



Spatial and temporal variability of river periphyton below a hypereutrophic lake and a series of dams



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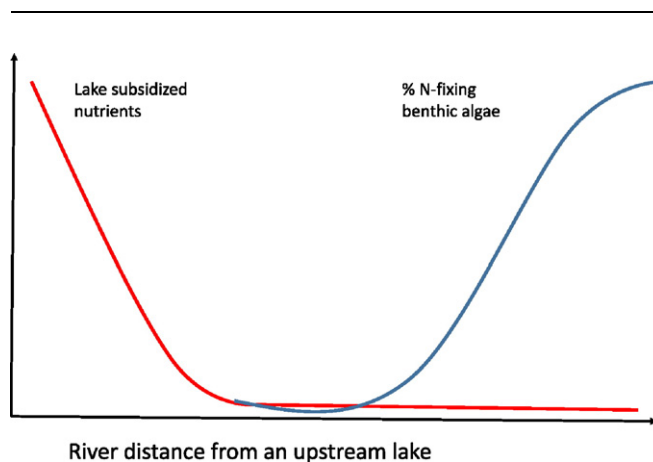
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HIGHLIGHTS

- Klamath River is an “upside-down” river originating from a hypereutrophic lake.
- Periphyton assemblages change seasonally with flow and longitudinally with nutrients.
- Longitudinally, nutrients and benthic N-fixers show an inverse relationship.
- This study can inform future nutrient reductions, flow regimes, and dam removals.

GRAPHICAL ABSTRACT



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ABSTRACT

Klamath River is described as an “upside-down” river due to its origins from the hypereutrophic Upper Klamath Lake (UKL) and hydrology that is heavily regulated by upstream dams. Understanding the lake and reservoir effects on benthic communities in the river can inform important aspects of its water quality dynamics. Periphyton samples were collected in May–November from 2004, 2006–2013 at nine long-term monitoring sites along 306 river km below UKL and a series of dams ($n = 299$). Cluster analysis of periphyton assemblages identified three statistically different periphyton groups (denoted Groups 1–3). Group 1 occurred primarily in the upstream reach for June–October and had a higher percentage of sestonic species, including the cyanobacteria *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*, consistent with the presence of upstream reservoirs. Group 2 had the highest relative biomass of diatoms and lowest relative biomass of cyanobacteria. Sites in the lower reach of the Klamath River fell into Group 2 in May–June and transitioning into Group 3 for July–October. Group 3 was dominated by nitrogen (N)-fixing species, including three diatoms (*Epithemia sorex*, *Epithemia turgida*, and *Rhopalodia gibba*) with cyanobacterial endosymbionts and the cyanobacterium *Calothrix* sp. Periphyton assemblages were strongly associated with temporal variations in flow conditions (e.g., decreasing flow from spring to fall) and spatial gradients in nutrient concentrations (e.g., decreasing from upstream to downstream). The inverse longitudinal relationship between periphyton biomass and nutrients may be explained by

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the ability of benthic N-fixers (Group 3) to overcome N limitation. Overall results showed a strong inverse relationship between the relative biomass of N-fixers and nitrogen concentrations and flow. This long-term dataset provides valuable insight into Klamath River's seasonal and longitudinal patterns of benthic algal communities and associated environmental variables. Our findings can inform river management decisions such as reducing upstream nutrient loads, setting flow regimes, and potential dam removals.

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1. Introduction

Stream ecologists have increasingly recognized lentic-lotic linkages as an integral part of river ecosystems (Jones, 2010, Lamberti et al., 2010, Steinman and Rosen, 2000, Ward and Stanford, 1983). Longitudinal ecosystem processes such as nutrient cycling (Arp and Baker, 2007) and organic matter processing (Larson et al., 2007) in a river can be interrupted by presence of either natural or man-made lentic systems resulting in predictable changes in their physical, chemical, and biological features below such impoundments. It is expected that environmental conditions right below dams will be characterized with stable flows and temperature, increased nutrients, organic matter, primary and secondary productivity, which then decrease in a downstream direction (Ellis and Jones, 2013, Ward and Stanford, 1983). The magnitudes of the disruption depend on many factors including the landscape position of the lentic systems (Martin and Soranno, 2006) and discharge regime (Benenati et al., 2000). Most research on lentic-lotic interactions focused on inlet and outlet comparison with a few sites downstream of the lentic system (Arp and Baker, 2007, Myers et al., 2007). In a comprehensive review on lentic-lotic interactions, Jones (2010) recognized how little is known about the spatial and temporal variability of the lake effects on downstream reaches and even less about longitudinal changes of biological communities in regulated rivers (Ellis and Jones, 2013). Arp and Baker (2007) estimated that nutrient cycling disrupted by lentic systems in a river may not be fully recovered within 20 km downstream. Ellis and Jones (2013) concluded that regulated rivers might have two recovery gradients: a thermal gradient of a few hundred kilometers downstream and a resource subsidy gradient of 1–4 km downstream. Understanding spatial and temporal variability of the lake effects on outlet streams can inform both important aspects of water quality dynamics and potential management actions (e.g., reducing upstream nutrient loads, setting flow regimes, dam removals) to improve their water quality.

The ecology of streams and rivers is often defined by their periphyton communities, which are known to be valuable indicators of ecosystem status due to their ecological and biogeochemical importance, sensitivity to human-induced changes in water quality, and ubiquitous distribution across ecosystems (McCormick and Stevenson, 1998). Predictable relationships between periphyton abundance, taxonomic composition, nutrient content and water quality have been identified in a variety of lotic ecosystems, including their effect on large diel fluctuations in pH and dissolved oxygen (DO). For example, nutrient enrichment of the South Umpqua River, Oregon was linked to periphyton growth and large diel fluctuations in pH and DO 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 (Biggs and Smith, 2002, Hart et al., 2013, Pan et al., 2004, Stancheva et al., 2013, Weillhoefer and Pan, 2006). Despite the value of periphyton communities as bioindicators, most of their properties have been established from studies in free-flowing streams and rivers conforming to the river continuum concept (RCC, Vannote et al., 1980) while research on spatial and temporal variation of periphyton assemblages in response to lake effects is limited. Cattaneo (1996) found that sestonic and periphyton biomass decreased with distance from lakes while Vadeboncoeur (1994) concluded that stream algae increased downstream but the concentration of lake algae depended on river discharge, which defined the distance

for recovery. Robinson and Kawecka (2005) compared benthic diatom assemblages in a small alpine stream/lake network and found that species compositions changed longitudinally. Cortez et al. (2012) observed variable longitudinal trends in benthic algal assemblages dependent on flow regulation.

Klamath River is heavily regulated by upstream dams and is described as an “upside-down” river due to its origins from Upper Klamath Lake (UKL, Fig. 1), a shallow and hypereutrophic lake dominated during summer by planktonic bloom-forming cyanobacteria (e.g., *Aphanizomenon flos-aquae*) (Eilers et al., 2004, Eldridge et al., 2013, Kann and Smith, 1999). 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). Downstream reservoirs Copco and Iron Gate are dominated during the summer by planktonic bloom-forming toxic cyanobacteria (e.g., *Microcystis aeruginosa*) (Jacoby and Kann, 2007). Not surprisingly, the Klamath River, especially its headwaters and mid sections, is heavily impacted by the UKL and downstream reservoirs, resulting in generally low TN:TP ratios (Oliver et al., 2014). These conditions favor the development of N-fixing algae such as cyanobacteria (e.g., *Calothrix* sp., *Rivularia* sp.) and benthic diatoms from the family Epithemiaceae (e.g., *Epithemia* and *Rhopalodia*). Diatoms are not capable of fixing nitrogen, but some taxa have gained such capacity by hosting N-fixing unicellular cyanobacterial endosymbionts, which allow them to live in N-poor environments (Peterson and Grimm, 1992, Stancheva et al., 2013). Research via nitrogen fixation assays and genomic DNA analysis indicates that cyanobacterium-like structures referred to as ‘spheroid bodies’ (SBs) possess nitrogen capacity (Prechtel et al., 2004). Prechtel 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. N-fixing periphyton can be important component of river food webs (Power et al., 2009, 2013) in Northern California and the Pacific Northwest where nitrogen can be the limiting nutrient in streams (Hill and Knight, 1988, Omernick, 1977). Therefore, our main objective was to characterize patterns of periphyton assemblages in response to lake effects on a large spatial scale and with a long-term dataset. Specifically, we wanted to examine if % benthic N-fixing algae can be used to assess lake effects on a large outlet river.

2. Methods

2.1. 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, which is regulated at Link Dam to provide water to the Klamath Irrigation Project. Water released from Link Dam flows through the Link River and Lake Ewauna, where the Klamath River proper begins (Fig. 1). From this point, the river flows through a series of impoundments, including Keno, J.C. Boyle, Copco 1, Copco 2, and Iron Gate Reservoirs (built between 1917 and 1962). One of these dams (Keno) maintains water surface elevation for irrigation diversions while the others are used for hydropower generation. From Iron Gate Dam, the tallest (53 m) and largest ($72 \times 10^6 \text{ m}^3$)

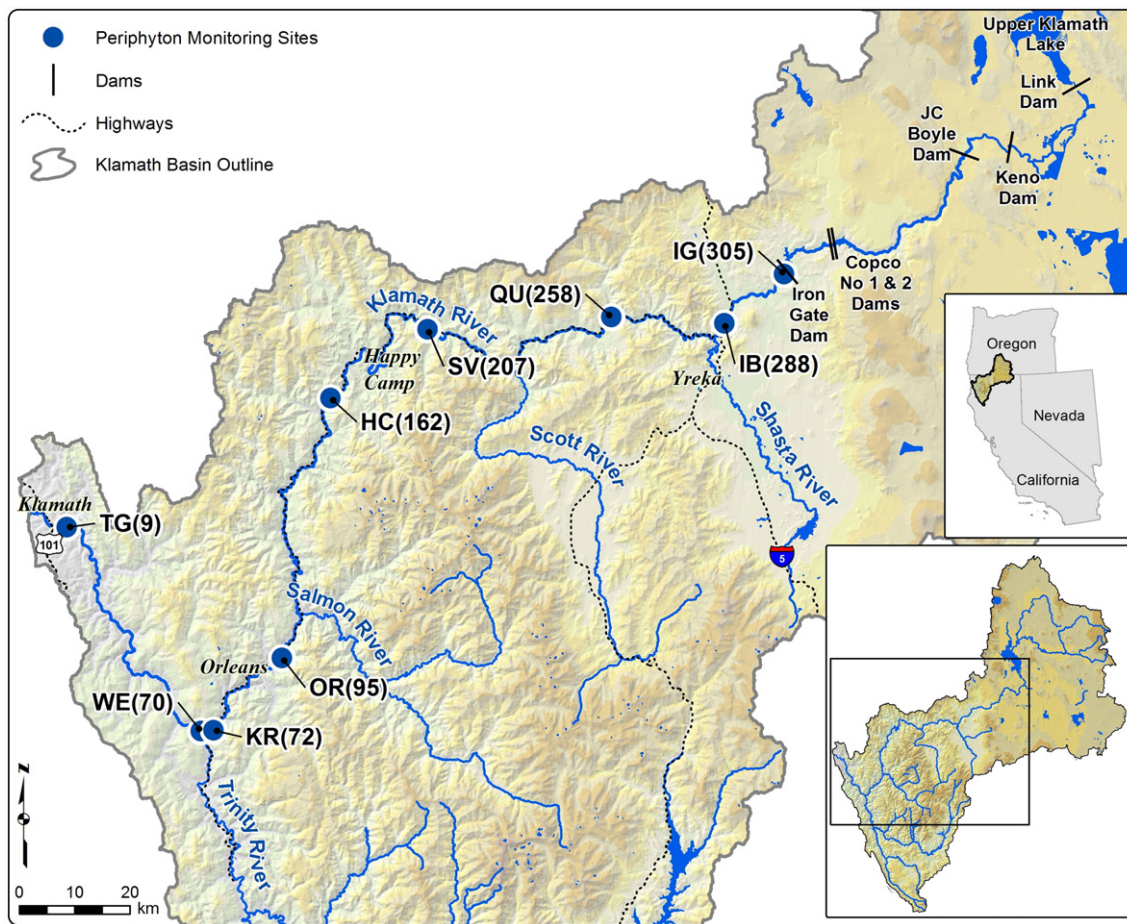


Fig. 1. Location of nine long-term periphyton monitoring sites on the Klamath River (river kilometer in parentheses), watershed boundary (inset on the lower right), and location on the California-Oregon border (inset on the right).

hydropower reservoir, near-surface (0–12 m depths) water is released into the river below which flows to the Pacific Ocean for 306 km, mostly through a confined canyon. The Klamath River watershed is 40,632 km² (2/3 is publically owned) with an elevation range from sea level to 4322 m (CA NCRWQCB 2010). The watershed geology is quite diverse and ranges from volcanic deposits in the upper half of the basin with more gentle slopes (above our study area) to sedimentary rocks in the lower half, which has steeper slopes. Land use in the upper basin is >50% agriculture and rangeland while the lower basin is predominantly forested (>50%) (Oliver et al., 2014). At higher elevations the vegetation is dominated by conifers while at lower elevation grow hardwood forests and shrubs. 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 nine long-term monitoring sites in the middle and lower mainstem Klamath River (i.e., below Iron Gate Dam, 305 river km, and Turwar, 9 river km upstream of the Klamath Estuary) (Fig. 1). Watershed areas for the nine sampling sites (from upstream to downstream) ranged between 11,992 km² and 31,339 km² with an elevation gradient of 661.1 m to 6.7 m.

2.2. Environmental data sources

2.2.1. Hydrologic and meteorological data

Streamflow data were obtained from USGS (2014). For periphyton sites located away from USGS stream gages, discharge was estimated using a watershed area accretion method similar to that used by other researchers in the study area (PacifiCorp, 2004, TetraTech, 2009,

Asarian et al., 2010). The total watershed area contributing to the ungauged accretions (areas of gaged tributaries were excluded) between each mainstem USGS gage (IG, SV, OR, and TG) was determined using GIS, and the ratios of individual areas to the total accretion area were calculated. Accretions for reaches between the mainstem gages were developed by calculating the difference between the five-day moving averages of the upstream gage, downstream gage, and any gaged tributaries within the reach. The accretion volume was then attributed to the periphyton monitoring stations in proportion to their watershed area. Daily mean air temperature and precipitation data for several Remote Automated Weather Stations (RAWS) were obtained from the RAWS USA Climate Archive (Western Regional Climate Center, 2014). A meteorological station was assigned to each water quality monitoring station according to proximity (longest distance was 48.3 km) and elevation.

2.2.2. Water temperature

Continuous (i.e., hourly or sub-hourly) water temperature data were collected by the Karuk Tribe (2011); Yurok Tribe (2008), and U.S. Fish and Wildlife Service (Ward and Armstrong, 2010) using multi-parameter sensors. We calculated daily summary statistics (i.e., mean) when at least 80% of daily measurements were present. For a few stations, daily means were estimated from daily minimum and maximum water temperature obtained from the USGS (2014).

2.2.3. Nutrients and chlorophyll a

Nutrient samples were generally collected at the same location as the long-term periphyton stations, although in some cases a nearby station was utilized. Grab samples for nutrient and planktonic chlorophyll

a analyses were collected with a churn splitter and shipped to the processing lab on ice the day of sampling (Yurok Tribe, 2008). Data were collected by a variety of entities such as Karuk Tribe (2011); Yurok Tribe (2008), and U.S. Fish and Wildlife Service (USFWS). Sampling frequency varied by station and year, but was generally monthly in 2004 and bi-weekly for 2006–2013. Parameters analyzed followed standard protocols (APHA, 1992) and included ammonia (NH_3), nitrate-plus-nitrite ($\text{NO}_3^- + \text{NO}_2^-$), total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP), and planktonic chlorophyll *a* (Chl *a*). Some data collection entities did not analyze TN, in which case TN was calculated as Total Kjeldahl Nitrogen (TKN) + $\text{NO}_3^- + \text{NO}_2^-$. Total inorganic nitrogen (TIN) was computed as $\text{NH}_3 + \text{NO}_3^- + \text{NO}_2^-$.

2.2.4. Matching periphyton samples to environmental data

Periphyton samples were assigned environmental data based on the average of environmental values encompassing a 14-day period (the 12 days preceding the periphyton sample, the day of the periphyton sample, and the day following) because periphyton samples were not collected on the same date as nutrient samples. In most cases the 14-day average was composed of a single nutrient sample (although sometimes up to four samples) and 14 days of streamflow, water temperature, and meteorological data. The 14-day period was chosen to correspond with the generally bi-weekly nutrient sampling and to be relevant to periphyton growth. Missing environmental data (14%, primarily water chemistry) were either left missing (5%) or imputed (9%) using regressions. For example, for water temperature, linear predictions were developed between daily average water temperature of stations within 48 river km and daily average water temperature of a particular station needing imputation. All regression models were statistically significant ($p \leq 0.05$) and had R^2 values between 0.50–0.95.

2.2.5. Field sampling

Periphyton samples were collected approximately monthly in May through November in 2004 and 2006–2013 at up to nine long-term monitoring sites (Fig. 1), with three to nine sites sampled per year and generally three to six samples collected per year per site ($n = 299$). Pilot sampling began in 2004 to inform the development of a Total Maximum Daily Load study (CA NCRWQCB, 2010) but a long-term monitoring program was not established until 2006. The periphyton sampling protocol (Yurok Tribe, 2008) was adapted from techniques recommended by U.S. EPA (Peck et al., 2006) and U.S. Geological Survey (Porter et al., 2008). Samples were collected by the Yurok Tribe (2008), Hoopa Tribe, Karuk Tribe (2011), Watercourse Engineering Inc., MaxDepth Aquatics (Eilers, 2005), and the CA NCRWQCB (2010). The sampling locations met the following criteria: depth of 0.30–0.61 m, velocity of 0.30–0.61 m s^{-1} , and no shading. Representative cobbles were selected from the stream bed at each sampling location, avoiding the extremes of algal cover. For each cobble, a 2.54 cm by 7.62 cm microscope slide (96.75 cm^2 area) was held against it while the remainder of the cobble was scrubbed off with a brush. Then the slide was removed and the periphyton was scraped with a razor blade and a toothbrush. Two samples (one for algal speciation and one for benthic Chl *a*), each composed of one (years 2004 and 2006) or five cobbles (years 2007–2013), were collected at every sampling location. Samples for algal speciation were preserved in Lugol's iodine (note: both 1-cobble and 5-cobble samples were used in this analysis because ordination plots revealed no effect of collection method on species composition).

2.2.6. Laboratory analysis

Samples for microscopic determination of periphyton density and biomass were processed according to APHA Standard Methods (APHA, 1992). Permanent microscope slides were prepared from each sample by filtering an appropriate aliquot of the sample through a 0.45 μm membrane filter (APHA, 1992, McNabb, 1960). A section was cut out and placed on a glass slide with immersion oil to make the filter

transparent, then placing a cover slip on top and applying clear nail polish to the periphery for permanency. Algal units (cells, colonies, or filaments) were identified and counted along a measured transect of the microscope slide with a Zeiss standard microscope (1000 \times , phase contrast). A minimum of 100 live (i.e., with intact chloroplasts) algal units were identified and counted in each sample (APHA, 1992). Average biovolume (μm^3) estimates of each species were obtained from measurements of each alga. Analyses were based on relative species biovolume (referred to as biomass throughout the paper for ease of interpretation), except for the data summary in Table 1 where absolute species biomass was also used.

2.2.7. Periphyton metrics

To characterize samples in terms of algal autecology, species were grouped into functional groups (e.g., heterotrophs, nitrogen (N)-fixers, pollution tolerant, etc.). The total relative biomass of each functional group for each sample was used to calculate autecological metrics, which were compiled from the literature and available databases (Asarian et al., 2014).

2.2.8. Statistical analyses

To assess differences in environmental variables or periphyton metrics among sites, one-way Analysis of variance (ANOVA) was used. Statistically significant ($\alpha = 0.05$) results were further analyzed with Tukey HSD test to identify significant differences between pairs of sites. If test assumptions were not met even after data transformation, we used the non-parametric alternatives to ANOVA and Tukey HSD, Kruskal–Wallis and Kruskal multiple comparison tests, respectively. In addition, linear regressions analyses were performed on seasonal means of sampling sites in order to determine inter-annual relationships and relevant longitudinal trends. Subsequent to evaluation of regression assumptions the core growing season (June–September) means of benthic N-fixers proportion was logit-transformed and $\text{NO}_3^- + \text{NO}_2^-$ and flow were log-transformed. Linear regression models were then fit to the transformed variables.

We used Non-metric Multidimensional Scaling (NMDS) to characterize spatial (i.e., site-to-site) and temporal (i.e., 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 (Clarke, 1993). 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 (Clarke, 1993). The NMDS function was specified to run with 20 random starts in search of optimal solution with the lowest stress value. To explore the environmental variables that explained the patterns in the ordination of periphyton assemblages, a linear fitting function was used (Oksanen et al., 2013). This function finds the vector averages of the environmental variables and fits them in the ordination space defined by the species data (NMDS plot). The significance of each vector was tested using 1000 permutations.

Cluster analysis was used to identify groupings in the periphyton assemblages. Cluster analysis, using the average linkage method, was based on Bray–Curtis similarity coefficient after exclusion of rare species (<1% relative biomass) and log-transformation of the data to down-weight the effect of dominant species.

To find the species most responsible for the differences among the clusters, indicator species analysis (Dufrene and Legendre, 1997) was performed. We calculated relative biomass (RB) of each species for each group. The higher RB 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

Table 1
Frequency, biomass, and % biomass (i.e., relative biomass) for the ten species in Klamath River periphyton samples with the highest mean biomass and relative biomass (top 10 species were the same for both metrics, though their order is somewhat different), sorted by relative biomass. Minimum biomass for each species was zero. n = number of observations, Med. = median, S.D. = standard deviation, Max = maximum.

Species	n	% freq.	Biomass (% of total)				Biomass ($10^{10} \times \mu\text{m}^3 \text{m}^{-2}$)			
			Mean	Med.	S.D.	Max	Mean	Med.	S.D.	Max
<i>Epithemia sorex</i> Kützing	182	61	23.7	10.4	27.9	92.8	126.1	10.8	214.9	1205.9
<i>Cocconeis placentula</i> Ehrenberg	275	92	13.9	4.2	18.5	80.4	14.5	9.3	19.7	193.6
<i>Cymbella affinis</i> Kützing	168	56	9.1	2.6	15.1	81.5	45.3	1.8	130.7	994.0
<i>Gomphoneis herculeana</i> (Ehrenberg) Cleve	155	52	8.5	2.9	12.8	82.2	35.7	2.6	85.8	865.3
<i>Diatoma vulgare</i> Bory	157	53	5.5	1.1	11.5	73.8	19.7	1.0	64.8	767.5
<i>Ulnaria ulna</i> (Nitzsch) Compère	176	59	4.3	2.3	5.8	32.3	18.8	3.9	40.0	299.2
<i>Nitzschia frustulum</i> (Kützing) Grunow	295	99	3.7	2.0	4.5	28.9	9.6	3.4	27.7	379.5
<i>Diatoma tenuis</i> Agardh	137	46	3.0	0.0	7.4	52.0	8.4	0.0	23.6	215.8
<i>Calothrix</i> sp.	57	19	3.0	0.0	9.7	59.8	10.2	0.0	33.5	280.0
<i>Navicula tripunctata</i> (Müller) Bory	138	46	2.1	0.0	3.6	28.1	2.3	0.0	4.1	24.0

is indicative of its faithfulness to the group. The indicator species value is a product of the relative frequency and relative biomass 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 differed among sites or clusters, 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. The statistical significance ($\alpha = 0.05$) of the R statistic was evaluated with 999 permutations. ANOSIM with Euclidean distance was also used to compare environmental variables (e.g., alkalinity, air and water temperature, benthic Chl *a*, precipitation, flow, site-normalized flow (% annual median), SRP, TP, NH_3 , $\text{NO}_3^- + \text{NO}_2^-$, TIN, and TN) among sites or cluster groups after all variables were normalized. In addition, to test for significant differences ($\alpha = 0.05$) in individual environmental variables among clusters, we used Kruskal multiple comparison tests. All data analyses were performed in R (R Development Core Team, 2012).

3. Results

3.1. Spatial and temporal patterns of environmental conditions

Many environmental variables changed longitudinally in the Klamath River. For instance, during the June–October season when most periphyton samples were collected, flow increased longitudinally due to tributary inputs while nutrient concentrations (e.g., SRP and NO_3^-) decreased longitudinally as did planktonic Chl *a* to a lesser extent (Fig. 2). Site-normalized flow (i.e., flow as a ratio of a site's annual median flow) decreased longitudinally because flows at upstream sites are more constant through the year, in part due to regulation by dams. Water temperature exhibited a more subtle longitudinal pattern with lowest temperatures at the most upstream site (IG), rising to QU and remaining high until OR and then decreasing to TG upstream of the estuary. ANOSIM results revealed no significant differences in environmental variables among sites ($R = -0.021$, $p > 0.05$). However, based on Kruskal multiple comparison tests, almost all individual variables differed significantly ($p \leq 0.05$) among sites. In general, the four most upstream sites (IG, IB, QU, and SV) differed from the four most downstream sites (OR, KR, WE, and TG). For example, the upstream sites had significantly ($p \leq 0.05$) higher nutrient concentrations than the downstream sites.

Seasonal patterns were readily apparent as well. Flow decreased from May through September before rising slightly in October. Water

temperature peaked in August at all sites. At most sites, NO_3^- concentrations were lowest in July and August, with highest values occurring in October. As a result of these longitudinal and seasonal patterns, many environmental variables were highly correlated.

3.2. Periphyton assemblage characterization

Periphyton assemblages in the Klamath River were dominated by diatoms. On average, diatoms comprised 92.2% of sample's relative biomass, followed by cyanobacteria (7.0%). None of the other algal groups (e.g., cryptophytes or greens) contributed to an average of more than 1% of sample's relative biomass. There were a total of 127 species found in the samples. The mean species richness was 18. The ten taxa with the highest mean biomass included nine diatoms such as *Epithemia sorex* Kützing, *Cymbella affinis* Kützing, and *Gomphoneis herculeana* (Ehrenberg) Cleve and one cyanobacterium *Calothrix* sp. (Table 1). Among these top ten taxa, one diatom (*E. sorex*) with cyanobacterial endosymbionts and heterocystous *Calothrix* sp. possess the ability to fix nitrogen. The most frequently observed species (*Nitzschia frustulum* (Kützing) Grunow) had low mean absolute biomass and low mean relative biomass compared to the other nine species (Table 1).

Seventy percent of relative biomass of species was sensitive to nutrient enrichment. Nitrogen autotrophs of low organic nitrogen (taxa generally intolerant to organically-bound nitrogen) accounted for a mean of 39.2% and nitrogen autotrophs of high organic nitrogen (taxa tolerant to organically-bound nitrogen) accounted for a mean of 37.2%. Alkaliphilous taxa comprised, on average, the highest sample biomass (45.8%) followed by alkalibiontic taxa (33.0%). Benthic N-fixers comprised 29.7% of sample biomass while sestonic N-fixers contributed only 1.3%. Nitrogen-fixers included five taxa of cyanobacteria (the benthic *Calothrix* sp. and *Rivularia* sp., and the sestonic (i.e., free-floating) *Anabaena flos-aquae* (Linnaeus) Brébisson, *Aphanizomenon flos-aquae* (Linnaeus) Ralfs, and *Gloeotrichia echinulata* (Smith) Richter), and three species of diatoms with cyanobacterial endosymbionts (*E. sorex*, *E. turgida* (Ehrenberg) Kützing, and *Rhopalodia gibba* (Ehrenberg) Müller).

3.3. Major periphyton assemblage groups in the Klamath River

The cluster analysis identified three major groups of periphyton assemblages, which were significantly different ($p \leq 0.001$) from each other based on ANOSIM and pairwise comparisons. Group 1 had the highest percentage (3.6%) of sestonic species and both diatoms and cyanobacteria had intermediate biomass (mean 92.9% and 6.3%, respectively). The most abundant species in this group were *Cocconeis placentula* Ehrenberg (mean 30.9% biomass), *Diatoma vulgare* Bory (mean 9.7% biomass), and *G. herculeana* (mean 9.0% biomass). The best indicator species for this group included *C. placentula* (indicator

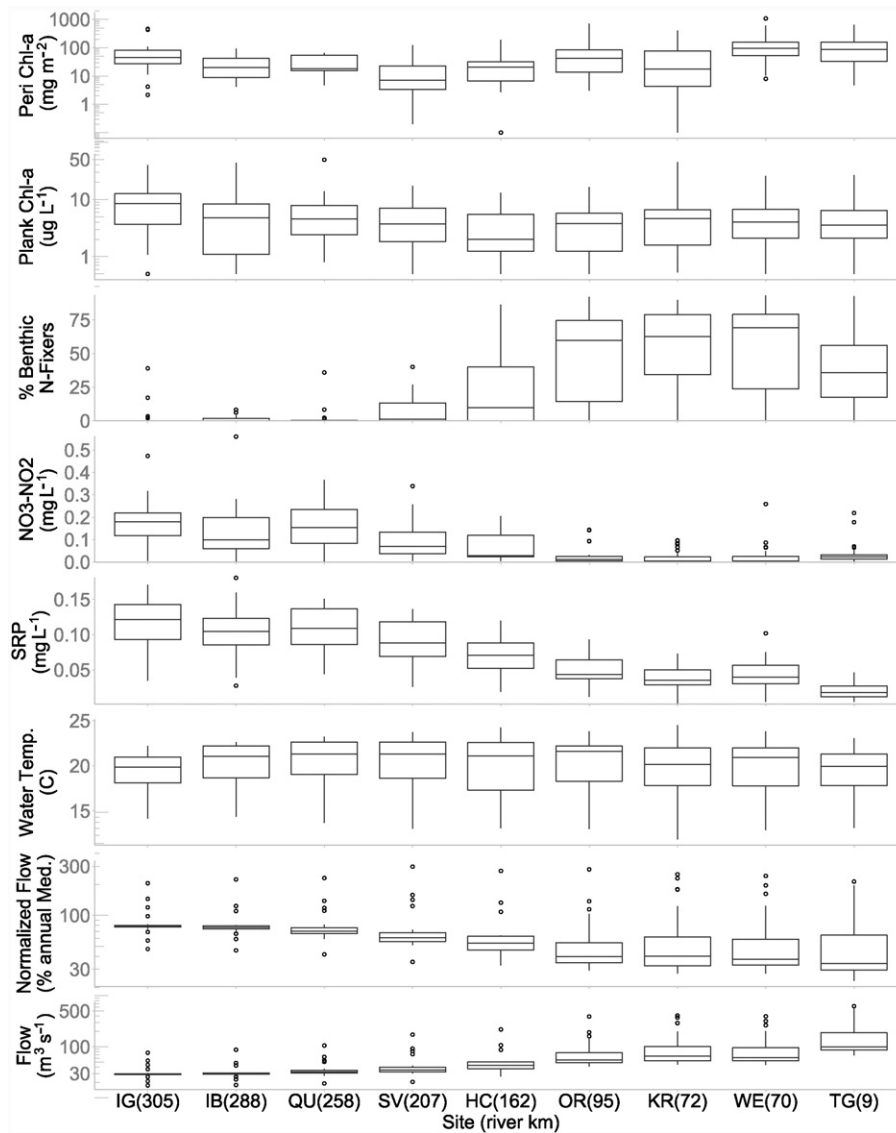


Fig. 2. Boxplots of periphyton metrics (top three panels) and environmental parameters (bottom five panels) for individual sites on the Klamath River for the months of June–October over nine year period (note: only downstream sites were sampled in May and there was only one sample per site in November). 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. Abbreviations: Peri Chl a, periphytic chlorophyll a [mg m^{-2}]; Plank Chl-a, planktonic chlorophyll a [$\mu\text{g L}^{-1}$]; $\text{NO}_3\text{-NO}_2$, nitrate–nitrite [mg L^{-1}]; SRP, soluble reactive phosphorus [mg L^{-1}].

value 0.80), *Navicula veneta* Kützing (indicator value 0.79), *Gomphonema angustatum* (Kützing) Rabenhorst (indicator value 0.55), and *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot (indicator value 0.55). In addition to diatoms, this group included two sestonic species of cyanobacteria *Aphanizomenon flos-aquae* (indicator value 0.14) and *M. aeruginosa* Kützing (indicator value 0.07). More than half of species biomass belonged to taxa which live in somewhat degraded conditions (mean 55.9% biomass). This group contained some pollution tolerant diatoms (e.g., *N. veneta*) and potential toxin producers (e.g., *M. aeruginosa*). Spatially, almost all upstream sites (IG, IB, QU, SV) belonged to this group. Temporally, 1/3 of all samples collected in August, September and October were in this group.

Group 2 was dominated by diatoms (mean biomass 96.3%), which reached their highest relative biomass here (compared to the other two groups), while cyanobacteria were least abundant in this group (1.9% biomass). The most abundant species in this group were *C. affinis* (mean 23.0% biomass), *Diatoma tenuis* Agardh (mean 13.8% biomass), and *G. herculeana* (mean 10.6% biomass). The best indicators

were *Achnanthes minutissimum* (Kützing) Czarnecki (indicator value 0.89), *D. tenuis* (indicator value 0.86), *Encyonema minutum* (Hilse) Mann (indicator value 0.78), and *Nitzschia dissipata* (Kützing) Grunow (indicator value 0.70). Species in this group were somewhat tolerant to nutrient and organic enrichment (mean 30.7% biomass). Spatially, this group included 1/3 of samples collected at downstream sites (OR, KR, WE, TG). Temporally, all May (note: May samples were collected only at downstream sites) and the majority of June samples were in this group.

The most dominant species in Group 3 included a diatom with cyanobacterial endosymbionts (*E. sorex*, mean 48.2% biomass) and another diatom *C. affinis* (mean 8.1% biomass). The N-fixing cyanobacterium *Calothrix* sp. had its highest mean relative biomass (5.4%) in this group compared to the other two groups. The best indicator species for this group was *E. sorex* (indicator value 0.88). The remaining indicators were all diatoms plus two cyanobacteria (*Calothrix* sp., indicator value 0.27 and *Rivularia* sp., indicator value 0.04), and one green alga (*Scenedesmus quadricauda* (Turpin) Brébisson, indicator value 0.31). In

addition to *E. sorex*, *Calothrix* sp., and *Rivularia* sp., other N-fixing species in this group included *R. gibba* (indicator value 0.20). Species in this group included eutrophic taxa (69.6% biomass), beta-mesosaprobic taxa (73.6% biomass) which live under 70–80% DO saturation and 2–4 mg L⁻¹ BOD, nitrogen autotrophs at low organic nitrogen (taxa generally intolerant to organically-bound nitrogen, 62.6% biomass), alkalibiontic (56.7% biomass), benthic N-fixers (60.2% biomass). Spatially, this group included approximately 2/3 of samples collected at downstream sites (OR, KR, WE, TG). Temporally, approximately 2/3 of all August, September and October samples were in this group.

ANOSIM results revealed no significant differences in environmental variables among cluster groups ($R = -0.001$, $p > 0.05$). However, Kruskal multiple comparison tests revealed significantly less nutrients (e.g., NH₃, NO₃⁻ + NO₂⁻, TIN, TN, TP, and SRP) at downstream sites (Groups 2 and 3) compared to upstream ones (Group 1).

3.4. Spatial variation of periphyton assemblages in the Klamath River

The ordination plot revealed a longitudinal gradient in periphyton species composition from upstream (upper right corner, Fig. 3) to downstream sites (left side and bottom, Fig. 3). Upstream sites (upper right corner, Fig. 3) were more similar to each other than to downstream sites (left side and bottom, Fig. 3). 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 Fig. 3). These longitudinal changes illustrated by the NMDS plots correspond to the three major periphyton groups identified by the cluster analysis.

Overall, based on ANOSIM results, there was a significant difference in species composition among sites ($R = 0.31$, $p = 0.001$). However, adjacent sites were more similar to each other than to more distant sites. Pairwise comparisons revealed that the four most upstream sites (IG, IB, QU, and SV within a distance of 98 km) and the most downstream one (TG) had significantly different ($p = 0.001$) species compositions from all other sites. The two middle sites (SV and HC within a distance of 45 km) were similar to each other but only site HC (162 river km) shared similar species with the downstream sites OR, KR, and WE (within a distance of 25 km). These results were significant even after adjusting the p -values with the Bonferonni correction ($p = 0.001$).

Individual autecological metrics superimposed on the NMDS plot (not shown) revealed that the relative biomass of N-fixers and nitrogen

autotrophs at low organic nitrogen conditions increased longitudinally, while sestonic species (most common at the two most upstream sites) and very tolerant to nutrient and organic enrichment species decreased longitudinally. Kruskal-Wallis test results showed significant differences ($p \leq 0.05$) in % benthic N-fixers among sites. The four upstream sites (IG-SV) had significantly less ($p \leq 0.05$) benthic N-fixers compared to the four downstream sites (OR-TG) with site HC (162 river km) showing intermediate relative abundances of benthic N-fixers (Fig. 2).

Abbreviations: Chl *a*, benthic chlorophyll *a* [mg m⁻²]; flow [m³ s⁻¹]; NO₃, nitrate-nitrite [mg L⁻¹]; site-normalized flow (% of median flow); SRP, soluble reactive phosphorus [mg L⁻¹]; water T, water temperature [°C]. See text for details.

3.5. Temporal variation of periphyton assemblages in the Klamath River

There appears to be a seasonal gradient in the periphyton assemblages from late spring-early summer (May–June) assemblages (lower right corner, Fig. 3) to late summer-early fall (August–October) assemblages (upper left corner, Fig. 3). Total biomass tended to increase in fall, and the relative biomass of N-fixers and nitrogen autotrophs at low organic nitrogen conditions increased toward the end of the summer and early fall when sestonic species (most common at the two most upstream sites) and species 'very tolerant' to nutrient and organic enrichment were also abundant.

Seasonally, the highest relative biomass of benthic N-fixers was observed in July–September at downstream sites. Nitrogen-fixers exhibited an interesting upstream migration with the progression of the summer (Fig. 4). While benthic N-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 benthic N-fixers were again constrained to downstream sites.

Benthic N-fixers, flow, and nitrate exhibited strong longitudinal and seasonal patterns. The apparent association between increasing upstream to downstream relative biomass of benthic N-fixers and decreasing NO₃⁻ + NO₂⁻ was stronger for the seasonal means ($r^2 = 0.74$, $p < 0.001$, Fig. 5) than it was for the individual samples. In contrast, the relationship between the June–September seasonal mean relative biomass of benthic N-fixers vs. flow revealed that while no overall linear relationship existed, flow was negatively associated with benthic N-fixers within many sites (see DWLS smoother; Fig. 6) indicating the effect of inter-annual flow variation on benthic N-fixers.

3.6. Periphyton assemblages and environmental variables

Environmental variables that correlated highly with the ordination space defined by species composition and that could potentially explain their patterns included TN, NO₃⁻ + NO₂⁻, SRP, and TP (Group 1), flow (Group 2), water temperature and benthic Chl *a* (Group 3) (Fig. 3, Table 2). Nutrients (e.g., TN, NO₃⁻ + NO₂⁻, SRP, and TP) defined a longitudinal gradient from upstream nutrient-rich sites (Group 1 in Fig. 3) to downstream sites (Groups 2 and 3 in Fig. 3) with lower nutrient concentrations. Water temperature and flow defined a second seasonal gradient from samples with low temperature and high flow (i.e., spring and early summer, Group 2) to samples with high temperature and low flow (i.e., late summer and fall, Group 3) as indicated by the opposite direction of their corresponding vectors (Fig. 3). Benthic Chl *a* concentrations also followed this second gradient, which captured the seasonal succession in periphyton assemblages from low biomass (i.e., spring and early summer, Group 2) to samples with high biomass (i.e., late summer and fall, Group 3).

4. Discussion

The 'lake and reservoir effects' are clearly reflected by the periphyton assemblages in the upper portion of the Klamath River (Asarian et al., 2014), an observation better conforming to the predictions of the serial

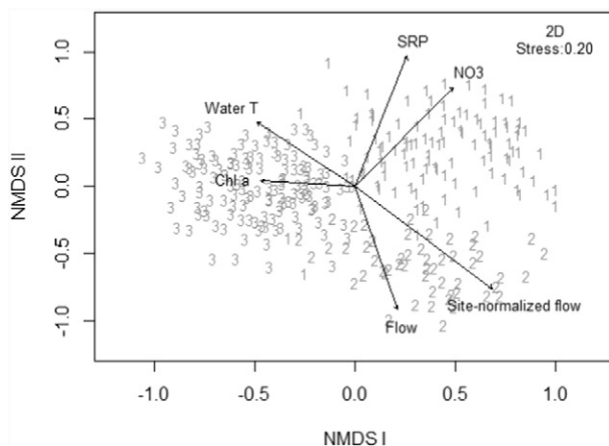


Fig. 3. Non-metric Multidimensional Scaling (NMDS) showing the relative similarity of periphyton assemblages for each sample, symbolized by the three major groups identified using cluster analysis. The distance between symbols indicates the relative similarity of the samples. Vectors indicate the significant environmental variables ($p < 0.05$) and their direction of largest change. Due to high correlation between some variables and ease of representation, only selected variables are displayed in the figure (see Table 2 for a complete list).

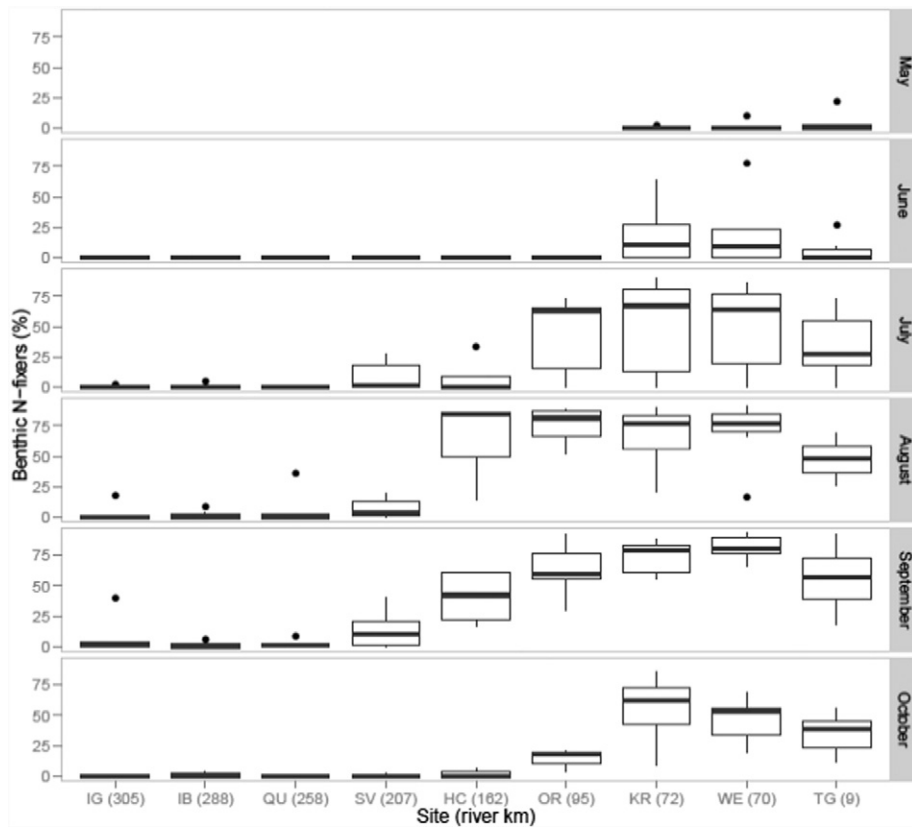


Fig. 4. Boxplots of percent biomass of benthic nitrogen-fixing periphyton species, by site (columns) and month (rows), for nine year period. Klamath River sites are arranged in downstream order (IG at left is most upstream, TG at right is most downstream). Note: only one sample per site in November.

discontinuity concept (SDC, Ward and Stanford, 1983) than to the RCC (Vannote et al., 1980). Similar to the SDC predictions, our results showed that nutrients and planktonic Chl *a* were higher at upstream sites. This finding was supported by the cluster analysis, which identified three statistically different periphyton groups where all upstream sites (IG, IB, QU, SV, most of HC) were grouped together (Group 1). Unlike the other two groups, Group 1 had the highest percentage (4.4%) of

sestonic species including *Aphanizomenon flos-aquae* and *M. aeruginosa*, two species of bloom-forming cyanobacteria that are prevalent directly upstream in the Iron Gate reservoir (Kann and Asarian, 2007) and Upper Klamath Lake (Kann and Smith, 1999, Eilers et al., 2004, Eldridge et al., 2013, Oliver et al., 2014). Although bloom-forming cyanobacteria decrease in the riverine section between Upper Klamath Lake and the upper river impoundments, they again increase

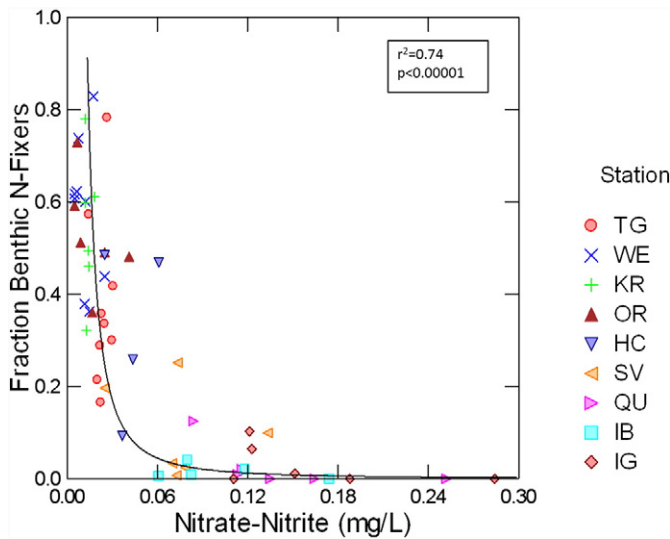


Fig. 5. Relative biomass of benthic N-fixers (%/100) vs. nitrate-nitrite ($\text{NO}_3^- + \text{NO}_2^-$) concentration (mg L^{-1}) for June–September seasonal means at Klamath River sites. Linear model was fit to log-transformed $\text{NO}_3^- + \text{NO}_2^-$ and logit-transformed relative benthic N-fixers; graph shown here is untransformed.

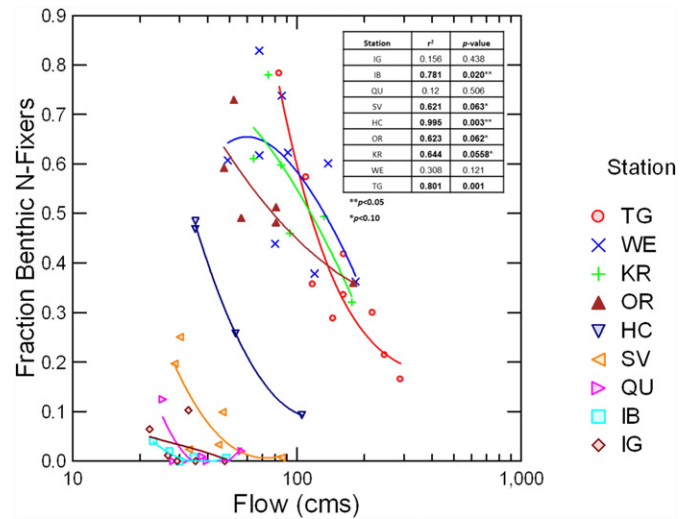


Fig. 6. June–September seasonal mean relative biomass of benthic N-fixers (%/100) vs. log June–September seasonal mean discharge ($\text{m}^3 \text{s}^{-1}$) at Klamath River sites shown with a Distance-Weighted Least Squares (DWLS) smoother fit to each site. The inset table shows r^2 and p -values for station linear models fit to log-transformed flow and logit-transformed relative biomass of benthic N-fixers.

Table 2

Results from the environmental vector fitting in the ordination space of the NMDS plot with variable scores along the two ordination axes (NMDS I–II), goodness-of-fit statistic (r^2) and its significance (p-value). Results sorted on r^2 .

Variable name	NMDS I	NMDS II	r^2	p-Value
Site-normalized flow (% of median flow)	0.678	−0.735	0.617	0.001***
Total nitrogen (mg L^{-1})	0.368	0.930	0.600	0.001***
Soluble reactive phosphorus (mg L^{-1})	0.290	0.957	0.584	0.001***
Total phosphorus (mg L^{-1})	0.283	0.959	0.575	0.001***
Flow ($\text{m}^3 \text{s}^{-1}$)	0.227	−0.974	0.514	0.001***
Total inorganic nitrogen (mg L^{-1})	0.573	0.819	0.477	0.001***
Nitrate–nitrite (mg L^{-1})	0.569	0.823	0.462	0.001***
Alkalinity ($\text{mg L}^{-1} \text{CaCO}_3$)	−0.476	0.880	0.381	0.001***
Water temperature ($^{\circ}\text{C}$)	−0.736	0.677	0.273	0.001***
Benthic chlorophyll <i>a</i> (mg m^{-2})	−0.986	−0.165	0.122	0.001***
Ammonia (mg L^{-1})	0.570	0.822	0.120	0.001***
Planktonic chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	−0.083	0.997	0.100	0.001***
Air temperature ($^{\circ}\text{C}$)	−0.112	0.994	0.059	0.001***
Precipitation (mm)	0.478	−0.878	0.003	0.730

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

p-Values based on 1000 permutations.

in the lacustrine habitat of Copco and Iron Gate reservoirs (Kann and Asarian, 2007), and such sestonic species (i.e., *Microcystis*) have been observed settling onto periphyton in the upper river reaches as well as transported downstream to the river estuary (Otten et al., 2015). Similar to the SDC predictions, benthic macroinvertebrates also show longitudinal patterns, with higher percent composition of filter-feeders in upstream and middle reaches (where Group 1 occurred in our study) than downstream (Malakauskas and Wilzbach, 2012). In addition, the assemblages in Group 1 were dominated by pollution tolerant species (e.g., *C. placentula*, TP optima $82.5 \mu\text{g L}^{-1}$; *N. veneta*, TP optima $58.21 \mu\text{g L}^{-1}$; *R. abbreviata*, TP optima $62.81 \mu\text{g L}^{-1}$) (Potapova et al., 2004).

The 'lake and reservoir effects' in the Klamath River diminish with distance downstream. The downstream reaches of the Klamath River are characterized with high periphyton biomass and assemblages dominated by N-fixing diatoms (e.g., *E. sorex*) and cyanobacteria (*Calothrix* sp.) that reflect overall N-limited conditions (Hill and Knight, 1988, Power et al., 1988, Peterson and Grimm, 1992). Indeed, our results revealed significantly less nutrients (e.g., NH_3 and SRP) in downstream reaches (Groups 2 and 3) than upstream (Group 1). This inverse relationship between periphyton biomass and nutrients can be explained by the ability of benthic N-fixers to overcome nitrogen limitation. A number of studies have shown inverse relationships between nitrogen concentrations and the relative abundance of N-fixers (Porter et al., 2008, Stancheva et al., 2013, Carpenter et al., 2014), endosymbiont biomass (Stancheva et al., 2013), and the number of endosymbionts within a diatom cell (DeYoe et al., 1992). The inverse relationship between periphyton biomass and nutrients may be amplified by sampling design and the sampling protocol used in this study. The protocol targets microscopic diatoms from cobble substrates which may adequately characterize downstream periphyton assemblages; however, the high nutrient concentrations in the upper stream reaches in the Klamath River, support an extensive amount of filamentous algae (e.g., *Cladophora* sp.) and macrophytes (e.g., *Potamogeton* sp., *Elodea* sp.). To capture filamentous algae and macrophytes as well as periphyton attached to their surface may require a reach-scaled sampling design. For instance, Stevenson and Bahls (1999) proposed a rapid semi-quantitative sampling protocol to capture reach-scale periphyton biomass. Periphyton biomass including filamentous macroalgae can be estimated in multiple sampling locations along multiple-transects in a stream reach by immersing a clear-bottom bucket with 50-dot grid. The protocol used in this study is not designed to adequately characterize filamentous algae or macrophytes and consequently sampled epilithic periphyton biomass in the upper streams may not reflect all primary producers in the river ecosystem. 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 our scientific understanding of the Klamath River.

Our study found that benthic N-fixers, which dominated Group 3, were largely constrained to the downstream reaches of the river (below site HC, 162 river km). These patterns in N-fixer biomass 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) especially during the summer and fall seasons (Oliver et al., 2014). Nitrate concentrations in the lower reaches of the Klamath River often drop below 0.05 mg L^{-1} 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). Similar to results from a review paper evaluating 22 streams where N-fixation was highest between June–August (Marcarelli et al., 2008), the Klamath River N-fixers also occurred primarily in the summer (July–September). Marcarelli and Wurtsbaugh (2006) 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).

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 (when river flows are higher) had more diatoms, fewer cyanobacteria, and a lack of N-fixers compared to late summer–fall assemblages (when river flows are lower), 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*). Low flows can have positive effect on algal growth by supplying nutrients, while high flows can have negative effects by scouring attached algae (Stevenson, 1996). In the Klamath River, high flows from snow melt and precipitation in spring and early summer result in the lowest periphyton biomass. After storm events, the periphyton assemblages are reduced to a thin film of scour-resistant diatoms (Steinman and McIntire, 1990, Mulholland et al., 1991). There were significant reductions ($p \leq 0.05$) in benthic Chl *a* before and during the monsoon floods in a New Mexico river network (Tornés et al., 2015). Similar to our findings, a study conducted in the nearby North Umpqua River also found that streamflow was the most important variable structuring periphyton assemblages (Carpenter et al., 2014). However, periphyton assemblages might not respond to high flows as well as individual species, which can better withstand such disturbances (Stevenson, 1990). The positive effect of low flows in summer and fall result in higher periphyton biomass because of increased nutrient supply (Pan et al., 1996, Porter et al., 2008, Stevenson et al., 2008). In addition, the seasonal effects of flow on periphyton assemblages we also observed that year-to-year variability in flow influenced benthic N-fixer proportion with higher flows leading to an overall lower proportion of benthic N-fixers.

The seasonal shift in species composition also coincides with longitudinal declines in nutrients, which are more pronounced in the summer and fall (Oliver et al., 2014). 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 biomass of N-fixers in our study were recorded in 2009, 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., 2013).

5. Conclusion

The 'lake and reservoir effects' are clearly reflected by the periphyton assemblages in the heavily regulated Klamath River and these effects diminish in downstream direction, an observation better matching the predictions of the SDC (Ward and Stanford, 1983) than the RCC (Vannote et al., 1980). We found that periphyton assemblages in the river are associated with both seasonal variations in flow conditions (e.g., decreasing flow from spring to fall) and longitudinal gradients in nutrient concentrations (e.g., decreasing from upstream to downstream). These variations were most pronounced in late summer (after July) and at downstream locations (below site HC, 162 river km), which had lower nutrient concentrations but higher algal biomass. This inverse relationship between periphyton biomass and nutrients can be explained by the ability of benthic N-fixers to overcome nitrogen limitation. Inter-annual variations in flow also played a role in seasonal mean benthic N-fixer proportion with higher flows leading to a lower proportion of benthic N-fixers at most Klamath River sampling sites. The results from this study can benefit ongoing efforts to improve the Klamath River water quality conditions (e.g., nutrient criteria development) and ecological restoration (e.g., dam removal).

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