Responses of Bloom-Forming Phytoplankton Populations to Changing Reservoir Chemistry and Physics

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ABSTRACT

Phytoplankton populations are integral to the structure and function of aquatic ecosystems, and phytoplankton are an excellent study system for exploring ecological questions. Reservoirs often exhibit high horizontal (inflow to dam) and vertical (surface to sediments) environmental heterogeneity, which plays a large role in determining phytoplankton population dynamics. In this thesis, I explore how three bloom-forming phytoplankton taxa, the dinoflagellates *Peridinium* and *Gymnodinium,* and the cyanobacterium *Planktothrix*, respond to horizontal and vertical environmental gradients, respectively. First, I monitored recruitment, or the process of leaving the sediments and entering the pelagic life stage, of dinoflagellates across a horizontal reservoir ecosystem gradient. Surprisingly, coupling of dinoflagellate biology with reservoir physics and chemistry varied along this continuum; recruiting cells were sensitive to reservoir physics (e.g., flow rate, solar radiation) in the upstream riverine zone, while recruitment was related to reservoir chemistry (e.g., dissolved oxygen, nutrients) in the downstream lacustrine zone. This study indicates that upstream habitats should be monitored when studying reservoir phytoplankton dynamics. Next, I investigated the environmental drivers of the vertical distribution and biomass of a hypolimnetic cyanobacterial bloom over two consecutive summers. I collected high-resolution *in situ* phytoplankton data, and measured environmental variables throughout the water column. Across both years, the vertical distribution of this population was determined by light availability, while the

cyanobacterial biomass was predicted by both light and nutrients. These two studies demonstrate that changing physics and chemistry across environmental gradients can regulate phytoplankton dynamics in reservoirs, and phytoplankton monitoring should include more spatially comprehensive sampling approaches.

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Chapter 1. Introduction

Phytoplankton are the base of the aquatic food web in open waters. They are an incredibly diverse group of organisms, exhibiting a broad range of morphologies, life history strategies, and resource requirements (Harris 1980, Morris 1980, Reynolds 2006). Due to their diversity, short generation times, and high rates of dispersal, phytoplankton communities are very dynamic, and have long been used as a study system for examining population, community, and ecosystem ecology questions (e.g., Hutchinson 1951, Margalef 1980).

Phytoplankton can be major drivers of lake and reservoir water quality (Taylor 1979, Boyer et al. 2009). Under certain conditions, such as increased nutrient loading and water temperatures, phytoplankton populations can form blooms, or dense aggregations of one taxon that dominate the community (Oliver and Ganf 2000, Paerl and Huisman 2009, O'Neil et al. 2012). Phytoplankton blooms can decrease light availability for other species by creating surface scums, alter food webs, deteriorate water quality, and produce toxins (reviewed in Paerl et al. 2001). Therefore, understanding the ecology of phytoplankton populations is critical for predicting phytoplankton dynamics in the face of global change, which is increasing blooms in many waterbodies around the world (Paerl and Huisman 2009, Paerl et al. 2011).

Although both natural lakes and manmade reservoirs have experienced increasing phytoplankton blooms globally (Paerl and Huisman 2009), much less is known about phytoplankton ecology in reservoirs. Reservoirs are numerically abundant across the globe (Lehner et al. 2011) and are increasingly being constructed for their many ecosystem services, including drinking water, recreation, fisheries, and hydropower (Zarfl et al. 2015). Because they are human-made ecosystems, reservoirs have many different characteristics from natural lakes

(Thornton et al. 1990, Wetzel 1990). For example, reservoirs typically experience much shorter hydraulic residence times than natural lakes, increasing the relative rates of sedimentation and nutrient input from the catchment (Thornton et al. 1990). While lakes typically are deepest in the center, giving a "bowl" shape to the bathymetry, reservoirs are generally deepest at the dam, with a more "triangular" bathymetric shape. Reservoirs are commonly built by damming rivers, and often represent a continuum of river-to-lake habitat, rather than a lacustrine ecosystem. Consequently, exploring the factors driving phytoplankton populations in manmade reservoirs is essential in understanding the feedbacks between ecology and reservoir water quality.

In this thesis, I explore how horizontal and vertical gradients shape phytoplankton communities in two human-made reservoirs. Because reservoirs are manmade systems, the aquatic environment is horizontally variable along the inflow-to-dam gradient (Kimmel and Groeger 1984, Thornton et al. 1990, Wetzel 1990); environmental factors such as local flow rate, nutrient cycling, and light attenuation are highly variable along the gradient from the riverine to the lacustrine zones of a typical reservoir (Kimmel and Groeger 1984). The abiotic changes along this continuum can be highly important to structuring phytoplankton populations horizontally through a reservoir ecosystem (Lind 2002, Cunha and Calijuri 2011). In comparison, vertical gradients in reservoir habitat, such as variations in water temperature, nutrient concentrations, and light availability, can also structure phytoplankton populations (reviewed in Reynolds 2006). Physical movement of water, e.g., climate-driven mixing and stratification patterns, can affect movement of phytoplankton throughout the water column (Cushing 1989, Reynolds 2006), and during stratified periods, there is a temperature gradient from the surface to the sediments. This vertical gradient, often characterized by high light availability in the warm surface waters and low light in the nutrient-rich bottom waters, favors

different phytoplankton populations at different depths (Ganf and Oliver 1982, Reynolds 2006). Because reservoirs are common across the global landscape, and increasingly being constructed for their ecosystem services, it is important to understand the biological populations that form the base of reservoir food webs. Broadly, I am interested in exploring the ecosystem processes related to changes in reservoir phytoplankton ecology, looking across both horizontal and vertical scales.

In this thesis, I investigated the environmental factors driving two bloom-forming phytoplankton populations in two small, moderately eutrophic reservoirs in Virginia, USA. I explored the physical and chemical drivers of two bloom-forming phytoplankton populations in drinking water reservoirs, specifically examining how the physical location of the population in the reservoir mediates its ecological dynamics. In Chapter 2, I examine how the horizontal position of phytoplankton on a riverine to lacustrine gradient alters its sensitivity to physical vs. chemical forcing, and in Chapter 3, I study how the vertical position of phytoplankton in the water column is controlled by light and nutrient availability. As part of a collaborative research effort, I addressed the following major questions:

1. What are the drivers of dinoflagellate recruitment along a spatial reservoir ecosystem continuum? (Chapter 2)
2. What factors determine the biomass and vertical distribution of a hypolimnetic cyanobacterial bloom? (Chapter 3)

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Chapter 2. Spatial variation in dinoflagellate recruitment along a reservoir ecosystem continuum[1]

Hamre, K.D., Gerling, A.B., Munger, Z.W., Doubek, J.P., McClure, R.P., Cottingham, K.L., and Carey, C.C.

2.1 Abstract

Physical and chemical gradients across ecosystem continua, such as stream-to-lake habitats within human-made reservoirs, provide valuable opportunities to examine how plankton respond to changing environmental variables. We quantified the rate of dinoflagellate cells recruiting from the sediments into the water column at three sites in a small reservoir to test the hypothesis that plankton are controlled by different factors in the riverine and lacustrine zones of a reservoir ecosystem continuum. We predicted that dinoflagellate recruitment would be tightly coupled with changes in the physical environment in the riverine zone and most closely related to water chemistry in the lacustrine zone. For the dominant dinoflagellate genus in the reservoir, *Peridinium*, the flux of recruiting cells from the sed[1]iments accounted for a median of 16% and a range of 4 - 53% of increases in pelagic cell abundance throughout the summer. As predicted, *Peridinium* recruitment rates at the riverine site were highly correlated with physical variables, e.g., inflow rate and light availability, while at the lacustrine site, recruitment rates were highly correlated with water chemistry. The recruitment rate of the second most common genus, *Gymnodinium*, was not correlated with environmental variables, though

[1] A version of this chapter is currently under review at the *Journal of Plankton Research*.

Gymnodinium's much lower densities suggest that its dynamics were controlled by other factors during the monitoring period. Our results reveal that the physical-biological coupling controlling algal recruitment, which can potentially play a large role in pelagic population growth and bloom formation, can vary substantially on a spatial gradient within even a small reservoir.

Keywords: algal life history, ecological gradient, *Gymnodinium*, *Peridinium*, phytoplankton, population ecology

2.2 Introduction

Organisms that live along abiotic gradients within an ecosystem, also referred to as an ecosystem continuum, provide a rich opportunity to examine physical-biological coupling (e.g., Whittaker 1967, Hart and Finelli 1999, Carmack and Wassman 2006, Harrison et al. 2008). Gradients provide the opportunity to quantify not only how the abundance of a species changes with varying environmental conditions (e.g., Brown 1984, Tilman 1993, Austin 2002, McGill et al. 2007), but also how organisms within the same species may respond differently to changing physical and chemical factors (Austin 2002, Oksanen and Minchin 2002, Vonlanthen et al. 2009). The effects of gradients on populations have been extensively studied along salinity gradients in estuaries (e.g., Bouvier and del Giorgio 2002, Crump et al. 2004, Harrison et al. 2008, Campbell and Kirchman 2013) and littoral-to-pelagic gradients within lakes (e.g., Schindler et al. 1996, Vonlanthen et al. 2009). However, less is known about how changing environmental conditions along a lotic to lentic gradient in reservoirs affect freshwater populations.

Many reservoirs are constructed by damming rivers, creating an ecosystem continuum of riverine to lacustrine conditions and providing an ideal opportunity to examine how environmental constraints may change within the same ecosystem (Thornton et al. 1990, Wetzel 1990). In a typical run-of-the-river reservoir, the physical and chemical environment exhibits marked changes from the upstream riverine zone to the downstream lacustrine zone near the dam (Kimmel and Groeger 1984, Thornton et al. 1990, Wetzel 1990). In the riverine zone, inflow dynamics dominate the local environment: this reservoir zone experiences shorter local hydraulic residence times, higher sedimentation, and more flushing of nutrients relative to downstream

(Kimmel and Groeger 1984). In contrast, the lacustrine zone experiences longer local residence times and is characterized by a deeper, lake-like basin with less temporal variability in physical conditions. As the name implies, the transitional zone is characterized by intermediate local residence times, sedimentation, and nutrient flushing. Thus, the reservoir continuum provides an excellent opportunity to investigate how population dynamics vary with environmental conditions.

Here, we investigated how the responses of two phytoplankton taxa to environmental conditions vary along a reservoir ecosystem continuum. While most studies exploring population responses to environmental gradients in reservoirs have been conceptual (e.g., Kimmel and Groeger 1984, Thornton et al. 1990, Wetzel 1990), recent empirical work indicates that the importance of different environmental drivers controlling phytoplankton population growth changes along this continuum (Cunha and Calijuri 2011, Rychtecky and Znachor 2011). In the riverine zone, algal population growth is often limited by light due to high flow and sedimentation rates (Kimmel and Groeger 1984, Lind 2002, Cunha and Calijuri 2011), while in the lacustrine zone, algal population growth may be limited by internal nutrient dynamics (Kimmel and Groeger 1984, Lind 2002). In the transitional zone, primary production may be affected by both upstream (physics) and downstream (nutrient cycling) controls (Kimmel and Groeger 1984, Lind 2002). We predicted that phytoplankton population dynamics would be tightly coupled with changes in the physical environment in the riverine zone and most closely related to water chemistry in the lacustrine zone. In the transitional zone of the reservoir, recruitment patterns would be driven by both physical and chemical conditions.

We tested this hypothesis using two dinoflagellate taxa, *Peridinium* and *Gymnodinium*. Dinoflagellates are common in many aquatic systems and can be observed in all three reservoir

zones because of their cosmopolitan distribution (e.g., Pollinger 1988). Dinoflagellates can play a critical ecological role because they are a high-quality food source to higher trophic levels (Breteler et al. 1999) and are often mixotrophic (Stoecker 1999). They are the dominant taxa that produce harmful algal blooms in estuaries and oceans (Paerl 1988, Anderson et al. 2002, Hallegraeff 2003, Sellner et al. 2003), and occasionally produce blooms in freshwater systems (Nakamoto 1975, Yamada et al. 1998, Rengefors and Legrand 2001, Hirabayashi et al. 2007). Because dinoflagellate blooms can cause taste and odor problems in drinking water (Anderson et al. 2002), understanding the factors that control their population dynamics is important for the management of drinking water reservoirs.

Dinoflagellate life cycles are complex (Pfiester and Anderson 1987), and certain critical steps in their life history may play disproportionately large roles in their population dynamics (Park and Hayashi 1993, Nehring 1996, Sanderson and Frost 1996). For example, pelagic populations are subsidized by recruitment from dormant cysts in the sediments (Park and Hayashi 1993, Sanderson and Frost 1996). These cysts can overwinter from preceding years and provide a seed bank of genetic variability for future populations (Pfiester and Anderson 1987, Rengefors 1998). Successful recruitment from the sediments to the water column typically plays an important role in increasing pelagic populations (Park and Hayashi 1993, Nehring 1996, Sandersen and Frost 1996).

Past work suggests that dinoflagellate recruitment can be stimulated by light (e.g., Binder and Anderson 1986, Anderson et al. 1987, Pfeister and Anderson 1987, Rengefors et al. 2004), water column mixing (Hansson et al. 1994, Hansson 1995), and water temperature (Rengefors and Anderson 1998, Rengefors et al. 2004), but is inhibited by anoxia (Rengefors and Anderson 1998). Most of these factors vary longitudinally along a reservoir ecosystem continuum, as well

as temporally within a season, creating the opportunity to evaluate their combined effects on dinoflagellate recruitment. Although multiple studies have examined the drivers of dinoflagellate recruitment in marine systems and natural freshwater lakes, much less is known about dinoflagellate recruitment in reservoirs.

To examine changes in *Peridinium* and *Gymnodinium* population dynamics along the reservoir ecosystem continuum, we measured recruitment and pelagic populations for both taxa in a drinking water reservoir throughout one summer season. We quantified the rate of cells leaving the sediments in the riverine, transitional, and lacustrine zones of the reservoir, and examined how recruitment rates in the different zones responded to temporal changes in physical and chemical variables throughout the sampling period.

2.3 Methods

2.3.1 Study site

Falling Creek Reservoir (FCR) is a small, eutrophic impoundment located in Vinton, Virginia, USA (37.30^{o}N, 79.84^{o}W). The reservoir is a drinking water source owned and managed by the Western Virginia Water Authority. The reservoir catchment is primarily forested, and includes one primary inflow stream that contributes ~95% of incoming water to FCR (Figure 2.1; Gerling et al. 2016). The inflow discharge rate is measured every 15 minutes on an INW Aquistar PT2X pressure sensor (INW, Kirkland, WA, USA) at a weir on the stream located ~250 m before it enters the reservoir.

Falling Creek Reservoir represents a continuum of a lotic to lentic ecosystem, with a shallow, more “riverine” zone (maximum depth in thalweg = 4 m) near the inflow, a

"transitional" zone (maximum depth = 7 m), and a deep "lacustrine" zone (maximum depth = 9.3 m) at the reservoir deep hole (Figure 2.1).

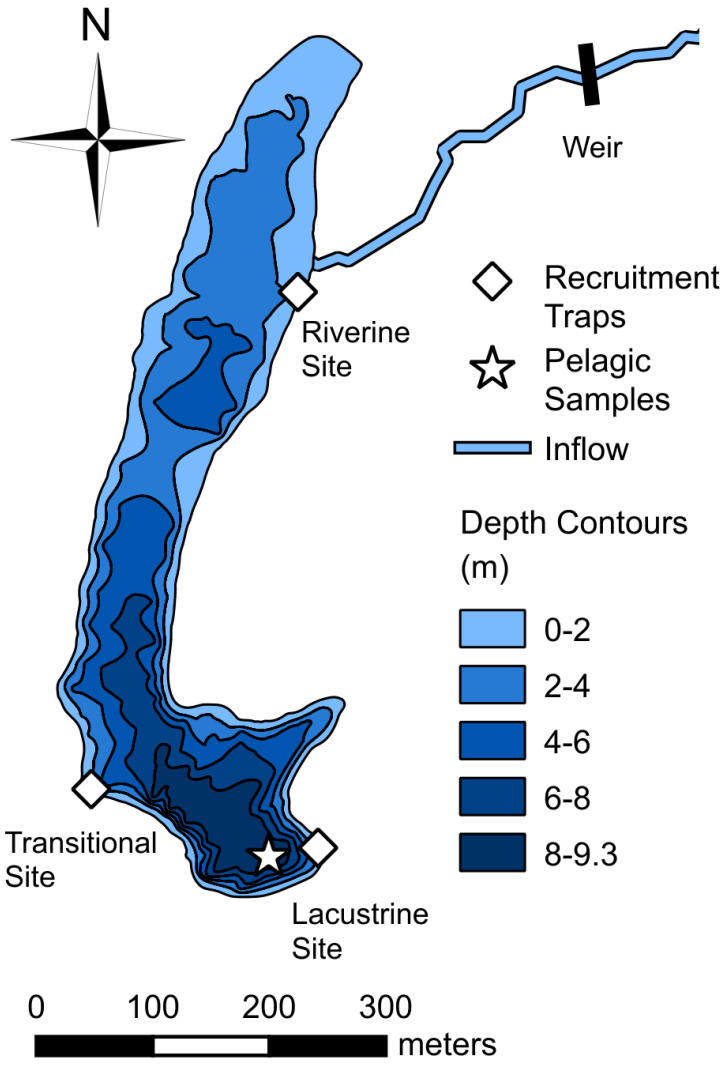

Figure 2.1. Bathymetric map of Falling Creek Reservoir, Vinton, Virginia, United States. Two replicate recruitment traps were deployed at each of the three reservoir zones: riverine, transitional, and lacustrine. The location of water chemistry and pelagic dinoflagellate sampling is denoted by a star. The inflow rate was measured at the weir entering the reservoir.

2.3.2 Dinoflagellate recruitment and pelagic population sampling

In 2014, we sampled dinoflagellate recruitment in FCR throughout the summer stratified period (6 June through 22 September) by collecting cells recruiting from the sediments into algal recruitment traps. Each recruitment trap consisted of a 90 mm-diameter transparent glass funnel attached to a white 250-mL plastic collection bottle, following the design of Carey et al. (2008). The traps were weighted and suspended ~20 cm above the sediments, with the opening of the funnel oriented downwards to collect organisms leaving the sediments, following previously used recruitment trap methods (Carey et al. 2008, Hansson 1996a).

We deployed traps at three sites that corresponded to the reservoir's riverine, transitional, and lacustrine reservoir zones (Figure 2.1). Each recruitment site contained duplicate traps hanging ~1 m apart. Previous studies have demonstrated that most dinoflagellate recruitment takes place from shallow sediments (Anderson and Armbrust 1987, Hansson et al. 1994), so we suspended the replicate recruitment traps from metal garden hooks (hereafter, recruitment trap masts) over ~1 m deep sediments. To stabilize the masts, we drilled holes in the bottom of 19-L plastic buckets, inserted the bottom prongs of the masts through the bucket bottoms, sank the buckets into the sediments, and then filled buckets with large rocks to anchor the masts. Masts were deployed in early May, giving disturbed sediments 4 weeks to stabilize before recruitment traps were hung on the masts on 6 June.

In this study, we defined the recruitment period as the duration of time extending from the day the traps were deployed until the day before they were sampled. We collected the contents of the recruitment traps weekly until late August, and then every 7-14 days through the end of September as recruitment rates decreased in late summer. On each sampling day, a snorkeler removed the trap by plugging the funnel stem to prevent contamination from surface

waters and bringing the sample bottle to the surface. The contents of the sample bottle were poured into a dark brown 250-mL plastic bottle and preserved immediately with Lugol's iodine solution. To prevent biofilm accumulation inside the recruitment traps, we replaced the trap bottles on each sampling day with fresh 250-mL bottles filled with tap water. Glass funnels were wiped clean or new funnels were substituted to ensure that they remained transparent throughout the monitoring period. After the cleaned recruitment traps were ready for deployment, we again plugged the funnel opening to prevent the tap water from leaking out while slowly lowering replacement traps back into position underwater. We took care not to disturb the sediments around the traps during deployment or sample collection. The recruitment trap mast in the lacustrine zone fell over on 13 August, and the mast in the riverine zone fell on 15 September; we were not able to reinstall these traps without disturbing the sediments.

We calculated recruitment rates into each trap according to standard methods (Hansson 1996a, Hansson 1996b, Carey et al. 2008). First, we quantified the number of cells of the two dominant dinoflagellate taxa (*Peridinium* and *Gymnodinium*) by subsampling the contents from each recruitment sample. We first homogenized the sample by stirring with a pipettor, then pipetted 0.1 mL subsamples into a Palmer-Maloney counting chamber. Using a Nikon Eclipse TS100 inverted microscope at 100x magnification, we counted multiple transects in the counting chamber. Ten subsamples were enumerated per recruitment trap; all microscopy was conducted by K.D.H. These counts were then converted to dinoflagellate recruitment rates in units of cells m^{-2} day^{-1} for each taxon.

To estimate the pelagic densities of each genus, we collected integrated phytoplankton samples approximately biweekly throughout the monitoring period from the deep hole (denoted by a star in Figure 2.1). Pelagic samples were collected with a plastic tube sampler (9 cm

diameter). We lowered the bottom of the tube to 4 m depth and capped the top of the tube to create a vacuum and ensure that no water was lost as we retrieved the tube bottom. The integrated epilimnetic water was added to a bucket and mixed thoroughly to homogenize the sample. We added 250 mL of this water to a dark brown sample bottle, which was preserved with Lugol's solution as described above. These samples were stored out of direct sunlight until microscopic analysis. Because the epilimnion was well-mixed through the summer (based on thermal profiles), we assume that these samples from one site are representative of pelagic dinoflagellate populations throughout the reservoir. We estimated pelagic dinoflagellate densities by settling 25 to 50 mL of preserved sample into a 5-mL counting chamber and counting cells on the Nikon Eclipse TS100 inverted microscope at 100x magnification following the protocol of Utermöhl (1958).

2.3.3. Environmental monitoring

To investigate which environmental variables might drive dinoflagellate recruitment, we collected physical, chemical, and weather data in the reservoir catchment and at the deep hole throughout the monitoring period. Thus, this study is focused on how dinoflagellate responses to the same set of measured environmental variables varied along the reservoir ecosystem continuum, and not on how the environmental variables themselves changed among reservoir zones – we did not monitor each variable in each reservoir zone. This approach is consistent with previous recruitment studies that compared recruitment data from multiple benthic sites with one pelagic site within a water body (e.g., Hansson 1994, Rengefors et al. 2004, Carey et al. 2014).

Catchment-scale variables included weekly mean inflow rate, mean residence time, and mean solar radiation. We used daily flow averages from the inflow weir to calculate mean

inflow rate and mean hydraulic reservoir residence time for each recruitment period, following the methods described by Gerling et al. (2016). Additionally, because light availability can be a major factor in driving recruitment patterns (e.g., Binder and Anderson 1986, Anderson et al. 1987), we obtained incoming shortwave radiation (solar irradiance) data on a 15-minute time scale from the Roanoke Regional Airport, located ~15 km from FCR. Similar to the procedure for daily flow data, we averaged the shortwave radiation measurement from noon on each day in a recruitment period to estimate the mean noon solar irradiance. Using radiation measured at noon vs. averaged over a longer midday period did not alter the findings from the statistical analyses.

Environmental data collected weekly at the reservoir deep hole included temperature, dissolved oxygen, turbidity, Schmidt stability, thermocline depth, Secchi depth, and nutrients. We used a 4 Hz CTD (Conductivity, Temperature, and Depth) SBE 19plus profiler (Seabird Electronics, Bellevue, Washington, USA) to collect high-resolution (~0.1 cm resolution) profiles of temperature, dissolved oxygen, and turbidity at the approximate midpoint of each ~7 d recruitment period. We then subsampled these CTD profiles to extract the temperature, dissolved oxygen, and turbidity as potential predictors of recruitment at 1.0 m depth to match the depth of recruitment traps. In addition, we used the full CTD temperature profiles to calculate reservoir Schmidt stability and thermocline depth using the *rLakeAnalyzer* package (Winslow et al. 2015), which calculates physical limnology metrics in the R statistical environment (R version 3.2.0, R Core Development Team 2015). On each sampling date, we also estimated water transparency using a Secchi disk and collected water samples to measure total and soluble fractions of nitrogen (N) and phosphorus (P).

For nutrient sampling, we used a 4 L Van Dorn sampler (Wildco Supply Company, Yulee, Florida, USA) to collect water from 1.6 m depth, which corresponded to the depth of the drinking water intake valve closest to the recruitment trap depth. After this water was homogenized in a bucket, a subsample was collected for total nutrient analysis and a second subsample for soluble nutrient samples was syringe-filtered in the field with 0.7 μm Whatman GF/F filters, both in acid-washed bottles. All samples were then frozen until laboratory analysis on a Lachat flow-injector analyzer (Lachat ASX 520 Series, Lachat Instruments, Loveland, Colorado, USA). Total N (TN) and total P (TP) concentrations were analyzed using USGS method I-4650-03, and soluble samples for ammonium (NH_4^+), nitrate-nitrite (NO_3^--NO_2^-), and soluble reactive P (SRP) were analyzed following the Quik-ChemMethod 10-115-10-1-B. We used these nutrient concentrations to calculate the molar ratios of TN:TP and dissolved inorganic N (DIN, the sum of NH_4^+ and NO_3-$^-NO_2^-$) to dissolved inorganic P (DIP, estimated as SRP).

2.3.4 Statistical analyses

To evaluate the temporal coherence (*sensu* Kratz et al. 1987) in recruitment rates within and across the riverine, transitional, and lacustrine sites, we calculated Spearman's nonparametric correlation of recruitment rates for all possible pairs of sites for each taxon (following Carey et al. 2008, 2014).

To test the prediction that dinoflagellate recruitment is more closely related to the physical environment in the riverine zone and to water chemistry in the lacustrine zone, we quantified the association between the monitored environmental variables and the temporal recruitment patterns of each taxon in each reservoir zone. First, we ln-transformed the recruitment rates for each taxon to meet the assumption of normality. Then, because many of our

environmental variables were closely correlated (| r | = 0.60 – 0.84), we used principal components analysis (PCA) in the R *vegan* package (Oksanen et al. 2015) to summarize the variability in the weekly averages for the catchment variables and the deep-hole environmental variables at the midpoint of each recruitment period. To interpret the resulting principal components (PC), we plotted the rank-ordered loading values for each variable, and used the inflection points in these plots to determine which variables loaded most heavily onto each PC. Finally, we calculated Pearson correlations between mean ln-transformed (n = 2 replicates) recruitment rates in each sample site and the scores from PC 1 and 2 to evaluate the associations between recruitment and potential multivariate drivers in each zone of the reservoir.

2.3.5 Contribution of recruiting cells to pelagic populations

To estimate the importance of recruitment to pelagic dinoflagellate populations, we calculated the percent recruitment contribution during sample periods when the pelagic population increased using standard methods (Carey et al. 2008, Karlsson-Elfgren et al. 2003). First, we used *Peridinium* and *Gymnodinium* recruitment rates to estimate the total number of cells entering the epilimnion during each recruitment period: we converted the median recruitment rate across all six traps (in cells m^{-2} day^{-1}) to total recruiting cells (cells day^{-1}) by multiplying recruitment rates by the area of FCR in the 0-2 m depth stratum. This assumes that the recruitment rates observed in traps at 1 m depth were representative of recruitment throughout FCR's 0-2 m stratum, a reasonable – and conservative – assumption, since reservoirs typically have steep bathymetry between the littoral and pelagic zones. Pelagic samples were typically collected on the same days as environmental samples, not recruitment trap samples, so we used daily estimates of dinoflagellate recruitment to estimate the total recruitment from 0-2 m

during each pelagic sample. Next, we multiplied the observed pelagic density of *Peridinium* or *Gymnodinium* (in cells L^{-1}) by the total epilimnetic volume to estimate the abundance of total epilimnetic cells, and then calculated the net change in pelagic density from the previous sampling period (the change in total epilimnetic cells). Finally, we calculated the relative contribution of recruitment to the change in the pelagic population using the following equation:

$$\text{recruitment contribution} = (\text{median total recruitment from 0-2 m}) / (\Delta \text{ total epilimnetic cells}) \quad \text{(eqn. 1)}$$

where changes in pelagic *Peridinium* or *Gymnodinium* populations not explained by recruitment rates were attributed to reproduction, cell death (e.g., due to grazing, lysis, etc.), and/or cell loss (e.g., due to discharge from the reservoir, forming cysts and leaving the pelagic life stage, etc.).

To provide some measure of uncertainty around the % recruitment contribution, we repeated the calculation using the minimum and maximum observed recruitment across all traps in each recruitment period.

2.4 Results

2.4.1 Recruitment patterns and contribution to pelagic populations

Recruitment rates of *Peridinium* and *Gymnodinium* varied spatially and temporally through the monitoring period (Figure 2.2), with pulses of high recruitment occurring at different times at each site throughout the summer. *Peridinium* recruitment rates were up to ~2-10 × higher than *Gymnodinium* (Figure 2.2), and *Peridinium* composed the majority of all recruiting dinoflagellate cells (50-100% of total cells in every trap each week) in Falling Creek Reservoir.

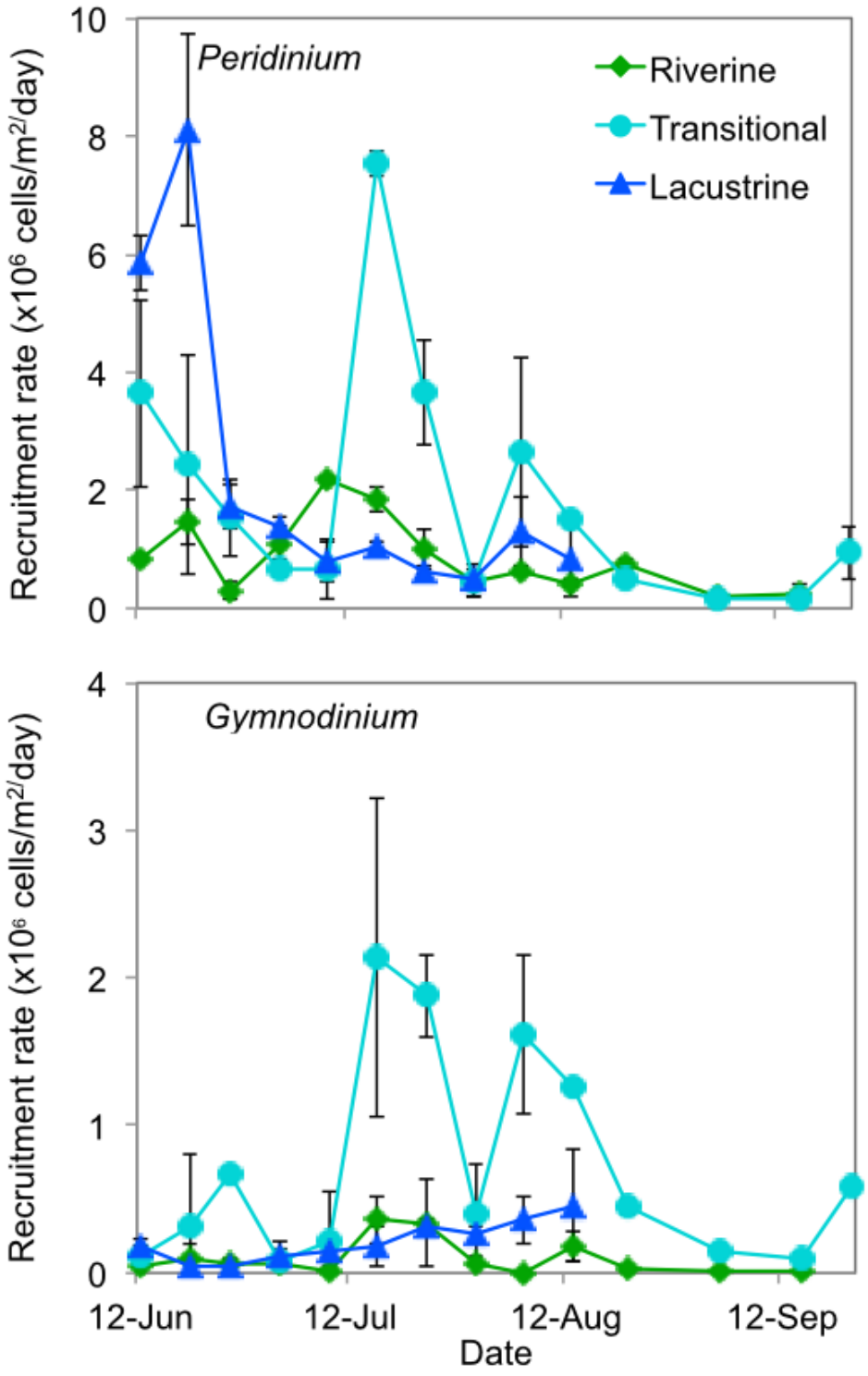
Peridinium
Riverine
Transitional
Lacustrine
Recruitment rate (x10⁶ cells/m²/day)
10
8
6
4
2
0
Gymnodinium
Recruitment rate (x10⁶ cells/m²/day)
4
3
2
1
0
12-Jun
12-Jul
12-Aug
12-Sep
Date

Figure 2.2. Mean ± 1 standard error of recruitment rates of *Peridinium* (top) and *Gymnodinium* (bottom) at three reservoir zone sites in Falling Creek Reservoir during summer 2014; note the different y-axis scales. The riverine and lacustrine series are truncated because their recruitment trap masts fell down during the sampling period.

Throughout the summer, *Peridinium* recruitment rates of replicate traps within a reservoir site were more closely correlated than recruitment rates among sites (Figure 2.3), even though the maximum distance between sites was <1 km. The highest correlation was observed for replicate recruitment traps within the riverine site; traps within the transitional and lacustrine sites were also highly correlated. Sites furthest from each other (riverine and lacustrine) were the least correlated (Figure 2.3). Importantly, *Peridinium* recruitment rates within sites did not exhibit significant temporal autocorrelation at 1 and 2 week lags (Pearson's r, $p > 0.06$ for all replicates).

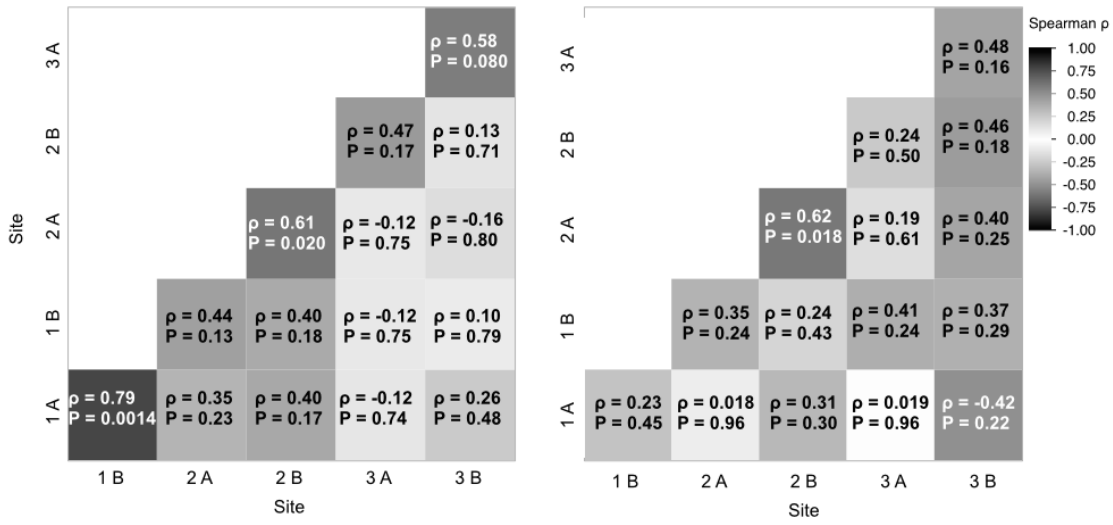

Figure 2.3. Spearman's ρ nonparametric correlations of *Peridinium* (left) and *Gymnodinium* (right) recruitment rates among all recruitment trap replicates in Falling Creek Reservoir in summer 2014. Site 1 = riverine zone, site 2 = transitional zone, and site 3 =

lacustrine zone; letters A and B denote replicate traps within each site. Darker colors indicate more closely correlated replicates, with white denoting Spearman's ρ correlations of ρ = 0 and black denoting ρ = ±1.0. Sites 1 (n = 13) and 3 (n = 10) have fewer sample periods than Site 2 (n = 14) because the recruitment trap masts fell over.

In contrast, *Gymnodinium* recruitment patterns were not closely correlated between site replicates within either the riverine or the lacustrine zone (Figure 2.3). *Gymnodinium* recruitment in the transitional zone was correlated between replicates (Figure 2.3); this site also generally exhibited the highest rates of *Gymnodinium* recruitment through the summer. No other traps among the three different sites exhibited strong correlations. *Gymnodinium* recruitment rates were not autocorrelated between replicates (Pearson's r, $p > 0.12$ for all replicates), but mean *Gymnodinium* recruitment did exhibit temporal coherence at the lacustrine site ($p = 0.019$), preventing us from comparing mean *Gymnodinium* recruitment with PC scores to analyze *Gymnodinium* along the reservoir continuum.

Recruitment patterns and pelagic populations appear to have been more closely synchronized at the beginning of the monitoring period for both *Peridinium* and *Gymnodinium* than at the end of the summer (Figure 2.4). For both taxa, pulses in recruitment lined up with peaks in pelagic populations in late June and mid-July; in August and September, the correspondence between changes in recruitment rates and pelagic abundances was weaker. These patterns were robust regardless of if the reservoir-wide minimum, median, and maximum recruitment rates were being examined. Overall, the median recruitment rate was closer to the minimum rate than to the maximum rate during most recruitment sample periods (Figure 2.4), but weeks with the highest maximum recruitment rates were closely coupled with peaks in pelagic populations for both genera (Figure 2.4).

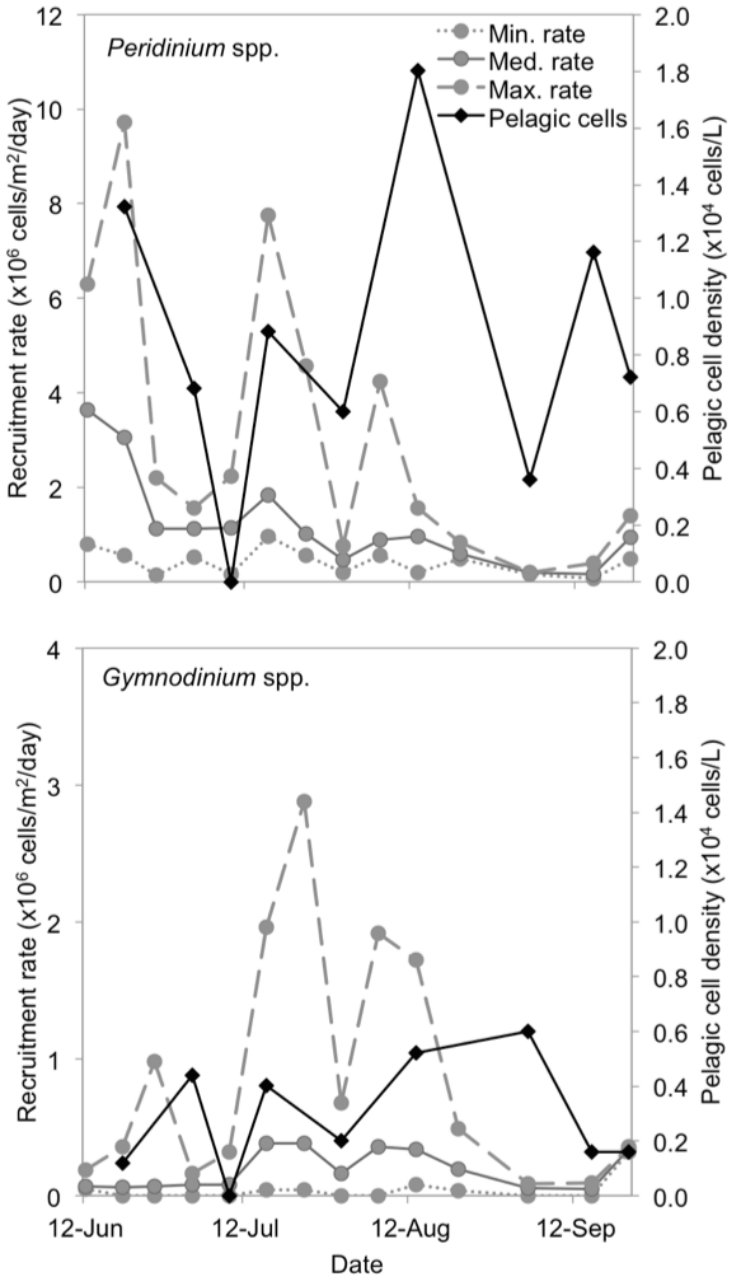

Figure 2.4. Minimum, median, and maximum observed recruitment rates (gray lines, calculated across all standing traps at that time period) and pelagic cell concentrations (black line) of *Peridinium* (top) and *Gymnodinium* (bottom) in Falling Creek Reservoir during summer 2014. Note that the recruitment rate scales differ between panels.

Recruitment from the sediments contributed to the pelagic population growth of both dinoflagellate taxa. In summer 2014, there were three sample periods when the pelagic

Peridinium population increased, allowing us to estimate the contribution of recruiting cells to pelagic populations. Median *Peridinium* recruitment rates contributed 6-16% of pelagic population growth in summer 2014 (Appendix A). *Gymnodinium* pelagic populations increased in four sample periods, and *Gymnodinium* recruitment contributed 3-150% of population growth (Appendix A). The 150% contribution estimate was observed in the week of 18 August and was due to recruitment rates that were higher than the pelagic population increase, indicating that some recruiting cells did not remain in the pelagic population (e.g., due to grazing, senescence, discharge from the reservoir, etc.).

2.4.2 Environmental conditions in the reservoir

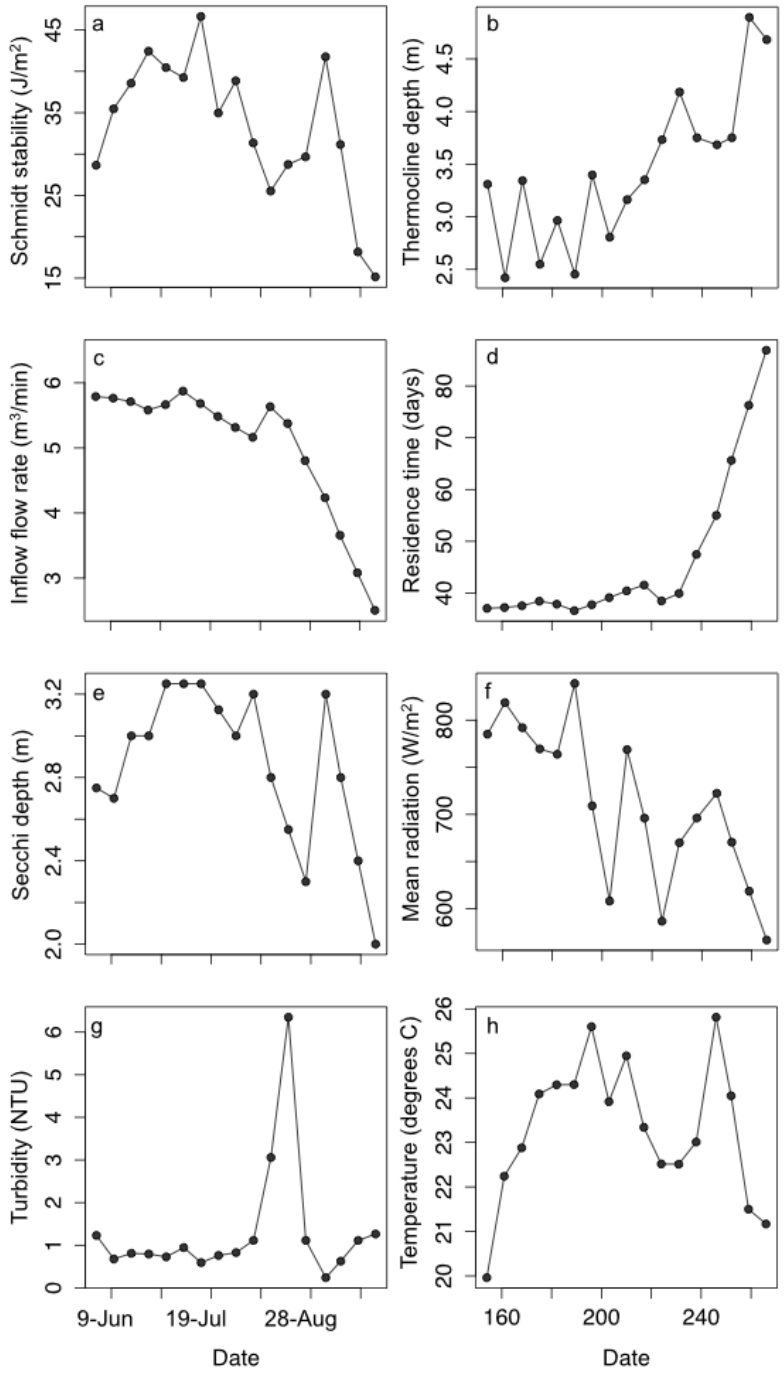

Figure 2.5. Reservoir physical variables: Schmidt stability (a), thermocline depth (b), inflow flow rate (c), residence time (d), Secchi depth (e), mean radiation (f), turbidity (g), and temperature (h) in Falling Creek Reservoir during summer 2014. Variables (a – e) strongly loaded onto PC1.

We observed seasonal changes in physics and water chemistry at the reservoir's deepest site throughout the monitoring period (Figures 2.5, 2.6). Water temperature at the depth of the recruitment traps warmed throughout June to August before decreasing in September (Figure 2.5h). Throughout the summer, FCR exhibited stratified conditions, with a well-mixed epilimnion extending to ~3 to ~4 m depth (Figure 2.5b). Schmidt stability decreased toward the end of the monitoring period as the reservoir approached fall turnover (Figure 2.5a). Due to dry summer conditions, inflow stream flow rate was lowest toward the end of the summer (Figure 2.5c), resulting in an increase in hydraulic residence time (Figure 2.5d). In contrast, Secchi depth and mean noon solar radiation did not follow a clear seasonal pattern (Figure 2.4e,f): both were highest in mid-summer (early July) and fluctuated toward the end of the monitoring period. Total and soluble N:P ratios, as well as concentrations of total and dissolved N and P fractions, were dynamic through the sampling season, yet suggested N limitation because DIN:DIP ratios were consistently <4 (Figure 2.6g). Epilimnetic dissolved oxygen concentrations fluctuated throughout the summer but remained above 7 mg/L (Figure 2.6f).

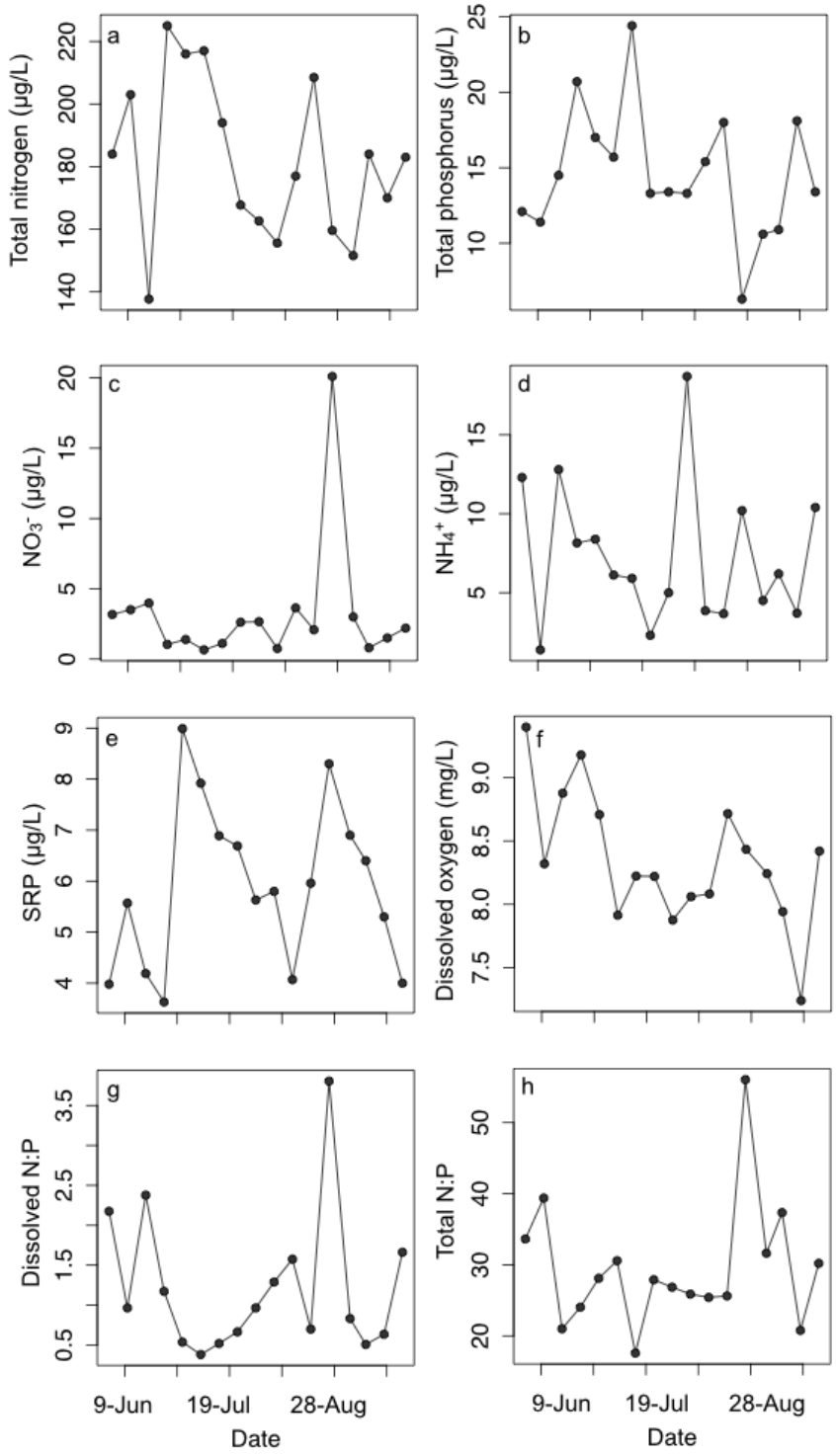

Figure 2.6. Water chemistry variables: Total nitrogen (a), total phosphorus (b), nitrate (NO_3^-, c), ammonium (NH_4^+, d), soluble reactive phosphorus (SRP, e), and dissolved oxygen (f) concentrations, and dissolved (g) and total (h) N:P ratios in Falling Creek Reservoir, summer

2014. Dissolved and total N:P fractions (g, h) were included in the PCA and strongly loaded onto PC2.

The PCA partitioned the physical and chemical variables into two separate multivariate axes (Figure 2.7, Appendix B), which together explained 67.5% of the variance. Physical environmental variables loaded strongly on PC1 (Figure 2.7, Appendix B). Inflow rate, solar radiation, Schmidt stability, and Secchi depth loaded positively onto this axis, while mean residence time and thermocline depth loaded negatively. In comparison, chemical environmental variables loaded strongly on PC2 (Figure 2.7, Appendix B). Dissolved oxygen and N:P ratios loaded positively onto this axis, while Secchi depth loaded negatively.

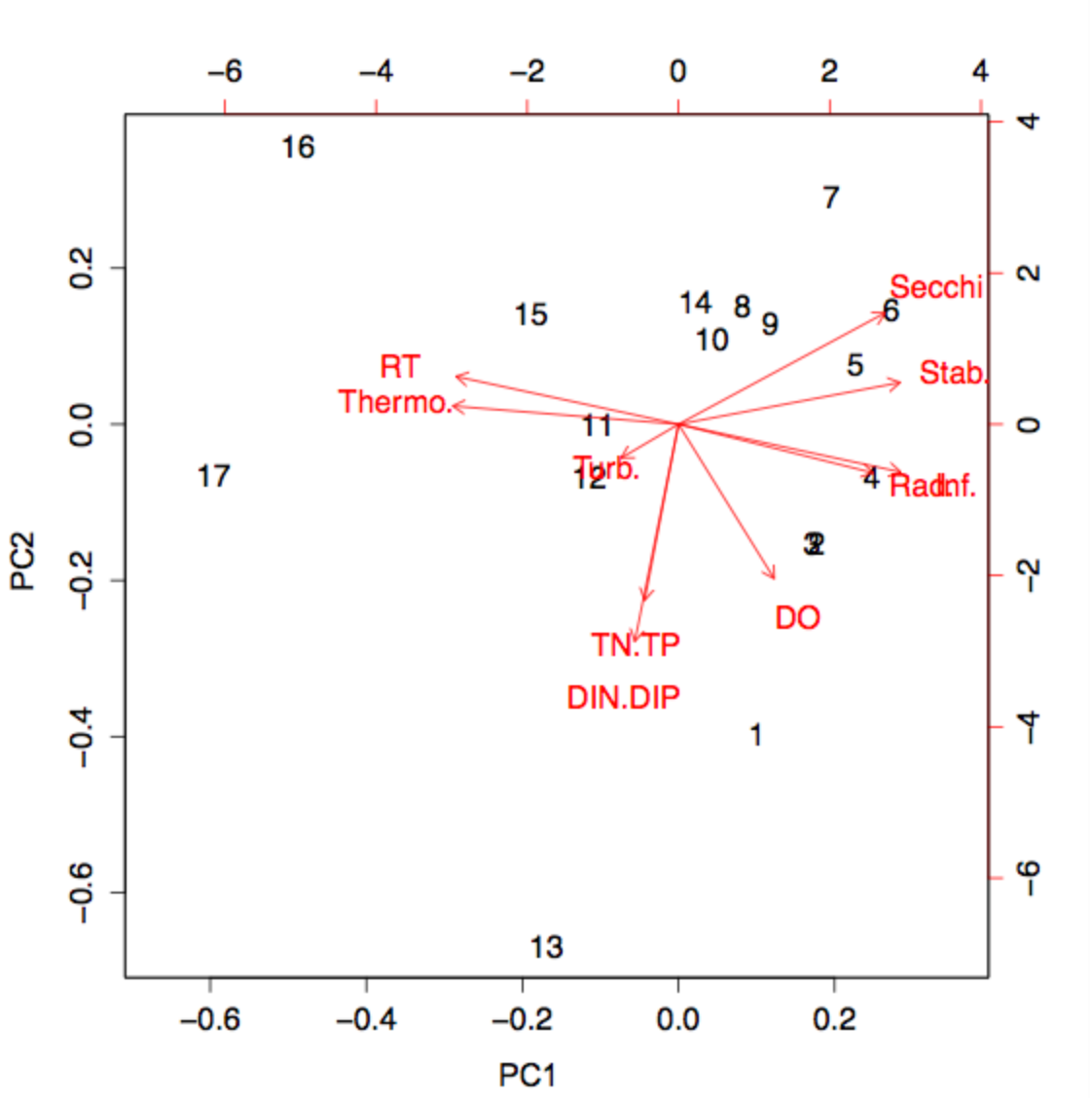

Figure 2.7. Biplot of observations (i.e., recruitment sample periods, numbered 1-17 in chronological order, weeks 2 and 3 overlap) and eigenvectors of environmental variables throughout the recruitment monitoring period in FCR. Bottom and left axes are normalized PC scores for the observations; top and right axes are loadings for the environmental variables; length of the red arrows represents the weight of their loadings onto PC1 (which explains 47.5% of variance) and PC2 (20% of variance). Environmental variables (clockwise from top) are abbreviated as: Secchi = Secchi depth; Stab. = Schmidt stability; Inf = mean inflow flow rate; Rad = mean radiation; DO = dissolved oxygen; TN.TP = total N:P; DIN.DIP = soluble N:P; turb.=turbidity; Thermo. = thermocline depth; and RT = mean residence time.

Plotting the PC scores for each sample period with the eigenvectors of environmental variables illustrates the multivariate changes in the FCR environment over the monitoring period (Figure 2.7): the position of each sample period relative to environmental loadings highlights the temporal variability in FCR and how the relative importance of environmental variables changed through the summer. For example, the variability among sampling periods was driven more by nutrients and dissolved oxygen earlier in the season, but then became dominated by physical drivers (radiation, inflow rate, Schmidt stability, and Secchi depth) during mid-summer. Throughout the rest of the summer, with the exception of week 13 (25-Aug – 1-Sep), physical drivers, primarily residence time, thermocline depth, and turbidity, dominated reservoir dynamics (Figure 2.7).

2.4.3. Relationship between recruitment and environment

Peridinium recruitment patterns in the three reservoir zones indicate differential sensitivity of *Peridinium* recruitment to physical and chemical environmental factors along a riverine-to-lacustrine reservoir continuum (Figure 2.8). Consistent with expectations, ln-transformed recruitment rates of *Peridinium* were positively correlated with the physical variables that loaded on PC1 at the riverine site and with the chemical variables that loaded on PC2 at the lacustrine site (Figure 2.8), but not vice versa. At the transitional site, *Peridinium* recruitment rates were weakly positively related to both PC1 and PC2 (Figure 2.8). We did not repeat these analyses for *Gymnodinium* due to lack of coherence between replicates within a site (Figure 2.3) and temporal autocorrelation at the lacustrine site.

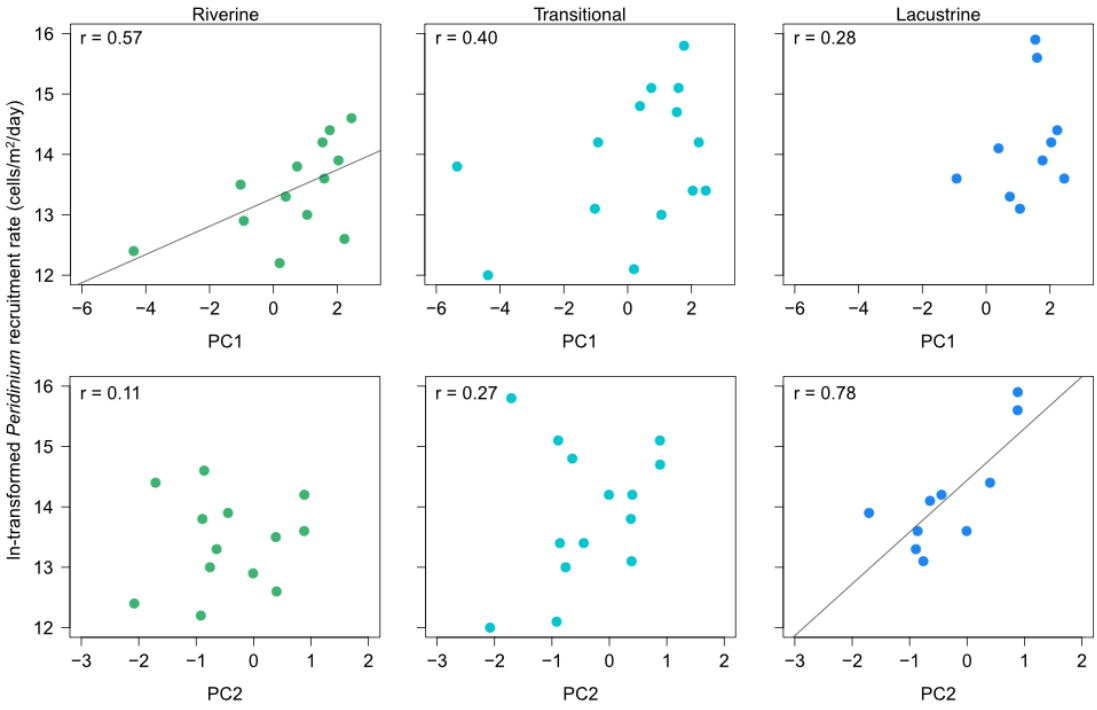

Figure 2.8. Ln-transformed mean *Peridinium* recruitment rates for each reservoir zone (lacustrine: left panels; transitional: center panels; and riverine: right panels) and PC scores (PC1: top panels, physical drivers; PC2: bottom panels, chemical drivers). Regression lines are shown for relationships with r > 0.5.

2.5 Discussion

An improved understanding of the factors controlling phytoplankton population dynamics is needed to better predict blooms in drinking water reservoirs. Our study demonstrates that recruitment from the sediments, a life history stage that is often overlooked in phytoplankton models, was an important factor promoting the pelagic population growth of two cosmopolitan dinoflagellate taxa in FCR. Our calculations indicate that median *Peridinium* recruitment contributed 6-16% of pelagic population increases, and median *Gymnodinium* recruitment contributed 2-106% of its population growth (Appendix A). Previous studies have estimated that algal recruitment rates contribute <10% of pelagic populations (Trimbee and Harris 1984,

Barbiero and Kahn 1994, Hansson et al. 1994), but recruitment of some cyanobacteria can contribute up to ~45% of pelagic populations (reviewed by Carey et al. 2014). Though our estimates of recruitment contribution were highly variable each week and only span one summer season, our data suggest that recruitment plays a larger role subsidizing dinoflagellate pelagic populations in FCR than in other freshwater systems. Dinoflagellates have slow division rates relative to other phytoplankton (Reynolds et al. 2006), so cells recruiting from the sediments may be provide an especially important subsidy for their pelagic populations.

Moreover, our results suggest that recruitment may be particularly important in reservoirs with short residence times (mean = 47 days in FCR in summer 2014), compared to natural lakes. Our estimates of the contribution of recruitment to pelagic populations were conservative because we assumed that recruitment from sediments deeper than 2 m was negligible; FCR's thermocline fluctuated between 2.5 m and 3.5 m during the high recruitment period in early summer (Figure 2.5a), and Hansson et al. (1994) found that recruitment of the dinoflagellate *Ceratium hirundinella* below the thermocline was negligible during summer stratification. Hence, recruitment likely occurred from the 2 – 3.5 m strata in FCR, contributing additional recruiting cells to the pelagic population. Furthermore, we estimated the importance of recruitment using the median recruitment rate calculated for each sample period, but if recruitment rates were spatially heterogeneous, then benthic subsidies to pelagic populations may actually be higher than our estimates. These estimates would be improved by including more pelagic sample sites to address horizontal patchiness in pelagic populations; however, we note that many previous studies have also only used one pelagic site to estimate recruitment contribution from multiple littoral recruitment traps (e.g., Hansson et al. 1994, Rengefors et al. 2004, Carey et al. 2014).

Throughout the monitoring period, *Peridinium* spp. was the dominant recruiting dinoflagellate taxon and had up to 50 × higher recruitment rates and 7 × higher pelagic abundances than *Gymnodinium* (Figures 2.2, 2.4). *Peridinium*'s dominance is likely due to multiple factors, which may include differences in the abundance of dormant cysts in the seed bank or different growth rates in the pelagic zone. Though both genera have been placed in the same Reynolds phytoplankton functional classification (Reynolds et al. 2002, Niesel et al. 2007), a survey of 24 German reservoirs over five years revealed that *Peridinium* and *Gymnodinium* very rarely co-occurred at high biovolumes (Niesel et al. 2007). Niesel et al. (2007) concluded that this was due to slight differences in their realized niche, and competition may also prevent the two genera from co-occurring. In these systems, *Gymnodinium* was more likely to dominate when TP concentrations were <10 μg/L and had a narrower pH tolerance (pH 6 – 7) than *Peridinium* (Niesel et al. 2007). These results support *Peridinium*'s dominance in FCR, which exhibited TP concentrations between 11 and 24 μg/L (Figure 2.6) and pH concentrations between 6.2 and 8.2 in summer 2014 (C.C.C, unpublished data).

The strong temporal coherence within our reservoir sites but lack of coherence among sites indicate that *Peridinium* recruitment is likely driven by similar environmental cues within a site but different environmental variables among sites. In contrast, *Gymnodinium* recruitment may be driven by factors other than environmental cues, or be responding to environmental cues at much finer spatial scales than *Peridinium*. Dinoflagellate recruitment can be heavily influenced by internal regulation, i.e., endogenous clocks within resting cysts (Andersen et al. 1987, Rengefors and Andersen 1998), which we did not measure but may have influenced recruitment patterns of *Gymnodinium* recruitment in FCR. Because *Gymnodinium* was not controlled by site-specific variables, we focused on *Peridinium* to test our hypothesis that

recruitment in the riverine zone would respond to physical variables, while recruitment in the lacustrine zone would be driven by changes in chemistry.

Changes in physical and chemical conditions are usually confounded in natural systems because they generally occur concurrently. Here, the unsupervised PCA separated the physical and chemical variables, providing an opportunity to examine how *Peridinium* responded to physical vs. chemical drivers across a reservoir continuum. Our results suggest that this dinoflagellate population may be driven by different factors in different reservoir zones, which has important implications for algal management in reservoirs. More broadly, our study contributes to the growing literature (e.g., Lind and Barcena 2003, Cunha and Calijuri 2011, Rychtecky and Znachor 2011) that has observed very different phytoplankton dynamics in the riverine vs. lacustrine zones of a reservoir.

The two dinoflagellate taxa exhibited substantial variability in recruitment rates among reservoir zones (Figure 2.3). In the riverine zone of the reservoir, variability in *Peridinium* recruitment was most closely related to physical conditions (Figure 2.8). This is likely because phytoplankton growth in the upstream riverine zone is often driven by physical variables, such as light availability and flow rate (Kimmel and Groeger 1984, Lind 2002, Cunha and Calijuri 2011). The riverine zone is usually shallower and more narrow than transitional and lacustrine zones; therefore, inflow flow rate should be relatively more important. Although we assumed a well-mixed epilimnion based on nutrient and thermal profiles, it is possible that nutrients were less limiting at the riverine site because of inflowing nutrient-rich stream water, thus reducing the relative sensitivity of algal recruitment to changing nutrient dynamics in comparison to physical conditions.

In the lacustrine zone, *Peridinium* recruitment rates were less sensitive to physics and instead more closely related to changes in nutrient chemistry (Figure 2.8). As expected, *Peridinium* recruitment rates were strongly related to PC2 scores ($r = 0.78$), which were driven by N:P ratios and dissolved oxygen concentrations, in spite of the shorter recruitment time series at this site. Reservoir lacustrine zones experience longer local residence times than upstream regions, and nutrient supply in this zone is usually driven more by internal nutrient cycling than external inflows (Kimmel and Groeger 1984). Thus, algal growth in downstream lacustrine zones is less closely coupled with reservoir physics and tends to be driven by internal nutrient chemistry (Kimmel and Groeger 1984, Lind 2002, Cunha and Calijuri 2011). Dinoflagellate growth is often limited at low (<16) N:P ratios (reviewed by Elser et al. 1990), and FCR experienced strong N limitation (dissolved N:P < 4) in summer 2014. Consequently, it follows that *Peridinium* recruitment rates in the lacustrine zone were highest during periods heavily influenced by increasing N:P ratios.

Our environmental variables were measured either at the reservoir deep hole (Secchi depth, nutrient chemistry, temperature, turbidity, and dissolved oxygen), or at the whole-reservoir scale (incoming solar radiation, inflow flow rate, reservoir residence time, Schmidt stability, and thermocline depth). Thus, our results reveal varied responses of dinoflagellates to the same core set of environmental drivers, and not changes in those environmental variables, along the reservoir continuum. Further investigation should include sampling environmental variables in each reservoir zone to improve our understanding of the factors driving dinoflagellate recruitment along the reservoir continuum. For example, our estimates of residence time were calculated for the whole basin, but local residence time in each zone is likely substantially different. Additionally, our data only span one summer, similar to the vast majority

of recruitment studies (reviewed in Carey et al. 2014), but a longer field study would provide greater insight into recruitment dynamics, especially for rare taxa, such as *Gymnodinium*, which may have very different densities between years (Hansen and Carey 2015).

2.5.1 Conclusions

Our results demonstrate that recruitment plays an important role in pelagic population dynamics of the two dominant dinoflagellates in Falling Creek Reservoir. Because dinoflagellates can form problematic blooms that represent a major water quality concern (Nakamoto 1965, Paerl 1988, Yamada et al. 1998, Hirabayashi et al. 2007), understanding the factors driving dinoflagellate recruitment, as well as the importance of recruitment to pelagic bloom formation, may improve reservoir management. In reservoirs with short residence times, such as FCR, pelagic phytoplankton population dynamics may depend heavily on recruitment, and response of recruitment patterns to environmental factors may be extremely variable spatially. We note that the maximum distance between reservoir recruitment trap sites in FCR was <1 km, indicating that the controls of dinoflagellate population dynamics can change rapidly over a short distance along the reservoir continuum. Consequently, we advocate consideration of both the spatial heterogeneity of environmental conditions and the changing response of algal recruitment along the reservoir continuum to improve management of dinoflagellate blooms in reservoirs.

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Chapter 3. *In situ* fluorometry reveals a persistent, perennial hypolimnetic cyanobacterial bloom in a seasonally anoxic reservoir[2]

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3.1 Abstract

Cyanobacterial blooms are increasing in freshwater waterbodies worldwide due to anthropogenic forcing. While most blooms occur at the water's surface, some cyanobacterial taxa, such as the toxin-producing *Planktothrix* spp., are able to modify their buoyancy to access high nutrient concentrations at deeper depths, creating large accumulations of biomass in the middle of the water column. However, despite that metalimnetic cyanobacterial blooms occur in many waterbodies, not much is known about the factors controlling their depth and biomass. Here, we used *in situ* fluorometry to examine the vertical distribution and[2] magnitude of *Planktothrix* biomass for two consecutive summers in a seasonally anoxic reservoir. We also collected depth profiles of light, temperature, and nutrients to determine which environmental drivers were most important for predicting *Planktothrix* biomass. In both summers, *Planktothrix* dominated the reservoir phytoplankton community, exhibiting a large (~100 μg/L), persistent bloom below the thermocline that lasted for ~100 days. The hypolimnetic bloom consistently exhibited its maximum biomass at the depth reached by 0.5-2.9% of incident light, as indicated

[2] A version of this chapter is in preparation for submission to *Freshwater Science*.

by both observed and modeled light profiles. Consequently, light availability was likely the most important factor driving the vertical distribution of the stratified *Planktothrix* bloom, and both light and nutrients together were strong predictors of cyanobacterial biomass at three hypolimnetic sample depths, explaining 71-93% of the variation in biomass. Our data suggest that the *Planktothrix* remained in the hypolimnion to maximize nutrient availability, while progressing slightly upward in the water column through each summer in response to light limitation. In contrast to previous studies focused on *Planktothrix* in the metalimnion, our findings demonstrate that *Planktothrix* can also dominate in the hypolimnion in low light and anoxic conditions, and form persistent blooms that last for multiple months. Consequently, as cyanobacterial blooms become more prevalent, it is critically important to monitor cyanobacterial blooms both at the surface *and* at depth in freshwater ecosystems.

3.2. Introduction

Cyanobacterial blooms are increasing in frequency and severity in freshwater lakes and reservoirs worldwide (e.g., Heisler et al. 2008, O'Neil et al. 2012, Visser et al. 2016), resulting in detrimental consequences for water quality and ecosystem functioning (Paerl et al. 2001, Ibelings and Chorus 2007, Paerl and Otten 2013). In addition, cyanobacterial blooms can affect freshwater food webs by altering nutrient availability and outcompeting other phytoplankton (Paerl and Otten 2013, Carey et al. 2014, Cottingham et al. 2015). Consequently, understanding the factors controlling cyanobacterial dominance in freshwater ecosystems is important for managing water quality and ecosystem functioning.

While cyanobacteria with gas vesicles typically accumulate at the water's surface in thermally-stratified lakes (Walsby et al. 1995, Walsby et al. 1997, Paerl et al. 2001), some cyanobacterial taxa form dense populations in the metalimnia of lakes and reservoirs. These blooms are generally located at or above the depth reached by 1% of incident light reaching the water's surface, which is considered the minimum amount of light needed for photosynthesis (Ryther 1956, Reynolds 1987, Mur et al. 1999). Little is known about why these deep accumulations of cyanobacteria form, as most research on cyanobacteria has focused on surface scums (reviewed in Paerl et al. 2001). It is thought that these metalimnetic taxa modify their buoyancy and grow at relatively low light conditions at depth in thermally-stratified systems to maximize both nutrient availability in bottom waters as well as light availability from above (Reynolds 1987, Mur et al. 1999). Thus, the vertical distribution of metalimnetic cyanobacteria may be predicted by light and nutrient conditions, though the relative importance of these drivers remains unknown.

Planktothrix is a common cyanobacterial genus that forms dense metalimnetic populations (Reynolds 2006, Camacho et al. 2000, Walsby and Schanz 2002, Briand et al. 2005, Jacquet et al. 2005). The incidence of *Planktothrix* in the metalimnion of European lakes has been increasing during recent decades (Jacquet et al. 2005, Ernst et al. 2007), which is of growing concern because *Planktothrix* spp. can produce the toxin microcystin (Christiansen et al. 2003, de Figueiredo et al. 2004, Ernst et al. 2007). High concentrations of *Planktothrix* can threaten ecosystem functioning and water quality, so it is important to understand the environmental conditions promoting *Planktothrix* blooms at depth. Furthermore, determining the factors controlling the incidence and depth of cyanobacteria in deeper waters, where blooms are not typically expected, would provide insight to the drivers of cyanobacterial dominance in freshwaters.

Increasing nutrient concentrations and seasonal hypolimnetic anoxia may promote *Planktothrix* blooms in freshwater systems. *Planktothrix* are favored by high total phosphorus (TP) concentrations (Anderson et al. 1987, Steinberg and Hartmann 1988) and thermally stratified conditions, and also need a nitrogen (N) source from the environment because it is a non-N-fixing taxon. Many eutrophic freshwater ecosystems experience anoxia in their bottom waters during summer stratification (reviewed by Jenny et al. 2016), which can cause internal nutrient (nitrogen, N, and phosphorus, P) loading from the sediments (Boström et al. 1988, Søndergaard et al. 2003). In these eutrophic, anoxic systems, *Planktothrix* may have a competitive advantage over other phytoplankton. Their ability to alter their vertical position within the water column during stratified periods (Reynolds 1987, Dokulil and Teubner 2000), as well as their adaptation to low light levels (Oberhaus et al. 2007, Bonilla et al. 2012), allow them to remain in the metalimnion and access nutrients available below the thermocline (Reynolds

1987). Consequently, as hypolimnetic anoxia becomes more frequent and severe due to anthropogenic activity (Jiménez Cisneros et al. 2014), many lakes and reservoirs will experience increased internal nutrient loading (Søndergaard et al. 2003), potentially increasing the incidence of *Planktothrix* blooms.

Because most *Planktothrix* research has been based off laboratory cultures (Walsby et al. 2004, Oberhaus et al. 2007), surface water samples (Rojo and Cobelas 1994, Briand et al. 2008), or homogenized water column samples (Rücker et al. 1997, Legnani et al. 2005), field studies on *Planktothrix* dynamics below the thermocline are needed to improve our understanding about the drivers and persistence of metalimnetic *Planktothrix* blooms (Davis et al. 2003, Jann-Para et al. 2004, Walsby et al. 2004, Jacquet et al. 2005). Here, we monitored *Planktothrix* population dynamics throughout the entire water column during two consecutive summers in a moderately eutrophic reservoir that experiences seasonal hypolimnetic anoxia. We used *in situ* fluorometry to determine the vertical distribution of *Planktothrix*, and measured light, temperature, and nutrient profiles to examine which environmental factors were most important in determining the magnitude and depth of cyanobacterial biomass in both years. We used this dataset to address two questions: first, what is the vertical distribution of cyanobacteria throughout the summer stratified period in each year?; and second, which environmental factors are most important in driving *Planktothrix* biomass at depth? Our overarching goal was to improve our understanding of the drivers of *Planktothrix* blooms in stratified, high-nutrient freshwater systems.

3.3 Methods

3.3.1 Study site

We monitored *Planktothrix* populations in Beaverdam Reservoir (BVR), a dimictic reservoir located in Vinton, Virginia, USA (37.316474, -79.818826), that is owned and managed by the Western Virginia Water Authority (WVWA). The reservoir has a surface area of 0.39 km^2 and a maximum depth of 13 m at full pond (Figure 3.1). Though it is not directly used as a drinking water source, BVR has an outflow pipe that flows into Falling Creek Reservoir, a drinking water source for residents of Roanoke, Virginia (Gerling et al. 2016). BVR was built in 1872 and currently has a completely forested catchment, though the land surrounding the reservoir was used for agriculture before it was abandoned in the 1930s (WVWA, unpublished data). BVR experiences hypolimnetic anoxia during summer stratification, leading to internal loading of nutrients (N and P).

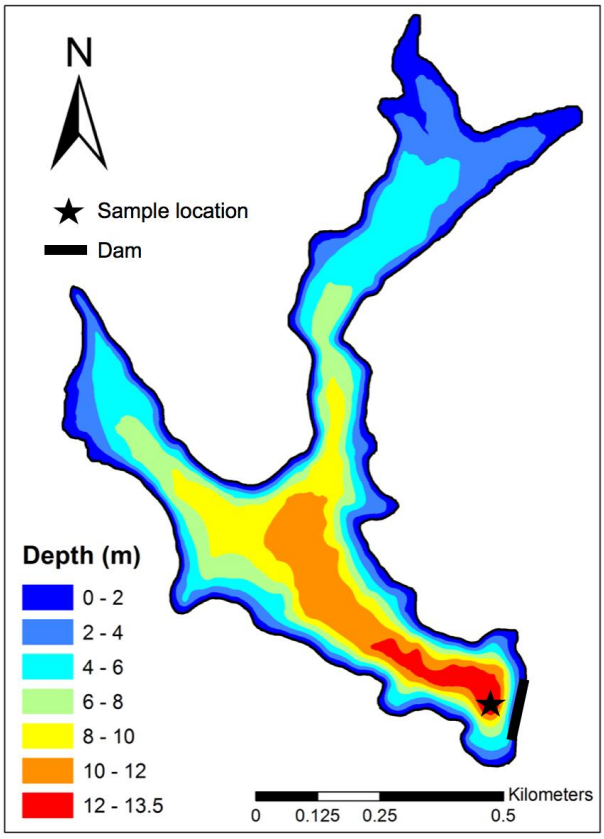

Figure 3.1. Beaverdam Reservoir (BVR) bathymetric map at full pond (37.316474, -79.818826).

3.3.2 Field methods: Routine monitoring

We collected physical and chemical depth profiles in BVR every 1-2 weeks during May-August and every 2-3 weeks during September during both 2014 and 2015. All profiles were sampled at the reservoir's deepest site near the dam (Figure 1). On each sample date, we used a 4 Hz CTD (Conductivity, Temperature, and Depth) SBE 19plus profiler (Seabird Electronics, Bellevue, Washington, USA) to collect ~1 cm water column profiles of water temperature, dissolved oxygen concentration (SBE 43 probe), turbidity, and conductivity. Secchi depth was

also measured on every sampling day. In 2015, we measured depth profiles of photosynthetically active radiation (PAR) at 1 m depth resolution with an LI-COR LI-250 underwater light meter (Lincoln, Nebraska, USA); water column PAR profiles were not collected in 2014.

In both years, we collected water samples with a 4-L Van Dorn sampler (Wildco Supply Company, Yulee, Florida, USA) for analysis of total and soluble fractions of N and P. In 2014, water samples were collected at 0.1, 4, 8, and 12 m depth. In 2015, we increased our sampling resolution and collected samples at 0.1, 3, 6, 9, and 12 m depth. We homogenized each depth sample from the Van Dorn in a bucket and collected 125 mL of this water for total N and P. For soluble nutrient samples (ammonium (NH_4^+), nitrate-nitrite (hereafter referred to as NO_3^-), and soluble reactive phosphorus (SRP)), we first syringe-filtered 125 mL of this water with 0.7-µm Whatman GF/F filters into acid-washed bottles. We froze all N and P samples until laboratory analysis.

We collected high-resolution profiles of phytoplankton biomass using a calibrated Fluoroprobe (Moldaenke, Schwentinental, Germany), a submersible fluorometer that uses fluorescence at multiple wavelengths to measure the *in situ* biomass of cyanobacteria, diatoms, chlorophytes, cryptophytes, and total chlorophyll *a* in µg/L (Moldaenke 2007). Fluoroprobe measurements have been closely correlated with phytoplankton counts and chlorophyll *a* derived from spectrophotometry and microscopy in a range of lakes (e.g., Gregor and Marsalek 2004, Ghadouani and Smith 2005, Catherine et al. 2012, Pannard et al. 2015). Fluoroprobe depth profiles were collected at the same location and frequency as the CTD profiles, with the fluorometer set to collect a reading every 3 seconds, yielding ~20-40 cm depth interval resolution. In both summers, we used the Van Dorn sampler to collect water samples from the chlorophyll maximum in the water column observed by the Fluoroprobe and used a Nikon

Eclipse TS100 model inverted microscope at 100x-400x magnification to identify the dominant phytoplankton taxa. In 2015, we collected water samples for manual (filtered) chlorophyll-*a* and pheophytin analysis from the same depths as the water chemistry samples on three sample days in August and September when cyanobacterial biomass was at high levels. We brought ~500 mL of water from each sampling depth back to the laboratory, filtered this water through 0.7-µm Whatman GF/C filters, and froze filters until laboratory analysis.

3.3.3 Laboratory methods

We analyzed water chemistry samples for total and soluble fractions of N and P to investigate relationships between nutrient concentrations and cyanobacterial biomass. We analyzed TN and TP concentrations following USGS method I-4650-03, and analyzed NH_4^+, NO_3^--NO_2^-, and SRP according to the Quik-ChemMethod 10-115-10-1-B using a Lachat flow-injector analyzer (Lachat ASX 520 Series, Lachat Instruments, Loveland, Colorado, USA).

We measured manual chlorophyll-*a* samples using spectrophotometry, following standard methods for the spectrophotometric analysis of chlorophyll *a* after ethanol extraction (American Public Health Association 1998). We extracted chlorophyll from the filters for 24 hours in 10 mL of 96% ethanol buffered with $MgCO_3$, then removed the filters from the test tubes and placed tubes in a centrifuge for ten minutes. After samples were centrifuged, we recorded absorbance at 664, 665, and 750 nm on a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan), then acidified samples with 0.1mL of 0.1 N HCl. At 2-3 minutes after acidification, we recorded absorbance at the same wavelength, and used these values to calculate the proportion of living versus nonliving chlorophyll following Wetzel and Likens (1991).

3.3.4 Reservoir thermal structure

We used CTD thermal profiles to calculate the thermocline depth, as well as the depth of the top and bottom of the metalimnion layer, on each sample date. We used *rLakeAnalyzer* (Winslow et al. 2015) to calculate these metrics in the R statistical environment (R v. 3.2.0 R Development Core Team 2015).

3.3.5 Modeling underwater PAR

Previous studies have demonstrated that light availability at depth is an important driver of metalimnetic cyanobacterial biomass (e.g., Fee 1976, Mur and Schreurs 1995). Secchi disk measurements are often used to estimate underwater light availability, but this method assumes consistent attenuation of light throughout the water column. Because BVR experiences substantial internal loading of metals (WVWA, unpublished data), as well as high hypolimnetic turbidity during summer stratification, using epilimnetic Secchi measurements to estimate light availability in the reservoir hypolimnion would not reflect the rapid attenuation of light at the thermocline. Moreover, our Secchi measurements in 2014 and 2015 were always shallower than the thermocline; therefore, extrapolating Secchi measurements to predict the hypolimnetic light environment would have been inappropriate.

To overcome this issue, we created a simple model to estimate hypolimnetic light attenuation in BVR in 2014, when PAR was not directly measured with an underwater light meter. We used turbidity and conductivity measurements collected by the CTD in 2015 to predict light attenuation, validated the model by comparing with PAR profiles from 2015, and then used the model to estimate hypolimnetic light attenuation in 2014. Because the spectral characteristics of the hypolimnion were dominated by reduced metals and particles, we modeled

hypolimnetic light availability at depth *z*, or the log-transformed percent of incident light at the surface reaching *z*, as a function of turbidity and conductivity measurements following previous studies (Blom et al. 1994, Davies-Colley and Smith 2001, Van Duin et al. 2001, Oliver et al. 2010). Turbidity from sediments and dissolved organic matter are major sources of light attenuation (Dodds and Whiles 2010), and these parameters have been commonly used to predict light attenuation (Austin 1973, Di Toro 1978, Van Duin et al. 2001). In systems with high concentrations of metals, conductivity can also affect the underwater light climate (Oliver et al. 2010). Hence, as turbidity and conductivity increased in the BVR hypolimnion throughout summer stratified seasons, light availability decreased. We were primarily interested in hypolimnetic light attenuation patterns, so we excluded the epilimnion (depths shallower than 4 m) from our light model. We also excluded depths below 9 m, because PAR measurements below 9 m in 2015 were almost always zero. Therefore, our model is applicable only for the 4-9 m zone of Beaverdam Reservoir, which encompasses the depths where *Planktothrix* was observed during both years of monitoring.

First, we used turbidity and conductivity data to model the percent of surface light reaching different depths in the hypolimnion during summer 2015 (Oliver et al. 2010); turbidity and conductivity at the same depth and time were not correlated ($r = 0.15$). We focused on the percent of surface light in the water column and not raw PAR observations so that the model would not be affected by day-to-day variability in PAR due to weather. We used all observations from below the mean summer thermocline depth (4 m) for which we had PAR, conductivity, and turbidity measurements ($n = 67$). We log-transformed the percent of surface light and used multiple linear regression to predict light in the hypolimnion of BVR for a specific hypolimnetic depth on any given day using R software. The best-fitting model ($R^2 = 0.80$, $p < 0.001$) was:

log(percent of surface light) = 2.18(± 0.18) - 0.13(± 0.023) × turbidity - 0.050(± 0.0038) × conductivity (eqn. 2)

with all light, turbidity, and conductivity observations from the same depth and day, and all parameter estimates ± 1 standard error. As a check on this model, we bootstrapped the observational data and found that light availability models created with only one randomly-chosen data point per day (vs. including all observations on a sampling day) were not statistically different (Cox Likelihood-Ratio test, $p = 0.59$).

Second, we used the model to predict the percent of surface light reaching our hypolimnetic sample depths for 2015 and compared it with the measured PAR profiles. In 2015, the predicted log-transformed percent of surface light was closely correlated with the observed values of light availability from the PAR profiles (Figure 3.2, $r = 0.86$). Finally, we then used the light availability model with 2014 turbidity and conductivity data to estimate the percent of surface light reaching different depths in the hypolimnion on each sample date in 2014. We used modeled light values, rather than observed values, in our 2015 analyses to maintain consistency between the years and to estimate light availability on the two sampling days when PAR profiles were not measured in that year.

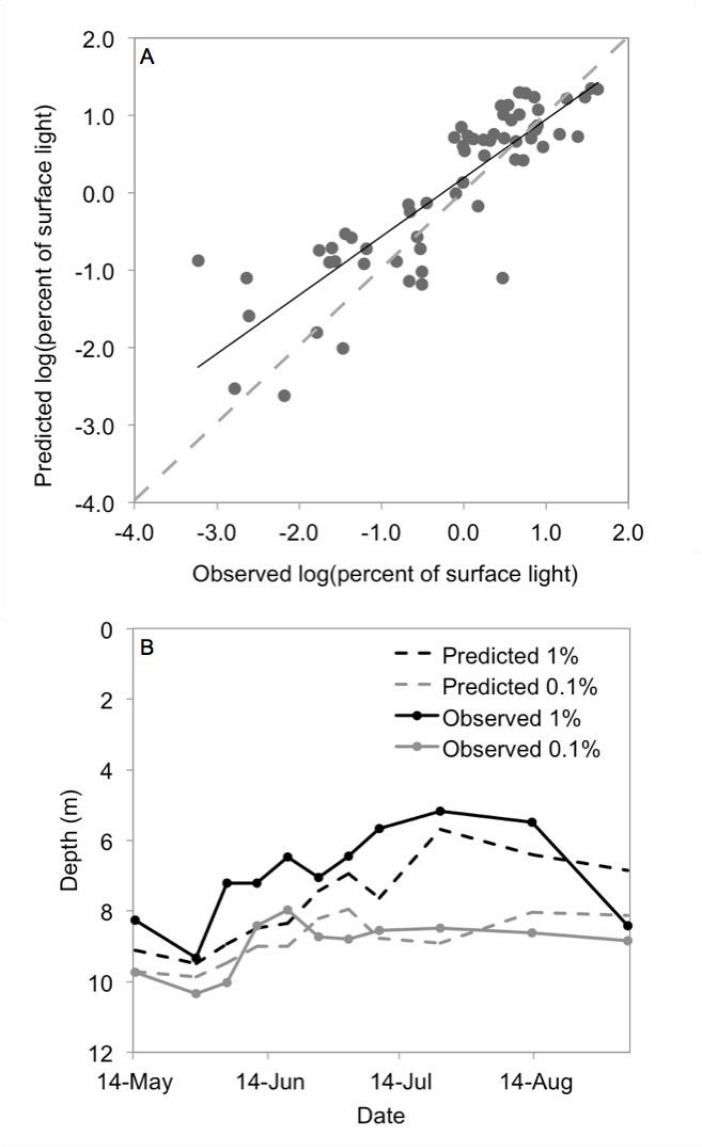

Figure 3.2. (A) Observed vs. predicted values of the log of percent of surface light in BVR water at 4-9 m depth, summer 2015. Solid line = linear trend; dashed line = 1:1 line. (B) Observed and predicted depths reached by 1% and 0.1% of surface light followed similar patterns in Beaverdam Reservoir in 2015. The depth reached by 1% of surface light predicted by the model was generally deeper than respective observed depth, indicating the model may slightly overestimate light availability at this depth.

3.3.6 Statistical analyses

We analyzed the relative importance of reservoir chemistry (total and soluble fractions of N and P) and physics (light estimated from the model described above and water temperature) as

potential drivers of cyanobacterial biomass (as determined by Fluoroprobe measurements) in BVR using a time series model. Because we collected water column water chemistry data at different hypolimnetic depths in both years (8 m in 2014 and 6 and 9 m in 2015), we focused on these three depths separately for our time series models; these were the only sample depths at which we observed a *Planktothrix* bloom throughout the two-year monitoring period.

The goal of the time series analysis was to determine the most important predictors of cyanobacterial biomass at the focal hypolimnetic depths throughout the summer season, not how the importance of the predictors changed week to week. Cyanobacterial biomass was autocorrelated on a one-week time lag (r = 0.81 at 8 m in 2014; r = 0.63 at 6 m and r = 0.47 at 9 m in 2015). Consequently, we used an autoregressive time series model approach with the entire dataset within one year and used AICc to compare alternate AR1 models with different environmental predictors. Because most of the candidate environmental predictors (light, nutrients, temperature) were significantly correlated, we compared all environmental predictors separately in models that included a cyanobacterial biomass AR1 term. We square root-transformed cyanobacterial biomass to meet the assumption of normality. The general form of the time series model was:

$$\text{sqrt cyanos}_{t+1} = \text{sqrt cyanos}_{t} + \text{environmental predictor}_{t} + \varepsilon \quad \text{(eqn. 3)}$$

where square root-transformed cyanobacterial biomass (abbreviated as sqrt cyanos) in the next week ($t + 1$) was the response variable, and square root-transformed biomass in the same week (t) as our environmental observations and an error term were the predictor variables. To include the entire sampling period for a year in the time series model, we linearly interpolated cyanobacterial biomass and environmental data in the early- and late-season periods when

samples were collected every 2-3 weeks so that our dataset was evenly spaced on a weekly time step. Because our dataset only had n=17 weeks for each year (with two weeks of interpolation), we were unable to use ARIMA models or other more data-intensive time series approaches.

We used AICc to choose the best-fitting model predicting cyanobacterial biomass for each focal hypolimmnetic depth. Finally, we compared predicted biomass from the best-fitting model to observed biomass in the reservoir hypolimnion measured by the Fluoroprobe.

3.4 Results

3.4.1 Cyanobacterial dominance in the reservoir

In both years, BVR experienced a persistent cyanobacterial bloom in the hypolimnion that lasted for most of the summer stratified period (Figure 3.3 A, B). In 2014, cyanobacteria appeared at ~10 m depth (2 m above the sediments) in June. Throughout the summer, the cyanobacterial bloom moved upward in the hypolimnion at a rate of approximately 0.5 m/week from May through August, (calculated using the depth of maximum cyanobacterial biomass), reaching ~7 m depth by September. The cyanobacterial bloom reached its peak biomass (92 μg/L) in late August and decreased quickly by the end of September, at which time no bloom was still detectable. In 2015, the cyanobacterial bloom began to form in late May, reaching its peak concentrations (96 μg/L) in late July, ~1 month before peak concentrations were observed in 2014. As in 2014, the depth of the cyanobacterial maximum moved upward in the hypolimnion, from ~9 to ~6 m, throughout the summer in 2015, even when concentrations decreased in August and September. From May 2015 to August 2015, the depth of maximum

cyanobacterial biomass moved upward at a rate of approximately 0.4 m/week. Similar to 2014, cyanobacterial biomass quickly decreased in September.

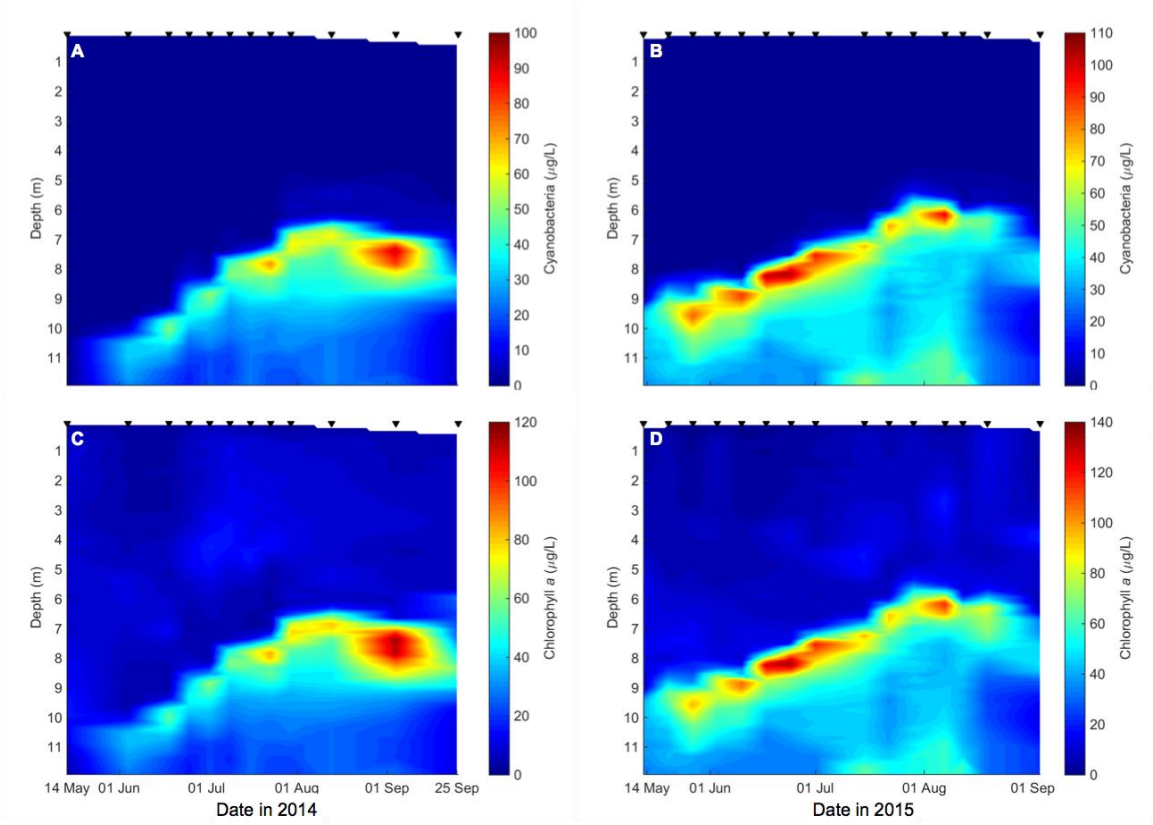

Figure 3.3. Cyanobacterial (A, B) and total chlorophyll *a* (C, D) biomass in summers 2014 (right column) and 2015 (left column) in Beaverdam Reservoir. Sampling dates are denoted by black triangles at tops of plots; space between dates is interpolated. White space at the top of plots indicates a slight decrease in water level in late summer. Note that the scales differ between cyanobacterial and total chlorophyll *a* concentrations, and between years.

Cyanobacteria in the hypolimnion dominated the phytoplankton community by biomass in both years (Figure 3.3), and patterns of total chlorophyll closely matched those of cyanobacterial biomass (Figure 3.3 C, D). Microscopic identification of hypolimnetic water samples revealed that the cyanobacterial community was dominated (>90% of cells) by *Planktothrix agardhii*. Total phytoplankton chlorophyll *a* in the epilimnion was much lower than

in the hypolimnion (<15 μg/L in both summers), and was primarily composed of green algae and diatoms (Appendix 1). No phytoplankton group (diatoms, green algae, or cryptophytes) other than the cyanobacteria exhibited concentrations higher than 32 μg/L throughout both summers (Appendix 1). On the three days in 2015 with manual (filtered) chlorophyll *a* samples, on average, 43% (± 12%, 1 S.D.) of chlorophyll *a* at 6 m was living, and 88% (± 4%, n = 2 replicates) of chlorophyll *a* at 9 m was living at the time of sample collection.

3.4.2 Reservoir environmental conditions

BVR exhibited strong thermal stratification and hypolimnetic anoxia in both summers (Figure 2). In both years, the water column was thermally stratified when cyanobacteria were first observed in the hypolimnion (Figures 3.3, 3.4). The thermocline depth ranged from 3.15 – 5.11 m in 2014 and 2.92 – 5.06 m in 2015. Consequently, the depth of maximum cyanobacterial biomass was always at least 2.6 m deeper than the thermocline in 2014, and at least 1.2 m below the thermocline in 2015, indicating that the cyanobacterial biomass was primarily hypolimnetic. Thermal stratification persisted throughout the sampling period in both years, and cyanobacterial biomass decreased before fall turnover in both 2014 and 2015. In 2014, hypolimnetic oxygen concentrations were already depleted (<5 mg/L) when monitoring began on 14 May, and the hypolimnion was completely anoxic (dissolved oxygen concentrations below the CTD's detection limit, 0.24 mg/L) by June. In 2015, anoxia throughout the hypolimnion was observed when monitoring began on 14 May.

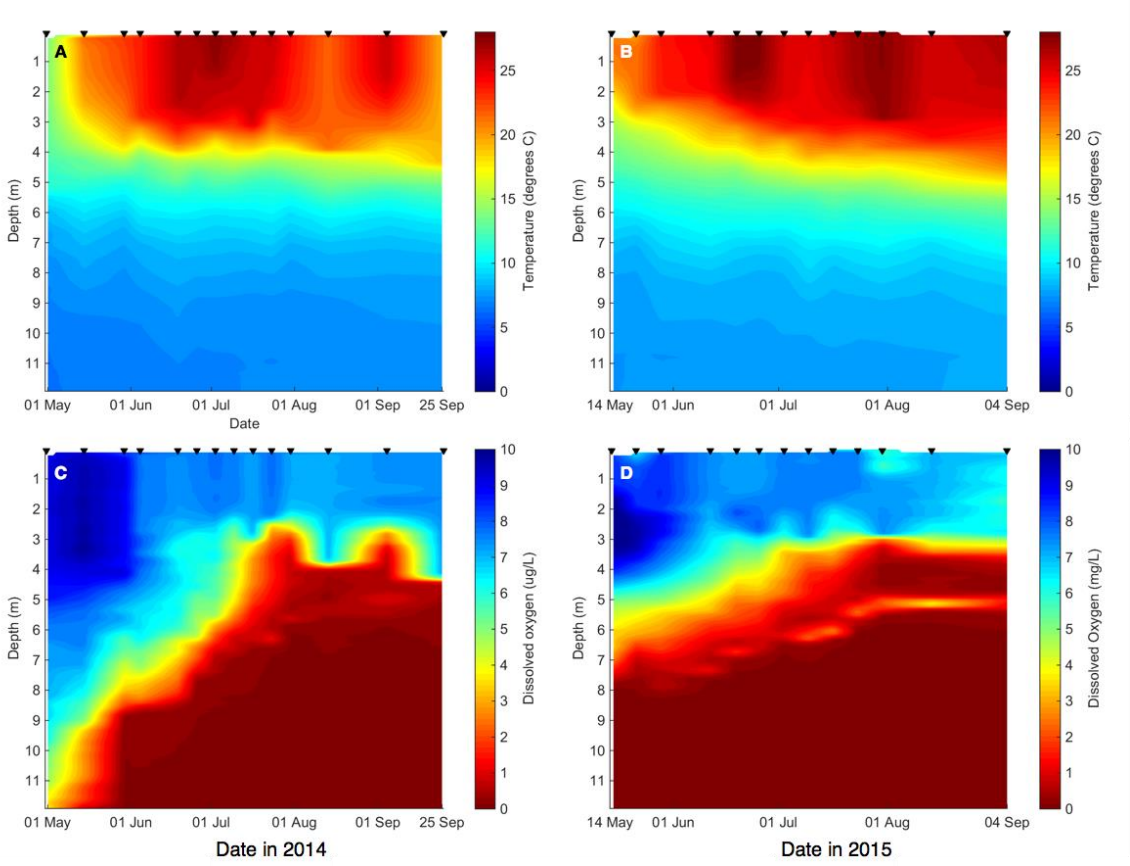

Figure 3.4. Water temperature (top row) and dissolved oxygen concentrations (bottom row) in Beaverdam Reservoir, in summers 2014 (left column) and 2015 (right column).

Anoxic conditions likely resulted in large internal nutrient loads from the sediments into the water column, as evident from the high concentrations of TP, TN, and NH_4^+ and low NO_3^- concentrations in the hypolimnion just above the sediments (Figure 3.5). Hypolimnetic nutrient concentrations followed similar patterns both years, with slightly higher total phosphorus concentrations in 2015 than 2014, reaching up to 93 μg/L in 2015 and 68 μg/L in 2014 (Figure 3.5I, J). SRP exhibited spikes in the epilimnion up to 100 μg/L in June in both years, likely due to nutrient runoff during storms (Figure 3.5G, H). Nitrate concentrations were low throughout the water column in both years (≤9 μg/L; Figure 3.5C, D) in comparison to NH_4^+ concentrations, which reached up to 1780 and 1420 μg/L at the sediments in 2014 and 2015, respectively (Figure

3.5A, B). Within the hypolimnion, the maximum N and P concentrations occurred closest to the sediments, below the depth of the cyanobacterial maximum.

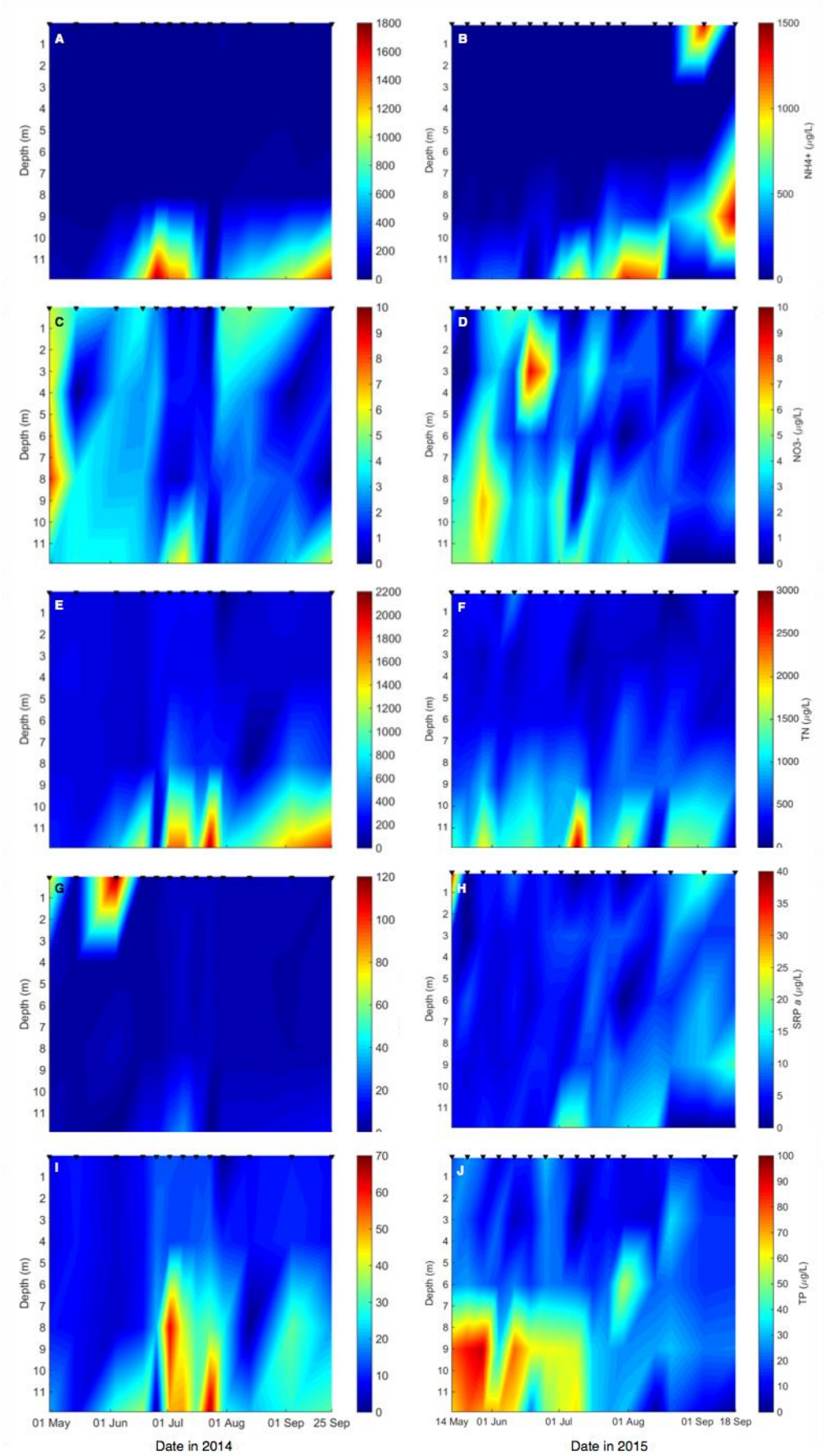
A
B
C
D
E
F
G
H
I
J
Depth (m)
NH4+ (µg/L)
NO3- (µg/L)
TN (µg/L)
SRP a (µg/L)
TP (µg/L)
01 May
01 Jun
01 Jul
01 Aug
01 Sep
25 Sep
14 May
18 Sep
Date in 2014
Date in 2015

Figure 3.5. In summer 2014 (left column) and 2015 (right column): Ammonium (NH_4^+; A, B), nitrate (NO_3^-; C, D), total nitrogen (TN; E, F), soluble reactive phosphorus (SRP; G, H), and total phosphorus (TP; I, J) in Beaverdam Reservoir. Sampling dates are denoted by black triangles at the top of each plot; the space between dates is interpolated. White space at the top of plots indicates a small decrease in water level in late summer. Note that the scales differ between variables and between years.

Conductivity and turbidity closely predicted the percent of surface light reaching the hypolimnion. We compared observed and predicted light in 2015, when both light datasets were available. Across all observations used in the model, observed and predicted log-transformed percent of surface light were closely correlated (Figure 3.2, $r = 0.86$). The model slightly overestimated light at low levels (<1% of incident surface light), but model predictions were very close to observed light at 1-100% of incident surface light (Figure 3.2, Appendix E), and hypolimnetic profiles of observed versus predicted percent of surface light were similar (Appendix F). Notably, the observed and predicted depths that 1% of incident light reached throughout the summer were closely correlated ($r = 0.69$), as were the observed and predicted depths of 0.1% of incident light (Appendix E, $r = 0.63$) in 2015.

The observed and predicted depths that 1% and 0.1% of surface light reached in the hypolimnion followed similar patterns through summer 2015 (Figure 3.2B). The observed depth reached by 1% of surface light was slightly shallower (mean 0.8 ± 0.3 m) than the depth predicted by the model, except on 4 September, when the predicted depth was 1.56 m deeper than the observed depth; the depth that the observed 0.1% of surface light reached was very similar to the predicted depth throughout the summer (Figure 3.2B).

3.4.3 Predictors of cyanobacterial vertical distribution and biomass

We found that the depth of the cyanobacterial biomass in the hypolimnion was strongly related to light availability, while the magnitude of cyanobacterial biomass was driven by both nutrients and light (Table 3.1). In both years, the cyanobacteria consistently exhibited their maximum biomass in the water column at the depth of 1% of predicted surface light (Figure 3.6), though the actual depth reached by 1% of surface light was likely slightly shallower (Figure 3.2B, Appendix F). The predicted 1% light depth was very strongly correlated with the depth of the cyanobacterial maximum biomass in 2014 (r = 0.95) and 2015 (r = 0.92).

Table 3.1 Time series models tested to estimate cyanobacterial biomass in BVR at 8m in 2014 and 6 and 9m in 2015, ranked by corrected AICc. The first model for each depth and year (shaded gray) is the best-fitting model, according to AICc. The response variable for all models is square root-transformed cyanobacterial biomass in the next sample period. The autocorrelation term "AR1" is the square-root transformed cyanobacterial biomass, lagged by one sampling period. TP (total phosphorus), TN (total nitrogen), NH_4^+ (ammonium), NO_3^- (nitrate), and SRP (soluble reactive phosphorus) and light (predicted percent of surface light) were used with the AR1 term to model cyanobacterial biomass the following week.

Year	Depth	Model	Equation	AICc
2014	8m	AR1 + TP	= -0.13 + 0.58 × (AR1) + 0.094 × (TP), R^2 = 0.93, p < 0.0001	52.20
2014	8m	AR1 + NO_3^-	= 4.25 - 1.1 × (NO_3^-) + 0.68 × (AR1), R^2 = 0.86, p < 0.0001	60.84
2014	8m	AR1 + TN	= -0.83 + 0.0097 × (TN) + 0.53 × (AR1), R^2 = 0.87, p < 0.0001	62.78
2014	8m	AR1 + light	= 5.35 + 0.23 × (AR1) - 0.45 × (light), R^2 = 0.87, P < 0.0001	63.14
2014	8m	AR1	= 1.065 + 0.83 × (AR1), R^2 = 0.79, p < 0.0001	69.03
2014	8m	AR1 + SRP	= 0.65 + 0.10 × (SRP) + 0.82 (AR1), R^2 = 0.79, p < 0.0001	72.19
2014	8m	AR1 + NH_4^+	= 0.1 + 0.84 × (AR1) - 0.0046 × (NH_4^+), R^2 = 0.79, p < 0.0001	72.35
2015	6m	AR1 + TN	= 3.98 + 1.16 × (AR1) - 0.013 × (TN), R^2 = 0.71, p = 0.0003	67.56
2015	6m	AR1 + light	= 3.19 + 0.40 × (AR1) + -0.59 × (light), R^2 = 0.70, p = 0.0004	68.16
2015	6m	AR1	= 0.70 + 0.75 × (AR1), R^2 = 0.59, p = 0.0005	69.72
2015	6m	AR1 + NH_4^+	= 2.28 + -0.18 × (NH_4^+) + 0.65 × (AR1), R^2 = 0.65, p = 0.0011	70.89
2015	6m	AR1 + SRP	= -0.29 + 0.19 × (SRP) + 0.76 × (AR1), R^2 = 0.61, p = 0.0021	72.39
2015	6m	AR1 + NO_3^-	= 1.65 - 0.40 × (NO_3^-) + 0.68 × (AR1), R^2 = 0.61, p = 0.0022	72.43
2015	6m	AR1 + TP	= 1.47 + 0.84 × (AR1) + -0.040 × (TP), R^2 = 0.60, p = 0.0025	72.81
2015	9m	AR1 + light	= 3.37 + 0.34 × (cyanos) + 11.17 × (light), R^2 = 0.85, p < 0.0001	38.38
2015	9m	AR1 + SRP	= 7.88 + 0.20 × (AR1) - 0.40 × (SRP), R^2 = 0.83, p < 0.0001	39.61
2015	9m	AR1 + NH_4^+	= 5.52 + 0.28 × (AR1) - 0.0056 × (NH_4^+), R^2 = 0.71, p = 0.0004	48.64

2015	9m	AR1 + TP	= 2.08 + 0.43 × (cyanos) + 0.032 × (TP), R^2 = 0.68, p = 0.0007	50.16
2015	9m	AR1 + NO_3^-	= 2.19 + 0.42 × (NO_3^-) + 0.45 × (AR1), R^2 = 0.62, p = 0.0017	52.54
2015	9m	AR1	= 2.21 + 0.66 × (cyanos), R^2 = 0.45, p = 0.0042	54.89
2015	9m	AR1 + TN	= 3.26 + 0.00021 × (TN) + 0.72 × (cyanos), R^2 = 0.51, p = 0.0098	56.83

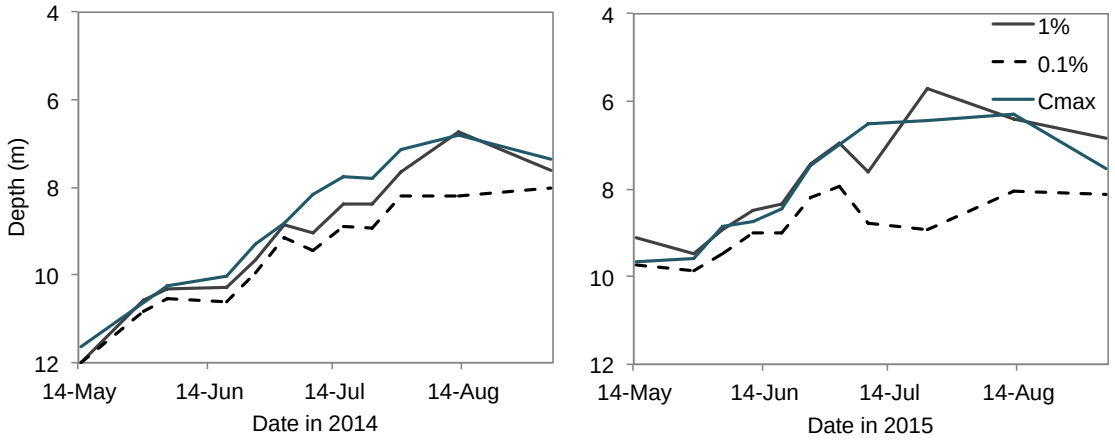

Figure 3.6. Depth of the maximum cyanobacterial biomass (Cmax), predicted depth reached by 1% of surface light, and predicted depth reached by 0.1% of surface light in summers 2014 and 2015.

The best-fitting models predicting cyanobacterial biomass indicate that different drivers were most important at different depths across the two years. AICc-ranking of models indicated that cyanobacterial biomass was best predicted by TP at 8 m in 2014 (R^2 = 0.93, p < 0.0001), TN at 6 m in 2015 (R^2 = 0.71, p = 0.0003), and percent of surface light at 9 m in 2015 (R^2 = 0.85, p < 0.0001) (Table 3.1). For 8 m in 2014, the TP model was 8.6 AICc units lower than the second-best model, indicating that it was the single best model for that depth (Burnham and Anderson 1998), but for 6 m in 2015, the light model was within <1 AICc unit of the TN model, indicating that both models were similar predictors of cyanobacterial biomass. For 9 m in 2015, the SRP model was only 1.2 AICc units greater than the best model, indicating that both light and SRP

models were also similarly important. For all three focal hypolimnetic depths, models with light and nutrient predictors performed substantially better than the null AR1 models without any environmental predictors.

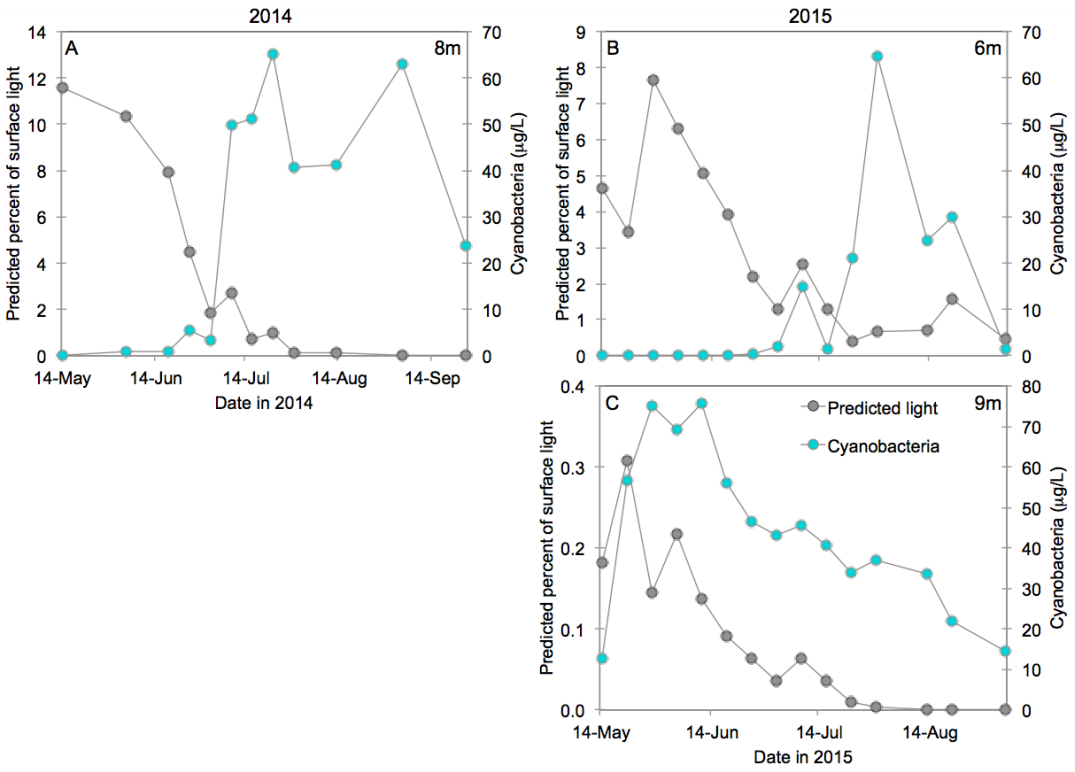

Figure 3.7. Cyanobacterial biomass and modeled percent of surface light at 8 m in 2014 (A), and 6 m (B) and 9 m (C) in 2015 in Beaverdam Reservoir.

Planktothrix in BVR appear to have exhibited a threshold response to light availabilities at ~1.65-1.85% of incident light. Above that light threshold, cyanobacterial biomass at 8 m in 2014 and 6m in 2015 were low (< 10 ug/L), but below that threshold, biomass sharply increased (up to 70 ug/L; Figure 3.7). At 9 m in 2015, light was consistently less than 1% of surface light, and cyanobacterial biomass closely (positively) parralelled patterns of light availability (Figure 3.7). Cyanobacterial biomass was not coupled closely with temperature (Appendix G) in the same way.

Using the best-fitting model at each depth (Table 3.1), modeled patterns of cyanobacterial biomass were similar to observed patterns (Figure 8). At 6 m in 2015, the high observed peak of cyanobacterial biomass on July 30 was not predicted by the model, indicating that there were other important drivers of cyanobacterial biomass. On all other dates, the best-fitting model for each focal depths yielded biomass patterns very similar to observed population dynamics.

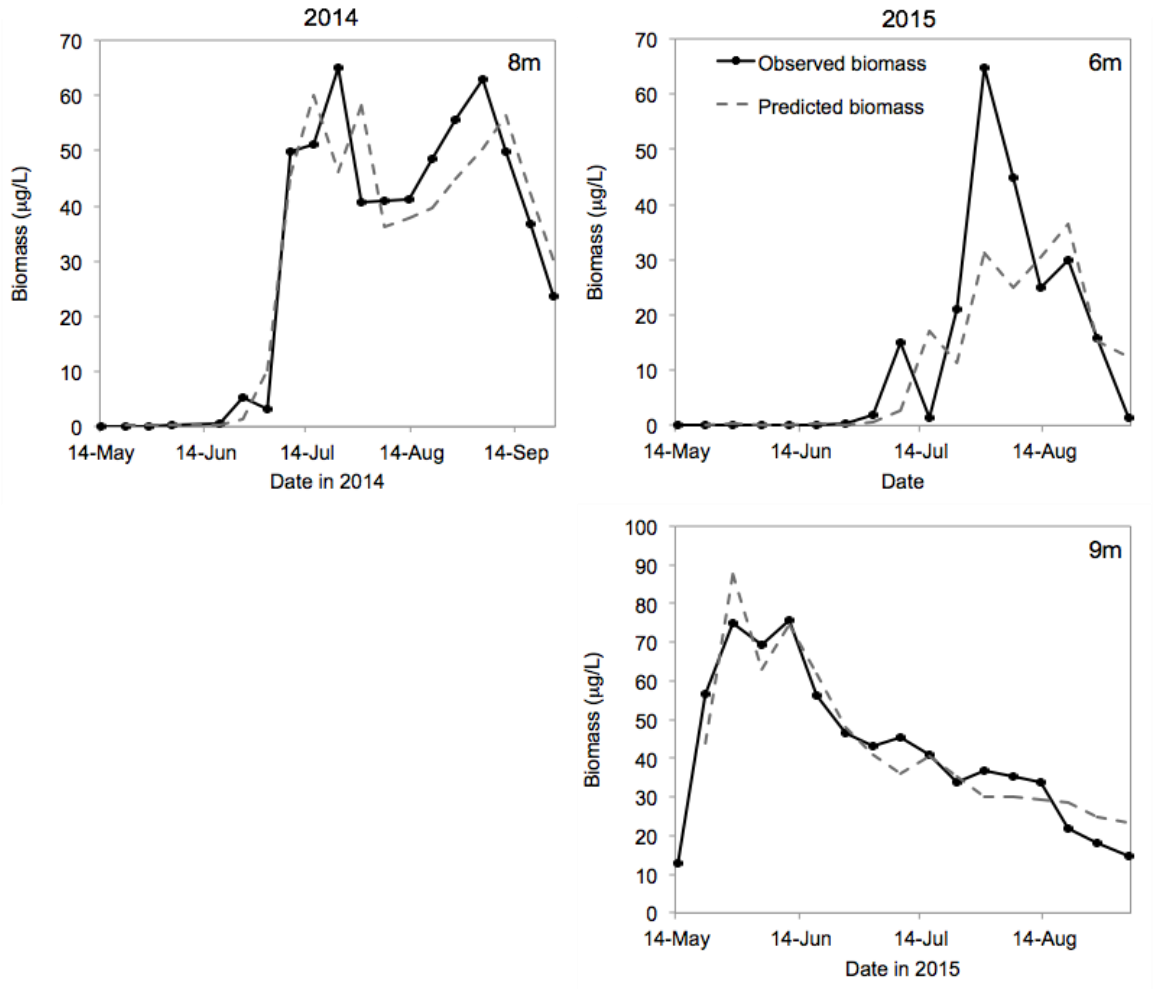

Figure 3.8. Observed and predicted cyanobacterial biomass at our sample depths (8 m in 2014, 6 and 9 m in 2015), using the best-fitting model (Table 3.1) for each sample depth. Cyanobacteria at 8 m in 2014 were modeled using TP and an autocorrelation term; cyanobacteria at 6 m in 2015 were modeled using TN and an autocorrelation term; cyanobacteria at 9 m in 2015 were modeled using predicted percent of surface light and an autocorrelation term. Note that the y-axis scale varies among depths.

3.5 Discussion

In both years, we observed very high cyanobacterial biomass in the hypolimnion of BVR, dominating the phytoplankton community in the entire water column. The cyanobacterial bloom, composed primarily of live *Planktothrix* remained in the hypolimnion for ~100 days in both summers. Cyanobacteria remained in the hypolimnion through both summers, but moved up in the water column slowly throughout each year (Figure 5), likely due to light limitation in upper waters, as evident from our light measurements and model (Figure 3.6). Sustained anoxia throughout the stratified period in both years (Figure 3.4) promoted high metal and nutrient release from the sediments (Figure 3.5), which in combination with increasing turbidity likely contributed to greater attenuation of light in the hypolimnion as the summer progressed, stimulating the cyanobacteria to move upwards.

Our data indicate that light was likely the most important predictor of cyanobacterial depth in 2014 and 2015. Interestingly, the direction of the light predictor terms in the time series model reveals that light was negatively associated with cyanobacterial biomass at 6 m and 8 m depths, but there was a positive relationship between light and cyanobacterial biomass at 9 m, our deepest sample depth with water chemistry data. These relationships indicate that at >1.65-1.85% incident light levels, light may be inhibiting *Planktothrix* populations, but stimulating *Planktothrix* at less than this threshold (Figure 3.7). Notably, in 2015, cyanobacterial biomass reached almost 80 μg/L at 9 m, where available light was less than 1% of surface light (Figure 3.7), which is typically considered the minimum light availability for phytoplankton photosynthesis (Ryther 1956).

The switch in the effect of light from being stimulatory in the deeper hypolimnion to inhibitory in the shallower hypolimnion was likely because the *Planktothrix* in BVR were adapted to the extremely low light conditions in the hypolimnion that resulted from high nutrient, metal, and particle concentrations at depth. The hypolimnion of BVR was nutrient-rich in both summers (Figure 3.5), providing a favorable environment for phytoplankton taxa able to modify their buoyancy and photosynthesize at low light levels (Reynolds 1987, Dokulil and Teubner 2000, Oberhaus et al. 2007, Bonilla et al. 2012). Because nutrient concentrations were high and phytoplankton growth is slow in cold, dark conditions, changes in light availability at our deepest sample depth (9 m) likely had large effects on cyanobacterial growth (Reynolds 1987, Mur et al. 1999).

Our data suggest that the compensation point for *Planktothrix* in BVR may be at or slightly below the depth of 1% of surface light, as indicated by *Planktothrix* biomass near this depth being higher than any other depth in the water column. The depth of maximum cyanobacterial biomass closely followed the depth reached by 1% of surface light in both years (Figure 3.6); however, our light model slightly overestimated light at low levels (Figure 2), so actual light availability was likely lower than 1% of surface light. These findings are similar to other studies that observed *Planktothrix* populations stratified at very low light levels (Bright and Walsby 2000, Camacho et al. 2000, Oberhaus et al. 2007). For example, Camacho et al. (2000) observed *Planktothrix* populations stratified at the depth reached by 0.5% surface light over a two-year study. Some phytoplankton taxa, such as *Planktothrix*, are likely able to successfully grow at depths deeper than 1% of surface light because of their physiology (El-Sayed et al. 1983, Camacho et al. 2000, Marra et al. 2014). For example, *Planktothrix* may increase the amount

chlorophyll per cell volume, as well as increase production of accessory pigments, to improve photosynthetic efficiency under low light conditions (Post et al. 1985, Reynolds 2006).

Though light availability in the hypolimnion was closely linked to the depth of the cyanobacterial bloom in both years, the magnitude of cyanobacterial biomass was likely driven by light and nutrients (Table 3.1). *Planktothrix* is not an N-fixing cyanobacterium, so high N and P concentrations together would likely substantially benefit the growth of this non-N-fixing taxon (e.g., Anderson et al. 1987, Steinberg and Hartmann 1988), as we observed in the hypolimnion of BVR. Remaining in the hypolimnion instead of the metalimnion (like *Planktothrix* populations reported in previous studies), may allow these cyanobacteria to access higher concentrations of nutrients than would be available deep below the thermocline. At all focal depths, cyanobacterial biomass was closely predicted by both nutrient and light models, suggesting that *Planktothrix* may be co-limited by both light and nutrients in this waterbody, as predicted from previous studies (Ryabov 2012, Arteaga et al. 2014).

In this study, *in situ* fluorometry allowed us to monitor the bloom at high depth resolution throughout each summer, collecting data from the whole-water column on the vertical distribution and magnitude of cyanobacterial blooms in Beaverdam Reservoir. The *in situ* fluorometer provides rapid, high-frequency *in situ* data, and may be more accurate than traditional methods, such as manually-filtered chlorophyll samples or microscopy counts (Gregor and Maršálek 2004, Catherine et al. 2012). However, this sensor may underestimate chlorophyll levels at high phytoplankton biomass (Gregor and Maršálek 2004), especially when the phytoplankton are dominated by colonial cyanobacteria (Gregor and Maršálek 2004), such as *Planktothrix*. If the Fluoroprobe underestimated cyanobacteria in BVR, it is possible that

cyanobacterial biomass was greater than observed values. Consequently, our estimates of the magnitude of the bloom may be conservative.

To estimate hypolimnetic light availability patterns in 2014, we created a model predicting the percent of surface light at different depths in the hypolimnion. Though predicted light patterns closely fit the observed light patterns in 2015 (Figure 3.2, Appendices E, F), our model overestimated light availability at low (0.1-1% of surface light) levels (Figure 3.2). Therefore, though we found the depth of maximum cyanobacterial biomass closely followed the predicted depth of 1% of surface light, actual light levels at these depths were likely <1% of surface light (Appendix F). Additionally, the model does not capture year-to-year variability that may have existed between 2014 and 2015. This model was created specifically for the hypolimnion of our focal reservoir and may not be applicable for other lakes and reservoirs.

3.5.1 Conclusions

We observed a perennial hypolimnetic cyanobacterial bloom persisting at and below the depth typically assumed to be the lower limit for phytoplankton population growth in aquatic ecosystems (Ryther 1956). *Planktothrix* biomass was highest at the depth reached by 1% of surface light, but cyanobacteria were also detected by the Fluoroprobe deeper in the water column at lower concentrations (Figure 3.3). Moreover, not only did phytoplankton grow below the depth of 1% of surface light, this population of *Planktothrix* had far higher biomass than any other phytoplankton taxon in this waterbody in both years. Our data add to the increasing evidence that some cyanobacteria may be better adapted to low light conditions in waterbodies than previously thought (e.g., Camacho et al. 2000). Consequently, as hypoxia and thermal stratification increase in frequency and intensity due to anthropogenic forcing, our data indicate

that it is critically important to monitor cyanobacterial blooms both at the surface *and* at depth in freshwater ecosystems.

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Chapter 4. Conclusions

Improving our understanding of the environmental factors controlling phytoplankton in reservoir ecosystems will allow us to better predict phytoplankton population dynamics in the face of global change. During my Master's research, I conducted two reservoir phytoplankton studies in Virginia, USA reservoirs. My findings demonstrate that whole-ecosystem phytoplankton monitoring across both horizontal and vertical gradients is necessary to understand the drivers and dynamics of phytoplankton populations in reservoirs.

Though algal monitoring is often incorporated into lake and reservoir management programs, the vast majority of phytoplankton monitoring focuses solely on pelagic populations at the deepest site of the waterbody. However, several important phytoplankton taxa, such as the dinoflagellates *Peridinium* and *Gymnodinium*, spend part of their life cycles in the sediments. Recruitment, or the life history process of leaving the sediments and entering the water column, can play an important role in subsidizing pelagic phytoplankton populations. Here, my estimates of the contribution of recruiting cells to pelagic dinoflagellate population growth indicate that recruitment may be especially important in reservoirs, likely because they are fast-moving systems relative to natural lakes; fast-flowing ecosystems with short water residence times will experience higher rates of flushing, and therefore high phytoplankton cell losses in the pelagic zone.

In addition, I observed different multivariate drivers of *Peridinium* recruitment along a reservoir ecosystem continuum. Changes in the coupling of biology to reservoir physics and chemistry along a very small spatial scale reveal the high sensitivity of recruiting cells to changes in reservoir chemistry (dissolved oxygen, nutrient concentrations) and physics (flow

rate, light, thermal stability). Recruitment rates were predicted by different factors based on where the population was located along this continuum: physical forcing dominated in upstream riverine sites, and nutrient ratios dominated in downstream lacustrine sites. These findings elucidate some of the complex physical, chemical, and biological processes that interact and drive phytoplankton populations along a spatial gradient in this reservoir, and emphasize the importance of considering both the whole life history cycle and the whole ecosystem – from the headwaters to the dam – in reservoir phytoplankton dynamics.

In addition to horizontal forcing, physics, chemistry, and phytoplankton populations also interact along vertical gradients through reservoir water columns. To investigate how reservoir chemistry and physics alter the vertical distribution of phytoplankton, I used *in situ* whole-water column fluorometry to monitor a phytoplankton bloom throughout two consecutive summers. Surprisingly, I observed substantial accumulations of the cyanobacterium *Planktothrix* in the hypolimnion of a reservoir. I found that light availability was the primary predictor of the *Planktothrix* vertical distribution, while the magnitude of its biomass was predicted by both light and nutrient availability. The *Planktothrix* bloom remained near or below the generally-accepted bottom depth of the euphotic zone (depth of 1% of surface light) through both summers.

My results suggest that *Planktothrix* remained at depth through each summer to maximize use of high hypolimnetic nutrient concentrations while still accessing sufficient light for photosynthesis. This cyanobacterial bloom would not have been detected if we monitored phytoplankton with other standard methods, such as integrated epilimnetic phytoplankton samples or surface grab sampling. Similar to Chapter 2, our results further emphasize the necessity of monitoring the entire water column when exploring phytoplankton dynamics, and the importance of both physical (light) and chemical (nutrients) variables in promoting

cyanobacterial blooms in a reservoir.

These study reservoirs offered an opportunity to investigate several phytoplankton ecology questions. Future phytoplankton research in these waterbodies could further explore interactions between light limitation, nutrient ratios, and phytoplankton populations. If logistically possible, the addition of multiple Fluoroprobe sampling sites along the Falling Creek Reservoir ecosystem continuum could reveal how upstream pelagic communities are related to, or different from, phytoplankton communities in the lacustrine zone. If my two thesis studies were continued, collecting environmental (nutrients, temperature, light) data at all FCR recruitment sites would likely be extremely informative. With continued monitoring of the *Planktothrix* bloom in BVR, expanding the sampling to include some upstream Fluoroprobe measurements, even just monthly or annually, would reveal the horizontal "range" of *Planktothrix* throughout the reservoir. However, I think that exploring the response of phytoplankton communities to whole-ecosystem manipulation, when possible, is the most pressing and ecologically interesting opportunity provided by these reservoirs and the Western Virginia Water Authority.

Though it is not possible to monitor the entire horizontal and vertical extent of every waterbody, my research emphasizes that, whenever possible, whole-ecosystem monitoring of reservoir phytoplankton populations, chemistry, and physics can lead to improved understanding of reservoir phytoplankton ecology. In Chapter 2, I observed that dinoflagellate recruitment was sensitive to very different environmental factors along a small (<1 km) spatial gradient. In Chapter 3, I observed a hypolimnetic cyanobacterial bloom whose vertical position in the water column was deeper than the typically accepted depth limit for photosynthesis. Overall, the take-home message from my thesis is that horizontal and vertical aquatic environmental heterogeneity

can play a large role in structuring phytoplankton populations at the whole-reservoir scale.

Appendix

Appendix A

Appendix A. Ranges of estimates of the contribution of recruitment to pelagic populations in Falling Creek Reservoir. For *Peridinium* (n = 3 sample periods when the pelagic population increased) and *Gymnodinium* (n = 4 sample periods), we used the minimum, median, and maximum recruitment rates observed each recruitment period. The median estimates are likely the most representative rates of the true importance of recruitment, and minimum and maximum estimates provide a measure of uncertainty

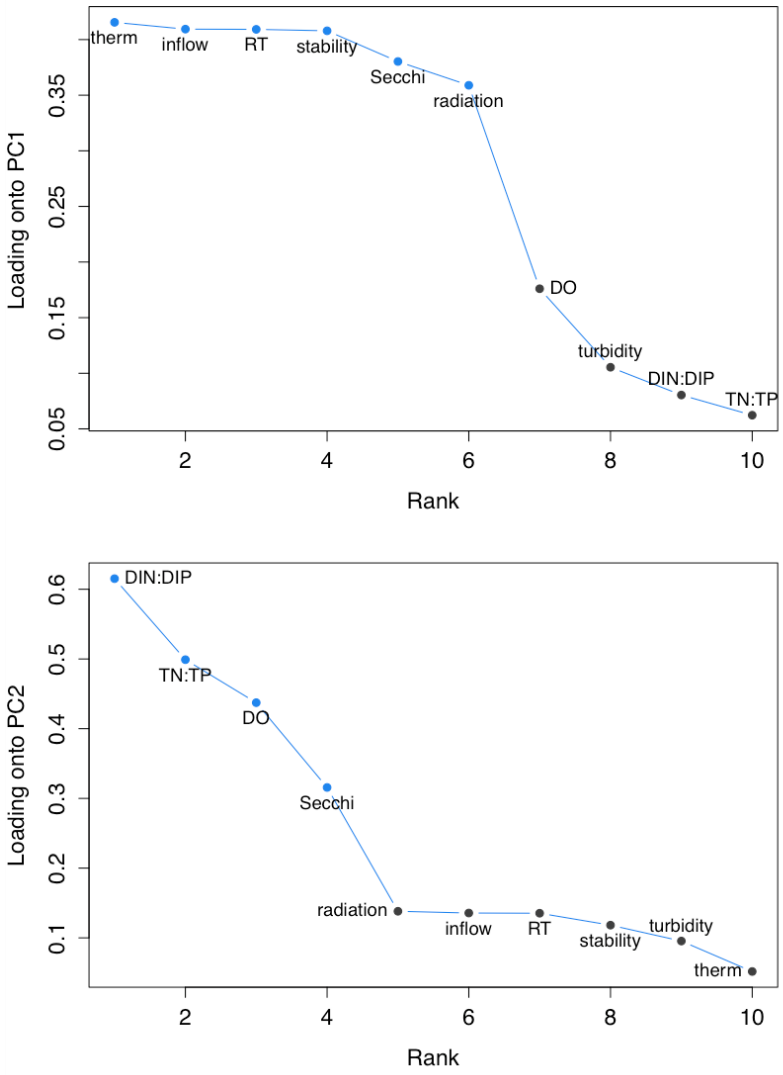
Genus	Minimum estimate	Median estimate	Maximum estimate
Peridinium	4-8%	6-16%	7-53%
Gymnodinium	0-18%	2-106%	8-468%

Appendix B

Appendix B. Loadings of environmental variables on principal components (PCs). Heavily-loaded variables are indicated in bold; the rank-ordered loading values for PC1 and PC2 are shown in Appendix 3

Variable	PC1	PC2
Average inflow flow rate (m^3/min)	**0.409**	0.136
Average radiation at noon (units)	**0.359**	0.138
Average residence time (days)	**-0.409**	-0.135
Dissolved N:P	-0.080	**0.615**
Dissolved oxygen (mg/L)	0.176	**0.437**
Schmidt stability	**0.408**	-0.118
Secchi depth (m)	**0.380**	**-0.316**
Thermocline depth (m)	**-0.415**	-0.052
Total N:P	-0.062	**0.499**
Turbidity (NTU)	-0.105	0.095
Variance explained	0.475	0.200

Appendix C



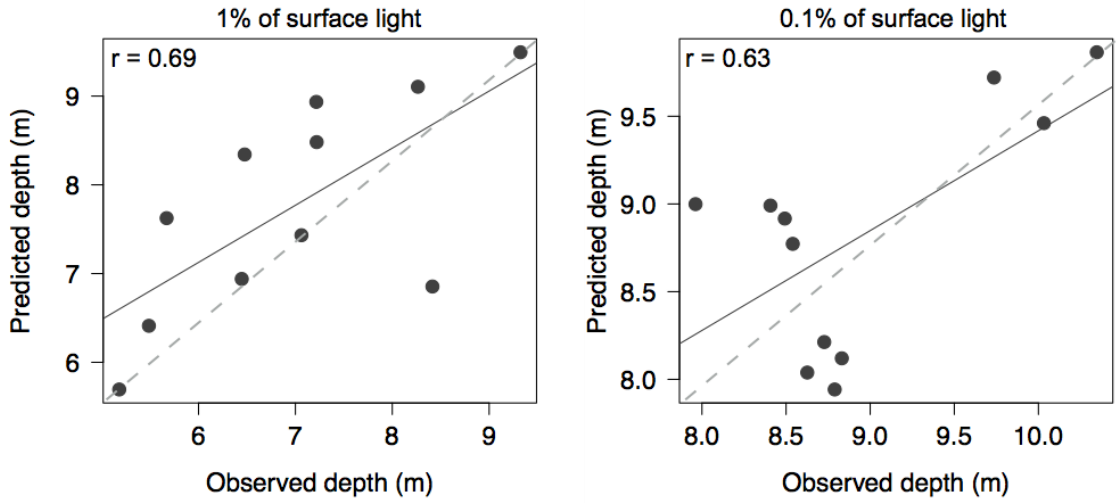
Appendix C. Rank-ordered loadings onto PC1 and PC2. We used these plots to identify which variables loaded heavily onto each principal component axis; blue points were heavily loading, grey points were not. Therm = thermocline depth; inflow = mean inflow flow rate; RT = residence time; stability = Schmidt stability; Secchi = Secchi depth; radiation = mean solar radiation; DO = dissolved oxygen; turbidity = turbidity; DIN:DIP = dissolved N:P; and TN:TP = total N:P

Appendix D

Appendix D. Median, mean (± SD), maximum observed biomass, and habitat (epilimnion or hypolimnion) of major phytoplankton taxa, across all observations obtained from the Fluoroprobe fluorometer (n = 616 in 2014, n = 890 in 2015) in two summers in Beaverdam Reservoir.

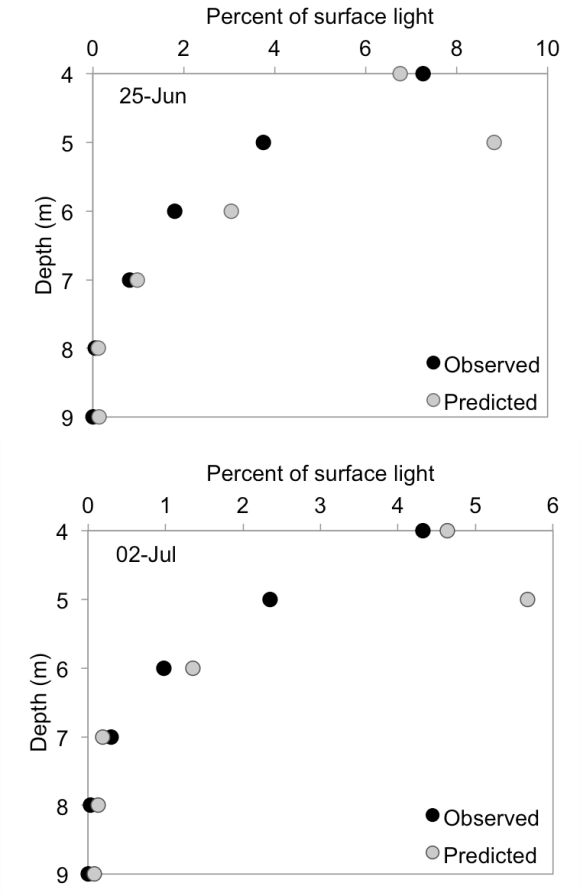
Year	Group	Median biomass (μg/L)	Mean (± SD) biomass (μg/L)	Maximum biomass (μg/L)	Habitat
2014	Total chlorophyll	9.01	15.9 ± 16.80	116.61	
2014	Green algae	2.82	3.23 ± 2.94	13.07	epilimnion
2014	Diatoms	1.6	2.27 ± 2.64	13.57	epilimnion
2014	Cryptophytes	0.57	2.01 ± 3.87	26.45	hypolimnion
2014	Cyanobacteria	0.44	8.38 ± 15.49	92.44	hypolimnion
2015	Total chlorophyll	11.35	24.47 ± 24.47	132.75	
2015	Green algae	1.92	2.03 ± 1.92	6.73	epilimnion
2015	Diatoms	1.38	1.92 ± 2.33	12.67	epilimnion
2015	Cryptophytes	1.45	3.00 ± 5.12	31.65	hypolimnion
2015	Cyanobacteria	0.8	16.61 ± 22.52	104.96	hypolimnion

Appendix E



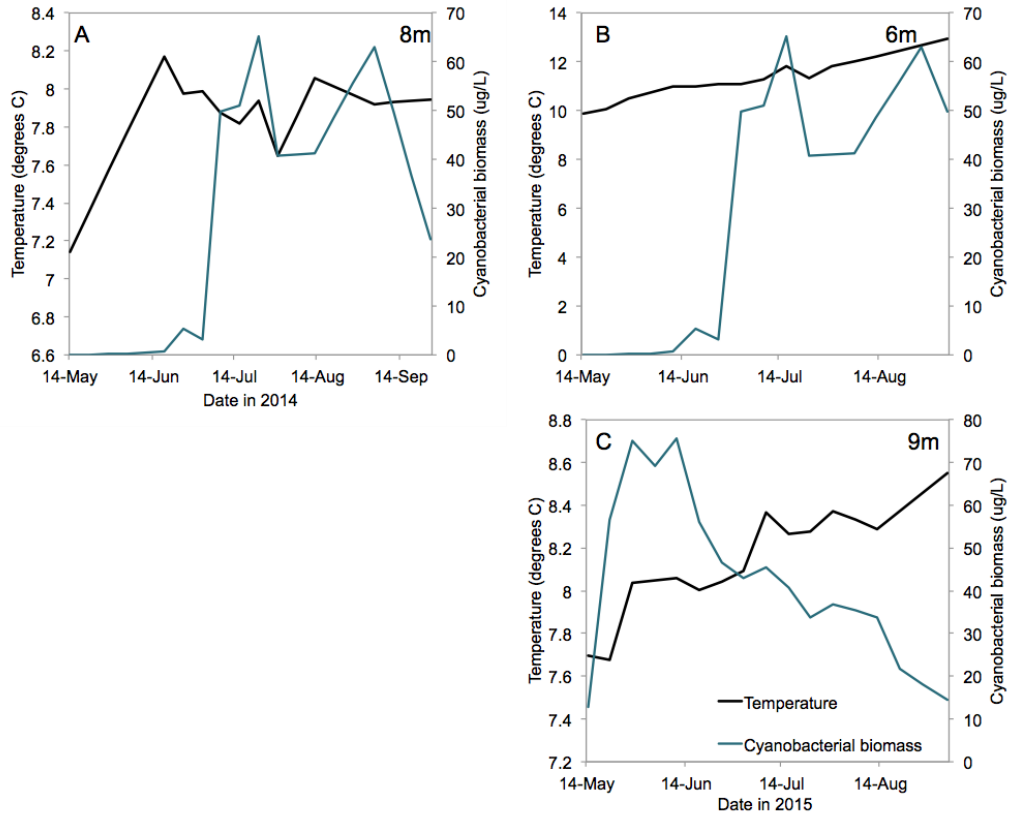
Appendix E. Observed and predicted depth of 1% (left) and 0.1% (right) of surface light were closely correlated in 2015. The dashed line in each plot is the 1:1 line; our light model slightly overestimates the depth reached by 1% of surface light in 2015.

Appendix F



Appendix F. Hypolimnetic profiles of observed and predicted percent of surface light on two typical sample days (top, June 25; bottom, July 2) in 2014. Epilimnetic depths were excluded from this figure because epilimnetic observations were not included in creating the model.

Appendix G



Appendix G. Cyanobacteria (green line) and temperature (black line) were not closely coupled the way that cyanobacteria and light were in BVR in 2014 (A) or 2015 (B, C).