Taxon-specific photosynthetic responses of attached algal assemblages to experimental translocation between river habitats

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Abstract: Attached algal and cyanobacterial taxa differ in their ability to exploit and tolerate the diversity of flow, irradiance, and temperature regimes typical of a heterogeneous riverscape. Understanding the drivers of the small-scale variation in algal taxonomic composition helps us predict the riverscape-scale effects of altered flow regimes, but microhabitat-scale variation in algal taxonomy complicates the interpretation of ecosystem-scale estimates of biomass or primary production. Using pulse-amplitude modulated (PAM) fluorometry, we performed 2 manipulative field experiments (in 2014 and 2015) to measure photosynthetic responses of algae and cyanobacteria to depth, temperature, and flow modifications. In 2014, we exposed 6 attached algal assemblages common to the South Fork Eel River (California, USA) to a 24-h incubation on either the river bottom (20 cm deep) or floating at the water surface. In 2015, we incubated 3 algal assemblages for 1 wk in either the thalweg or at the river's edge. For PAM measurements, we developed a novel method (Photosynthesis–Irradiance Periphyton Experimental System [PIPES]) for manipulating attached filamentous algae, a morphology common in aquatic habitats but underrepresented in photosynthesis experiments. To make the PIPES, we sandwiched thin (<1 mm) layers of filamentous attached algae between 2 layers of mesh so that the algae could be isolated and manipulated for repeated PAM measurements. In the 2014 experiment, incubating *Cladophora*, *Rivularia*, *Microcoleus*, and *Anabaena* at the water surface tended to decrease photosynthetic rates relative to submerged controls, whereas for *Nostoc*, the photosynthetic rates were higher in floating treatments. In the 2015 experiment, *Cladophora* and *Oedogonium* incubated in the warmer, low-flow river margin had persistently lower photosynthesis rates than their counterparts incubated in the thalweg. The PIPES method improves our ability to make PAM measurements on attached algae. PIPES can be used in conjunction with other methods to evaluate taxon-specific responses to environmental conditions and to help us predict how algal assemblages will shift in dominance under different river management regimes.

Keywords: photosynthesis, PAM, algae, cyanobacteria, *Cladophora*, *Microcoleus*, benthic, flow, temperature, microhabitat, electron transport rate

River environments are characterized by extreme spatial heterogeneity in abiotic factors (Frissell et al. 1986) that affect the growth and distribution of attached algae and cyanobacteria (hereafter, algae). At any given location, abiotic conditions fluctuate over time. For example, changes in solar radiation, habitat connectivity, or discharge alter light, temperature, velocity, shear stress, or substrate stability (Power and Stewart 1987, Resh et al. 1988). Attached algae can adjust to these

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changes in stresses and resource fluxes by modifying their physiology, morphology, or behavior (Bold and Wynne 1985, Stevenson et al. 1996). Algal taxa differ greatly in their tolerances of shear stress and temperature fluctuations and in their requirements for light and specific nutrients (Douglas 1958, Lowe and Pan 1996). Knowledge of how specific taxa respond to abiotic changes over hours, weeks, or seasons can help us predict which taxa will dominate assemblages under given environmental regimes.

At any time, spatial variation in algal composition on riverbeds reflects past events as well as present conditions. For instance, flood scour often determines the availability of bare substrate for algal colonization relative to substrates where algal residues persist from the previous summer. Annual variation in winter flows also determines which guilds of algae-grazers will dominate during the following summer (Wootton et al. 1996, Power et al. 2008). Riparian vegetation, channel and valley geomorphology, and daylength control light availability at the reach scale, which in turn leads to spatial and temporal variability in algal composition and photosynthesis (Hill et al. 1995, Finlay et al. 2011).

Changing flow and temperature regimes can markedly shift the autotrophic base of rivers in summer, which profoundly affects river food webs (Power et al. 2013, 2015). Some highly nutritious algae (e.g., diatoms) are foundational for river food webs, whereas other taxa (filamentous chlorophytes or cyanobacteria) are less nutritious and sometimes toxic (Vadeboncoeur and Power 2017). Even flow changes of ~20 cm/s have been shown to shift algal assemblages between filamentous chlorophytes and cyanobacteria (Hart et al. 2013). Differences among algal taxa in their photosynthetic response to changing abiotic conditions underlie emergent patterns in algal species composition.

Traditional tools, such as biomass monitoring and oxygen sondes, help us detect and understand mesoscale spatiotemporal variation in algal abundance and metabolism in rivers but are less effective for assessing variation on a smaller scale. For example, during summer low flow, microhabitat-scale variation in substrate, flow, and temperature (Biggs 1996) as well as immediate biotic pressures, such as competition and grazing (Lamberti and Resh 1983, Power 1995), produce a taxonomic mosaic of algae on the riverbed. River ecologists generally respond to this microhabitat-scale variation in algal taxa by increasing sample size to attempt to estimate average biomass or primary production. However, understanding the drivers of the small-scale variation in algal taxonomic composition on riverbeds helps us predict how altered flow regimes will ultimately manifest themselves in different algal assemblages with different ecological effects at the riverscape scale.

PAM fluorometry

Historically, there has been a dearth of tools for rapid assessment of photosynthetic performance by different algae under ranges of environmental conditions, but pulse-amplitude

Table 1. List of notations and acronyms related to photosynthetic processes and experimental tools used in this paper.

Term	Explanation
a	Alpha: The slope of the initial linear portion of the PE curve or RLC at low irradiance values. This parameter represents the light-use efficiency for photosynthesis at low light concentrations.
F_o	Minimum fluorescence of a dark-adapted alga. Calculated with weak light that is not strong enough to induce photosynthesis.
F_m	Maximum fluorescence of a dark-adapted alga. Calculated with a saturating pulse which closes all PSII reaction centers generating Chl *a* fluorescence.
F_v / F_m	Maximum quantum efficiency (electrons produced/photon absorbed) of PSII photochemistry: ($F_m - F_o$) / F_m.
PAM	Pulse-amplitude modulation.
PE	Photosynthesis irradiance: P is photosynthesis rate (mg C mg^{-1} Chl *a* h^{-1}), and E is radiant flux (μmol photons m^2/s).
PIPES	Photosynthesis-Irradiance Periphyton Experimental System: A method for manipulating filamentous algae, cyanobacteria, and their epiphytes to collect PAM fluorometry data.
P_{max}	Maximum photosynthetic rate (the asymptote of a PE curve).
PSII	Photosystem 2.
rETR	Relative electron transport rate: The rate of electron transport through photosystem II. PAM fluorometry estimates this value as a function of Chl *a* fluorescence and irradiance. Without calibration, the units are arbitrary. Hence the rate is relative.
$rETR_{max}$	Light-saturated relative electron transport rate (the asymptote of the RLC curve).
RLC	Rapid light curve: A type of PE curve generated with PAM fluorometry by sequentially acclimating algae to a series of 9 light intensities, where the duration of acclimation at each intensity ≤30 s.

modulated (PAM; see notation definitions in Table 1) fluorometry measures photosynthetic irradiance on intact biofilms rapidly and non-destructively (Consalvey et al. 2005) and can measure at small scales. Rapid light curves (RLCs) generated by PAM accurately detect diel patterns in photosynthesis (Chevalier et al. 2010). PAM fluorometry is an excellent tool for monitoring the photosynthetic performance of an alga before and after an experimental perturbation (Hancke et al. 2008).

PAM fluorometry is based on the relationship between Chl *a* efficiency and light intensity that was first described by collecting photosynthesis–irradiance (PE) curves. Algae use light as an energy source to fix C from CO_2, and they acclimate to the light environment by adjusting the amount of their cellular machinery devoted to harvesting light. The predictable relationship between the rate of C fixation and light is described by a PE curve, where P is the photosynthesis rate in mg C mg^{-1} Chl *a* h^{-1}, and E is the radiant flux of photons/unit area of a surface (μmol m^2/s; Falkowski and Raven 2007, Kirk 2011). At low light intensities, early and late in the day, there is a linear relationship between the rate of C fixation and light intensity. The slope of this linear portion of the curve, alpha (α), represents light-use efficiency at low light. Eventually, increases in light do not yield higher photosynthetic rates—the PE curve asymptotes because the cells lack the cellular capacity to use additional light. This maximum photosynthetic rate (P_{max}) of attached algae usually occurs around the highest light intensities that algae experience during the day, but maximum intensity varies among assemblages and across environments, depending on riparian and landscape shading, water depth, and self-shading within the mat (biofilm thickness). High light intensity can cause photoinhibition, a decline in photosynthetic efficiency stemming from Rubisco binding with O_2 instead of CO_2, oxidation of pigments by free radicals, and light being dissipated as heat by photoprotective carotenoids (Kirk 2011).

PAM fluorometry detects changes in chlorophyll fluorescence associated with the rate of electron transport in photosystem II (PSII) and generates RLCs based on relative electron transport rates (*r*ETR). These RLCs are analogous to PE curves based on gas exchange (White and Critchley 1999, Ralph and Gademann 2005). Because RLCs are faster to collect and do not disturb algae as much as PEs, RLCs enable experimental designs and data collection that would otherwise be impractical with PEs. However, natural algal biofilms can vary from almost-invisible, thin skins coating a rock to turfs many centimeters deep and filaments many meters long. This variation in biofilm thickness impedes comparison of different algal assemblages using PAM fluorescence because PAM is most accurate when used on biofilms of uniform thinness (Perkins et al. 2002, Consalvey et al. 2005). Standardizing PAM measurement is particularly challenging for filamentous morphologies, and these morphologies are underrepresented in PAM fluorometry experiments and data sets.

In the present study, we used PAM fluorescence to assess how different algal taxa responded to translocation among habitats in a heterogeneous environment. We measured PAM RLCs on replicate biofilms dominated by either cyanobacteria, diatoms, or filamentous green algae to assess how these algal taxa responded to experimental short-term (hours–days) manipulations of light and flow conditions in the South Fork Eel River (SF Eel River) of northwestern California, USA. Photosynthetic rates and RLC parameters provide a proximate metric of algal responses to stresses. We were particularly interested in developing tools to assess the environmental conditions that would foster dominance by different algal assemblages. Therefore, we developed the Photosynthesis–Irradiance Periphyton Experimental System (PIPES) to translocate algae. The PIPES establishes a more uniform biofilm, which helps standardize PAM fluorometry on filamentous algal asemblages. Our study was an exploratory experiment in which we chose the realism of in-situ incubations over the precise control possible with laboratory manipulations of flow and light. We expected that cyanobacterial taxa would be more tolerant of low-flow and high temperature conditions than either diatoms or chlorophytes. Predicting how changing environmental conditions will alter dominance among algal taxa will inform understanding, protection, and management of river ecosystems.

METHODS

To address our research objectives, we used PAM fluorometry in 2 field experiments to monitor the photosynthetic response of common attached algal assemblages in the SF Eel River to differences in their light and flow environment. Our experiments occurred at a sunlit, mainstem reach (drainage area ~153 km^2) of the SF Eel River within the Angelo Coast Range Reserve in Mendocino County, California, USA (Fig. S1A; lat 39.719255, long 123.652614). The 200-m study reach has a bed of sandstone and mudstone-shale, thinly overlain with winter-mobile gravels, pebbles, and cobbles. Our study took place during the summers of 2014 and 2015, 3 and 4 y, respectively, after the onset of a severe California drought that began in 2011. In 2014, algal assemblages were floated at the river's surface to assess the effect of light and temperature on their photosynthetic performance. In 2015, algal assemblages were translocated between habitats to understand how flow and temperature affect photosynthesis. For both these experiments, PIPES were used to make PAM fluorometry measurements.

Study site: The Eel River watershed

The SF Eel River watershed is under Mediterranean seasonality and receives most rainfall between October and April (USGS 2020). During normal winter flooding, bed scouring discharges (~120 m^3/s) are attained or exceeded in this reach of the SF Eel River every 1 to 2 y, but flow typically drops

below 1 m^3/s during summer. During summer low flow, velocities are too slow to mobilize even small bed sediments, and most river channel habitat consists of long shallow pools <2 m deep, connected by short riffles (Power 1990a, Finlay et al. 2002), and filamentous algal mats often detach from substrates and form floating mats that accumulate along channel margins.

Seasonal phenology of attached algal assemblages in the upper SF Eel River is well documented (Power 1990b, Power et al. 2008, 2013, Furey et al. 2012). As in many other temperate lakes and rivers worldwide (Whitton 1970, Dodds and Gudder 1992, Zulkifly et al. 2013), *Cladophora glomerata* (L.) Kützing, 1843 is a dominant macro-algal component of summertime attached algae assemblages in the rivers of northern California. In late spring, as days lengthen and flows warm and subside, profuse, vegetative growth of *C. glomerata* is initiated from basal *C. glomerata* cells that survived winter flood scour on stable boulder and bedrock substrates. New *C. glomerata* growth is green, but filaments develop a yellow hue as they became colonized by a monolayer of epiphytic diatoms. By midsummer, *C. glomerata* fronds and associated epiphytes cover much of the surface area of coarser (bedrock, boulder, large cobble) substrate on the SF Eel River's riverbed, and these assemblages turn rusty-red as later successional epiphytic diatoms form layers 5 to 10 cells deep on the *Cladophora* host (Power et al. 2009). Thus, green, yellow, and red *Cladophora* are identifiable successional stages, but multiple stages occur in the river at any given time because of spatial variation in flow, solar radiation, and other conditions.

Epilithic assemblages typically include several other taxa besides *Cladophora*, with assemblage composition influenced by abiotic conditions. In areas subjected to intense winter scour, epilithic assemblages are dominated by diatoms, shorter filamentous green algae (e.g., *Oedogonium* Link ex Hirn, 1900), or cyanobacteria. In high-flow riffles, epilithic diatoms cover rock substrates as thin, slippery skins, dominated by *Cocconeis placentula* Ehrenberg, 1838 and *Epithemia adnata* (Kützing) Brébisson, 1838. Rocks in riffles also support extremely adnate, N-fixing cyanobacteria in the genus *Rivularia* C. Agardh ex Bornet and Flahault, 1886. In sunny, fast-flowing reaches, disk-shaped colonies of *Rivularia* several mm in diameter but <0.1 mm thick may coalesce to larger dark black patches. Globulous soft, globulus firm, and ear-like colonies of *Nostoc* Vaucher ex Bornet and Flahault, 1886 spp. occur in riffle and pool habitats throughout the SF Eel River.

In recent years, potentially toxic cyanobacteria have become a more visible component of the attached algal mosaic in the SF Eel River. Incipient *Anabaena* Bory ex Bornet and Flahault, 1886 colonies begin as small, dark blue-green spots on *Cladophora* fronds. They spread vegetatively but are also motile and eventually completely blanket short, red *Cladophora* fronds, forming dense, mucous-rich, dark blue-green *Anabaena* spires (Bouma-Gregson et al. 2017, 2018). Another motile cyanobacterium, *Microcoleus* Desmazières ex Gomont, 1892 (*M. autumnalis* [Gomont] Strunecky, Komárek, and J. R. Johansen, 2013; basionym *Phormidium autumnale* Gomont, 1892) (Strunecký et al. 2013) occurs on rocks under a wide range of light conditions, from shaded tributaries to sunny mainstems, but seems to be restricted to faster-flowing sites.

PIPES

We developed PIPES to allow researchers to isolate algal taxa or assemblages and spread them in thin layers on a deployment system where they can be measured repeatedly with PAM. We constructed PIPES from 2 squares (5 × 5 cm) of 0.3-mm, no-see-um mesh (Seattle Fabrics, Seattle, Washington), a 5-cm segment of Schedule 40 polyvinyl chloride (PVC) pipe (inner diameter = 2.54 cm, outer diameter = 3.3 cm), and a soft plastic ring (a snap-cap lid with a 1.75-cm diameter hole in its center) that fit snugly on the end of the PVC pipe. Using fingers and forceps, we carefully spread, as uniformly as possible, a thin (<1 mm) layer of an algal assemblage over 1 square of no-see-um mesh. We then placed the 2nd piece of mesh on top of the algae and centered the algae-mesh sandwich over the lumen of an upright segment of PVC pipe. We affixed the plastic ring to the end of the pipe, holding the mesh in place without covering the algae (Figs 1A–E, 2A–F). We also constructed PIPES without a biofilm to provide a control for fluorescence of the mesh and for any fouling that occurred during deployment in the river. Critically, creating thin layers of algal biofilms reduces variability in RLC parameters resulting from variable biofilm structure.

PAM fluorometry

We used PAM fluorometry to compare the photosynthetic responses of SF Eel River attached algae to translocation between different habitats. We measured an RLC with a DIVING-PAM (WinControl 3.16 software; Walz, Effeltrich, Germany) for each isolated algal type before and after the experimental manipulations described below. Each sample was dark adapted (see below for methods of dark adaption for each experiment) prior to taking PAM measurements. We fixed the PAM fiber optic cable 3 mm above the sample, with an illumination area of 0.238 cm^2 and excitation wavelength of 655 nm. We set the gain to 2 because this setting consistently yielded minimum fluorescence values (F_o) values >300 fluorescence units across all algal assemblages. Immediately upon placing the fiber optic assembly on the dark-adapted algal sample, we recorded its maximum (F_m) and minimum (F_o) fluorescence. Maximum variable fluorescence (F_v / F_m = (F_m – F_o) / F_m) of a dark-adapted sample is a measure of the maximum quantum efficiency, electrons produced/photon absorbed by PSII (Genty et al. 1989, Schreiber et al. 1995). Next, we ran an RLC using 9 light intensity steps ranging from 0 to 1843 or 0 to 3711 μmol m^2/s,

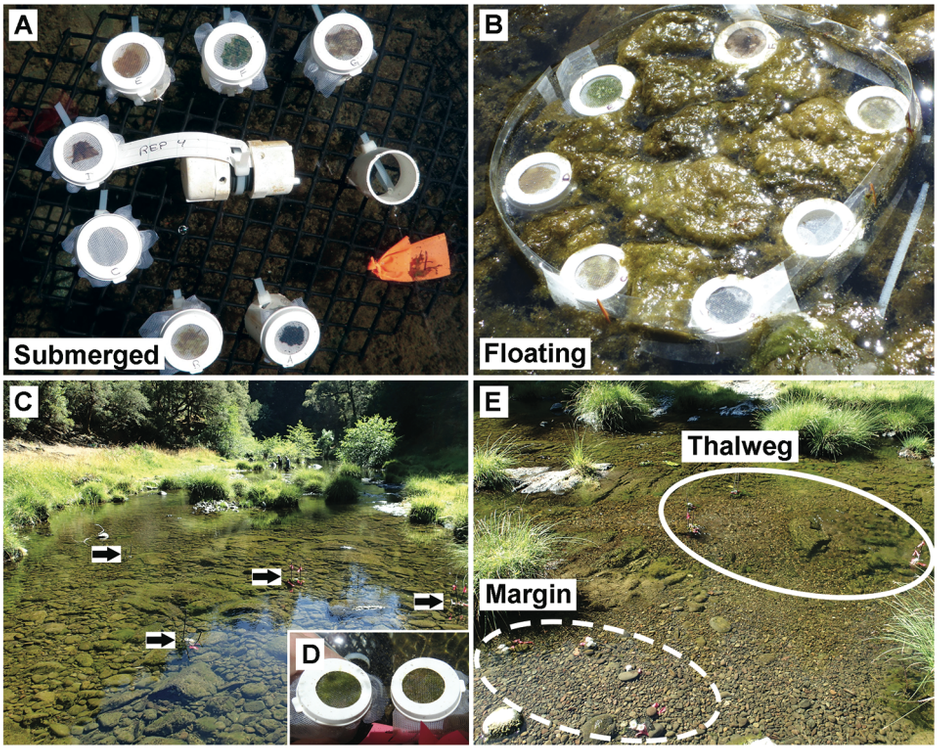

Figure 1. Photosynthesis-Irradiance Periphyton Experimental System (PIPES) deployments. Submerged (A) and floating (B) experimental treatments in 2014 (PIPES outer diameter 3.3 cm). Different taxa were placed on each PIPES for pulse-amplitude modulated fluorometry measurements, and the temperature logger was fixed to the center of the array. Experimental reach (South Fork Eel River, California, USA) used in 2015 (C), arrows indicate the location of samples. *Cladophora* and *Oedogonium* PIPES from 2015 (D). Thalweg and margin treatments from 2015 experiment (E).

for the 2014 and 2015 experiments, respectively. The algae were exposed to each light intensity for 20 s (RLC width = 20) before the application of a saturating pulse. This range of light intensities coincides with maximum solar irradiance intensities experienced by the benthic algae during the brightest part of the day, based on terrestrial photosynthetically active radiation sensors deployed in the nearby South Meadow at the Angelo Reserve (http://angelo.berkeley.edu/data/meteorological-data/).

From these data, we generated RLCs that were well described by the hyperbolic tangent function (Jassby and Platt 1976), which does not include a photoinhibition parameter. Light-saturated electron transport rate ($r\mathrm{ETR}_{max}$) is the asymptote of the RLC and analogous to maximum photosynthesis (P_{max}) of PE curves measured using oxygen or C. Again, light-use efficiency (α) is the slope of linear portion of the curve at low irradiance values. Maximum variable fluorescence (F_v / F_m) of a dark-adapted sample is proportional to the number of electrons generated by PSII for each photon absorbed. Any stress or damage to PSII will decrease F_v / F_m, therefore, it can be used to evaluate photosynthetic stress of an assemblage (Consalvey et al. 2005, Murchie and Lawson 2013).

24-h depth manipulation (2014)

To understand the effect of the warm stagnant conditions in floating algal mats on photosynthesis of various assemblages, we compared the RLC parameters for 6 algal assemblages in experimental arrays either on the river bottom (submerged control; Fig. 1A) or at the water surface (floating treatment; Fig. 1B) for 24 h. The taxa (referring to the visually dominant alga in an assemblage) used in this July 2014 experiment (Fig. 2A–F) were: yellow *Cladophora* (*C. glomerata* epiphytized by diatoms including *Rhoicosphenia* Grunow, 1860, *Gomphonema* Ehrenberg, 1832, and *Ulnaria* [Kützing] Compère, 2001); red *Cladophora* (*C. glomerata* heavily epiphitized by diatoms in the Rhopalodiaceae: *Epithemia turgida* [Ehrenberg] Kützing, 1844, *E. sorex* Kützing, 1844, and *E. gibba* Ehrenberg [Kützing], 1844); *Rivularia* (*Rivularia haematites* C. Agardh ex Bornet and Flahault, 1886); *Nostoc* (amorphous colonies of *Nostoc verrucosum* Vaucher ex Bornet and Flahault, 1886); the highly motile *Microcoleus*; and motile *Anabaena* (spires of *A. oscillarioides* Bornet and Flahault, 1886).

We carefully removed the algal assemblages of interest from rocks and sediments collected from the SF Eel River in the Angelo Coast Range Reserve on 22 July 2014. We removed

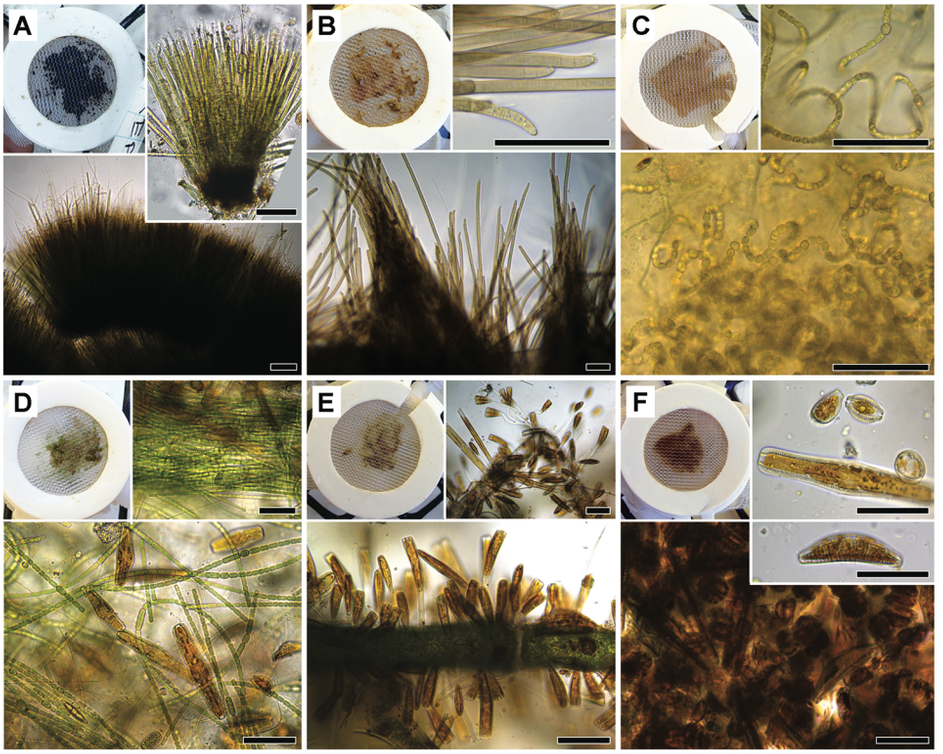

Figure 2. The algal and cyanobacterial taxa used in the 2014 experiments. Each panel shows a taxon contained within the Photosynthesis-Irradiance Periphyton Experimental System (PIPES), along with the representative microscopic views. A.—*Rivularia haematites* (Cyanobacteria). B.—*Microcoleus autumanalis* (Cyanobacteria). C.—*Nostoc verrucosum* (Cyanobacteria). D.—*Anabaena oscillarioides* (Cyanobacteria) with *Epithemia gibba* embedded in the mucilage. E.—*Cladophora glomerata* (yellow) epiphitized by diatoms such as *Rhoicosphenia*, *Gomphonema*, and *Ulnaria*. F.—*C. glomerata* (red) heavily epiphitized by diatoms in the Rhopalodiaceae (*Epithemia gibba*, *E. sorex*, and *E. turgida*. Scale bar = 50 μm for all images.

C. glomerata and *Anabaena* by detaching portions of filamentous fronds with forceps, and we removed the remaining more adnate epilithic assemblages by scraping or picking off colonies with spoons or forceps. We did not make precise flow and depth measurements at the harvesting sites, but we generally collected *Cladophora*, *Rivularia*, and *Anabaena* from shallow pools with water flowing <10 cm/s, whereas we collected *Nostoc* and *Microcoleus* from riffles with flows >10 cm/s, and collection depths were 20 to 50 cm.

After collection, we microscopically (Optiphot-2; Nikon, Tokyo, Japan) confirmed the composition of the assemblages, stored them overnight in river water in glass fingerbowls placed on ice in an opaque cooler, and constructed the PIPES for each algal assemblage the following day. Identification followed Diatoms of North America (https://diatoms.org) and taxonomy texts (Komárek and Anagnostidis 2007, Komárek 2013, Wehr et al. 2014).

Each experimental unit consisted of an array of 7 PIPES, of which 6 each had a different algal assemblage and 1 was a PIPES without algae (fluorescence control), affixed with nylon cable ties to a 30 × 30-cm square of black plastic mesh (Vexar®; MasterNet, Mississauga, Ontario). We arranged PIPES in a circle on the mesh, with the position of each assemblage on a given array determined randomly (Fig. 1A–E). We attached a DS1922L iButton® temperature logger (Maxim Integrated, San Jose, California), encased in a flow-through PVC capsule, in the center of each array to measure temperature at 15-min intervals throughout the ~24-h experiment. We randomly assigned 3 arrays to a floating treatment and 3 arrays to submerged controls on the bottom of the stream to create 3 blocks of paired treatments. We placed each submerged control array (depth = 20 cm) within 30 cm of a floating treatment array (algae covered by <1 cm of water) in the same stream microhabitat. We used nylon cable ties to anchor each array to a piece of rebar driven into the streambed.

Time course of deployment and measurements We constructed experimental arrays in the field under low light, early in the afternoon of 23 July 2014. We kept algae in the dark except when we were actively constructing the PIPES. After assembling the algal arrays, we submerged all 6 arrays on the bottom of the river for 1 to 1.5 h. We then sequentially

covered the arrays in aluminum foil for 1 to 1.5 h to dark-adapt the algae immediately before taking initial PAM measurements.

After the dark adaptation on the riverbed, we retrieved a single covered array, placed it in a bin of fresh river water, and took it to the nearby PAM sampling station on the river bank. We uncovered each PIPES on the array, one at a time, immediately before placing the PAM fiber optic sample holder over the center of the algal assemblage. During this process (~4 min/PIPES), we kept the entire array shaded. We repeated this process for all 6 arrays. Initial (d 0, 23 July 2014) PAM measurements commenced at 1652 h (Array 1, Block 1), and we completed them by 2030 h (Array 6, Block 3). As soon as we had collected data for each array, we returned that array to the river and deployed it according to its assigned experimental treatment, either floating or submerged in the water column. The experimental deployment lasted ~24 h, and we repeated PAM measurements at the end of that time.

Daily patterns in sun exposure are not uniform throughout the streambed owing to partial shading by riparian trees and valley walls. Thus, each array experienced slightly different light patterns, but paired arrays experienced the same light regime. On d 1 (24 July 2014), arrays 5 and 6 (Block 3) experienced full sunlight beginning at 1207 h, and arrays 1 and 2 (Block 1) were illuminated by 1254 h. However, arrays 3 and 4 (Block 2) were not fully illuminated until 1308 h.

We were strongly interested in the effect of increased light and high temperatures on the floating arrays. Therefore, we decided to maximize the period of sun exposure on d 1, and we rearranged the sequence in which we collected data relative to d 0. We began collecting RLCs (according to PAM methods described above) on 24 July 2014 at 1640 h (Block 3) and finished collecting data at 2000 h (Block 2).

We used response ratios to evaluate effects of experimental treatments on RLC parameters for the different algae because we were interested in the relative magnitude of the treatment effect, rather than any absolute parameter values. We calculated response ratios (Hedges et al. 1999) by dividing the parameter value on a given day by the initial measurement taken on d 0, then taking the natural log of this value: $\ln(\text{parameter value}_{\text{day\_n}} / \text{initial parameter value}_{\text{day\_0}})$. To confirm response ratio data met parametric model assumptions, we used the Shapiro–Wilk test to check for normality and Levene's test to check for homogeneity of variance.

We analyzed the response ratio data to assess treatment effects and examine potential taxa-specific responses. First, we used 2-way analysis of variance (ANOVA) models on the response ratios to test how the submerged and floating treatment affected the different RLC parameters for each of the 6 algal taxa. We then used paired *t*-tests to compare the difference in RLC parameter values in the submerged and floating treatments for each algal type, with *p*-values adjusted (false discovery rate; Benjamini and Hochberg 1995) to account for multiple comparisons. The taxa represented 3 different taxonomic or functional groups: Chlorophyta/Bacillariophyceae (*Cladophora* with epiphytic diatoms), motile cyanobacteria (*Anabaena*, *Microcoleus*), and non-motile cyanobacteria (*Nostoc*, *Rivularia*). We created a custom orthogonal planned-contrasts matrix to compare Chlorophytes to all cyanobacteria and motile cyanobacteria to non-motile cyanobacteria by using *t*-tests.

Multi-day manipulation of temperature and flow environment (2015)

In 2015, we wanted to track the photosynthetic response of algae to different micro-habitats for longer than 24 h, so we manipulated the temperature and flow environment of 3 taxa, incubating them at either the river's wetted edge (margin treatment) or the deepest part of the main channel (thalweg control), for 6 d (Fig. S1). The experimental reach was ~65 m long and contained 4 replicate sites, each with a margin treatment and a thalweg control, and was ~100 m downstream from the 2014 experimental site (Fig. S1). Wetted channels were 10 to 15 m wide at all sites.

We conducted the 2015 experiment early in the summer (June), which meant fewer algal assemblages were available than in the 2014 experiment, so we focused on 3 taxa: epilithic diatom assemblages (primarily *Navicula* Bory de Saint-Vincent, 1822; *Ulnaria*; *Cocconeis* Ehrenberg, 1837; and *E. adnata*) and 2 Chlorophyte species, unepiphytized (green) *C. glomerata* and *Oedogonium*. In early June, neither of these chlorophytes had many epiphytes. We collected cobbles (45–64 mm diameter) covered with epilithic diatom assemblages within 2 m of the thalweg location at each site, and we collected *Cladophora* and *Oedogonium* from the thalweg ~10 m upstream of site 1. *Cladophora*, *Oedogonium*, and cobbles were all collected in water 20 to 30 cm deep. We confirmed taxa by microscopy, as described in the 2014 experiment.

On 8 June 2015, we collected algae and constructed 48 PIPES, 24 with *Oedogonium* and 24 with green *Cladophora*, and we also collected 24 cobbles covered with epilithic diatoms. We affixed the *Cladophora* and *Oedogonium* PIPES to Vexar mesh, as described in the 2014 experiment. We left the diatom biofilms on cobbles intact and simply moved cobbles to the appropriate margin or thalweg treatment.

At each of the 4 sites (Fig. S1), we placed 3 samples of each algal taxon (*Cladophora*, *Oedogonium*, epilithic diatoms) where flow was maximal (thalweg control) and similar to the collection locations, and we set another 3 samples of each taxon in low-flow areas near the edge of the wetted river channel (margin treatment) (Fig. S1). We placed cobbles and PIPES 4 to 6 cm beneath the water surface to minimize differences in light climate between the margin and the thalweg. Thalweg and margin placements were 5 to 7 m apart from one another at each site. Shading was minimal because this river segment runs approximately east to west, reducing

morning and afternoon shading from the canyon, and riparian trees are set well back from the summer-inundated channel.

We collected RLC parameters on 9, 10, 11, and 15 June 2015. We made measurements from 1300 to 2000 h on 9 June and 1000 to 1800 h on subsequent days, with the order of measurements randomized over site, algae, and treatment to control for diel changes in photosynthetic physiology of the algae. We dark-adapted samples for 15 to 30 min in a cooler of river water before we used PAM to measure maximum (F_m) and minimum (F_o) fluorescence and collect an RLC. After we made these measurements, we immediately returned the samples to their experimental location in the river.

In 2015, we calculated response ratios on the RLC parameters, averaged together the response ratios from the 3 samples/taxon at each site, tested for normality and equality of variance (as described above), and conducted 2 statistical tests on the response ratios at the 4 margin and 4 thalweg sites. First, we used mixed-effect linear models to test for main effects of treatment (margin or thalweg) and algal taxon type on photosynthetic characteristics, using site as a random effect to account for repeated measurements over time. Second, for each algal taxon, we used 2-way ANOVA to test for interactions between treatment and date (with date as a categorial variable) to assess whether the response to the experimental manipulation changed over time.

We measured temperature and flow at the 4 sites in the 2015 experiment. We deployed iButtons at each site and treatment location for the duration of the experiment to collect discrete temperature measurements every 10 min. To assess if the temperature regimes were different among sites, we conducted separate 1-way ANOVAs and Tukey's honestly significant difference (HSD) tests for the daily minimum, maximum, and mean temperatures calculated from the iButton data. We measured water velocity immediately in front of PIPES at each thalweg and margin location once a day at 0.6 the total water depth on 10, 11, and 15 June with a pygmy flow meter (Gurley Precision Instruments, Troy, New York). Because many flow velocities measured 0 cm/s, we used a nonparametric Mann–Whitney test to determine differences in water velocity between the margin and the thalweg. We calculated all statistics, for both 2014 and 2015 experiments, with the *lme4* and *lmerTest* packages in R 3.6.2 (R Project for Statistical Computing, Vienna Austria). Code for these analysis can be found at https://github.com/keithbg/PAM_Angelo_Analyses.

RESULTS

24-h depth manipulation (2014)

Visual and microscopic examination of experimental arrays confirmed that the target algal assemblages persisted over the experimental deployment (Fig. S2). Fouling of the mesh was minimal during the experiment, and the blank treatments without algae had no detectible fluorescence signal at the time of deployment (d 0) or when they were retrieved (d 1).

Temperature varied over the course of the day. All experimental arrays experienced overnight temperatures of ~19°C (Fig. 3). As the river warmed on the morning of 24 July (d 1), all submerged arrays experienced similar temperature trends. Temperatures of the floating arrays began to diverge from each other and from the submerged arrays after 1100 h, when direct sunlight first hit portions of the pool surface. Temperatures at the water surface were 0.5 to 2°C higher than at the river bottom during the afternoon of 24 July (Fig. 4).

Within each taxon, initial RLCs (collected before the translocation was imposed) did not differ between PIPES assigned to floating and submerged treatments, except for yellow *Cladophora* (Fig. 4). The yellow *Cladophora* assigned to the floating treatment had higher maximum variable fluorescence (F_v / F_m), higher α, and higher $r\mathrm{ETR}_{max}$ than PIPES assigned to the submerged treatment for the same taxon (Fig. S3), although no treatment had been imposed yet. Maximum variable fluorescence was higher for both yellow and red *Cladophora* than for all 4 cyanobacteria taxa (Fig. S3).

We used response ratios to compare photosynthetic parameters for individual PIPES before and after imposing the experimental treatment. Algal identity had an effect on the response ratios for F_v / F_m, α, and $r\mathrm{ETR}_{max}$ (Table S1). Except for *Nostoc*, F_v / F_m and α declined for both floating and submerged arrays during the experiment (Figs 5, S3).

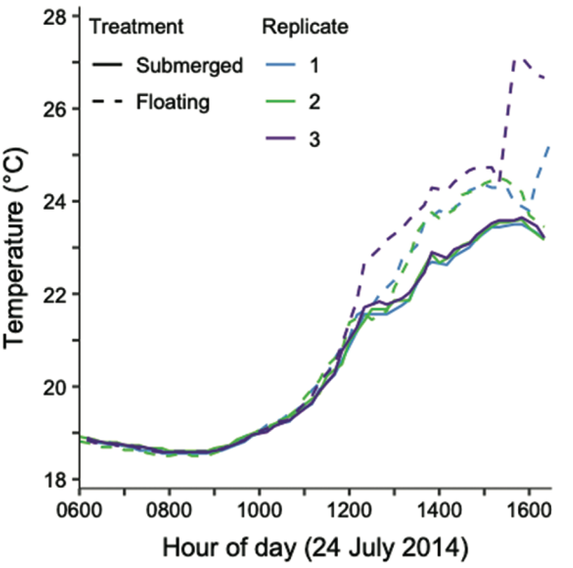

Figure 3. Water temperature profiles from temperature loggers in 2014 showing increased temperatures in floating treatments (n = 3) compared to submerged controls (n = 3) in the South Fork Eel River, California, USA. Temperature was recorded every 15 min.

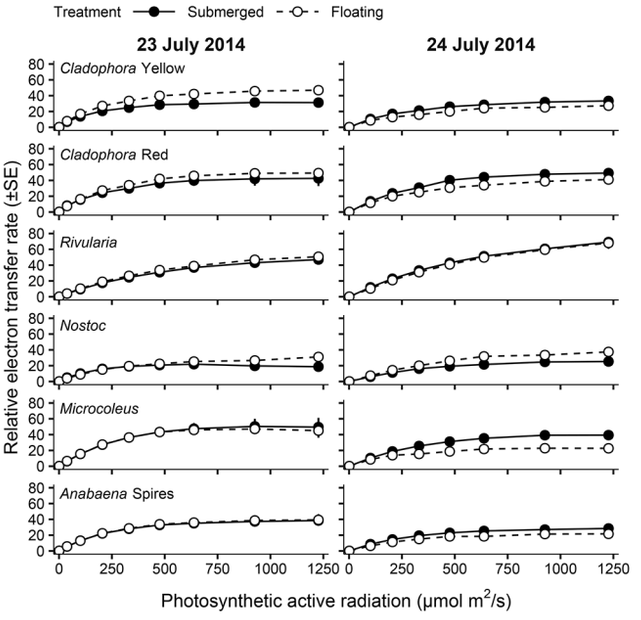

Figure 4. Rapid light curves for the 6 algal taxa in the floatation experiment in 2014. Algal taxa were floated or submerged in the South Fork Eel River, California, USA, for 24 h. Curves show the mean (±SE) relative electron transport rate of the 3 replicates for each measurement day. Black circles represent submerged controls, and white circles represent floating treatments.

Floating the algae at the surface generally lowered response ratios for $r\mathrm{ETR}_{max}$, had a marginal negative effect for α, and had no effect for F_v / F_m (Table S1). The minimal or positive response of *Nostoc* to the floating treatment (Fig. 5) likely drove the algae by treatment interaction effect for F_v / F_m and α.

Paired *t*-tests to identify which algal taxa were responding to the difference in depth did not suggest a difference in mean parameter values between treatments, possibly because of low replication. The functional group statistical tests found no differences between the response of the *Cladophora*/diatom assemblage and that of cyanobacteria for any parameters (Table S2). However, in the comparison between motile and non-motile cyanobacteria, there was a strong interaction between treatment and motility for α, because *Nostoc* had a high α response ratio in the floating treatment, but for $r\mathrm{ETR}_{max}$ there was only a treatment effect, with non-motile cyanobacteria having substantially highe $r\mathrm{ETR}_{max}$ response ratio values (Table S2, Fig. 5).

Multi-day manipulation of temperature and flow environment (2015)

Temperature and flow conditions varied between the margin and thalweg locations. During the 6-d 2015 experiment, water velocities were 10 to 20× faster ($p < 0.05$) in the thalweg than in the margin (Fig. 6A). Among the 4 sites, the daily mean temperatures were more variable in the margin (20.9–23.4°C) than in the thalweg (21.1–22.4°C) (Fig. 6B). The margin of site 4 was warmer than all other margins, and the margin at site 3 was warmer than the margin at site 2 (Tukey's HSD: $p < 0.05$; Fig. 6B). Daily mean temperatures were higher in the margin ($p < 0.05$) than the thalweg. This trend was driven by site 4, which was located where the river channel was widest (~15 m) and had the greatest temperature differences between the warm margin and cool thalweg. Among sites 1 to 3, there was no difference in daily mean temperatures between the margin and the thalweg ($p > 0.05$). In contrast, average daily minimum temperatures were much lower ($p < 0.05$) in the margin (18.9°C) than in the thalweg (19.9°C), and average daily maximum temperatures were higher in the margin (27.2°C) than in the thalweg (24.6°C), regardless of whether analyses included sites 1 through 4 or only sites 1 through 3. The greatest effect of our manipulation was on the minimum and maximum temperatures the algae experienced each day, whereas daily mean temperatures were similar between treatments.

The RLCs for algae assigned to thalweg and margin treatments were similar immediately before the experimental manipulation on 9 June (Fig. 7). During the experiment, *Cladophora* and *Oedogonium* incubated in the margin treatment had consistently lower F_v / F_m and α than their counterparts in the thalweg control (Table S3, Figs 8, S4). *Oedogonium* in the margin also had substantially lower $r\mathrm{ETR}_{max}$ than in the thalweg, but $r\mathrm{ETR}_{max}$ was similar between treatments for *Cladophora*. For diatoms on cobbles, while F_v / F_m and α were lower in the margin, $r\mathrm{ETR}_{max}$ response ratios in the 2 habitats were similar but with the margin being slightly higher (Figs 8, S4). Algal identity and the habitat placement had a substantial effect on the response ratios for F_v / F_m, α, and $r\mathrm{ETR}_{max}$ (Table S4). There was an interaction effect between algal identity and treatment on $r\mathrm{ETR}_{max}$ due to the higher $r\mathrm{ETR}_{max}$ response ratios of diatoms on cobbles in the margin habitat. Sampling date did not affect RLC parameters within treatment ($p > 0.05$; Table S3), nor was there an interaction between treatment and sampling date. Thus, the responses of each alga to the habitat manipulation did not change over time ($p > 0.05$; Table S3).

DISCUSSION

We designed samplers (PIPES) that could overcome some of the inherent difficulties of PAM analysis while still exposing algae to realistic changes in abiotic conditions across small spatial scales in situ. Sandwiching the biofilms between mesh allowed us to assess RLC parameters of biofilms of similar thickness, both among taxa and between days. The array structure allowed us to expose different taxa to altered light, flow, and temperature environments. By using PIPES and PAM analysis, we were able to assess whether the taxonomic composition of algal biofilms affected their response

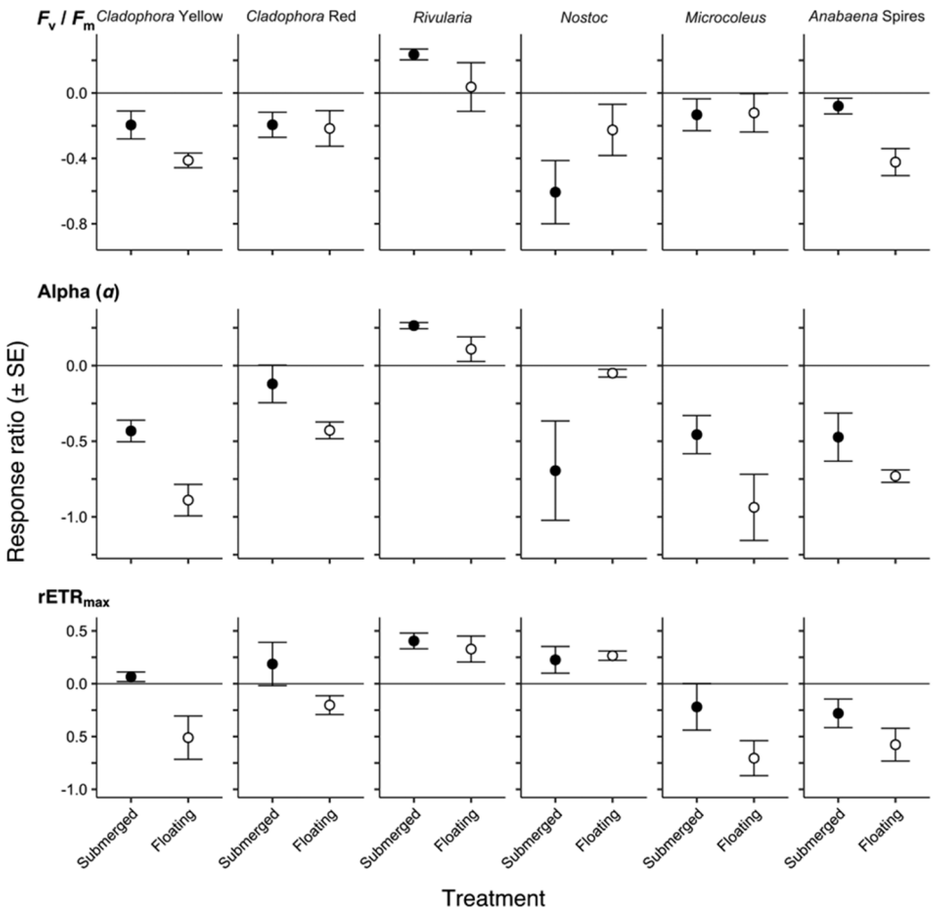

Figure 5. Response ratios of pulse-amplitude modulated fluorometry parameters, mean (±SE) of the 3 replicates, for the 6 algal taxa in 2014. Algal taxa were floated or submerged in the South Fork Eel River, California, USA, for 24 h. Top panel: F_v / F_m, the maximum quantum yield for Photosystem 2. Middle panel: alpha (α), the light-use efficiency coefficient. Bottom panel: Maximum electron transport rate (rETR$_{max}$) of the algae. Submerged controls are shown as black circles, and floating treatments are shown as open circles.

to potential stressors associated with different microhabitats in the SF Eel River.

PAM fluorometry can assess, with seductive rapidity, the photosynthetic competency of primary producers, but interpreting those data presents challenges. The relative ease of data acquisition is substantially offset by the sensitivity of the algal response to antecedent light conditions. PAM fluorometry is an excellent tool for detecting algal responses to chemical stressors and light gradients (Jones et al. 1999, Kühl et al. 2001) and for detecting diel rhythms in photosynthesis (Ensminger et al. 2001). Using PAM to assess differences among algal assemblages in the field is more difficult. Biofilm thickness is nonuniform, and many biofilm denizens are highly motile, actively migrating in the biofilm in response to light availability. Both these factors add variation to field-based PAM measurements and can make interpreting PAM data challenging.

We used response ratios to describe the changes of individual PIPES to the experimental manipulation because photosynthesis-irradiance parameters are very sensitive to light exposure immediately before PAM analysis. We were interested primarily in the magnitude of the response to the translocation treatment, not the temporal dynamics of individual algal biofilms.

Small-scale variation in depth, temperature, and flow in a cobble-bedded river create heterogeneous resource regimes for microalgae, which can influence algal assemblage composition. Attached algal photosynthesis responded rapidly and persistently to vertical (depth) and horizontal translocation to different microhabitats within the SF Eel River. The strength and direction of the photosynthetic response to altered flow, temperature, and light at new sites was taxon specific. Maximum photosynthetic rates of the chlorophytes, *Cladophora* and *Oedogonium*, declined when they were presumably stressed by exposure to high temperature at the water surface or the slow flow environment at the river's edge. Responses of cyanobacteria to warming were less clear, as physical translocation of cyanobacteria caused short-term photosynthetic rates to increase, decrease, or remain unchanged, depending on the taxon. The rapidity and persistence

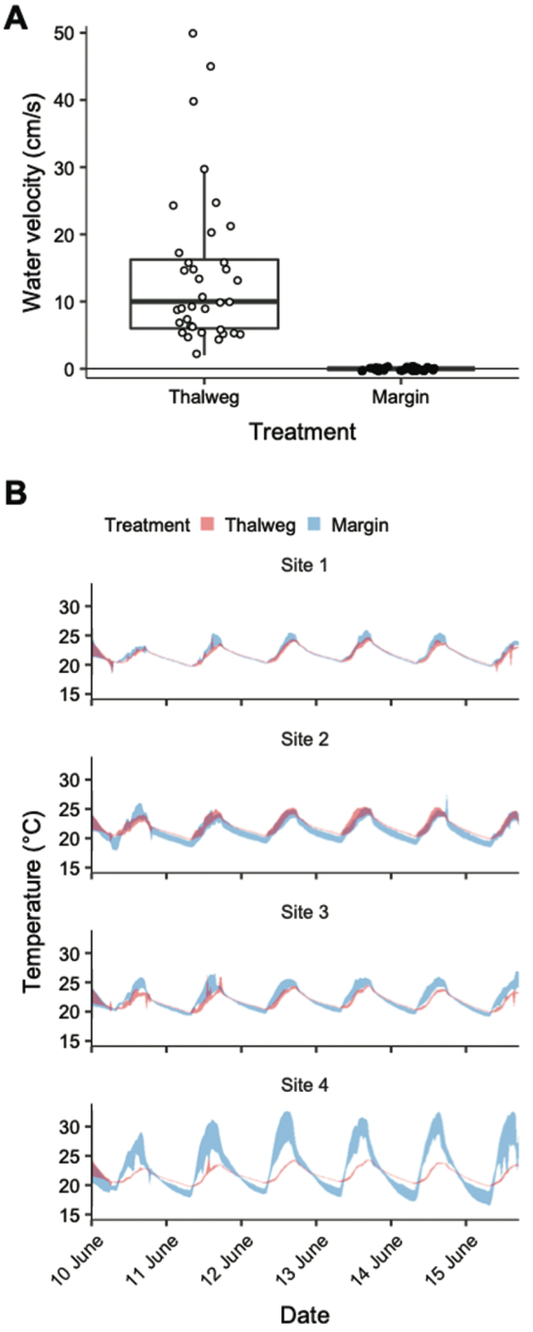

Figure 6. A.—Water velocities at 4 replicate margin and thalweg sites in the South Fork Eel River, California, USA, in 2015. Boxplots show the 25th and 75th percentiles (interquartile range), and the horizontal line gives the median. Whiskers extend 1.5× the interquartile range. Velocities were taken at 0.6 the depth in front of each experimental site and were higher at thalweg sites (black circles) compared to margin sites (white circles). B.—Temperature variation at the 4 replicate sites. The colored band spans the minimum and maximum temperature recorded at each site every 10 min. The red bands are margin treatment sites, and the blue bands are thalweg control sites.

of the taxon-specific photosynthetic response to changes in microhabitats may influence how structurally complex riverbeds develop a taxonomic mosaic of algal assemblages.

Effects of depth manipulation on different taxa

In the 2014 experiment, there was a decline in variable fluorescence, $r\mathrm{ETR}_{max}$, and α in most biofilms between d 0 and d 1. This decline occurred irrespective of experimental manipulation, suggesting that the different light conditions imposed on the algae before the short dark adaptation affected the photosynthetic parameters. On d 0, we kept the biofilms in near-darkness for long periods until we ran the RLCs. Thus, the algae exhibited some of the RLC attributes typically associated with daybreak, such as high variable fluorescence (Serôdio et al. 2005, Laviale et al. 2009). The decline in RLC parameters over 1 d might also represent a handling impact on the biofilms, which our experiments did not address. Future short-term translocation experiments should include a 1 to 2 d pre-exposure of the PIPES to the reference condition, in this case the submerged control, before taking the initial PAM measurements. Placing control PIPES at the original collection location, rather than translocating the control to a reference site, could also be considered for future experiments.

The maximum variable fluorescence was lower in cyanobacteria than in the chlorophytes before and after experimental manipulation in 2014. This systematic variation between phyla is expected owing to fundamental differences in the cell structure and photosystem organization of cyanobacteria compared to cholorophytes. For instance, phycobilisomes contribute to background fluorescence of dark-adapted cyanobacteria, which leads to underestimates of maximum variable fluorescence in cyanobacteria (Campbell et al. 1998, Schuurmans et al. 2015, Ogawa et al. 2017).

Floating the algae substantially affected photosynthetic parameters (Figs 4, S2). One possibility is that, relative to the submerged treatments, the floating experimental arrays experienced a beneficial increase in a limiting resource: light. In most cases, the slope of the RLC (α) declined in the floated treatments relative to the submerged controls (Fig. 5), which is an expected response to acclimation to high light. However, if floating alleviated light limitation, then we would also expect increases in $r\mathrm{ETR}_{max}$ of algae in the floating arrays relative to their submerged counterparts. These increases never occurred (Fig. 5). Therefore, we cannot conclude that the algae responded positively to the increase in light associated with flotation. A 2nd possibility is that the increase in light experienced by the floated algae represented a stress. If so, we might see some evidence of photoinhibition. Attached algae rarely exhibit photoinhibition as a downturn in a RLC (Perkins et al. 2002), and we intentionally focused on obtaining accurate measurements of $r\mathrm{ETR}_{max}$ and α by limiting the RLCs to environmentally relevant

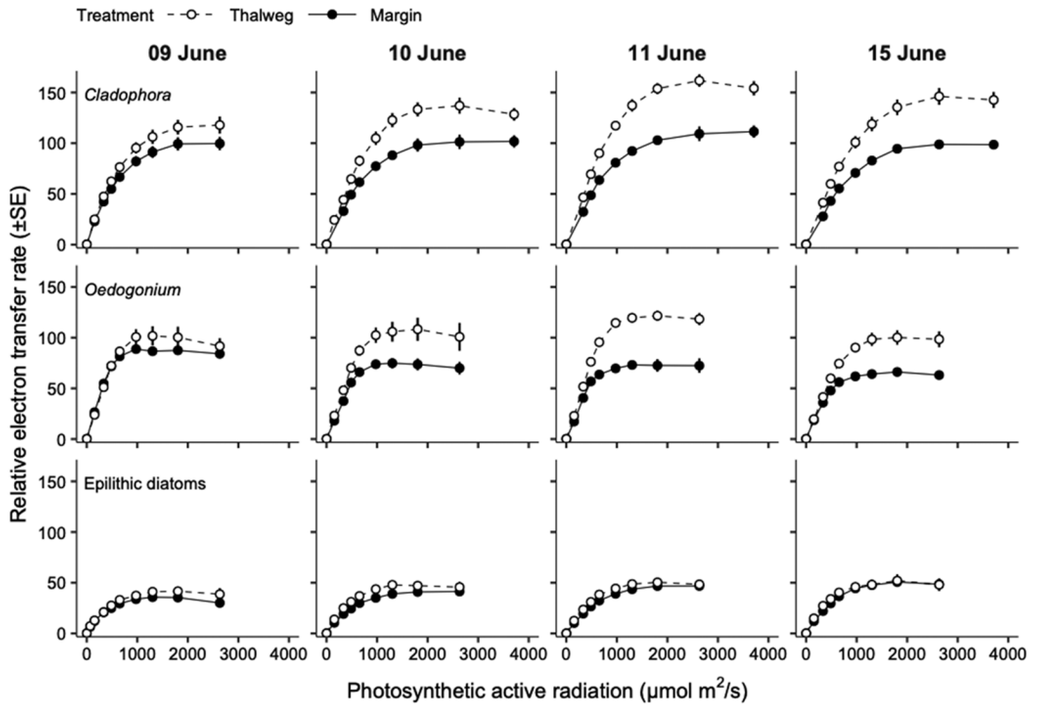

Figure 7. Rapid light curves for the *Cladophora*, *Oedogonium*, and epilithic diatoms deployed in margin and thalweg habitats in the South Fork Eel River, California, USA, in 2015. Curves show the mean (±SE) relative electron transport rate (*r*ETR) of the 4 replicate sites for each measurement day. Black circles represent thalweg controls, and white circles represent margin treatments.

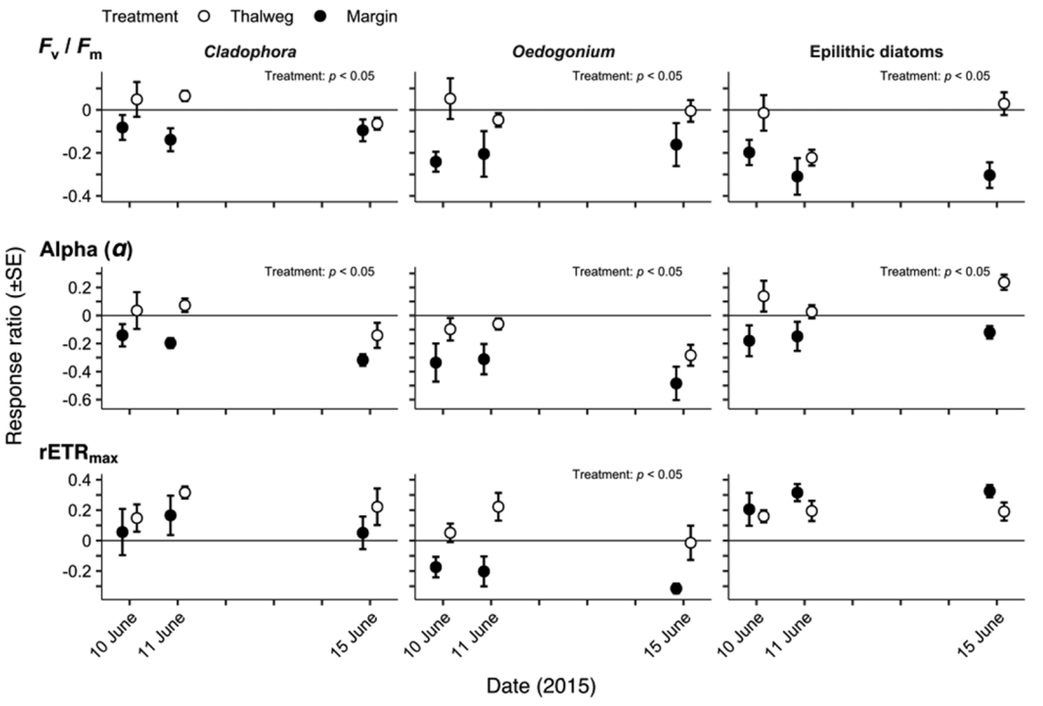

Figure 8. Response ratios of pulse-amplitude modulated (PAM) fluorometry parameters for *Cladophora*, *Oedogonium*, and epilithic diatoms deployed in margin and thalweg habitats in the South Fork Eel River, California, USA, in 2015. Top panel: F_v / F_m, the maximum quantum yield for Photosystem 2. Middle panel: alpha (α), the light-use efficiency coefficient. Bottom panel: Maximum relative electron transport rate ($r\mathrm{ETR}_{max}$) of the algae. Thalweg controls are shown in black circles, and margin treatments are shown in open circles. The mean (±SE) of the 4 replicate sites is plotted. The top right corner of panels indicates if the treatment had a substantial effect ($p < 0.05$) on the PAM parameter. There was no effect of measurement date, nor was there an interaction effect between measurement date and treatment for any of the parameters.

surface light intensities (<1500 μmol m^2/s photosynthetically active radiation). We cannot rule out photoinhibitory cellular damage or an increase in photorespiration. However, these data do not provide strong evidence of a stress response by the floating algae to high light per se. In attempting to assess whether the increase in light associated with flotation was a boon or a stress to the algae, it is important to understand that the magnitude of difference in light exposure between the floating and submerged treatments was a small fraction of the variation in light exposure over the course of the day because the depth of water over the surface of the submerged controls was only 20 cm.

Algae in the floated arrays experienced higher afternoon temperatures than algae incubated near the riverbed (Fig. 3), and the data are consistent with this increase in temperature imposing a stress on the assemblages dominated by green algae and diatoms. Values of $r\mathrm{ETR}_{max}$ and α decreased in the floating treatments relative to the submerged controls for both red *Cladophora* and yellow *Cladophora*, although replication was low. Incubating the algae at the surface caused these assemblages to use light less efficiently at both high and low light intensities. Temperature conditions at the surface are ecologically relevant for these algae because, during maximum summer growth, oxygen bubbles become entrapped in *Cladophora* fronds, resulting in extensive floating surface scums (Fig. 1B). Water trapped in these surface scums has been previously shown to be up to 8 to 10°C warmer than the surrounding river water (Power 1990a, Power et al. 2015). *Cladophora* becomes increasingly bleached as the cells senesce, cell walls collapse, and cell contents leak (Power et al. 2015). Our experimental flotation of the yellow and red *Cladophora* suggests that conditions on the surface compromise the photosynthetic competency of *Cladophora*, which likely contributes to the physical deterioration of floating aggregations.

Cyanobacterial responses to flotation were taxon specific. Floating PIPES of the cyanobacteria *Nostoc* and *Rivularia* had no effect on $r\mathrm{ETR}_{max}$ (Fig. 5). For *Nostoc*, but not *Rivularia*, floating increased light use efficiency at limiting irradiances (α). These 2 taxa may have a broader temperature tolerance than the *Cladophora* in the SF Eel River. Interestingly, the motile cyanobacteria taxa that have recently become more noticeable in the SF Eel River, *Anabaena* and *Microcoleus*, appeared to exhibit stress responses to floatation. For both motile taxa, $r\mathrm{ETR}_{max}$ and α trended lower in the floating arrays relative to the submerged control arrays. The stress may have been associated with temperature or reduced water flux and may have been similar to, though less pronounced than, the response by the *Cladophora*/diatom assemblage. Cyanobacteria are thought to be more tolerant of high temperatures than eukaryotic algae (Reynolds 2006, Paerl et al. 2011), but that was not evident for these 2 motile species. *Anabaena* and *Microcoleus* are highly motile, and the biofilms in the floated treatment appeared to thin slightly over the experiment. Thus, the apparent reduction in $r\mathrm{ETR}_{max}$ may be associated with less algal biomass under the fiber optic probe at the end of the experiment, and further investigation of PIPES is necessary to understand how motile cells move within PIPES and affect PAM results.

Effects of flow and temperature manipulation on different taxa

The 2015 experiment manipulated flow and temperature conditions by translocating algae between river margin and thalweg habitats. The light environment an alga inhabits can affect photosynthetic parameters. Algae acclimated to high light environments often have lower values of α than those grown in the shade (Stevenson et al. 1996). However, there were similar light environments between the margins and the thalweg in the 2015 experiment. Therefore, the substantial decline in α for all algae incubated in river margins was not caused by differences in light exposure and is likely the result of the flow and temperature differences between the treatments. In addition, the decreased F_v / F_m in 2015 for all algal groups moved to the river margin was expected because of stress from low flows and temperature extremes, and this decrease should not have been influenced by light.

There are limits to the physiological plasticity of algae, and our manipulations show that changing the physical location of the algae, even by a few meters, can compromise their photosynthetic performance. It is likely that the algae could not fully acclimatize to the slower flow velocity in the margin. Stagnant water decreases algal production (Whitford 1960, Whitford and Schumacher 1964), and even small velocity increases, such as 0 to 2.5 cm/s, have been shown to increase photosynthetic rates (Pfeifer and McDiffett 1975). Slow or stagnant flows in the river margin thicken the boundary layer around algal assemblages, reducing total nutrient delivery rate, nutrient uptake, and metabolic waste removal.

We cannot determine the mechanism underlying the effect of temperature on photosynthetic performance from these data. Considering that the average daily temperatures were similar between the margin and thalweg, the more extreme temperatures in the margin are a more likely mechanism for affecting photosynthetic efficiencies. Daily temperatures during the hottest part of the day may damage photosystems, as exposing algae to stressful high temperatures for ~1 h can prevent photosystem recovery (Salleh and McMinn 2011). The maximum temperatures in the margin, however, were below reported optimal temperatures for *Cladophora* and other chlorophytes (Dodds and Gudder 1992, Lürling et al. 2013). Only the margin of site 4 exceeded 30°C, a threshold that often negatively affects *Cladophora* (Bellis 1968, Dodds and Gudder 1992, Hayakawa et al. 2012). Therefore, the high absolute temperature values in the experiment were unlikely to stress photosynthesis. Instead, the cellular resources that had to be allocated to maintaining photosynthesis in more

variable temperature regimes in the margins may have impaired the overall physiological performance of the algal groups.

The immediate effects of the translocation experiment in 2015 persisted for the duration of the experiment, indicating that responses of algal photosystems could not fully compensate for the negative stress imposed by our treatments. Photosystems respond to changing conditions at multiple timescales (Raven and Geider 2003). Within seconds to minutes, photosynthetic regulation involves adjusting enzymes and electron pathways without synthesizing or degrading molecules. On the scale of hours to days, cells can acclimate to new environmental conditions by adjusting the molecular composition and cellular abundance of their photosystems. Our experimental manipulations show that both rapid photosystem regulation and longer photosystem acclimation >6 d were not able to maintain higher photosynthetic efficiencies for the algae translocated to the river margin.

Conclusion

The temperature and flow differences between our treatments in both experiments show the strong effects of changing flow regimes on benthic algae in rivers, and our results emphasize that, under different flows, velocity and temperature changes differentially affect the photosynthetic physiology of algal taxa. In arid climates, dams and water diversions affect summer base flows and temperature conditions of river habitats (Lytle and Poff 2004, Dewson et al. 2007). Small velocity changes of a few cm/s and minor changes in the temperature regime can persistently affect the physiology of algal taxa, which could change the outcome of species interactions and lead different taxa to dominate. Though many factors contribute to the growth and accrual of algae, photosynthesis is foundational to algal fitness, and differences in photosynthetic efficiency may contribute to different spatial arrangements of algal assemblages and taxonomic dominance. Managing base flows to prevent critical shifts in algal assemblage composition, which may affect aquatic food webs, requires understanding these small-scale environmental controls and physiological mechanisms that affect algal growth rates and fitness. Such understanding is particularly important given how climate change, drought, and water extraction are dramatically altering discharge and channel hydraulics in the SF Eel River and other rivers throughout the world.

In midsized, clear-water rivers like the Eel, attached algae form the trophic basis of production for complex riverine food webs upon which fishes, riparian animals, and humans depend (Vadeboncoeur and Power 2017). Algal taxonomic composition changes in space and time in response to variation in resources, grazers, and abiotic stresses, and algal taxa vary in their contribution to the food web. For instance, diatoms are packed with lipids that promote the rapid growth and development of primary consumers. Green algae are often difficult to ingest and not particularly nutritious. Cyanobacteria can be a rich source of protein and N or can be full of mucous and toxins. The relative abundance and distribution of these different algae will determine the accessibility of primary production to animals in the river. Advances in estimation of stream metabolism using in-situ oxygen probes have revolutionized our understanding of factors controlling primary production at the reach scale (Genzoli and Hall 2016, Hall et al. 2016), but these studies do not identify species-specific responses to stressors or environmental controls. The PIPES that we have developed are a new tool for photosynthetic measurements on filamentous attached algae that can target specific taxa. If used in conjunction with more established methods, such as in-situ oxygen probes, PIPES can help us understand the processes determining algal distributions. Our exploratory experiments evaluated the costs and benefits of different microhabitats for algal taxa that vary markedly in their effects on river ecosystems. Although refinements and tests of repeatability are necessary, our results indicate that the combination of PAM fluorometry and PIPES can reveal the metabolic consequences of environmental heterogeneity for the taxonomic structure, productivity, and distribution of attached algal assemblages.

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