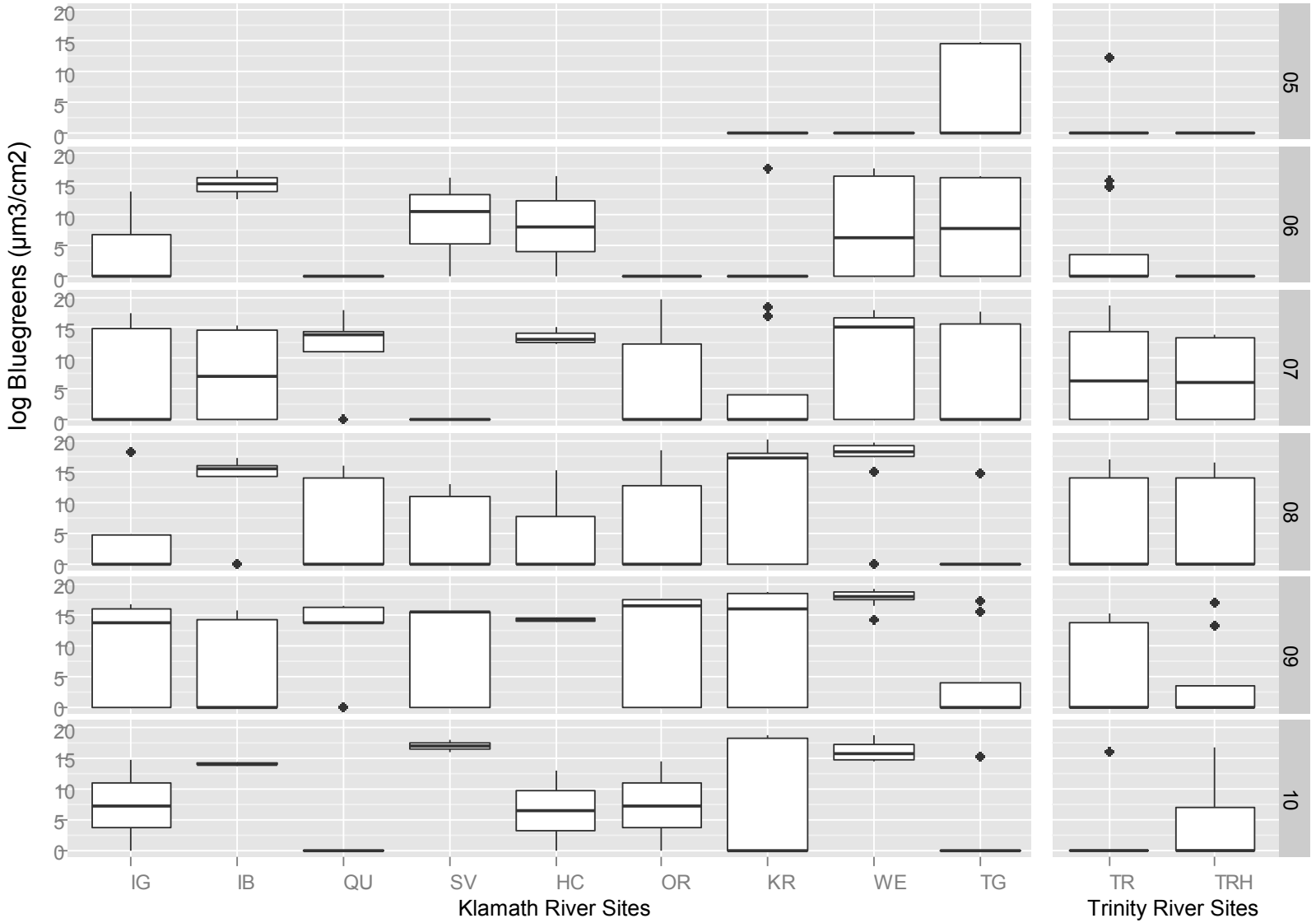

Figure 24. Boxplot of cyanobacteria biomass, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). Y-axis is log-transformed. See Table 1 for key to site codes.

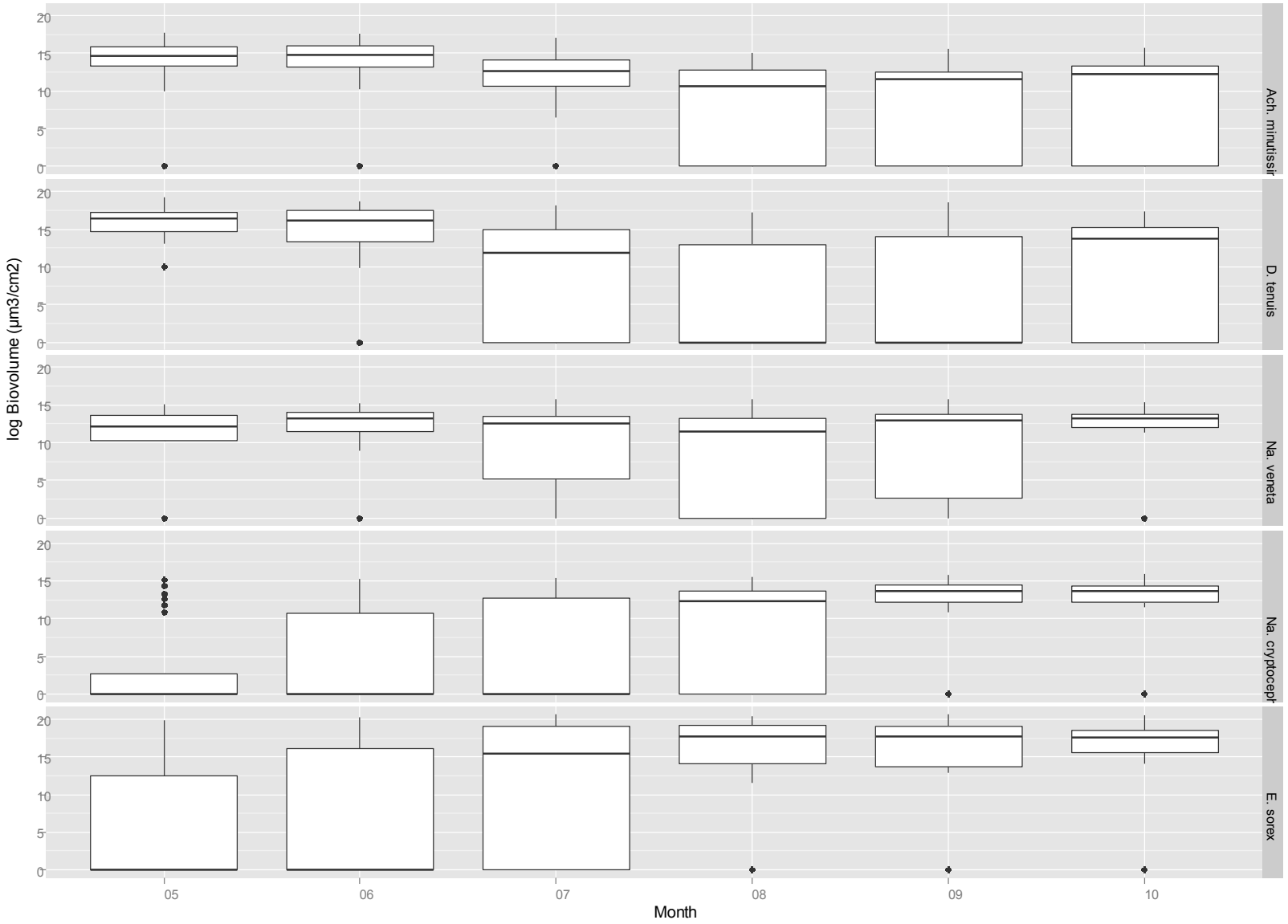

Figure 25. Boxplot of biomass of dominant periphyton species, by month (rows). Y-axis is log-transformed. See Table 1 for key to site codes.

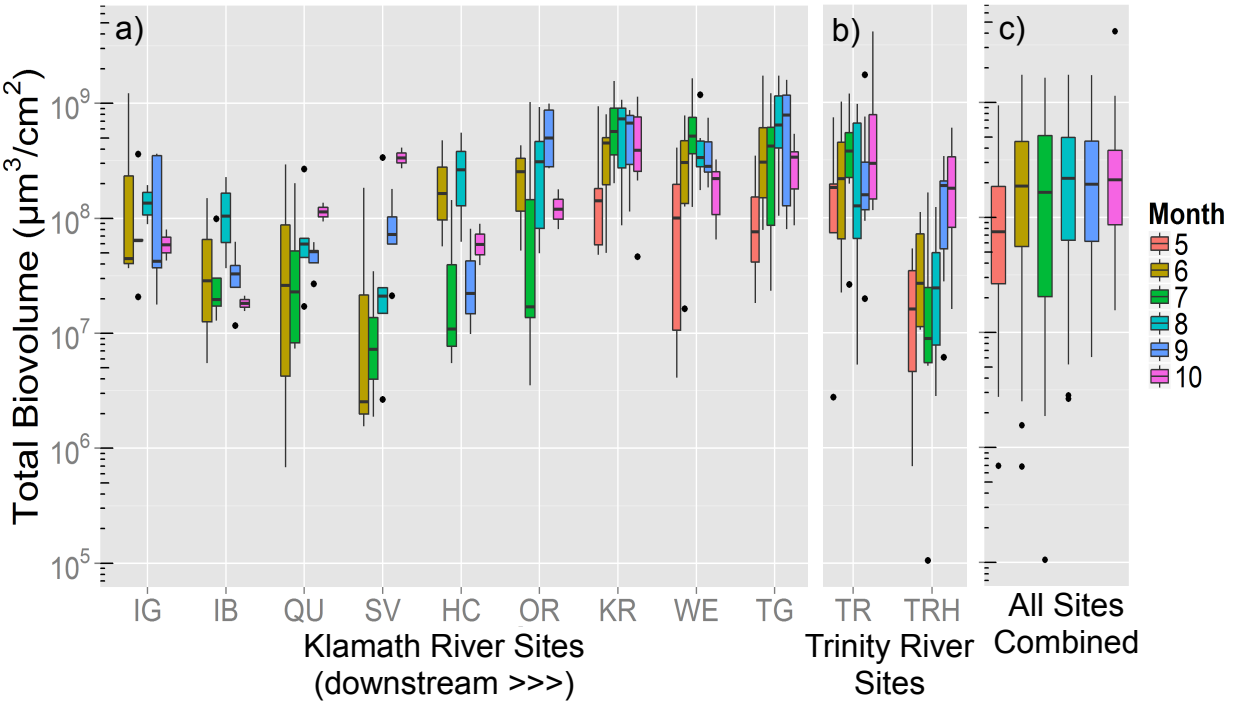

Figure 26. Boxplot of total periphyton biomass, by month, for individual sites on the (a) Klamath and (b) Trinity rivers, and (c) all sites combined. See Table 1 for key to site codes.

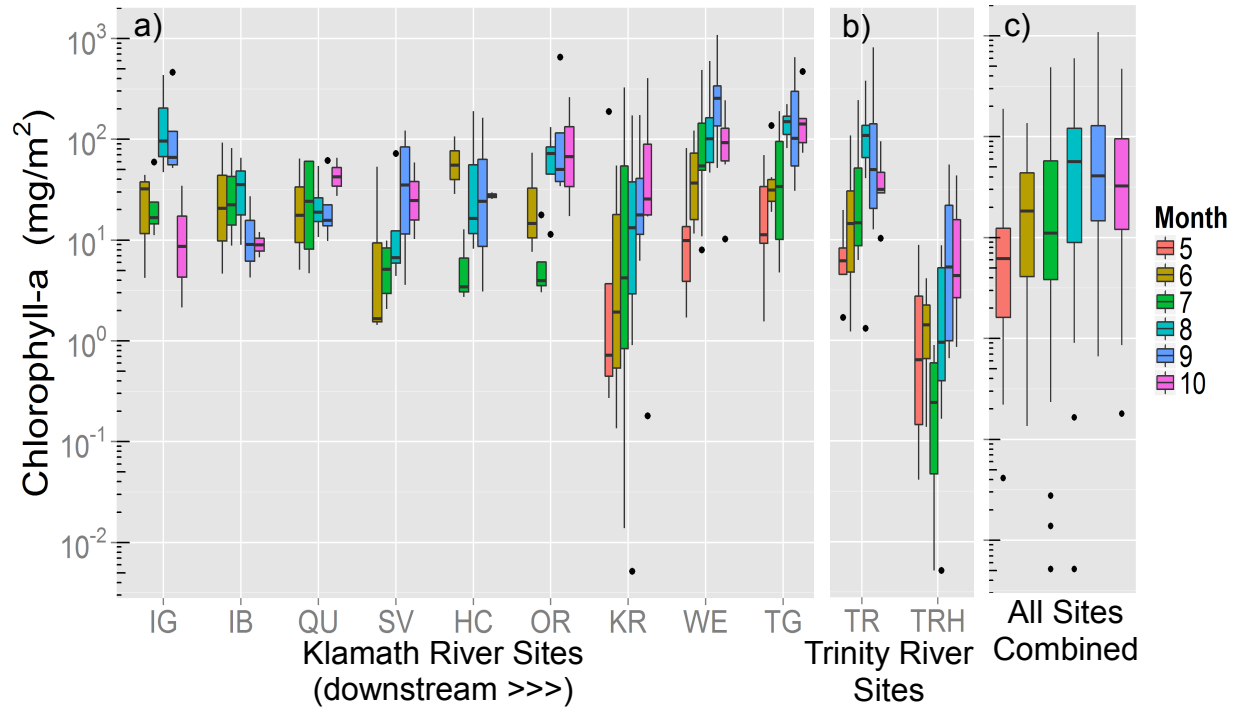

Figure 27. Boxplot of periphyton chlorophyll-*a*, by month, for individual sites on the (a) Klamath and (b) Trinity rivers, and (c) all sites combined. See Table 1 for key to site codes.

4 DISCUSSION

Periphyton assemblages in the Klamath River show a clear longitudinal pattern. Vannote et al. (1980) proposed rivers as a continuum of longitudinally changing environmental conditions and biological communities (River Continuum Concept (RCC)). According to this concept, rivers originate from mountain headwaters with heavy canopy cover and limited algal growth due to light limitation, through mid-sized river sections with higher benthic algal production because of nutrient inputs from upstream organic matter processes and lateral inputs from floodplains as well as tributaries, to large river sections with abundance of nutrients and phytoplankton. Unlike most river ecosystems, the Klamath River originates from a hyper-eutrophic lake (Upper Klamath Lake) dominated during the summer by planktonic bloom-forming cyanobacteria (e.g., *Aphanizomenon flos-aquae*) (Kann and Smith 1999, Eilers et al. 2004, Eldridge et al. 2013). The excessive nutrient loading to the lake is due to a combination of natural factors such as its large watershed with phosphorus-rich volcanic terrains and anthropogenic factors such as drainage of natural wetlands for agriculture, livestock grazing, removal of riparian vegetation, and stream bank erosion (ODEQ 2002). Not surprisingly, the Klamath River, especially the headwater and mid sections of the river, are heavily impacted by the Upper Klamath Lake and downstream hydroelectric reservoirs. Such 'lake and reservoir effects' are clearly reflected by the periphyton assemblages in the upper portion of the study reach below Iron Gate Dam extending downstream to Happy Camp. Cluster analysis based on periphyton assemblages identified three statistically different periphyton groups (ANOSIM R=0.70, p=0.001). All sampled upstream sites (IG, IB, QU, SV, most of HC) were grouped together (Group 3). The assemblages in this group were dominated by pollution tolerant taxa (e.g., *Cocconeis placentula*, TP optima 82.5 µg/L; *Navicula veneta*, TP optima 58.21 µg/L; *Rhoicosphenia abbreviata*, TP optima 62.81 µg/L; Potapova et al. 2004). Unlike the other two groups, Group 3 had the highest percentage (4.4%) of sestonic species including *Aphanizomenon flos-aquae* and *Microcystis aeruginosa,* two species of bloom-forming cyanobacteria that are prevalent directly upstream in Iron Gate Reservoir. Such sestonic species have been observed settling onto periphyton in the upper river reaches (Figure 28). Benthic macroinvertebrates also show longitudinal patterns, with higher percent composition of filter-feeders in upstream and middle reaches (where Group 3 occurred in our study) than downstream (Malakauskas and Wilzbach 2012).

Figure 28. *Microcystis aeruginosa* colonies (bright green color) entrained onto periphyton below Iron Gate Dam, September 2007.

The 'lake and reservoir effects' in the Klamath River diminish with distance downstream. In its downstream reaches, the Klamath River is characterized with typical Western streams/rivers periphyton 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). It is well documented that heterocystous cyanobacteria are capable of fixing nitrogen under oxygenated conditions (see review by Adams and Duggan 1999). These taxa possess the enzyme nitrogenase in differentiated cells called heterocysts. Heterocysts provide fixed nitrogen to adjacent vegetative cells in the filament while the vegetative cells provide carbon and reductant to heterocysts for this energy expansive process. The development of heterocysts and their abundance per filament are in response to deprived nitrogen conditions in the environment. For example, the genus *Calothrix* is commonly observed in streams and rivers with limited nitrogen concentrations (Power et al. 1988, Peterson and Grimm 1992, Grimm and Petrone 1997, Munn et al. 2002). In addition to its N-fixing capacity, *Calothrix* is also grazer-resistant in streams, which was attributed to its unique growth form (Power et al. 1988). The trichomes (i.e., filaments) in *Calothrix* taper from their base, where the terminal heterocysts are, to their tip. The cell division is commonly restricted to the trichome's base. The tapered trichomes often form tufts embedded with copious mucilage, which allows grazers to remove only the distal portions of the trichomes but leave the basal regenerated portions intact.

Diatoms from the family Epithemiaceae (e. g., *Epithemia* and *Rhopalodia*) contain N-fixing unicellular cyanobacterial endosymbionts, which allow them to live in N-poor environments (Peterson and Grimm 1992, Stancheva et al. 2013). Eukaryotes such as diatoms are not capable of fixing nitrogen, but some taxa have gained such capacity by hosting N-fixing cyanobacteria as endosymbionts. The cyanobacterium-like structure in *Rhopalodia* is referred to as 'spheroid bodies' (SBs). Research indicates that the SBs possess nitrogen capacity via nitrogen fixation assays and genomic DNA analysis of the SBs (Prechtl et al. 2004). Despite the fact that most research has focused on *Rhopalodia gibba,* a recent study suggested that spheroid bodies in diatoms from the family Epithemiaceae, including *Epithemia sorex* and *E. turgida,* were derived from a single endosymbiotic cyanobacterium (Nakayama et al. 2011). The SBs are genomically close to *Cyanothece*, the closest relative of the SB ancestor. Prechtl et al. (2004) reported that the SBs cannot survive outside the host cells, suggesting that the SBs may be in the process of becoming a permanent N-fixing organelle in eukaryotes. Nitrogen has been reported as the limiting nutrient in streams in Northern California and the Pacific Northwest (Hill and Knight 1988, Omernick 1977), and N-fixing periphyton can be important components of river food webs (Power et al. 2009, 2013).

Our study found that N-fixers, which dominated Group 1, were constrained to the downstream reaches of the river. Similar to results from a review paper evaluating 22 streams (Marcarelli et al. 2008; N-fixation was highest between June-August) the Klamath River N-fixers also occurred primarily in the summer (July-September). Marcarelli and Wurtsbaugh (2006) also experimentally demonstrated in a subalpine oligotrophic Idaho stream that N-fixing rates of periphyton assemblages were significantly higher in treatments with warmer temperature and enriched P. The relative abundance of N-fixers (Porter et al. 2008, Stancheva et al. 2013) and endosymbiont biomass (Stancheva et al. 2013), as well as the number of endosymbionts within a diatom cell (DeYoe et al. 1992) increased with decreasing nitrogen concentrations. Short-term *in situ* nutrient enrichment experiments in a Wyoming stream indicated that PO_4^{3-} addition

stimulated N-fixation in epilithic assemblages and in contrast, NO_3^- enrichment inhibited N-fixation and the inhibitory effects on N-fixation by NO_3^- enrichment was stronger than the stimulatory effects of phosphorus enrichment (Kunza and Hall 2013). These patterns in N-fixer abundance relative to nitrogen availability are consistent with observed nitrogen concentrations in downstream reaches of the Klamath River, which are far lower than those upstream near Iron Gate Dam (Asarian et al. 2010). Nitrate concentrations in the lower reaches of the Klamath River often drop below 0.05 mg/L during July through September (Asarian et al. 2010), indicating that decreased nitrogen availability may explain the seasonal and longitudinal patterns of N-fixer dominance of the periphyton community. Nitrogen budgets for the Klamath River showed that the most downstream reach (i.e., from WE to TG) was the only reach with net-negative retention of nitrogen for June through October, indicating that N-fixation is likely occurring (Asarian et al. 2010).

The longitudinal pattern of benthic algal productivity predicted by the RCC (Vannote et al. 1980) has been observed in rivers (Cushing et al. 1983) but such patterns have not been well defined in periphyton assemblages. It has been documented that periphyton assemblages respond to water quality gradients across a region (Stevenson et al. 2008; Potapova and Charles 2002, 2007). Pan et al. (2012) sampled 20 sites along each of seven large rivers in Oregon and Washington, and found that longitudinal patterns in benthic diatom assemblages, as indicated by standardized Mantel r of association between benthic diatom assemblage similarity and spatial river distance among sites, were only evident in one of the rivers (r=0.69). In that river, the longitudinal shift in benthic diatom assemblages was strongly associated with a phosphorus gradient (Pan et al. 2012). The longitudinal patterns in both nutrients and periphyton assemblages observed in the Klamath River in this study corroborate previous literature that periphyton assemblages respond to water quality gradients. These relationships will be tested further in the second phase of this study which will utilize a variety of multivariate statistical techniques.

Overall periphyton biomass increased from upstream to downstream in the Klamath River (e.g., Figure 11), despite the decreasing upstream to downstream nutrient concentrations. Some of the downstream biomass increase is due to the ability of the N-fixers to overcome nitrogen limitation (Group 1 had greater biomass than Group 3); however, by design, the sampling protocol used in this study does not capture all primary producers in the river ecosystem. The protocol targets diatoms and samples only cobbles, so algae growing on other substrates such as macrophytes (Figure 29a), gravel, and boulders are not included. The protocol is not designed to adequately characterize filamentous algae (e.g., *Cladophora* sp.) and does not include macrophytes (e.g., *Potamogeton* sp. [Figure 29], *Elodea* sp.). *Cladophora* sp. (Figure 30) can grow in dense mats but these patches are easily missed by the sampling protocol that targets epilithic diatoms. Quantitative data are lacking, but qualitative observations indicate that macrophytes dominate the algal/plant community of the Klamath River between Iron Gate Dam (Figure 29) and the Scott River confluence, whereas downstream the composition shifts towards periphyton although macrophytes do still occur in quiescent areas such as backwaters and channel margins (PacifiCorp 2005). Malakauskas and Wilzbach (2012) also noted macrophytes lining the riverbanks from Iron Gate Dam to Independence Creek (river mile 94). Macrophyte dominance in this upper reach is attributed to channel substrate stability (PacifiCorp 2005) which is due to a combination of natural (i.e., historical lack of gravel) and human-caused factors (i.e., dams and reservoirs reducing hydrologic variability, interrupting sediment transport, and resulting in streambed armoring) (USDOI and CDFG 2012). The lack of information regarding the

distribution and ecological significance (e.g., effects on water quality, food webs, and fish habitat) of aquatic macrophytes and filamentous algae is a notable gap in scientific understanding of the Klamath River.

Figure 29. Dense mats of macrophyte *Potamogeton pectinatus* in the Klamath River: (a) at Brown Bear river access, approximately 9 miles downstream of site QU (Quigley's), August 21, 2013, (b) several miles downstream of site IG (Iron Gate Dam), August 29, 2012.

Figure 30. Filamentous algae, likely *Cladophora* sp., growing on cobble near margin of the Klamath River between Happy Camp and Orleans, August 30, 2012.

Gross primary production (GPP) and net ecosystem production (NEP), which have been calculated based on continuous water quality sensors and are both dominated by the benthic community, decrease with distance downstream in the Klamath River (Genzoli 2013). The contrast of the decreasing longitudinal trends in GPP and NEP (Genzoli 2013) and the increasing longitudinal trend in periphyton biomass found in this study confirm that the periphyton sampling protocol is not representative of reach-averaged conditions. In addition to issues with substrate, macrophytes, and filamentous algae discussed in the previous paragraph, another potential contributor is that water depth increases with distance downstream in the Klamath River (Asarian et al. 2010), and therefore it is likely that the depth (1 to 2 feet) and velocity (1 to 2 feet per second) conditions targeted in the periphyton sampling protocol may represent a smaller fraction of the river's cross sectional width at downstream sites than at upstream sites.

In addition to the effect of longitudinal gradient on the structure of periphyton assemblages in the Klamath River, they were also influenced by a seasonal change. Spring assemblages had more diatoms, fewer cyanobacteria, and a lack of N-fixers compared to late summer-fall assemblages, which saw a reduction in diatoms and an increase in cyanobacteria (including N-fixer *Calothrix* sp.) and N-fixers, especially diatoms with cyanobacterial endosymbionts (*E. sorex*). Our results are similar to an experimental study in an N-limited desert stream (Peterson and Grimm 1992), which found that *E. sorex* was an early successional species of nutrient-poor substrates, while *Calothrix* sp. developed later in the periphyton succession. This shift from diatoms to cyanobacteria was attributed to light changes within the periphyton assemblage (i.e., shading) and temporal increases in temperature. Indeed, Klamath River spring species were also indicative of high oxygen concentrations associated with low water temperature. A national survey found that diatom species indicative of high oxygen concentrations were negatively correlated with nitrogen concentrations (Porter et al. 2008). Temperature has a positive effect on N-fixation rates (Marcarelli and Wurtsbaugh 2006, 2007), which is supported by the fact that the highest relative abundances of N-fixers in our study were recorded in 2009 (Figure 19), an unusually warm year in the study area (Asarian and Kann 2013) and during which Upper Klamath Lake experienced peaks in algal biomass, microcystin concentrations, and nutrients (Eldridge et al. 2012).

Group 2 represented spring (May and June) samples dominated by diatom species somewhat tolerant to nutrient and organic enrichment. Diatoms are often the most diverse and abundant algal group in streams (Biggs 1996, Stevenson et al. 2010). The most abundant species in this group have cosmopolitan distribution without specific environmental preferences (Krammer and Lange-Bertalot 1986, 1991a, b). Diatoms dominate river ecosystems throughout the year due to their preferences for low nutrient (Borchardt 1996) and low light conditions (Hill 1996). Similar to our study, over 90% of algal biomass and diversity in streams can be comprised by diatoms (EPA EMAP, unpublished data). Their ease of sampling, short life cycles, and sensitivity to pollution make them good indicators of environmental conditions (Stevenson et al. 2010). Therefore, diatoms are widely used in regional and national bioassessment programs (EPA EMAP, EPA REMAP, USGS NAQWA).

In this study, periphyton metrics, except for % of N-fixers, did not reveal as clear spatial and temporal patterns as other studies. One possible explanation might be the fact that the 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. As a result of

these limitations, we still do not have autecological information for each taxon in the KR. Recently, several researchers used a weighted-averaging model to develop regional or system-specific algal metrics (Stevenson et al. 2008, Potapova and Charles 2002, 2007). For example, Munn et al. (2002) sampled benthic algae along an agricultural gradient in the Central Columbia Plateau, Washington, to develop benthic algal metrics for conductivity, phosphorus, and inorganic nitrogen. This approach is appropriate for well-defined environmental gradients and the KR represents an ideal system with a strong longitudinal nutrient gradient. Detailed nutrient and water quality information are available for the Klamath River (Asarian and Kann 2013, Watercourse Engineering 2013), and thus algal nutrient metrics (i.e., optima and tolerance) for common taxa in KR could be developed by using weighted-averaging methods.

This report describes spatial-temporal dynamics of the Klamath River periphyton assemblage for the years 2004 through 2012. Data regarding various environmental factors such as nutrients, hydrology, water quality, and climate are available for this same time period. In a follow-up analysis, we plan to use multivariate statistical analysis to determine the linkages between these environmental controlling factors and the resulting periphyton assemblages.

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6 ACKNOWLEDGMENTS

The report was prepared for the Klamath Basin Tribal Water Quality Work Group (Work Group) using funds awarded to the Work Group by the U.S. EPA Region 9 and administered by the Yurok Tribe. We thank all the entities who contributed data to this report, particularly the Yurok Tribe, Hoopa Valley Tribe, Karuk Tribe, Watercourse Engineering, PacifiCorp, and the North Coast Regional Water Quality Control Board. We also appreciate Mary Power, Rex Lowe, and Paula Furey for spreading their inspiration and enthusiasm for the study of periphyton.

APPENDIX A: PERIPHYTON SPECIES LIST AND TABLE OF AUTECOLOGICAL ATTRIBUTES

Table A1. Frequency and autecological attributes for species detected in 2004-2012 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.

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes													
				LT sites (n=332)	All sites (n=505)	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	4	8	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		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	85	119	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	37	67	2	2	3							3				1
Achnanthes minutissima	ACMN	diatom	Achnanthidium minutissimum (Kützing) Czarnecki	256	392	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	19	46	2	2	4	2	2	2	4	2	2	2	5			1
Amphora coffeiformes	AFCF	diatom	Amphora coffeaeformis (Agardh) Kützing	1	2	2	2	4	2	2	3	3	5	3	1				1
Amphora ovalis	AFOV	diatom	Amphora ovalis (Kützing) Kützing	12	20	2	2	4	2	2	2	2	5	1	3	4			1
Amphora perpusilla	AFPR	diatom	Amphora pediculus (Kützing) Grunow	110	184	2	2	4	2	2	2	2	5	3	3	4			1
Anabaena flos-aquae	ABFA	bluegreen	Anabaena flos-aquae (Linnaeus) Brébisson	2	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	26	50	2	2											1	2
Anomoeoneis vitrea	AOVT	diatom	Brachysira vitrea (Grunow) Ross		1	2	2	5	2	1	2	1	2	2	2				1
Aphanizomenon flos-aquae	APFA	bluegreen	Aphanizomenon flos-aquae (Linnaeus) Ralfs	34	48	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	17	37	1	2												1

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes												
				LT sites (n=332)	All sites (n=505)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Calothrix sp.	KXXX	bluegreen	Calothrix sp.	52	87	2	1												1
Chlamydomonas sp.	CHXX	green	Chlamydomonas sp.	3	8	1	2											1	2
Cladophora sp.	CFXX	green	Cladophora sp.	20	34	2	2										2	1	1
Cladophora sp.	CFX9	green	Cladophora sp.			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	11	11	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	295	455	2	2	4	2	2	3	2	5	2	3	4			1
Cosmarium sp.	CSXX	green	Cosmarium sp.		1	2	2												
Crucigenia quadrata	CGQD	green	Crucigenia quadrata Morren		1	2	2											1	2
Cryptomonas erosa	CXER	cryptophyte	Cryptomonas erosa Ehrenberg	5	7	1	2											1	2
Cyclotella meneghiniana	CCMG	diatom	Cyclotella meneghiniana Kützing	20	39	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	2	3	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	223	334	2	2	4	2	1	1	2	5	2	3	5			1
Cymbella cesatii	CMCS	diatom	Encyonopsis cesatii (Rabenhorst) Krammer		3	2	2	3	1	1	1	1	1	3	3				1
Cymbella cistula	CMCL	diatom	Cymbella cistula (Ehrenberg) Kirchner		1	2	2	4	2	1	2	2	5	1	3	5			1
Cymbella mexicana	CMMX	diatom	Cymbella mexicana (Ehrenberg) Cleve	6	11	1	2								3				1
Cymbella microcephala	CMMC	diatom	Encyonopsis microcephala (Grunow) Krammer	1	3	2	2	4	2	1	1	1	4	3	2				1
Cymbella minuta	CMMN	diatom	Encyonema minutum (Hilse) Mann	99	166	2	2	3	2						2				1
Cymbella sinuata	CMSN	diatom	Reimeria sinuata (Gregory) Kociolek et Stoermer	156	243	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	3	11	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	188	283	2	2	4	3	2	3	3	5	1	2				1
Diatoma tenue elongatum	DTTE	diatom	Diatoma tenuis Agardh		3	2	2	4	3	2	3	3	5	1	2				1
Diatoma vulgare	DTVL	diatom	Diatoma vulgaris Bory	157	243	2	2	5	2	2	2	2	4	1	3	4			1
Didymosphenia geminata	DDGM	diatom	Didymosphenia geminata (Lyngbye) Schmidt		1	2	2	6							3				1

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes												
				LT sites (n=332)	All sites (n=505)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft Benthic/Sestonic
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	222	346	2	1	5	2	1	2	2	5	2	3			1
Epithemia turgida	EPTR	diatom	Epithemia turgida (Ehrenberg) Kützing	36	56	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.		1	2	2											1
Fragilaria capucina mesolepta	FRCM	diatom	Fragilaria capucina var. mesolepta Rabenhorst	7	27	2	2	4	2						2			1
Fragilaria construens	FRCN	diatom	Staurosira construens (Ehrenberg) Williams et Round	43	68	2	2	4	2	1	1	2	4	1	3	5		1
Fragilaria construens venter	FRCV	diatom	Staurosira construens var. venter (Ehrenberg) Hamilton	81	127	2	2	4	2	2	1	2	4	1	3			1
Fragilaria crotonensis	FRCR	diatom	Fragilaria crotonensis Kitton	3	8	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	4	15	2	2	4	2	2	1	2	7	3	3			1
Fragilaria vaucheria	FRVA	diatom	Fragilaria vaucheriae (Kützing) Petersen	76	91	2	2	4	2	2	3	3	5	3	2	2		1
Fragilaria vaucheriae	FRVA	diatom	Fragilaria vaucheriae (Kützing) Petersen		17	2	2	4	2	2	3	3	5	3	2	2		1
Frustulia rhomboides	FSRH	diatom	Frustulia rhomboides (Ehrenberg) De Toni	1	1	1	2	2	1	1	1	1	1	2	3			1
Glenodinium sp.	GDXX	dinoflagellate	Glenodinium sp.	1	1	1	2											2
Gloeocystis sp.	GLXX	green	Gloeocystis sp.	1	1	2	2											1
Gomphoneis herculeana	GSHR	diatom	Gomphoneis herculeana (Ehrenberg) Cleve	145	207	2	2								3			1
Gomphonema acuminatum	GFAC	diatom	Gomphonema acuminatum Ehrenberg		2	2	2	4	2	1	2	2	5	2				1
Gomphonema angustatum	GFAN	diatom	Gomphonema angustatum (Kützing) Rabenhorst	215	334	2	2	4	2	1	1	1	1		2	5		1
Gomphonema clevei	GFCL	diatom	Gomphonema clevei Fricke	26	36	2	2								3			1
Gomphonema gracile	GFGC	diatom	Gomphonema gracile Ehrenberg emend Van Heurck		1	2	2	3	2	1	1	1	3	3	2			1
Gomphonema olivaceum	GFOM	diatom	Gomphoneis olivaceum (Hornemann) Dawson ex Ross and Sims	66	89	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	187	288	2	2	3	2	1	1	2	2	3	2			1
Gomphonema tenellum	GFTN	diatom	Gomphonema minutum (Agardh) Agardh	33	55	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

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes												
				LT sites (n=332)	All sites (n=505)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Gomphonema ventricosum	GFVT	diatom	Gomphonema ventricosum Gregory	107	156	2	2		1	1	1	1	1						1
Gyrosigma spencerii	GYSP	diatom	Gyrosigma spencerii (Smith) Griffith et Henfrey	12	18	1	2	4							2				1
Hannaea arcus	HNAR	diatom	Hannaea arcus (Ehrenberg) Patrick	17	22	1	2	6							3				1
Lyngbya sp.	LNXX	bluegreen	Lyngbya sp.	6	7	2	2												1
Melosira ambigua	MLAM	diatom	Aulacoseira ambigua (Grunow) Simonsen	1	1	2	2	4	2	2	3	2	5	1	3				2
Melosira granulata	MLGR	diatom	Aulacoseira granulata (Ehrenberg) Simonsen	21	26	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	42	72	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	8	21	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		1	1	2	4	2	2	3	3	4	3	2	3			1
Navicula cascadensis	NVCS	diatom	Navicula cascadensis Sovereign	2	5														
Navicula cryptocephala	NVCR	diatom	Navicula cryptocephala Kützing	188	295	2	2	4	2	2	3	3	7	2	3				1
Navicula cryptocephala veneta	NVCV	diatom	Navicula veneta Kützing	243	379	1	2	4	3	2	4	4	5	3	1	1			1
Navicula decussis	NVDC	diatom	Geissleria decussis (Hustedt) Lange-Bertalot et Metzeltin	15	32	2	2	4	2	1		1	4	3	3				1
Navicula graciloides	NVGC	diatom	Navicula cari Ehrenberg	30	44	1	2		2				7		2				1
Navicula gregaria	NVGR	diatom	Navicula gregaria Donkin	18	25	1	2	4	3	2	4	3	5	3	2	2			1
Navicula menisculus upsaliensis	NVMU	diatom	Navicula upsaliensis (Grunow) Peragallo	5	5	1	2	4	2			2			2				1
Navicula minima	NVMN	diatom	Eolimna minima (Grunow) Lange-Bertalot et Schiller	3	6	2	2	4	2	3	4	4	5	3	1	1			1
Navicula minuscula	NVML	diatom	Adlafia minuscula (Grunow) Lange-Bertalot	15	24	1	2	4	1			2	1	4	1				1
Navicula mournei	NVMO	diatom	Navicula mournei Patrick		1														
Navicula pupula	NVPP	diatom	Sellaphora pupula (Kützing) Meresckowsky	13	19	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		1	2	2	4	2	2	4	2	7	2	3	5			1
Navicula sp.	NVXX	diatom	Navicula sp.	40	55	1	2												1
Navicula tripunctata	NVTP	diatom	Navicula tripunctata (Müller) Bory	134	187	2	2	4	2	2	2	2	5	3	3	4			1

Species Name Assigned by Lab	Spec. Code	Group	Current Species Name	Freq.		Autecological Attributes												
				LT sites (n=332)	All sites (n=505)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Navicula viridula	NVVR	diatom	Navicula viridula (Kützing) Ehrenberg	29	46	2	2	4	2	2	2	3	5	1	2				1
Neidium affine	NDAF	diatom	Neidium affine (Ehrenberg) Pfitzer		3	2	2	3	2	1	1	1	4	1					1
Neidium sp.	NDXX	diatom	Neidium sp.		1	1	2												1
Nitzschia acicularis	NZAC	diatom	Nitzschia acicularis (Kützing) Smith	14	32	1	2	4	2	4	4	3	5	1	2	3			2
Nitzschia amphibia	NZAM	diatom	Nitzschia amphibia Grunow	75	124	2	2	4	2	3	3	3	5	3	2	2			1
Nitzschia capitellata	NZCP	diatom	Nitzschia capitellata Hustedt	35	68	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
Nitzschia communis	NZCM	diatom	Nitzschia communis Rabenhorst	110	156	2	2	4	2	4	3	4	5	4	1	1			1
Nitzschia dissipata	NZDS	diatom	Nitzschia dissipata (Kützing) Grunow	159	227	1	2	4	2	2	2	2	4	3	3	4			1
Nitzschia fonticola	NZFT	diatom	Nitzschia fonticola Grunow	2	3	2	2	4	2	2	2	2	4	1	3				1
Nitzschia frustulum	NZFR	diatom	Nitzschia frustulum (Kützing) Grunow	313	462	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	50	60														
Nitzschia linearis	NZLN	diatom	Nitzschia linearis (Agardh) Smith	28	36	2	2	4	2	2	2	2	4	3	2	5			1
Nitzschia microcephala	NZMC	diatom	Nitzschia microcephala Grunow	20	32	2	2	4	2	4	3	3	5	1	1	3			1
Nitzschia palea	NZPL	diatom	Nitzschia palea (Kützing) Smith	81	130	2	2	3	2	4	4	5	6	3	1	1			1
Nitzschia paleacea	NZPC	diatom	Nitzschia paleacea Grunow ex Van Heurck	168	256	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.	27	42	1	2												1
Nitzschia volcanica	NZVL	diatom	Nitzschia volcanica Sovereign	16	19														
No Algae Present	ZZZZ		No Algae Present		1														
Oscillatoria limosa	OSLS	bluegreen	Oscillatoria limosa (Dillwyn) Agardh		2	1	2												1
Oscillatoria sp.	OSXX	bluegreen	Oscillatoria sp.	70	104	1	2												1
Pediastrum boryanum	PSBR	green	Pediastrum boryanum (Turpin) Meneghini	3	3	2	2											1	2
Pediastrum tetras	PSTT	green	Pediastrum tetras (Ehrenberg) Ralfs	1	1	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	7	14														
Rhoicosphenia curvata	RHCU	diatom	Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	256	379	2	2	4	2	2	2	2	5	2	3	4			1
Rhopalodia gibba	RPGB	diatom	Rhopalodia gibba (Ehrenberg) Müller	41	73	1	1	5	2	1	3	2	5	3	2				1
Rhopalodia musculus	RPMS	diatom	Rhopalodia musculus (Kützing) Müller		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

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				LT sites (n=332)	All sites (n=505)	Motility	N-Fixer	pH	Salinity	N Uptake Metab.	Oxygen Tol.	Saprobic	Trophic	Moisture	Pollution Class.	Pollution Tol.	Nuisance	Eutrophic Soft	Benthic/Sestonic
Scenedesmus acuminatus	SCAC	green	Scenedesmus acuminatus (Lagerheim) Chodat	8	14	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											1	2
Scenedesmus quadricauda	SCQD	green	Scenedesmus quadricauda (Turpin) Brébisson	72	121	2	2											1	2
Schroderia sp.	SHXX	green	Schroderia sp.	1	2														
Selenastrum minutum	SLMN	green	Selenastrum minutum (Nägeli) Collins	8	11	2	2											1	2
Sphaerocystis schroeteri	SFSR	green	Sphaerocystis schroederii Chodat	2	2	2	2											1	2
Spirogyra sp.	SPXX	green	Spirogyra sp.	7	15	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	2	5	2	2	5	2	3	4	4	6	2	2				2
Stephanodiscus niagarae	STNG	diatom	Stephanodiscus niagarae Ehrenberg		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	72	112	2	2	5							3				1
Synedra radians	SNRD	diatom	Fragilaria radians (Kützing) Williams et Round		2	2	2	4							2				1
Synedra rumpens	SNRM	diatom	Fragilaria capucina var. rumpens (Kützing) Lange-Bertalot	18	42	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	187	302	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	3	5	2	2											1	2
Ulothrix sp.	ULXX	green	Ulothrix sp.	23	46	2	2										2		1
Ulothrix sp.	ULX9	green	Ulothrix sp.			2	2										2		1
Unidentified flagellate	MXFG	unknown	Unidentified flagellate	1	1														
Vaucheria sp.	VAXX	green	Vaucheria sp.		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

Attribute Name	Category Code	Category Name	Category Description
	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
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.

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APPENDIX B: BOXPLOTS OF PERCENT BIOMASS OF THE 10 MOST FREQUENT SPECIES, BY SITE AND MONTH

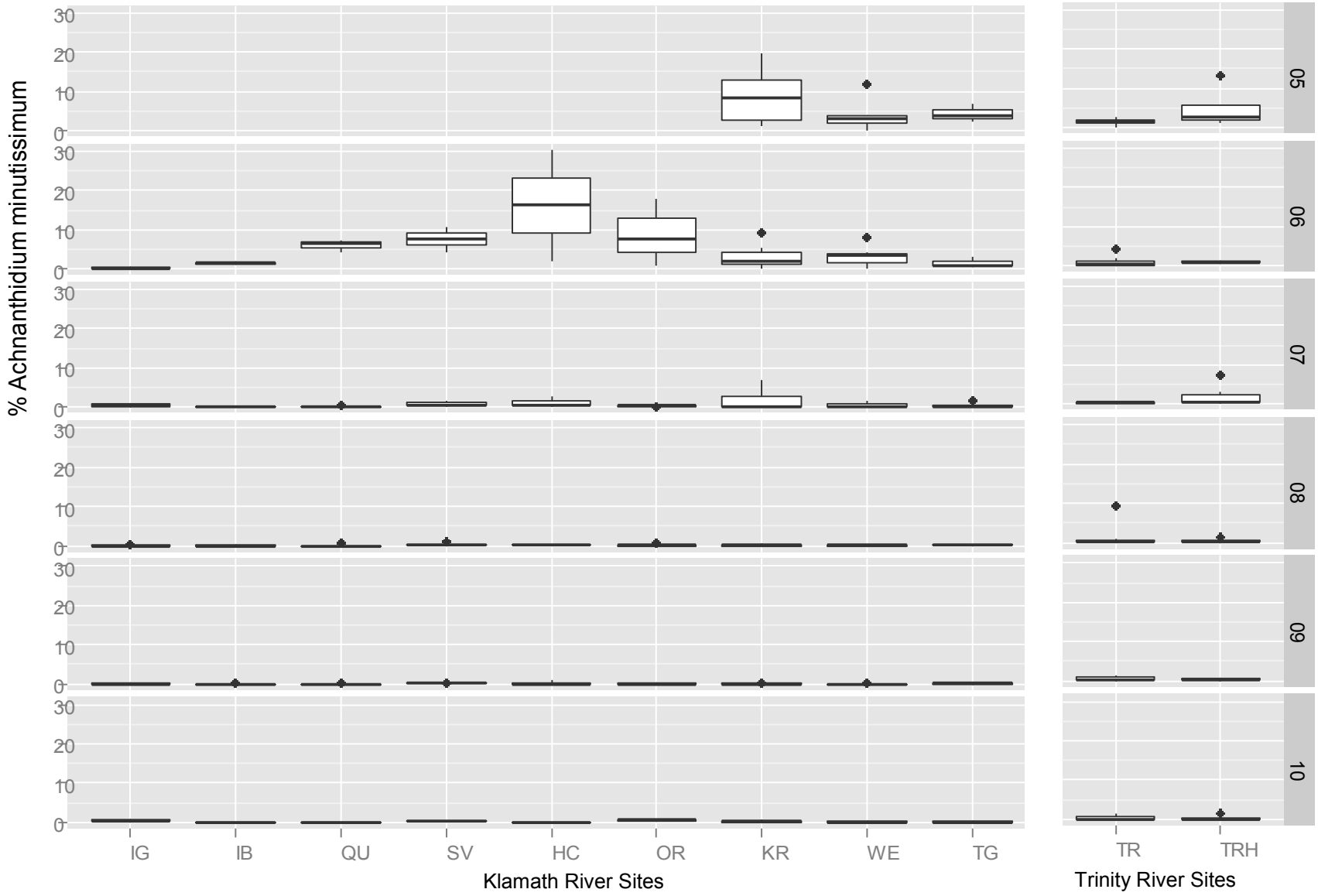

Figure B1. Boxplot of percent biomass of *Achnanthidium minutissimum* (Kützing) Czarnecki, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

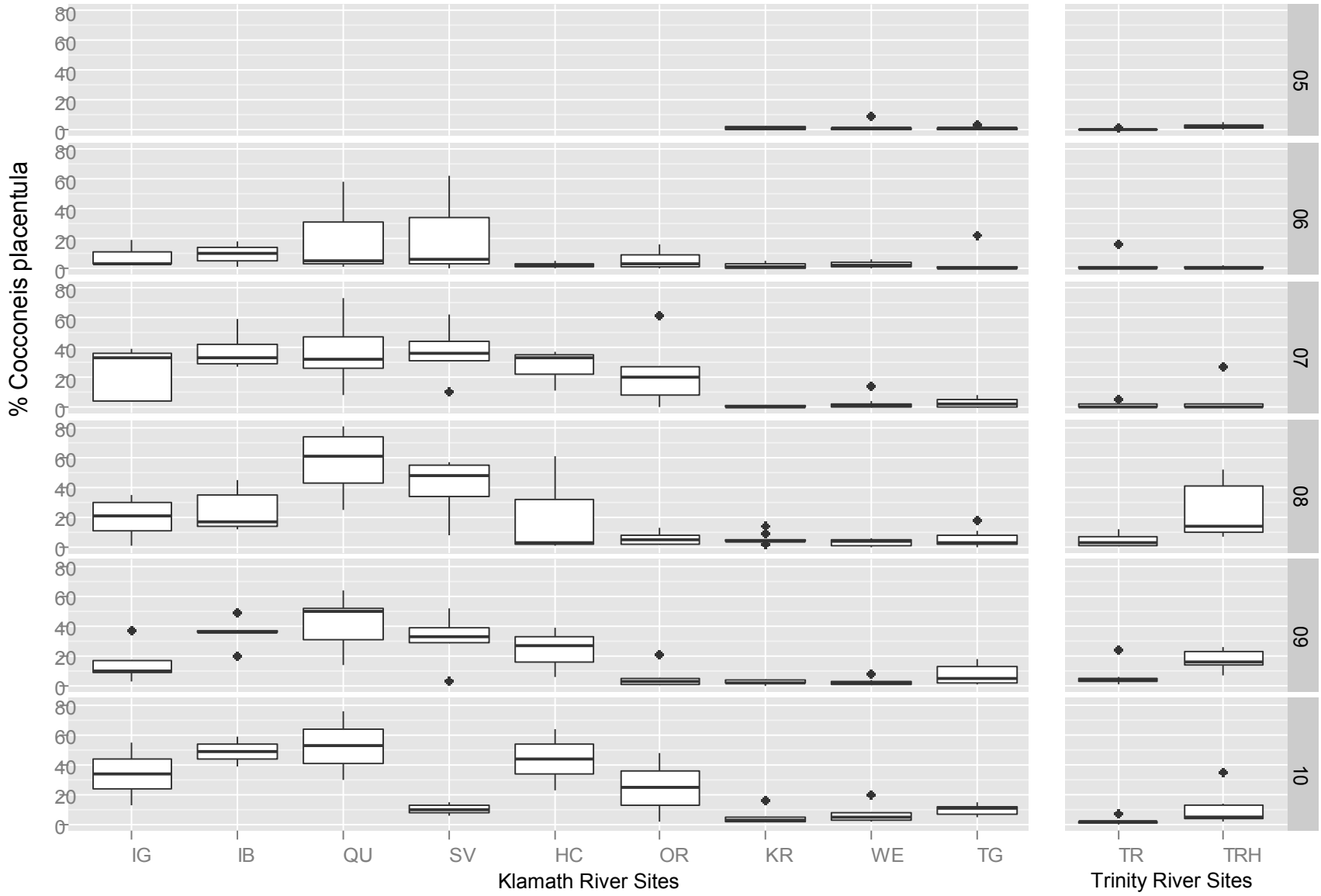

Figure B2. Boxplot of percent biomass of *Cocconeis placentula* Ehrenberg, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

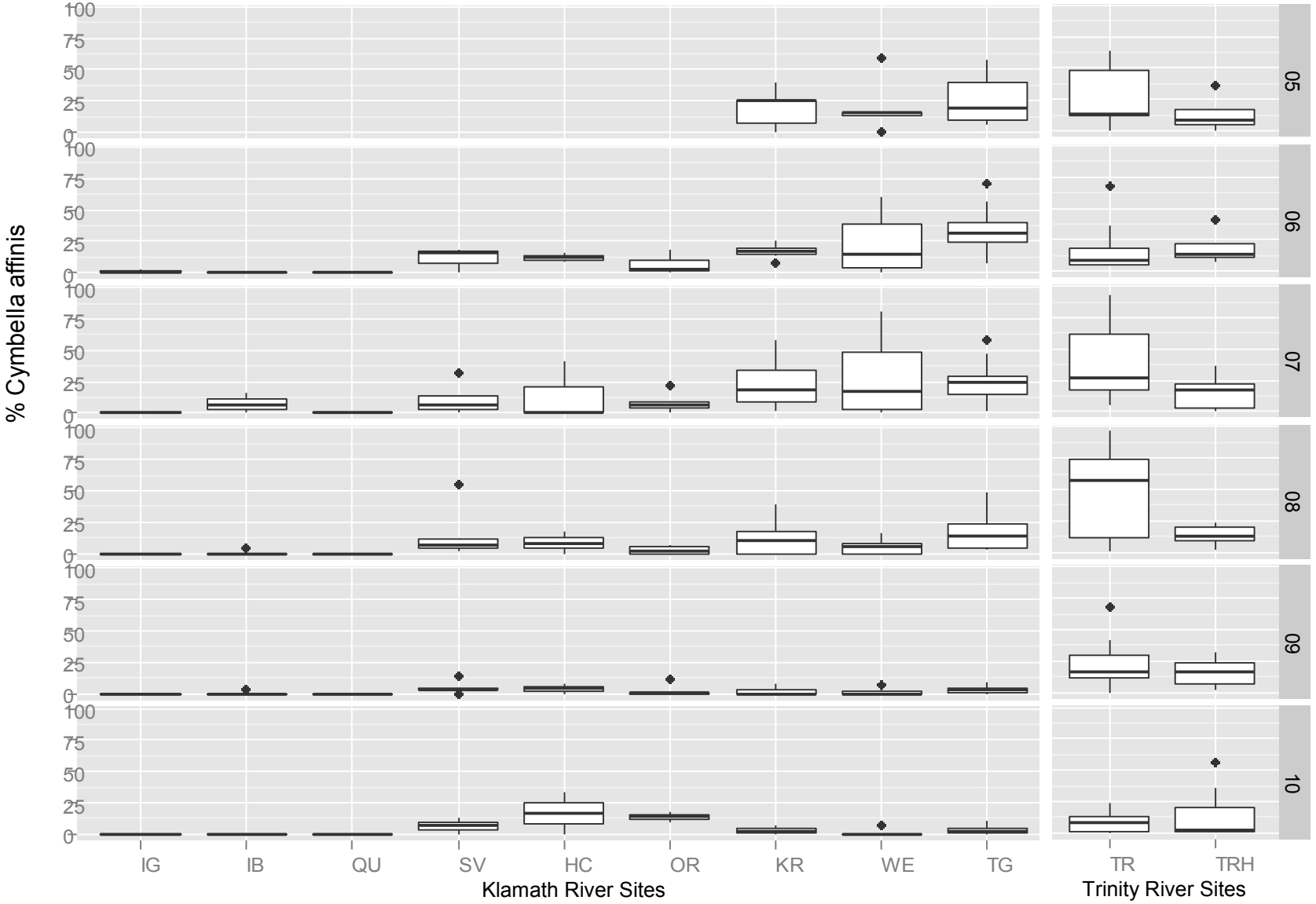

Figure B3. Boxplot of percent biomass of *Cymbella affinis* Kützing, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

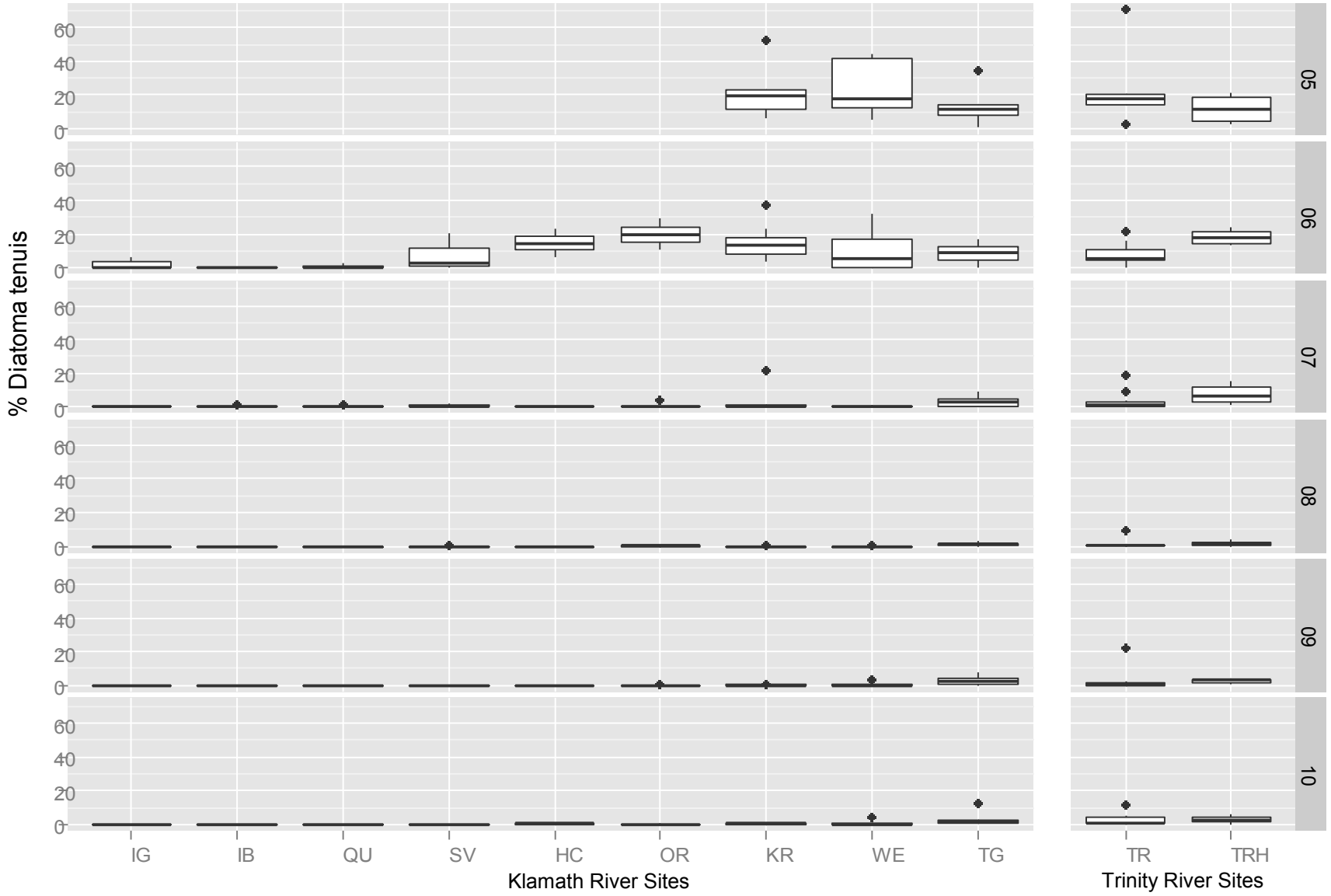

Figure B4. Boxplot of percent biomass of *Diatoma tenuis* Agardh, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

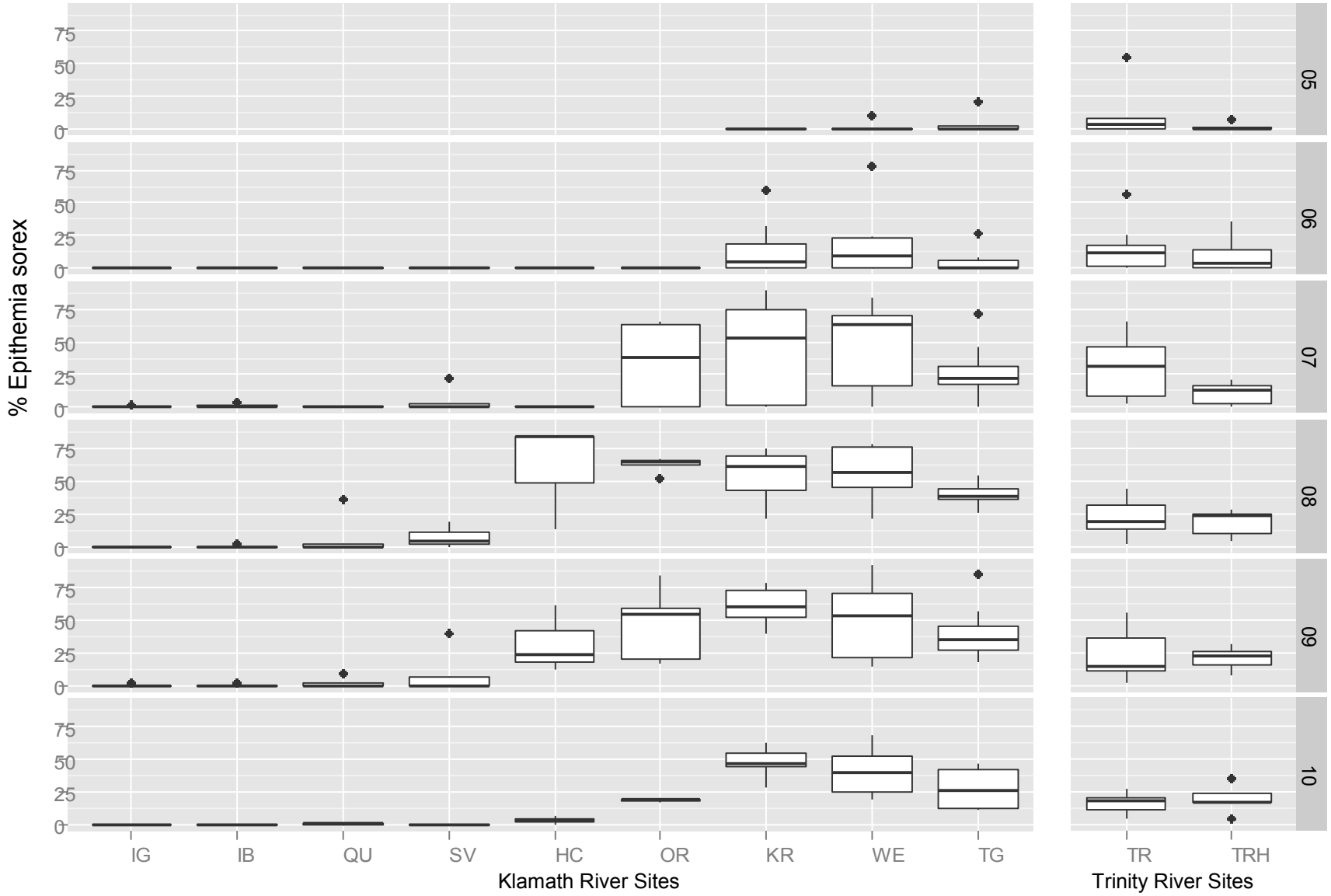

Figure B5. Boxplot of percent biomass of *Epithemia sorex* Kützing, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

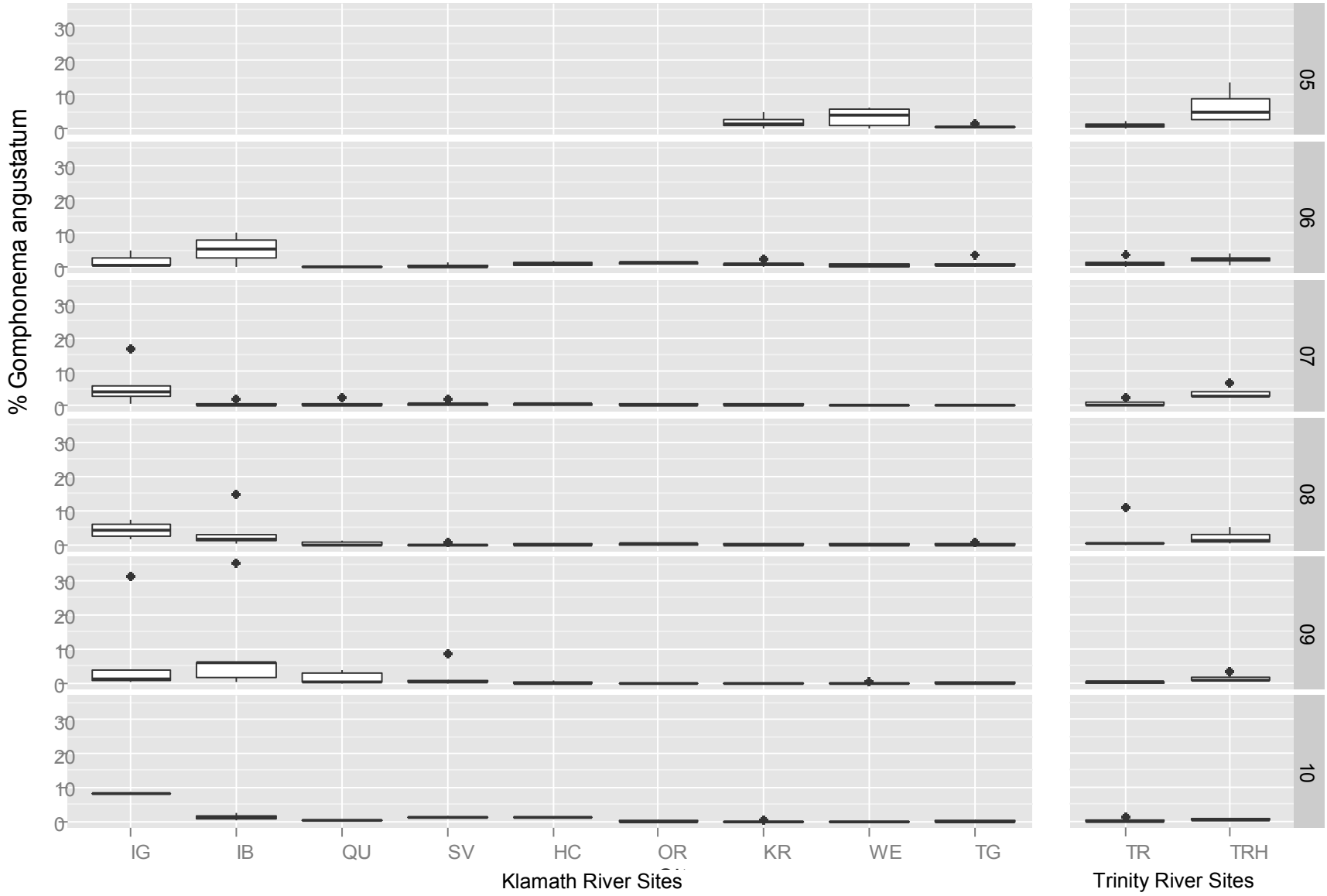

Figure B6. Boxplot of percent biomass of *Gomphonema angustatum* (Kützing) Rabenhorst, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

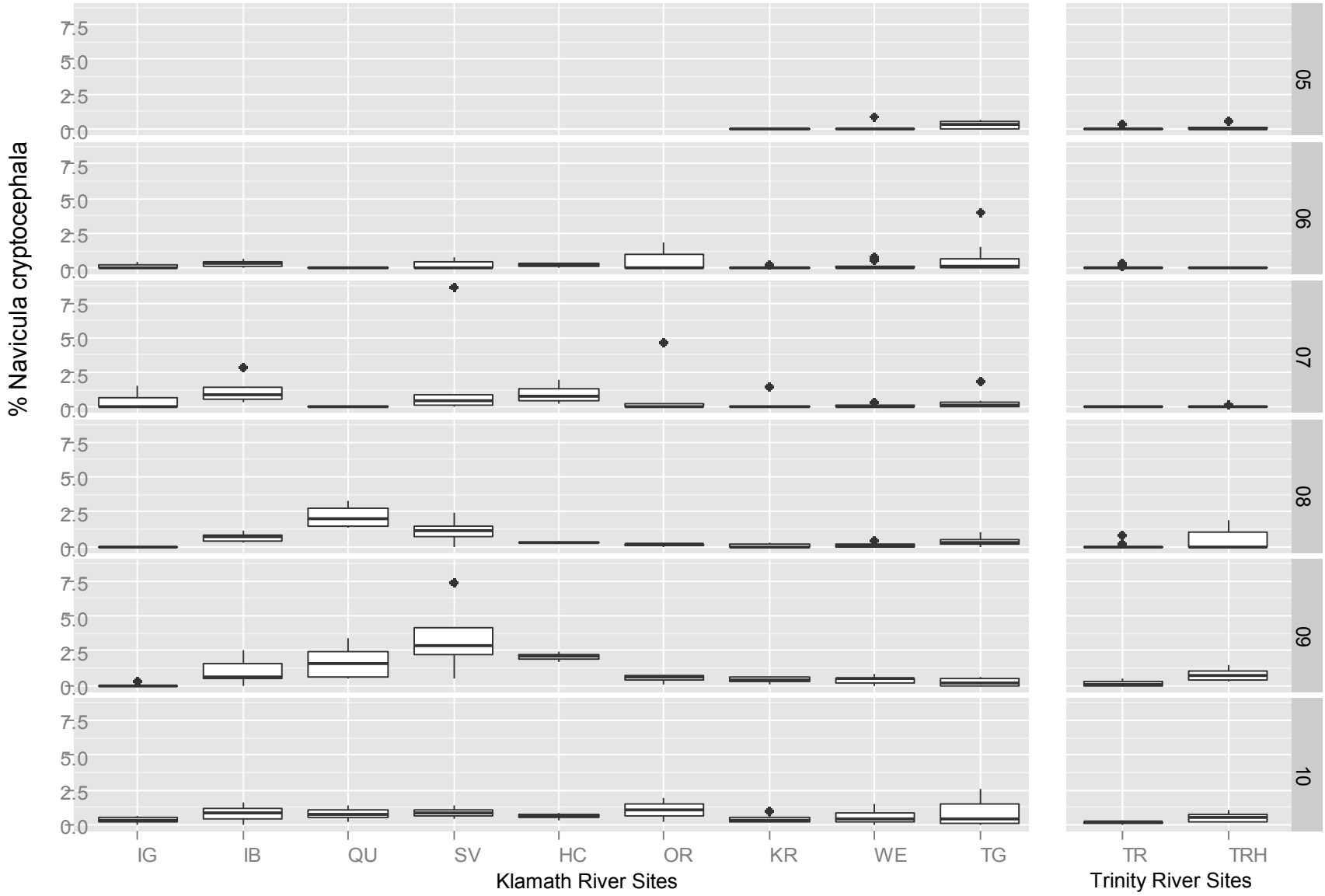

Figure B7. Boxplot of percent biomass of *Navicula cryptocephala* Kützing, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

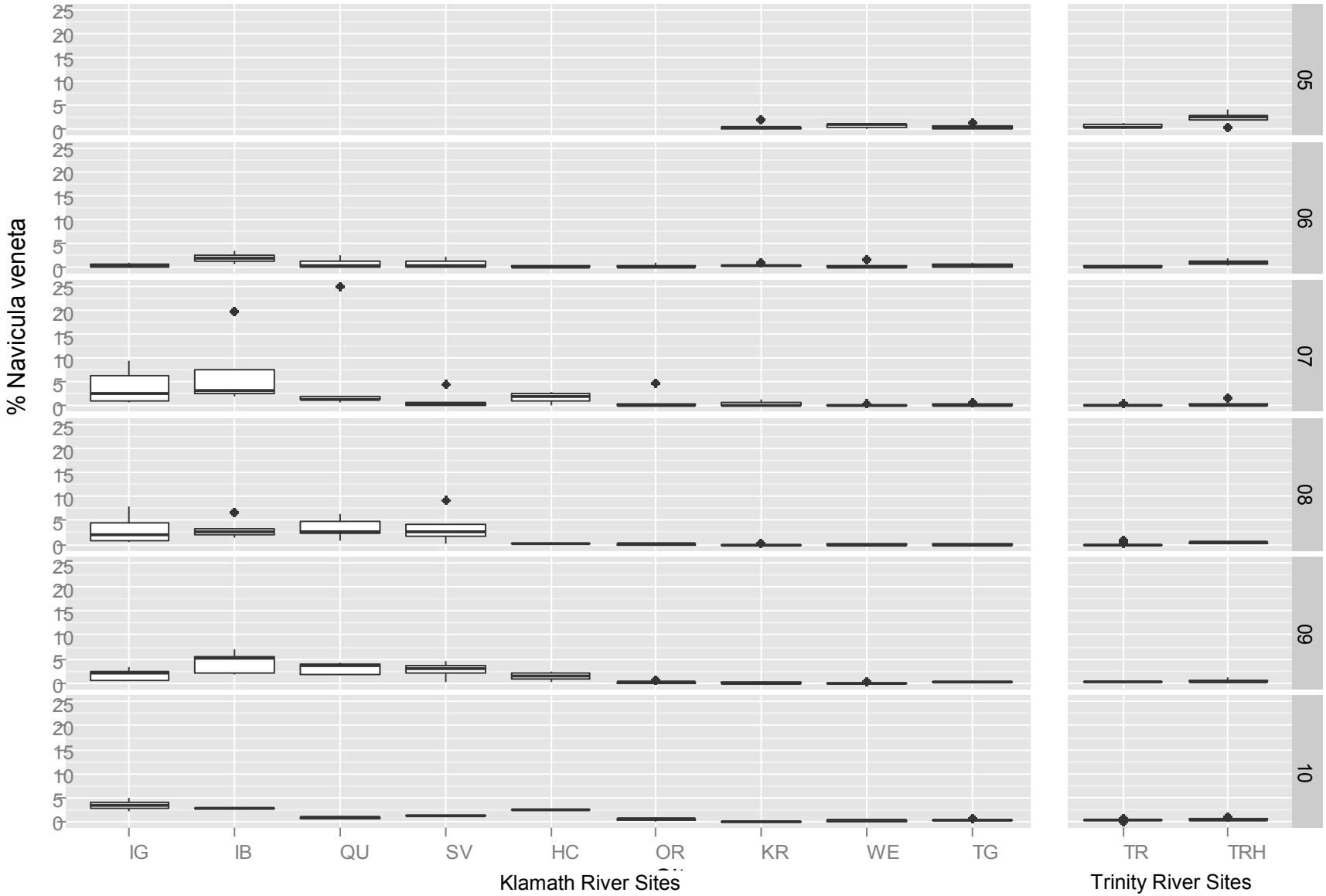

Figure B8. Boxplot of percent biomass of *Navicula veneta* Kützing, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

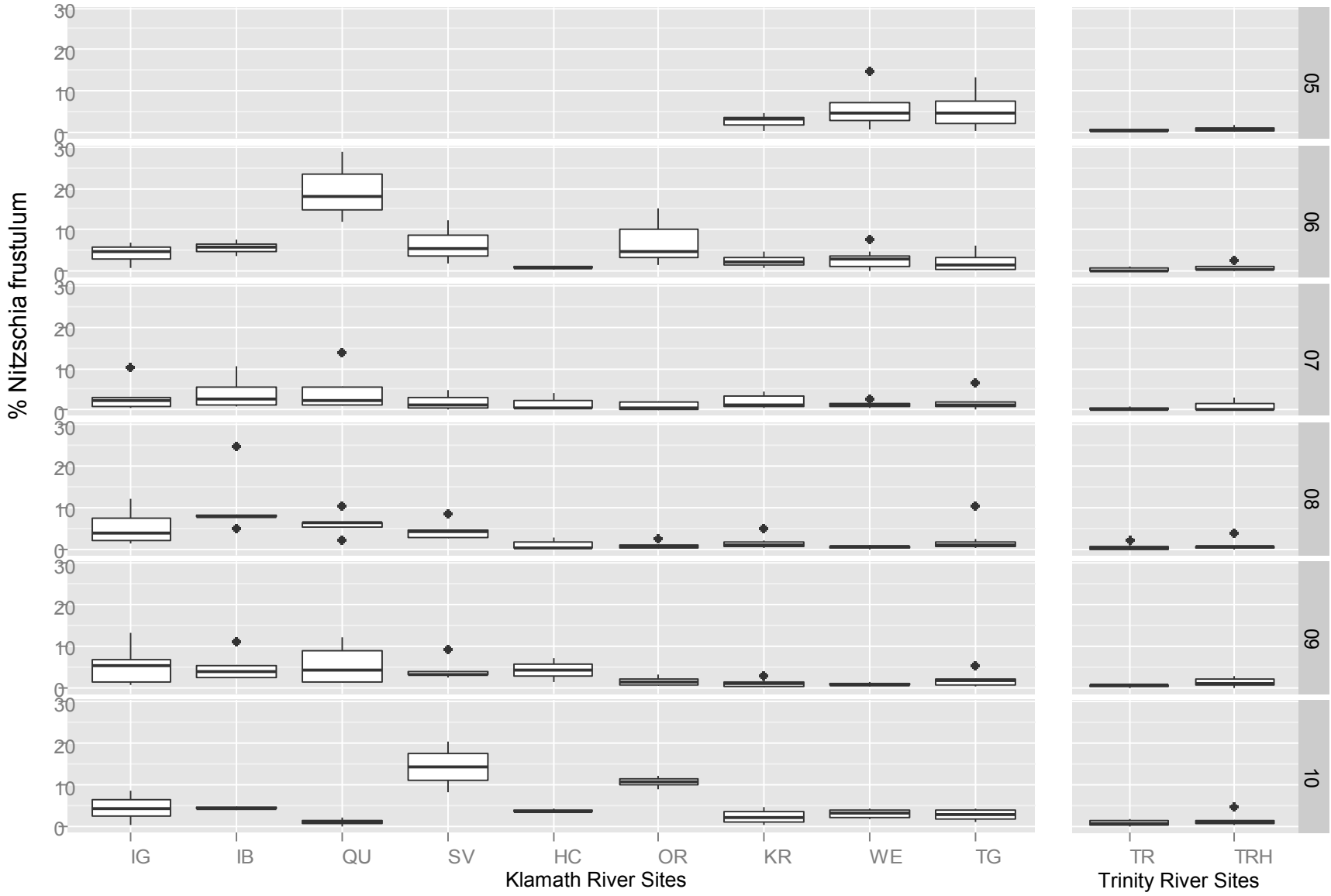

Figure B9. Boxplot of percent biomass of *Nitzschia frustulum* (Kützing) Grunow, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

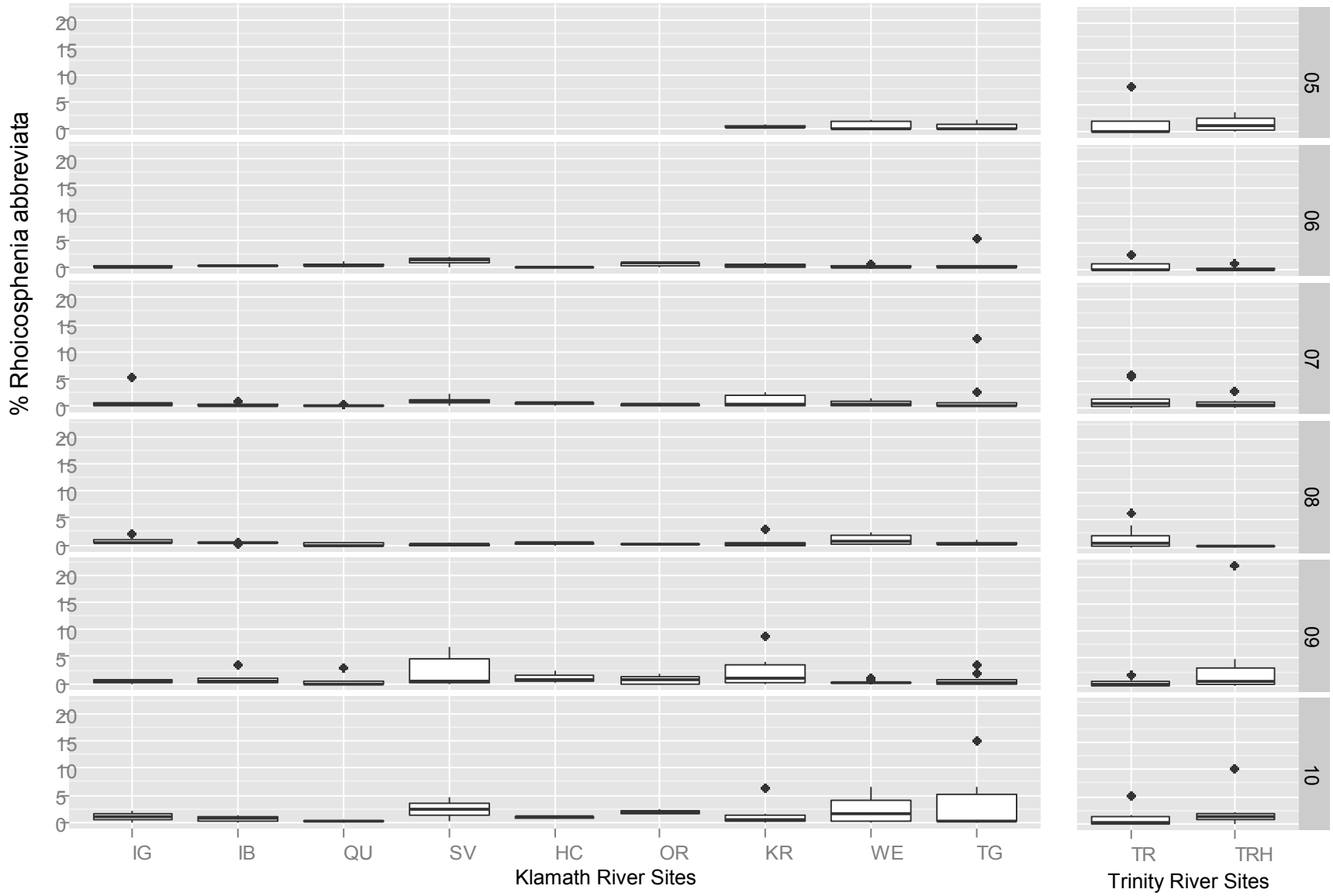

Figure B10. Boxplot of percent biomass of *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot, by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

APPENDIX C: SUPPLEMENTAL BOXPLOTS OF PERCENT BIOMASS FOR VARIOUS AUTECOLOGICAL METRICS

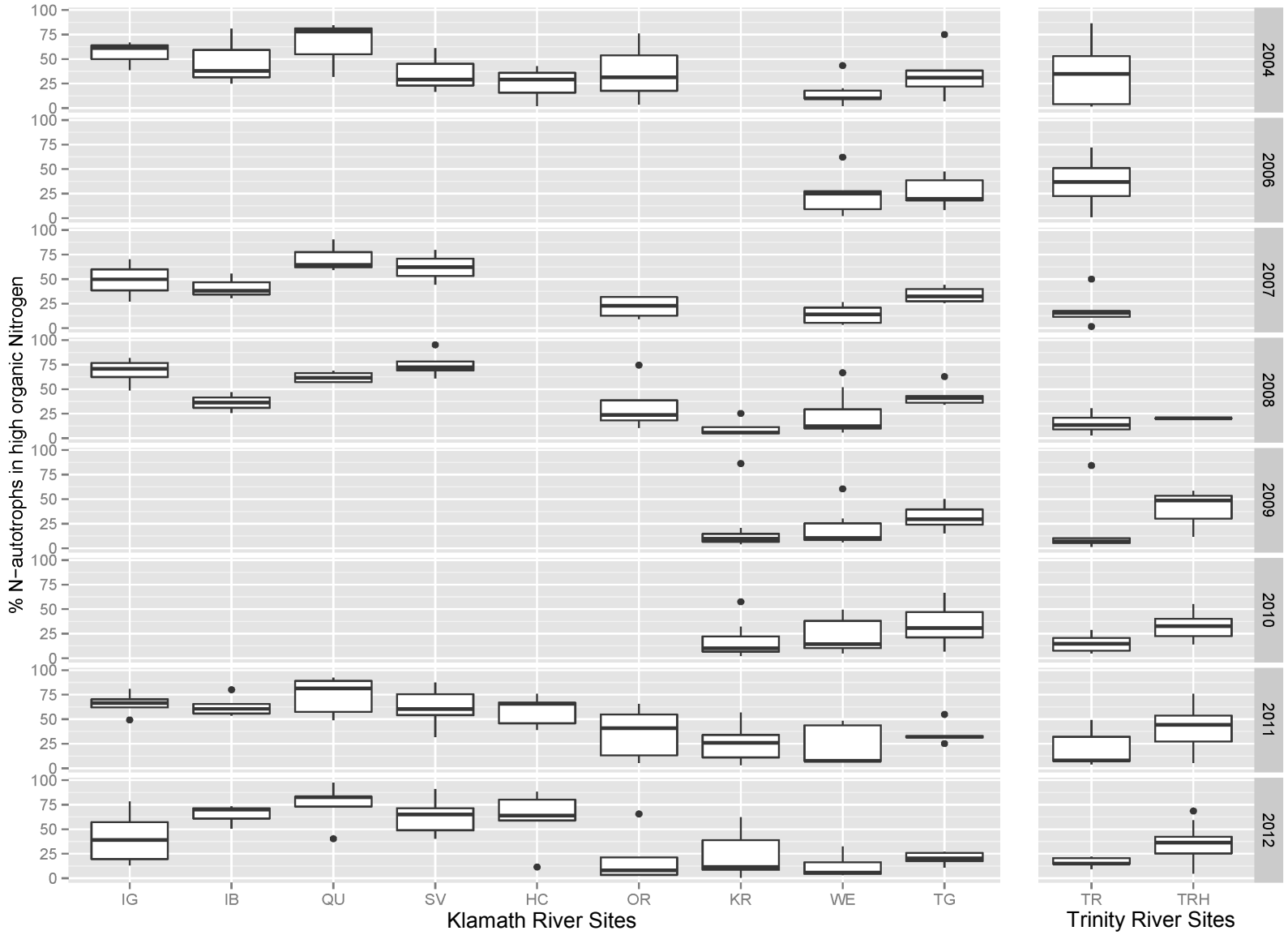

Figure C1. Boxplot of percent biomass of taxa that are nitrogen-autotrophs in high organic nitrogen conditions, by site (columns) and year (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

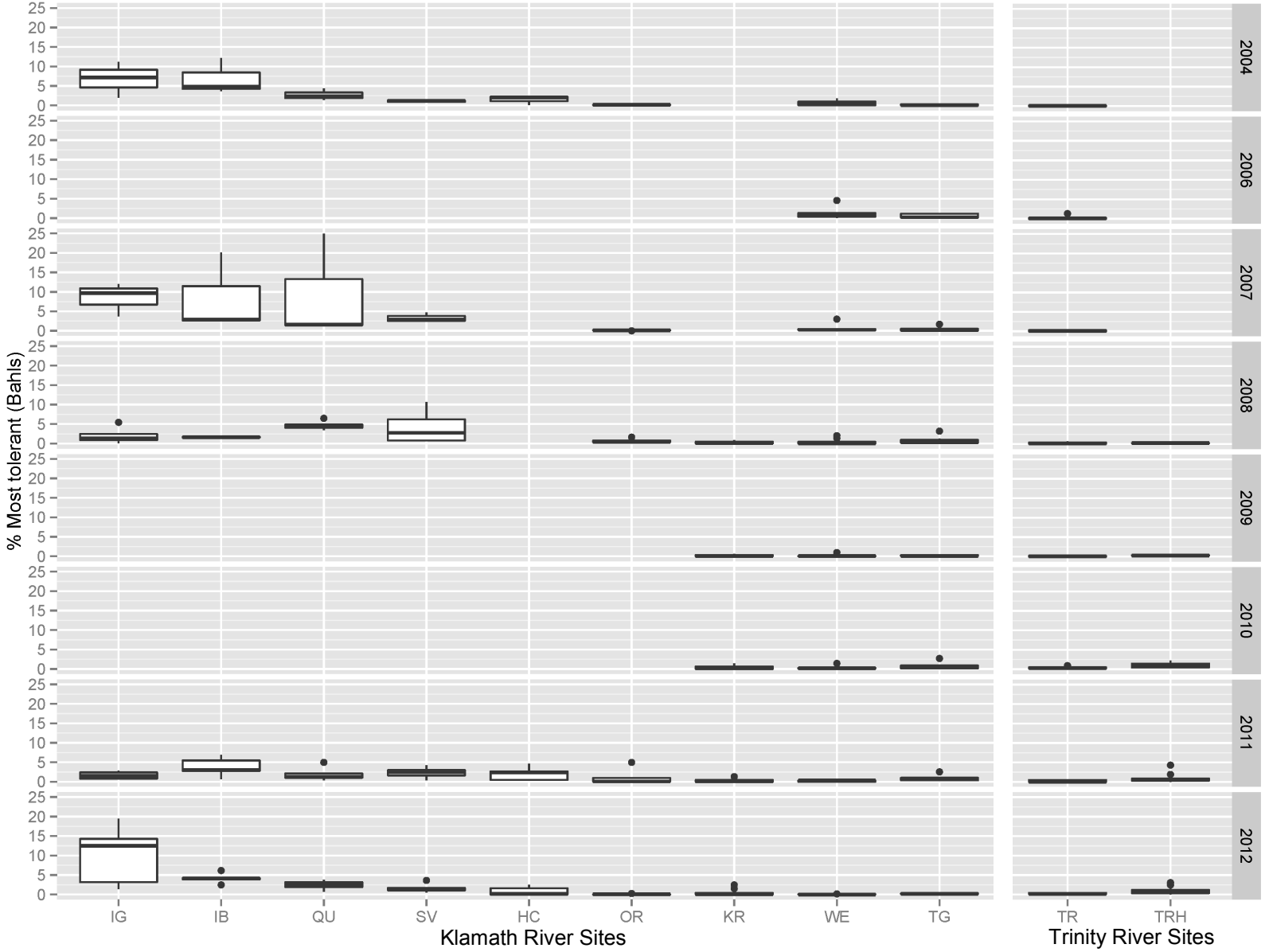

Figure C2. Boxplot of percent biomass of taxa that are most tolerant to pollution (Bahls 1993), by site (columns) and year (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.

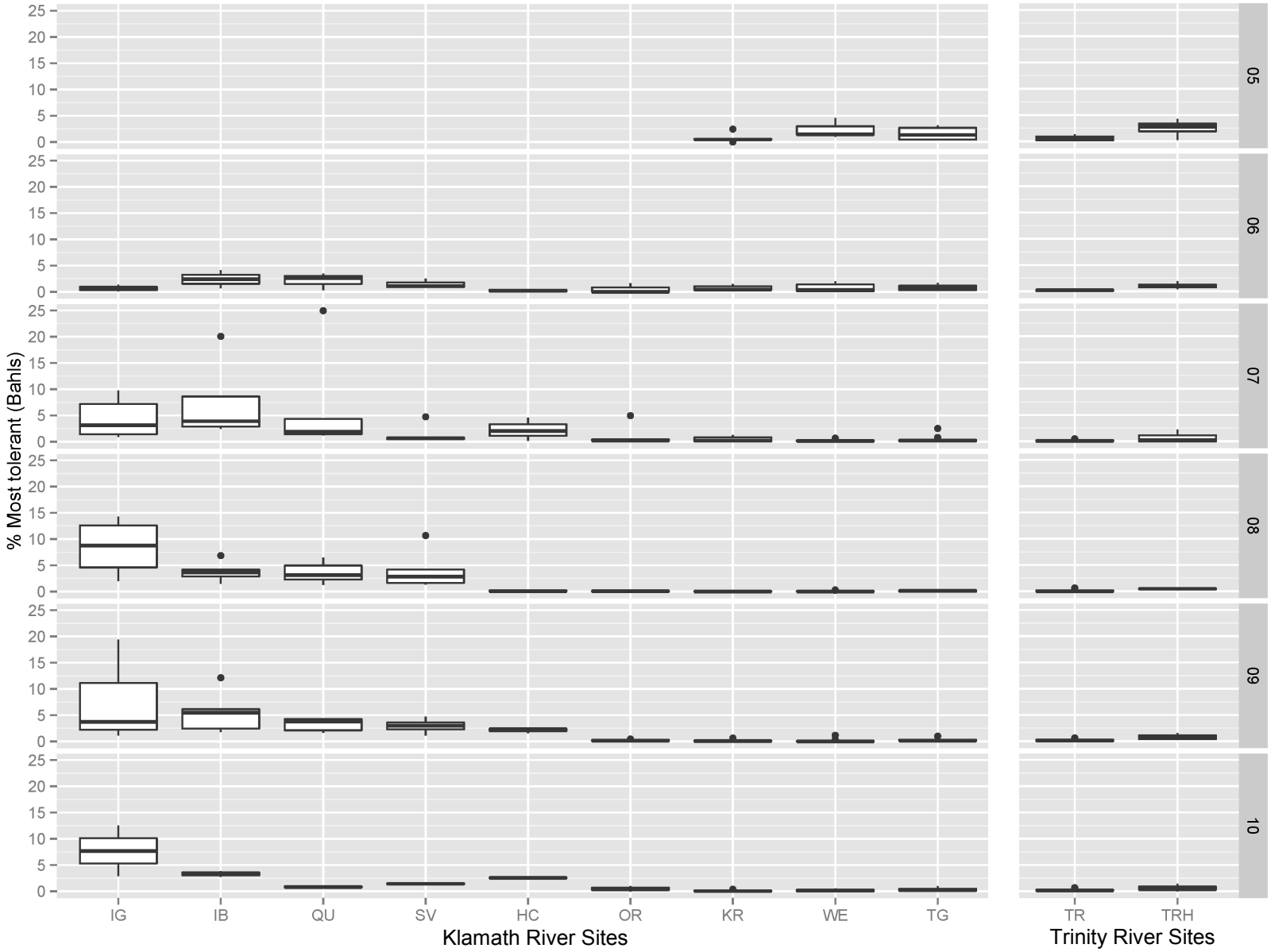

Figure C3. Boxplot of percent biomass of taxa that are most tolerant to pollution (Bahls 1993), by site (columns) and month (rows). Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). See Table 1 for key to site codes.