In situ fluorometry reveals a persistent, perennial hypolimnetic cyanobacterial bloom in a seasonally anoxic reservoir

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Abstract: Cyanobacterial blooms are increasing in waterbodies worldwide because of anthropogenic forcing. Most blooms occur at the water's surface, but some cyanobacterial taxa, such as *Planktothrix*, are able to modify their buoyancy to access more favorable growing conditions in deeper waters. Here, we used in situ fluorometry to examine the vertical distribution and biomass of *Planktothrix* in a seasonally anoxic reservoir for 3 consecutive summers. We also collected depth profiles of photosynthetically active radiation, temperature, and nutrients to evaluate which environmental drivers were most important for predicting *Planktothrix* biomass. In all 3 summers, *Planktothrix* dominated the phytoplankton community, exhibiting a large (concentrations ~100 μg/L), persistent (lasting ~100 d) bloom below the thermocline. The bloom consistently exhibited maximum biomass at or below the depth reached by 1% of surface light. Light availability probably was the most important factor driving the vertical distribution of the stratified *Planktothrix* bloom, and light, temperature, and nutrients together were strong predictors of cyanobacterial biomass in the hypolimnion, explaining 71 to 93% of the variation in biomass. Our data suggest that *Planktothrix* remained in the hypolimnion where nutrient availability was maximized, while progressing slightly upward in the water column through each summer in response to light limitation. Our findings demonstrate that *Planktothrix* can dominate in low light and anoxic conditions and can form persistent blooms that last for multiple months. As cyanobacterial blooms become more prevalent, monitoring cyanobacteria at the surface *and* at depth will become critically important in freshwater ecosystems.
Key words: *Planktothrix agardhii*, light limitation, nutrients, cyanobacteria, FluoroProbe, stratification, turbidity

Cyanobacterial blooms (i.e., a large aggregation of cyanobacterial biomass) 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 because of their toxins, scums, and noxious odors (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). Therefore, understanding the factors controlling cyanobacterial dominance in freshwater ecosystems is important for managing water quality and ecosystem functioning.

Cyanobacteria with gas vesicles typically accumulate at the water's surface in thermally stratified waterbodies (Walsby et al. 1995, 1997, Paerl et al. 2001), but some cyanobacterial taxa form dense populations in the metalimnia of lakes and reservoirs (Reynolds et al. 1987, Walsby and Schanz 2002, Kurmayer et al. 2016). These blooms generally are situated 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 et al. 1987, Mur et al. 1999). The depth reached by 1% of surface light is considered the approximate compensation point for phytoplankton growth, but some taxa adapted to life in deeper waters may be able to form populations below this depth (Reynolds 2006).

Most research on cyanobacteria has focused on surface scums, and less is known about the cyanobacteria that accumulate in deeper waters (reviewed by Paerl et al. 2001). These taxa are thought to modify their buoyancy to grow

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at relatively low light conditions at depth in thermally stratified systems to maximize access to nutrients in bottom waters and light availability/avoidance from above (Fee 1976, Reynolds et al. 1987, Mur et al. 1999, Kurmayer et al. 2016). The vertical distribution and biomass of deep-water cyanobacteria may be predicted by light and nutrient conditions, but the relative importance of these drivers remains unknown.

Planktothrix is a common cyanobacterial genus that forms dense metalimnetic populations (Camacho et al. 2000, Walsby and Schanz 2002, Briand et al. 2005, Jacquet et al. 2005, Reynolds 2006). Reports of *Planktothrix* in the metalimnion of European lakes have been increasing during recent decades (Jacquet et al. 2005, Ernst et al. 2007, Kurmayer et al. 2016), a situation of growing concern because some strains of *Planktothrix* can produce the toxin microcystin (Christiansen et al. 2003, de Figueiredo et al. 2004, Ernst et al. 2007). Toxins and secondary metabolites produced by *Planktothrix* may play a role in this taxon's ability to dominate an ecosystem (Kurmayer et al. 2016). *Planktothrix* blooms can persist for several months (Lindholm et al. 1999, Dokulil and Teubner 2012) and can occur in anoxic metalimnia (Pasztaleniec 2004). Understanding the environmental conditions promoting *Planktothrix* populations is important for preserving water quality and ecosystem functioning. Furthermore, identifying 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 in general.

Increasing nutrient concentrations and seasonal hypolimnetic anoxia, arising from anoxic remineralization of N and P, may promote *Planktothrix* blooms in freshwater systems. *Planktothrix* is favored by high total P (TP) concentrations (Steinberg and Hartmann 1988, Anderson et al. 2002) and thermally stratified conditions, and requires an external N source because it is a non-N-fixing taxon. Many mesotrophic and eutrophic freshwater ecosystems experience anoxia in their bottom waters during summer stratification (reviewed by Jenny et al. 2016), which can cause internal N and P loading from the sediments (Boström et al. 1988, Søndergaard et al. 2003). In these high-nutrient, anoxic systems, *Planktothrix* may have a competitive advantage over other phytoplankton. *Planktothrix* has the ability to alter its vertical position in the water column during stratified periods via buoyant gas vesicles (Reynolds et al. 1987, Dokulil and Teubner 2000, Walsby et al. 2004) and is adapted to low light levels via accessory photosynthetic pigments and increasing the concentration of pigments in each cell (Post et al. 1985, Reynolds 2006, Oberhaus et al. 2007, Bonilla et al. 2012). These traits enable it to remain in the metalimnion and to access nutrients available below the thermocline (Reynolds et al. 1987, Kurmayer et al. 2016).

As hypolimnetic anoxia becomes more frequent and severe because of 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. These blooms could occur in both temperate eutrophic and mesotrophic lakes, where *Planktothrix* may already be increasing (Posch et al. 2012, Kurmayer et al. 2016). In addition, many waterbodies with a history of agriculture and high nutrient loading in their catchment may experience legacy effects of this land use that will increase their susceptibility to blooms of cyanobacterial taxa (e.g., *Planktothrix*) that can stratify deep enough to access internally released nutrients during summer stratification.

Most studies of *Planktothrix* ecology have been conducted with 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 of *Planktothrix* dynamics below the thermocline are needed to improve our understanding of 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). During 3 consecutive summers, we monitored *Planktothrix* population dynamics throughout the entire water column in a moderately eutrophic reservoir that experiences seasonal hypolimnetic anoxia. We used in situ fluorometry to document 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. We used this data set to address 2 questions: 1) What is the vertical distribution of cyanobacteria throughout the summer stratified period in each year? 2) 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.

METHODS

Study site

We monitored *Planktothrix* populations in Beaverdam Reservoir (BVR), a dimictic reservoir in Vinton, Virginia, USA (lat 37.316474°N, long −79.818826°E), owned and managed by the Western Virginia Water Authority (WVWA). At full pond, the reservoir has a surface area of 0.39 km^2, a catchment area of 3.69 km^2, a maximum depth of 13 m (Fig. 1), and a residence time of ~0.9 y. During the monitoring period in our study, the water depth in BVR was controlled at ~12 m. BVR is not used directly as a drinking water source, but it has an outflow pipe that flows into Falling Creek Reservoir, a source of drinking water for residents of Roanoke, Virginia (Gerling et al. 2016). BVR was built in 1872 and currently has a completely forested catchment, but the land surrounding the reservoir was used for agri-

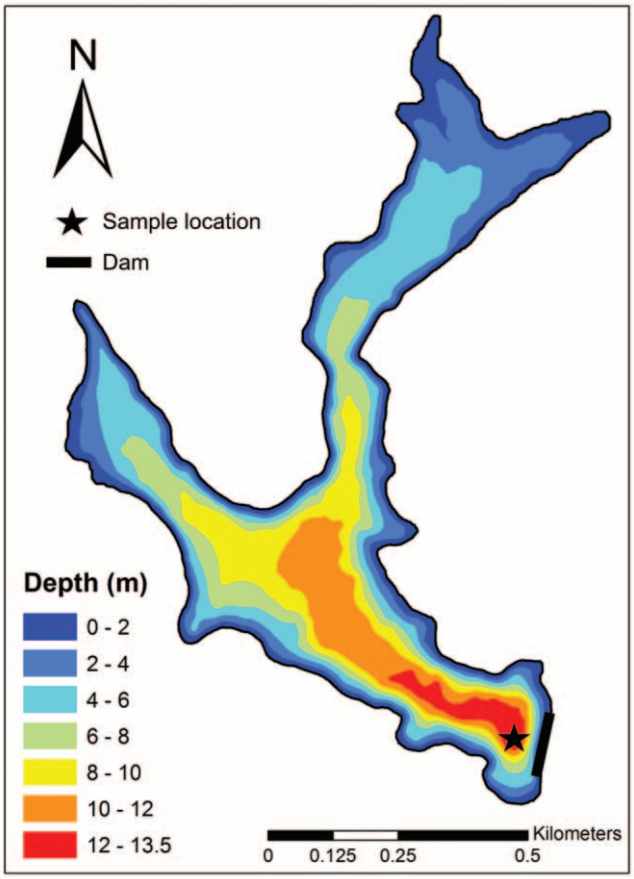

Figure 1. Beaverdam Reservoir (BVR) (lat 37.316474°N, long −79.818826°E) bathymetric map at full pond. The black line represents the reservoir's dam, and the star denotes the primary sampling site in this study.

culture before it was abandoned in the 1930s (Carey et al. 2018). BVR experiences hypolimnetic anoxia during summer stratification, leading to internal loading of N and P.

Field methods: routine monitoring

We collected physical and chemical depth profiles in BVR every 1 to 2 wk in May to August and every 2 to 3 wk in September 2014, 2015, and 2016. We sampled all profiles at the reservoir's deepest site near the dam (Fig. 1). We also collected one additional synoptic phytoplankton profile at another site in the reservoir on 16 September 2016 (Fig. S1). Routine monitoring of additional regular sampling locations would have improved our understanding of the cyanobacterial dynamics in BVR but was prevented by logistical constraints. On each sampling date, we used a 4 Hertz CTD (conductivity, temperature, and depth) SBE 19plus profiler (Seabird Electronics, Bellevue, Washington) to collect water-column profiles of temperature, dissolved O_2 (DO) concentration (SBE 43 probe), turbidity, and conductivity at ~1-cm intervals. The profiling rate was ~0.103 m/s. We also measured Secchi depth on every sampling date. In 2015 and 2016, we measured depth profiles of photosynthetically active radiation (PAR) at 1-m-depth resolution with an LI-250 underwater light meter (LI-COR, Lincoln, Nebraska). We did not collect water-column PAR profiles in 2014. We used CTD thermal profiles to calculate the thermocline depth (the depth of maximum temperature change) on each sampling date. We used *rLakeAnalyzer* (Winslow et al. 2015) to calculate this metric in the R environment (version 3.3.3; R Project for Statistical Computing, Vienna, Austria).

In all years, we collected water samples with a 4-L Van Dorn sampler (Wildco Supply Company, Yulee, Florida) for analysis of total and soluble fractions of N and P. In 2014, we collected water samples at 0.1-, 4-, 8-, and 12-m depth. In 2015 and 2016, 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 sampler in a bucket and collected 125 mL of this water for total N and P. For soluble nutrient samples (NH_4^+, NO_3^- + NO_2^- [hereafter NO_3^-], and soluble reactive P [SRP]), we syringe-filtered 125 mL of this water through 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 with a calibrated FluoroProbe (bbe Moldaenke, Schwentinental, Germany), a submersible fluorometer that uses fluorescence at multiple wavelengths to measure in situ concentrations of cyanobacteria, diatoms, chlorophytes, cryptophytes, and total chlorophyll *a* (Chl *a*) in units of µg Chl *a*/L, hereafter referred to as cyanobacterial and chlorophyll biomass. FluoroProbe measurements are closely correlated with Chl *a* and phytoplankton counts derived from spectrophotometry and microscopy, respectively, in a range of lakes (e.g., Gregor and Maršálek 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 s, yielding ~20- to 40-cm depth interval resolution. The profiling rate was ~0.056 m/s.

In all summers, we used the Van Dorn sampler to collect water samples from the Chl *a* maximum in the water column observed by the FluoroProbe and used a Nikon Eclipse TS100 model inverted microscope (Nikon, Tokyo, Japan) at 100 to 400× magnification to identify the dominant phytoplankton taxa according to Komárek and Komárková (2004). In 2016, we collected water with the Van Dorn sampler for filtered Chl *a* analysis twice per week at the depth of maximum Chl *a* concentration, as measured by the in situ FluoroProbe measurements. We brought ~500 mL of water from each sampling depth to the laboratory in opaque bottles, immediately filtered this water through 1.2-µm Whatman GF/C filters, and froze the filters until laboratory analysis.

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 US Geological Survey (USGS) method I-4650-03, and analyzed NH_4^+, NO_3^-, and SRP according to the QuikChem Method 10-115-10-1-B with a Lachat flow-injector analyzer (ASX 520 Series; Lachat Instruments, Loveland, Colorado).

In 2016, we estimated the proportion of cyanobacterial biomass that was living vs dead at the time of collection by analyzing Chl *a* samples by spectrophotometry, following standard methods for the spectrophotometric analysis of Chl *a* and pheophytin after ethanol extraction (APHA 1998). We extracted Chl *a* from the filters for 24 h 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 10 min. After centrifugation, we recorded absorbance at 664, 665, and 750 nm on a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan), then acidified samples with 0.1 mL of 0.1 N HCl. At 2 to 3 min after acidification, we recorded absorbance at the same wavelengths, and used these values to calculate the proportion of Chl *a* from active, living vs nonliving (senesced or decomposing) cells at the time of collection (Wetzel and Likens 1991).

Statistical analyses

We analyzed the relative importance of reservoir chemistry (total and soluble fractions of N and P) and physics (light availability and water temperature) as potential drivers of cyanobacterial biomass (in μg/L, as estimated from FluoroProbe measurements) in BVR using time-series modeling. We examined cyanobacterial and nutrient patterns throughout the water column in all 3 summers, but focused our time series on 2015 and 2016, primarily because we did not collect PAR profiles in 2014 and only had 1 hypolimnetic sampling depth (8 m) from that year. Within 2015 and 2016, we analyzed the environmental factors correlated with cyanobacterial biomass at 6- and 9-m depths, the only sampling depths at which we observed a *Planktothrix* bloom throughout 2015 and 2016.

The goal of the time-series analysis was to identify the most important predictors of cyanobacterial biomass at the focal hypolimnetic depths throughout the summer season, not how the importance of the predictors changed from week to week. Cyanobacterial biomass was autocorrelated on a 1-wk time lag ($r = 0.63$ at 6 m and $r = 0.47$ at 9 m in 2015; $r = 0.40$ at 6 m and $r = 0.74$ at 9 m in 2016). Therefore, we used an autoregressive (AR) time-series model approach with the entire data set within 1 y and used the corrected Akaike information criterion for small samples (AICc) to compare alternate AR1 models with different environmental predictors. Most of the candidate environmental predictors (light, nutrients, temperature) were significantly correlated, so we compared all environmental predictors separately in models that included a cyanobacterial biomass AR1 term. We $\sqrt{(x)}$-transformed cyanobacterial biomass to meet the assumption of normality. The general form of the time-series model was:

$$\sqrt{\text{cyanos}_{t+1}} = \sqrt{\text{cyanos}_t} + \text{environmental predictor}_t + \varepsilon, \quad \text{(Eq. 1)}$$

where $\sqrt{\text{cyanos}_{t+1}}$ is $\sqrt{}$(cyanobacterial biomass) in the next week (response variable), $\sqrt{\text{cyanos}_t}$ is $\sqrt{}$(cyanobacterial biomass) in the same week (t) as our environmental observations (predictor variables), and ε is an error term. 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 to 3 wk so that our data set was evenly spaced on a weekly time-step. Because our data set had only $n = 17$ wk for each year (with 2 wk of interpolation), we were unable to use an autoregressive integrated moving average (ARIMA) model or other more data-intensive time-series approaches. We did not include DO in our analyses, because DO concentrations at 9-m depth were below the limit of detection in both years, and DO concentrations at 6 m, when above the limit of detection, may have been affected by cyanobacterial activity.

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

RESULTS

Cyanobacterial dominance in the reservoir

Cyanobacteria in the hypolimnion dominated the phytoplankton community by biomass in all 3 y, and patterns of total Chl *a* (Fig. 2A–C) closely matched those of cyanobacterial biomass (Fig. 2D–F). Total Chl *a* was much lower in the epilimnion than in the hypolimnion (<20 μg/L in both summers), and was primarily composed of green algae and diatoms (Table S1). No phytoplankton group (diatoms, green algae, or cryptophytes) other than the cyanobacteria exhibited concentrations >44 μg/L throughout all summers (Table S1).

In all 3 years, BVR experienced a persistent cyanobacterial bloom in the hypolimnion that lasted for most of the summer stratified period (Fig. 2D–F). Microscopic inspection of hypolimnetic water samples revealed that the cyanobacterial community was dominated (>90% of cells) by *Planktothrix agardhii* in all 3 y. Manual Chl *a* samples collected twice weekly indicated that, on average, 70% (± 0.19 [SD], $n = 28$) of biomass measured at the depth of maximum cyanobacterial concentration was living at the time of collection throughout the summer, which indicates that cyano-

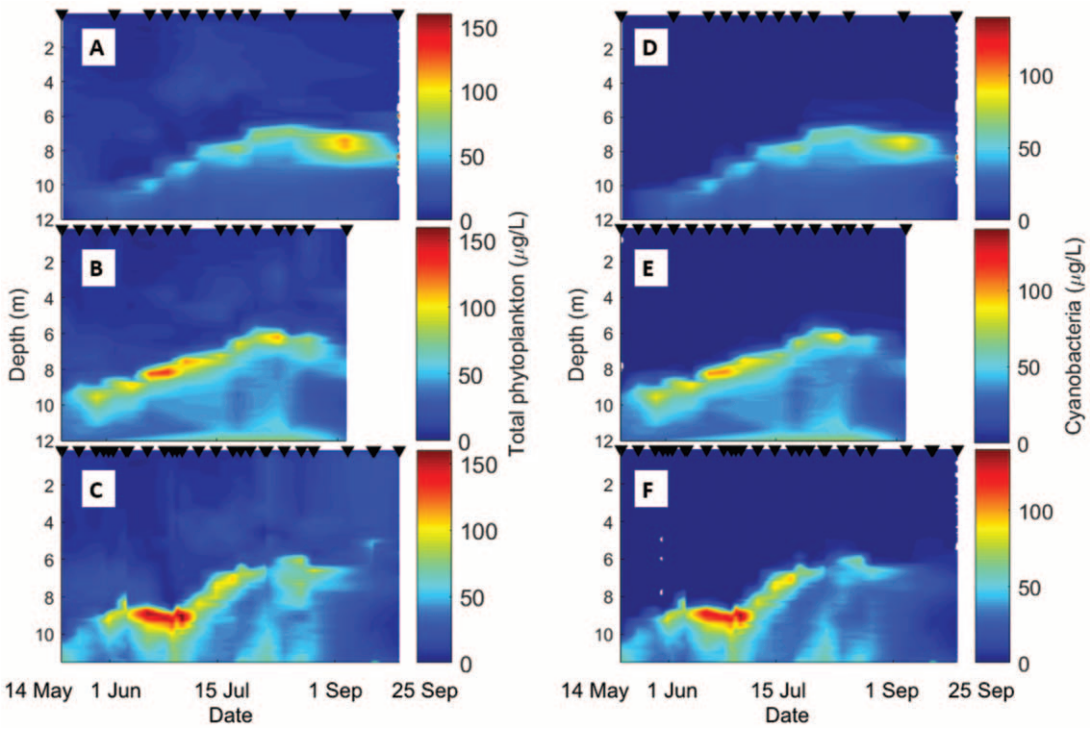

Figure 2. Total phytoplankton (as chlorophyll *a*; A–C) and cyanobacterial biomass (D–F) in summers 2014 (A, D), 2015 (B, E), and 2016 (C, F) in Beaverdam Reservoir. Sampling dates are denoted by black triangles at tops of plots; space between dates is interpolated. Note that the scales differ between cyanobacterial and total phytoplankton concentrations. FluoroProbe profiles were not collected in September 2015 because of sensor maintenance.

bacteria at these depths were active and alive, as opposed to senesced, decomposing cells having sunk from shallower depths.

In 2014, cyanobacteria appeared at ~10 m depth (2 m above the sediments) in June (Fig. 2D). The depth of maximum cyanobacterial biomass moved upward in the hypolimnion at a rate of ~0.5 m/wk from May through August, 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 the water column.

BVR exhibited hypolimnetic cyanobacterial blooms in 2015 and 2016, but cyanobacteria reached greater peak concentrations than in 2014. In 2015, the cyanobacterial bloom began to form in late May, reaching its peak concentrations (96 μg/L) in July, ~1 mo before peak concentrations were observed in 2014 (Fig. 2E). As in 2014, the depth of the cyanobacterial maximum moved upward in the hypolimnion throughout the summer in 2015 at a rate of ~0.4 m/wk from ~9 to ~6 m. Similar to 2014, cyanobacterial biomass decreased quickly in September. In 2016, the cyanobacteria bloom began to form in late May at ~9 m depth and reached a peak concentration of 140 μg/L in late June (Fig. 2F). The depth of the cyanobacterial bloom remained ~9 m until the bloom had reached peak concentrations. In late June 2016, the bloom began to move upward in the water column toward ~6 m at a rate of ~0.7 m/wk, and maximum concentrations decreased to ~60 to 90 μg/L through July. Cyanobacterial biomass remained <~60 μg/L for the rest of the summer, with the population decreasing to below detection by September. The additional FluoroProbe profile collected at the 2nd location on 16 September 2016 identified a maximum cyanobacterial concentration of 20.9 μg/L at 7.33-m depth, which was nearly identical to our regular sample site (20.7 μg/L at 7.36-m depth), suggesting that our focal site most likely represented typical reservoir conditions (Fig. S1).

Reservoir environmental conditions

BVR exhibited strong thermal stratification (Fig. 3A–C) and hypolimnetic anoxia in all summers (Fig. 3D–F). In all 3 y, the water column was thermally stratified when cyanobacteria were first observed in the hypolimnion (Figs 2D–F, 3A–C). The thermocline depth during the monitoring period ranged from 3.15 to 5.11 m in 2014, 2.92 to 5.06 m in 2015, and 4.5 to 7.4 m in 2016. Consequently, the depth of maximum cyanobacterial biomass was always ≥2.6 m deeper than the thermocline in 2014, ≥1.2 m below the thermocline in 2015, and ≥1.6 m below the thermocline in 2016, indicating that the cyanobacterial biomass was primarily hypolimnetic.

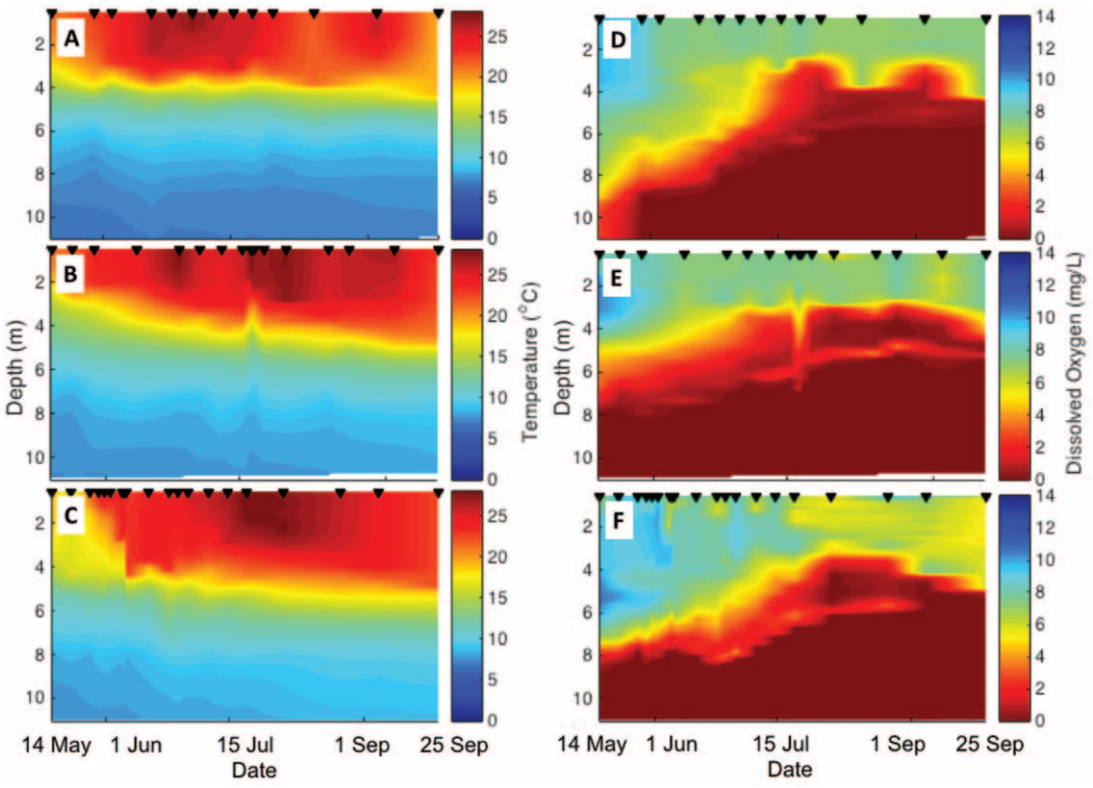

Figure 3. Water temperature (A–C) and dissolved O_2 (DO) concentrations (D–F) in Beaverdam Reservoir in summers 2014 (A, D), 2015 (B, E), and 2016 (C, F). Sampling dates are denoted by black triangles at tops of plots; space between dates is interpolated. Note that scales differ between variables and years.

Thermal stratification persisted throughout the sampling period in all 3 y, and cyanobacterial biomass decreased before fall turnover in all 3 y. In 2014, hypolimnetic O_2 concentrations were already depleted (<5 mg/L) when monitoring began on 14 May, and the bottom depths of the hypolimnion were completely anoxic by June (Fig. 3D). In 2015, anoxia (<0.5 mg O_2/L) throughout the hypolimnion was observed when monitoring began on 14 May (Fig. 3E), and in 2016, the hypolimnion had become anoxic by the time monitoring began on 26 May (Fig. 3F).

Release of nutrients from the sediments into the water column followed the development of anoxia, as evident from the high concentrations of TN (Fig. 4A–C), TP (Fig. 4D–F), SRP (Fig. 5A–C), and NH_4^+ (Fig. 5D–F), and low NO_3^- (Fig. 5G–I) concentrations in the hypolimnion just above the sediments. Hypolimnetic nutrient concentrations followed similar patterns in all 3 y, with slightly higher TP concentrations in 2015 than 2014 and 2016, reaching up to 93 µg/L in 2015, 68 µg/L in 2014, and 70 µg/L in 2016 (Fig. 4D–F). SRP concentrations were highest in the hypolimnion just above the sediments, reaching 20 µg/L in 2014 (Fig. 5A). NO_3^- concentrations were low throughout the water column in all 3 y (≤9 µg/L; Fig. 5G–I) in comparison to NH_4^+ concentrations, which were >1400 µg/L at the sediments in all 3 y (Fig. 5D–F). Within the hypolimnion, the maximum N and P concentrations occurred closest to the sediments, below the depth of the cyanobacterial biomass maximum.

PAR levels were low at our 9-m sampling depth in 2015 and 2016, but still detectable. In 2015, PAR at 9 m was between 0.0057 and 2.3 µmol s^{-1} m^{-2} (median = 0.054 µmol s^{-1} m^{-2}), and PAR at 6 m ranged from 3.6 to 49 µmol s^{-1} m^{-2} (median = 12.9 µmol s^{-1} m^{-2}). In 2016, PAR at 6 m was between 0.46 and 63 µmol s^{-1} m^{-2} (median = 4.54), and PAR at 9 m was between 0.0005 and 0.85 µmol s^{-1} m^{-2} (median = 0.106 µmol s^{-1} m^{-2}). Turbidity patterns in the reservoir largely followed the patterns of cyanobacterial concentrations in both 2015 and 2016 (Fig. S2A, B).

Predictors of cyanobacterial vertical distribution and biomass

The depth of the cyanobacterial biomass in the hypolimnion was strongly related to light availability, whereas the magnitude of cyanobacterial biomass was driven by temperature, nutrients, and light (Table 1, Fig. S3A–L). In both 2015 and 2016, the cyanobacteria consistently exhibited maximum biomass in the water column well below the depth of 1% surface light (Figs 6A, B, S3I–L). All other potential environmental predictors—thermocline depth, depths of maximum soluble and total nutrient concentrations, and depth of maximum hypolimnetic temperature—were not

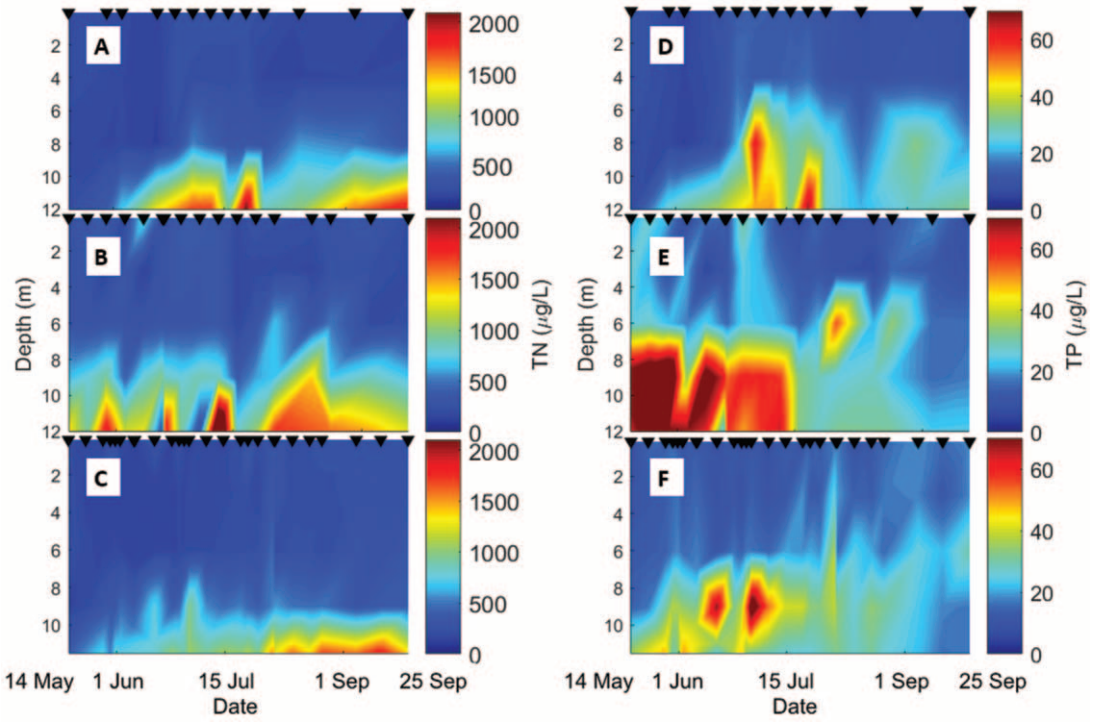

Figure 4. Total N (A–C) and total P (D–F) concentrations in Beaverdam Reservoir in summers 2014 (A, D), 2015 (B, E) and 2016 (C, F). Sampling dates are denoted by black triangles at the tops of plots; the space between dates is interpolated. Note that the scales differ between variables and between years.

correlated with the depth of the maximum cyanobacterial biomass (all $r \leq 0.08$, $p \geq 0.81$). Cyanobacterial biomass was negatively correlated with log(% surface light) at 6 m in both years when PAR was measured (both $\rho < -0.65$; Table S2), but these patterns were not evident at 9 m.

The best-fitting models predicting cyanobacterial biomass indicate that different drivers were most important at different depths between 2015 and 2016. AICc ranking of models indicated that cyanobacterial biomass in 2015 was best predicted by TN at 6 m (full model $R^2 = 0.81$, $p = 0.0001$) and by temperature at 9 m (full model $R^2 = 0.58$, $p = 0.009$) (Tables 1, S3). In 2016, cyanobacterial biomass at 6 m was best predicted by TP (full model $R^2 = 0.51$, $p = 0.0095$), and biomass at 9 m was best predicted by light (full model $R^2 = 0.71$, $p = 0.0007$). For 6 m in 2016, the TP model was within 1 AICc unit of the light and TN models, indicating that all 3 models were similar predictors of cyanobacterial biomass. For all focal hypolimnetic depths, models with light, temperature, and nutrient predictors performed substantially better than the null AR1 models without any environmental predictors (Table S3).

Using the best-fitting model at each depth identified by lowest AICc (Table 1), modeled cyanobacterial biomass was similar to observations (Fig. 7A–D). At 6 m in 2016, the high observed peak of cyanobacterial biomass on August 18 was not predicted by the strongest model (TP). Models using TP, light, or TN were equally strong predictors of cyanobacterial biomass at 6 m in 2016, indicating multiple important drivers of cyanobacterial biomass. On all other dates, the best-fitting model for each focal depth yielded biomass patterns very similar to observed population dynamics.

We excluded DO from our time-series analysis because measurements of DO concentrations at 9 m depth were below the limit of detection (0.24 mg/L) each summer, so should be interpreted with caution. However, cyanobacterial biomass was strongly negatively correlated with DO at 6 m in both 2015 ($\rho = -0.76$) and 2016 ($\rho = -0.69$).

DISCUSSION

In all 3 y, we observed very high cyanobacterial biomass in the hypolimnion of BVR. This population comprised most of the phytoplankton biomass throughout the entire water column. The cyanobacterial bloom, primarily live *Planktothrix*, remained in the hypolimnion for ~100 d in each summer. Cyanobacteria remained in the hypolimnion through summers 2014 to 2016, but moved up in the water column slowly throughout each year (Fig. 2A–F). Sustained anoxia throughout the stratified period in the 3 y (Fig. 3D–F) promoted high nutrient release from the sediments (Figs 4A–F, 5A–I), which in combination with increasing turbidity (Fig. S2A, B), probably contributed to greater attenuation of light in the hypolimnion as the summer progressed, stimulating the cyanobacteria to move upward.

The legacy of agriculture in BVR's catchment may play a role in promoting the current *Planktothrix* blooms. The

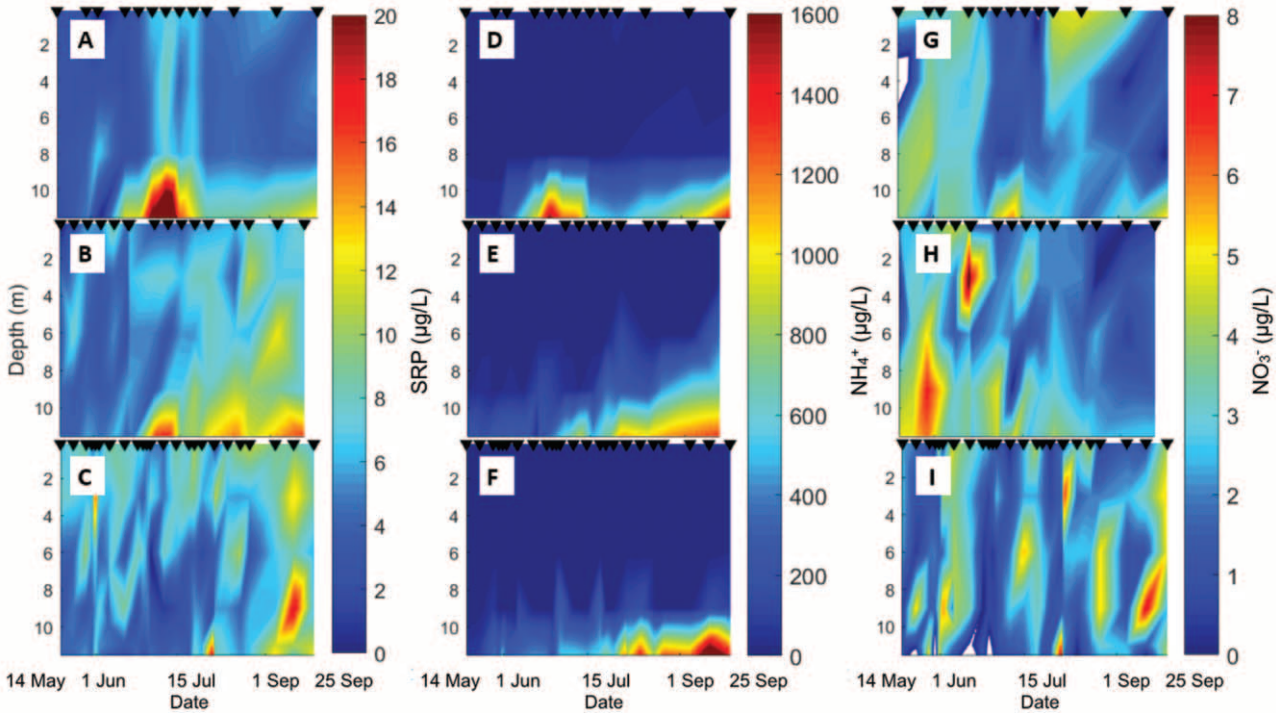

Figure 5. Concentrations of soluble reactive P (SRP) (A–C), NH_4^+ (D–F), and NO_3^- (G–I) concentrations in Beaverdam Reservoir in summers 2014 (A, D, G), 2015 (C, E, H) and 2016 (C, F, I). Sampling dates are denoted by black triangles at the tops of plots; the space between dates is interpolated. Note that the scales differ between variables and between years.

catchment of BVR is currently forested, but the land was used for agriculture in the 1800s before abandonment in the 1930s (Gerling et al. 2016). Despite reforestation, BVR experiences severe hypolimnetic anoxia and high internal nutrient loading during summer stratification, which is probably a result of a history of nutrient loading (Gerling et al. 2016). These high-nutrient hypolimnetic conditions provide the high N and P concentrations needed to support

Table 1. Comparison of time-series models to estimate cyanobacterial biomass (CB) in Beaverdam Reservoir. Three best-fitting models from each focal depth are shown; models are listed in descending order of best fit, as determined by corrected Akaike's information criterion (AICc). Cyanobacterial biomass is modeled at 6 and 9 m in 2015 and 2016. The response variable for all models is √(cyanobacterial biomass in the next sampling period). The autocorrelation term (AR1) is √(cyanobacterial biomass, lagged by 1 sampling period). Total P (TP), Total N (TN), and light (log[%surface light]) were used with the AR1 term to model cyanobacterial biomass the following week. * denotes the best-fitting model(s) at each of the depths, according to AICc value.

Year	Depth (m)	Model predictors	Time-series model equation	AICc
2015	6	AR1 + TN*	$CB = -2.84 + 0.53(AR1) + 0.012(TN)$, $R^2 = 0.81$, $p = 0.0001$	54.78
	6	AR1 + light	$CB = 1.24 - 1.48(light) + 0.69(AR1)$, $R^2 = 0.74$, $p = 0.0011$	56.99
	6	AR1 + TP	$CB = -2.25 + 0.13 \times (TP) + 0.60(AR1)$, $R^2 = 0.76$, $p = 0.0004$	58.13
	9	AR1 + temp*	$CB = 39.1 + 0.37(AR1) - 4.3(temp)$, $R^2 = 0.58$, $p = 0.0090$	49.60
	9	AR1 + light	$CB = 5.34 + 0.25(AR1) + 0.16(light)$, $R^2 = 0.12$, $p > 0.05$	53.82
	9	AR1 + TP	$CB = 3.60 + 0.022(TP) + 0.28(AR1)$, $R^2 = 0.29$, $p > 0.05$	56.87
2016	6	AR1 + TP*	$CB = -5.57 + 0.26(AR1) + 0.41(TP)$, $R^2 = 0.51$, $p = 0.0095$	72.55
	6	AR1 + light*	$CB = 1.19 + 0.28(AR1) - 0.98(light)$, $R^2 = 0.40$, $p = 0.045$	72.99
	6	AR1 + TN*	$CB = -6.04 + 0.21(AR1) + 0.038(TN)$, $R^2 = 0.49$, $p = 0.013$	73.38
	9	AR1 + light*	$CB = 4.67 - 0.68(light) + 0.77(AR1)$, $R^2 = 0.71$, $p = 0.00065$	63.17
	9	AR1 + TP	$CB = 0.28 + 0.087(TP) + 0.45(AR1)$, $R^2 = 0.72$, $p = 0.00025$	67.52
	9	AR1 + TN	$CB = -0.21 + 0.0047(TN) + 0.68(AR1)$, $R^2 = 0.65$, $p = 0.0012$	71.29

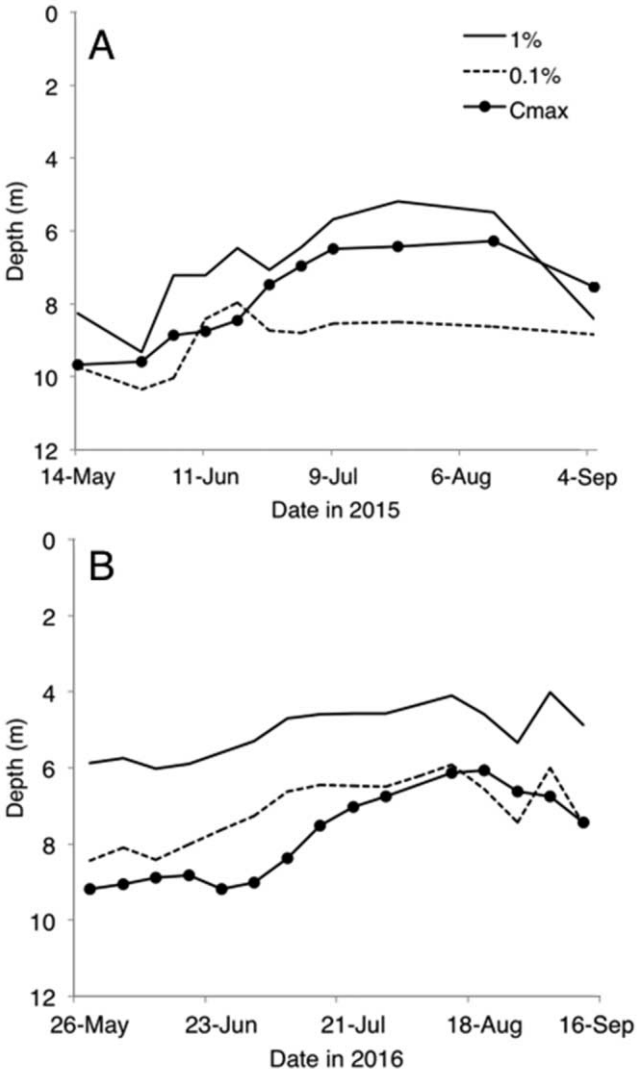

Figure 6. Depth of the maximum cyanobacterial biomass (Cmax), depth reached by 1% of surface light, and depth reached by 0.1% of surface light in summers 2015 (A) and 2016 (B).

the growth of *Planktothrix* populations below the thermocline. Similar conditions in other waterbodies, especially old reservoirs with agricultural landuse legacies, also may favor the development of *Planktothrix* blooms at depth.

Since monitoring of BVR cyanobacterial populations began in summer 2014, the magnitude of the bloom has increased each year (Fig. 2A–F). This increase may be related to the temperature of the hypolimnion, which also has increased with each consecutive year (Fig. 3A–C). The mean temperature at 6 m in 2016 (12.40°C) was 1.31°C warmer than in 2015 (11.09°C), and 2.62°C warmer than in 2014 (9.78°C). This observation suggests that the magnitude of cyanobacterial blooms in BVR probably are related to environmental cues, but with only 3 y of data, we are unable to perform a quantitative interannual comparison. We do not have ice-off data for the reservoir, and monitoring began after thermal stratification set up each year, so we are unable to compare the effects of the timing of stratification onset on the blooms among years. Despite some differences in the timing and peak magnitude of the cyanobacterial bloom among the 3 summers, the depth, range of biomass magnitude, pattern of upwards migration, and duration of the cyanobacterial blooms were generally consistent, compared to the high variability that is more typical of epilimnetic cyanobacterial blooms (e.g., Carey et al. 2014). These observations are based on just 1 focal sampling site, but data from our additional sampling location in 2016 suggests that the cyanobacterial population detected in our study extended beyond the deep hole of the reservoir (Fig. S1).

Light is frequently a driving factor in phytoplankton population growth and vertical distribution, and the typical expectation is that phytoplankton will remain at or above the depth reached by 1% of surface light (Ryther 1956). However, we observed living cyanobacterial population at light levels considerably lower than this threshold: cyanobacterial biomass reached ~90 μg/L at 9 m in 2015 and ~140 μg/L at the same depth in 2016, where light availability was <1% of surface light. These in situ observations strongly suggest that the compensation point for *Planktothrix* in BVR may be at or below the depth of 1% of surface light. Moreover, our data indicate that light was the most important predictor of cyanobacterial depth in 2015 and 2016 because the depth of maximum cyanobacterial biomass closely followed the depth reached by 1% of surface light in 2015 and the depth reached by 0.1% of surface light in 2016 (Fig. 6A, B).

These findings are similar to those of other investigators who 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 2-y study in a lake in Spain. Some phytoplankton taxa, such as *Planktothrix*, can grow successfully 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 of Chl *a*/cell volume and production of accessory pigments, such as phycobilins, thereby improving photosynthetic efficiency under low light conditions (Foy and Gibson 1982, Post et al. 1985, Reynolds 2006, Kurmayer et al. 2016). In another study examining *Planktothrix* biovolume in the hypolimnion of a temperate lake, *Planktothrix* were present at 9 m when light levels were <2 μmol m^{-2} s^{-1} (Zotina et al. 2003), indicating that PAR conditions experienced in BVR's hypolimnion (≤2.3 μmol m^{-2} s^{-1} in 2015 and ≤0.85 μmol m^{-2} s^{-1} in 2016 at 9 m) are comparable to suitable conditions for *Planktothrix* growth in other systems.

Light availability in the hypolimnion was closely linked to the depth of the cyanobacterial bloom in both years, but the

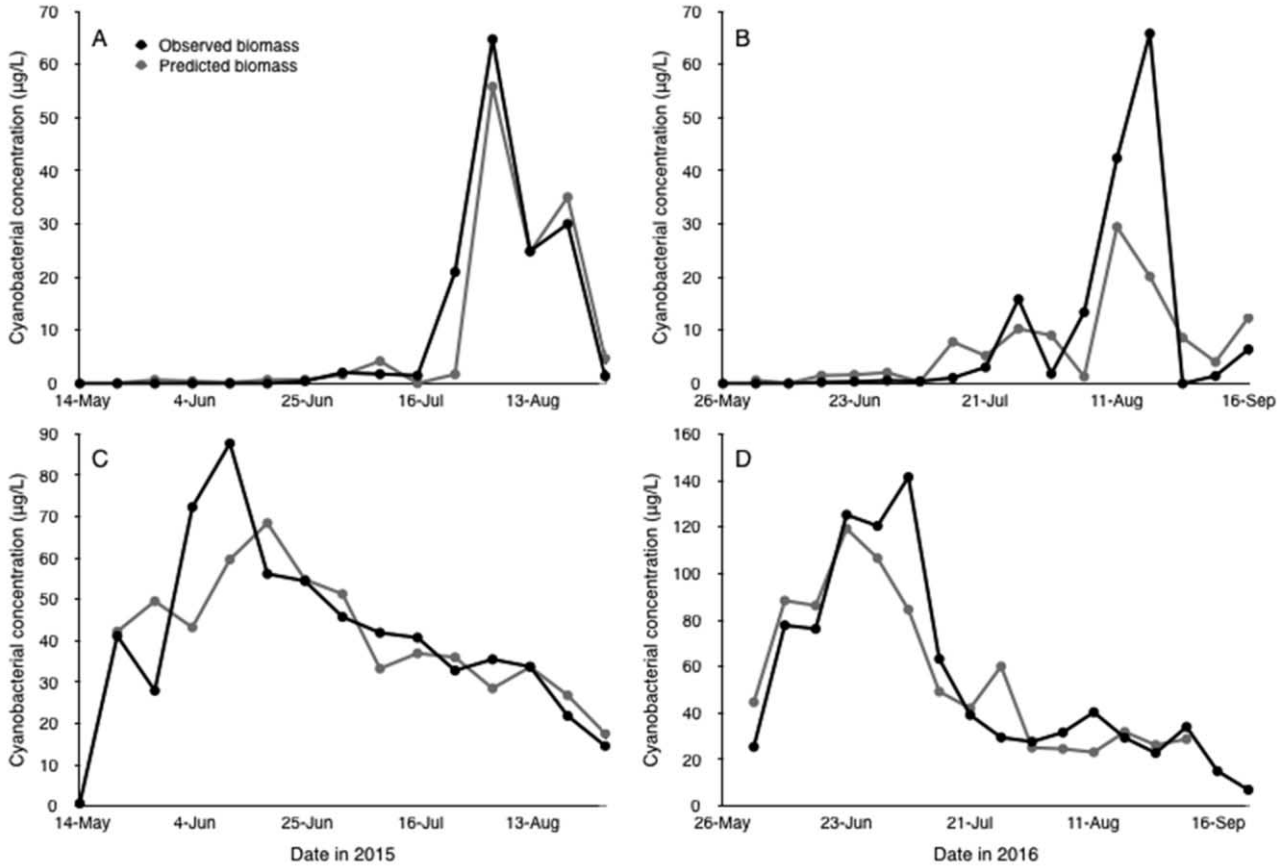

Figure 7. Observed and predicted cyanobacterial biomass at our sampling depths: 6 m in 2015 (A), 9 m in 2015 (C), 6 m in 2016 (B), and 9 m in 2016 (D), using the best-fitting model (Table 1) for each sampling depth. Cyanobacteria at 6 m in 2015 were modeled using TN and an autocorrelation term. Cyanobacteria at 9 m in 2015 were modeled using temperature and an autocorrelation term. Cyanobacteria at 6 m in 2016 were modeled using TP and an autocorrelation term. Cyanobacteria at 9 m in 2016 were modeled using light (log[% surface light]) and an autocorrelation term. Note that the *y*-axis scale varies among sampling depths and years.

magnitude of cyanobacterial biomass probably was driven by light, temperature, and nutrients together (Table 1). These environmental factors were highly correlated in all summers, indicating that cyanobacterial biomass in BVR probably responded to a combination of these factors, rather than a single predictor across all depths and times. This finding is similar to those of other investigators who have found that *Planktothrix* growth is closely related to both light and temperature when populations stratify at depth (Davis et al. 2003, Oberhaus et al. 2007) or is co-limited by light, temperature, and nutrients together (Davis et al. 2003, Oberhaus et al. 2007, Ryabov 2012, Arteaga et al. 2014). *Planktothrix* does not fix N, so high N and P concentrations together probably would substantially benefit the growth of this non-N-fixing taxon (e.g., Steinberg and Hartmann 1988, Anderson et al. 2002), as we observed in the hypolimnion of BVR. *Planktothrix* can be grazed by cladoceran zooplankton (Oberhaus et al. 2007), but very few crustacean zooplankton were present in the hypolimnion of BVR in 2015 or 2016 (Carey et al. 2018), leading us to conclude that grazing was not a significant cause of cyanobacterial depth in the BVR hypolimnion during our study.

Our study provides evidence that large, persistent cyanobacterial blooms can form in anoxic hypolimnia, where it is often assumed that phytoplankton biomass is low. Chl *a* and pheophytin analyses from 2016 demonstrate that biomass at the depth of maximum cyanobacteria was mostly living at the time of collection, as opposed to dead, senescing material that had sunk from shallower depths. Therefore, we find it surprising that we did not observe a detectable DO increase at the depth of maximum cyanobacterial biomass throughout each summer. Cyanobacteria in these depths probably were producing O_2, but it was immediately consumed by aerobic respiration or by chemical oxidation reactions. Moreover, the *Planktothrix* may have consumed most of the O_2 it produced. Thus, net primary production at 9 m was probably very close to 0, even though *Planktothrix* was living and photosynthesizing.

In situ fluorometry allowed us to monitor the bloom at high depth resolution throughout each summer by col-

lecting data on the vertical distribution and magnitude of cyanobacterial blooms from the whole water column in BVR. This technology enables rapid collection of near-real time data from multiple locations within a waterbody (Leboulanger et al. 2002, Gregor and Maršálek 2004), thereby allowing researchers to expand their monitoring to include more depths and more locations within a given ecosystem (Shade et al. 2009). Some phytoplankton taxa, such as *Planktothrix*, may vary greatly in their pigment concentrations depending on environmental conditions (Foy and Gibson 1982, Reynolds 2006, Oberhaus et al. 2007). This fact should be kept in mind when interpreting FluoroProbe results. However, previous authors have concluded that FluoroProbe measurements of *Planktothrix* are comparable to manually extracted Chl *a* measurements (Leboulanger et al. 2002, Jacquet et al. 2005). Moreover, Gregor and Maršálek (2004) and Catherine et al. (2012) found that in situ fluorometers may be more accurate than traditional methods, such as manually filtered Chl *a* samples or microscopy counts, for estimating phytoplankton biomass. FluoroProbes can underestimate Chl *a* concentrations at high phytoplankton biomass levels (Gregor and Maršálek 2004, Ghadouani and Smith 2005), which would suggest that our estimates of the magnitude of the bloom may be conservative.

Conclusions

We observed a perennial cyanobacterial bloom persisting at and below the depth typically considered to be the lower limit for phytoplankton population growth in aquatic ecosystems (Ryther 1956) in an anoxic hypolimnion. Moreover, phytoplankton grew below the depth of 1% of surface light, and this population of *Planktothrix* had far higher biomass than any other phytoplankton taxon in this waterbody in all 3 y. Our data add to the increasing evidence that some cyanobacteria may be better adapted to low light conditions and anoxia in waterbodies than previously thought (e.g., Camacho et al. 2000), even at depths below the often reported threshold of 1% of surface light. Cyanobacterial blooms in anoxic water have been observed in other freshwater ecosystems (e.g., Pasztaleniec 2004, Wilhelm et al. 2006), but they are usually shorter-lived and interannually inconsistent, even in waterbodies with higher O_2 concentrations. Our data indicate that it is critically important to monitor cyanobacterial blooms at the surface *and* at depth in freshwater ecosystems as the frequency and intensity of hypoxia and thermal stratification increase because of anthropogenic forcing.

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LITERATURE CITED

Anderson, D. M., P. M. Glibert, and J. M. Burkholder. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25:704–726.

APHA (American Public Health Association). 1998. Standard methods for the examination of water and wastewater. 20th edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Arteaga, L., M. Pahlow, and A. Oschlies. 2014. Global patterns of phytoplankton nutrient and light colimitation inferred from an optimality-based model. Global Biogeochemical Cycles 28:648–661.

Bonilla, S., L. Aubriot, M. C. S. Soares, M. González-Piana, A. Fabre, V. L. Huszar, M. Lürling, D. Antoniades, J. Padisák, and C. Kruk. 2012. What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? FEMS Microbiology Ecology 79:594–607.

Boström B., J. M. Andersen, S. Fleischer, and M. Jansson. 1988. Exchange of phosphorus across the sediment–water interface. Hydrobiologia 170:229–244.

Briand, E., M. Gugger, J. C. François, C. Bernard, J. F. Humbert, and C. Quiblier. 2008. Temporal variations in the dynamics of potentially microcystin-producing strains in a bloom-forming *Planktothrix agardhii* (cyanobacterium) population. Applied and Environmental Microbiology 74:3839–3848.

Briand, J. F., S. Jacquet, C. Flinois, C. Avois-Jacquet, C. Maisonnette, B. Leberre, and J. F. Humbert. 2005. Variations in the microcystin production of *Planktothrix rubescens* (Cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from laboratory experiments. Microbial Ecology 50:418–428.

Bright, D. I., and A. E. Walsby. 2000. The daily integral of growth by *Planktothrix rubescens* calculated from growth rate in culture and irradiance in Lake Zürich. New Phytologist 146:301–316.

Camacho, A., E. Vicente, and M. F. Miracle. 2000. Ecology of a deep-living *Oscillatoria* (= *Plankto-thrix*) population in the sulphide-rich waters of a Spanish karstic lake. Archiv für Hydrobiologie 148:333–355.

Carey, C. C., R. P. McClure, J. P. Doubek, M. E. Lofton, N. K. Ward, and D. T. Scott. 2018. *Chaoborus* spp. transport CH_4 from the sediments to the surface waters of a eutrophic reservoir, but their contribution to water column CH_4 concentrations and

diffusive efflux is minor. Environmental Science and Technology 52:1165–1173.

Carey, C. C., K. C. Weathers, H. A. Ewing, M. L. Greer, and K. L. Cottingham. 2014. Spatial and temporal variability in recruitment of the cyanobacterium *Gloeotrichia echinulata* in an oligotrophic lake. Freshwater Science 33:577–592.

Catherine, A., N. Escoffier, A. Belhocine, A. B. Nasri, S. Hamlaoui, C. Yéprémian, C. Bernard, and M. Troussellier. 2012. On the use of the FluoroProbe®, a phytoplankton quantification method based on fluorescence excitation spectra for large-scale surveys of lakes and reservoirs. Water Research 46:1771–1784.

Christiansen, G., J. Fastner, M. Erhard, T. Börner, and E. Dittmann. 2003. Microcystin biosynthesis in *Planktothrix*: genes, evolution, and manipulation. Journal of Bacteriology 185:564–572.

Cottingham, K. L., H. A. Ewing, M. L. Greer, C. C. Carey, and K. C. Weathers. 2015. Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling. Ecosphere 6:1–19.

Davis, P. A., M. Dent, J. Parker, C. S. Reynolds, and A. E. Walsby. 2003. The annual cycle of growth rate and biomass change in *Planktothrix* spp. in Blelham Tarn, English Lake District. Freshwater Biology 48:852–867.

de Figueiredo, D. R., U. M. Azeiteiro, S. M. Esteves, F. J. Gonçalves, and M. J. Pereira. 2004. Microcystin-producing blooms—a serious global public health issue. Ecotoxicology and Environmental Safety 59:151–163.

Dokulil, M. T., and K. Teubner. 2000. Cyanobacterial dominance in lakes. Hydrobiologia 438:1–12.

Dokulil, M. T., and K. Teubner. 2012. Deep living *Planktothrix rubescens* modulated by environmental constraints and climate forcing. Hydrobiologia 698:29–46.

El-Sayed, S. Z., D. C. Biggs, and O. Holm-Hansen. 1983. Phytoplankton standing crop, primary productivity, and near-surface nitrogenous nutrient fields in the Ross Sea, Antarctica. Deep Sea Research Part A. Oceanographic Research Papers 30:871–886.

Ernst, B., S. J. Hoeger, E. O'Brien, and D. R. Dietrich. 2007. Physiological stress and pathology in European Whitefish (*Coregonus lavaretus*) induced by subchronic exposure to environmentally relevant densities of *Planktothrix rubescens*. Aquatic Toxicology 82:15–26.

Fee, E. J. 1976. The vertical and seasonal distribution of chlorophyll in lakes of the Experimental Lakes Area, northwestern Ontario: implications for primary production estimates. Limnology and Oceanography 21:767–783.

Foy, R. H., and C. E. Gibson. 1982. Photosynthetic characteristics of planktonic blue-green algae: the response of twenty strains grown under high and low light. British Phycological Journal 17:169–182.

Gerling, A. B., Z. W. Munger, J. P. Doubek, K. D. Hamre, P. A. Gantzer, J. C. Little, and C. C. Carey. 2016. Whole-catchment manipulations of internal and external loading reveal the sensitivity of a century-old reservoir to hypoxia. Ecosystems 19:555–571.

Ghadouani, A., and R. E. Smith. 2005. Phytoplankton distribution in Lake Erie as assessed by a new in situ spectrofluorometric technique. Journal of Great Lakes Research 31:154–167.

Gregor, J., and B. Maršálek. 2004. Freshwater phytoplankton quantification by chlorophyll a: a comparative study of in vitro, in vivo and in situ methods. Water Research 38:517–522.

Heisler, J., P. M. Glibert, J. M. Burkholder, D. M. Anderson, W. Cochlan, W. C. Dennison, Q. Dortch, C. J. Gobler, C. A. Heil, E. Humphries, A. Lewitus, R. Magnien, H. G. Marshall, K. Sellner, D. A. Stockwell, D. K. Stoecker, and M. Suddleson. 2008. Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8:3–13.

Ibelings, B. W., and I. Chorus. 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: a review. Environmental Pollution 150:177–192.

Jacquet, S., J. F. Briand, C. Leboulanger, C. Avois-Jacquet, L. Oberhaus, B. Tassin, B. Vinçon-Leite, G. Paolini, J. Druart, O. Anneville, and J. F. Humbert. 2005. The proliferation of the toxic cyanobacterium *Planktothrix rubescens* following restoration of the largest natural French lake (Lac du Bourget). Harmful Algae 4:651–672.

Jann-Para, G., I. Schwob, and M. Feuillade. 2004. Occurrence of toxic *Planktothrix rubescens* blooms in lake Nantua, France. Toxicon 43:279–285.

Jenny, J. P., P. Francus, A. Normandeau, F. Lapointe, M. E. Perga, A. E. K. Ojala, A. Schimmelmann, and B. Zolitschka. 2016. Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. Global Change Biology 22:1481–1489.

Jiménez Cisneros B. E., T. Oki, N. W. Arnell, G. Benito, J. G. Cogley, P. Doll, T. Jiang, S. S. Mwakalila. 2014. Freshwater resources. Pages 229–269 *in* C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova, B. Girma, E. S. Kissel, A. N. Levy, S. MacCracken, P. R. Mastrandrea, and L. L. White (editors). Climate change 2014: impacts, adaptation, and vulnerability. Contribution of WGII to the 5th assessment report of the IPCC. Cambridge University Press, New York.

Komárek, J., and J. Komárková. 2004. Taxonomic review of the cyanoprokaryotic genera *Planktothrix* and *Planktothricoides*. Czech Phycology 4:1–18.

Kurmayer, R., L. Deng, and E. Entfellner. 2016. Role of toxic and bioactive secondary metabolites in colonization and bloom formation by filamentous cyanobacteria Planktothrix. Harmful Algae 54:69–86.

Leboulanger, C., U. Dorigo, S. Jacquet, B. Le Berre, G. Paolini, and J. F. Humbert. 2002. Application of a submersible spectrofluorometer for rapid monitoring of freshwater cyanobacterial blooms: a case study. Aquatic Microbial Ecology 30:83–89.

Legnani, E., D. Copetti, A. Oggioni, G. Tartari, M. T. Palumbo, and G. Morabito. 2005. *Planktothrix rubescens*' seasonal dynamics and vertical distribution in Lake Pusiano (North Italy). Journal of Limnology 64:61–73.

Lindholm, T., P. Öhman, K. Kurki-Helasmo, B. Kincaid, and J. Meriluoto. 1999. Toxic algae and fish mortality in a brackish-water lake in Åland, SW Finland. Hydrobiologia 397:109–120.

Marra, J., V. Lance, R. Vaillancourt, and B. Hargreaves. 2014. Resolving the ocean's euphotic zone. Deep Sea Research Part I: Oceanographic Research Papers 83:45–50.

Mur, R., O. M. Skulberg, and H. Utkilen. 1999. Cyanobacteria in the environment. Pages 15–40 *in* I. Chorus and J. Bartram (editors). Toxic cyanobacteria in water: a guide to their public health

consequences, monitoring and management. E and FN Spon, London, UK.

O'Neil, J. M., T. W. Davis, M. A. Burford, and C. J. Gobler. 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14:313–334.

Oberhaus, L., J. F. Briand, C. Leboulanger, S. Jacquet, and J. F. Humbert. 2007. Comparative effects of the quality and quantity of light and temperature on the growth of *Planktothrix agardhii* and *P. rubescens*. Journal of Phycology 43:1191–1199.

Paerl, H. W., R. S. Fulton, P. H. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. TheScientificWorld 1:76–113.

Paerl, H. W., and T. G. Otten. 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microbial Ecology 65:995–1010.

Pannard, A., D. Planas, and B. E. Beisner. 2015. Macrozooplankton and the persistence of the deep chlorophyll maximum in a stratified lake. Freshwater Biology 60:1717–1733.

Pasztaleniec, A. 2004. Vertical distribution of dominant cyanobacteria species in three lakes–evidence of tolerance to different turbulence and oxygen conditions. Polish Journal of Ecology 52:347–351.

Posch, T., O. Köster, M. M. Salcher, and J. Pernthaler. 2012. Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming. Nature Climate Change 2:809–813.

Post, A. F., R. de Wit, and L. R. Mur. 1985. Interactions between temperature and light intensity on growth and photosynthesis of the cyanobacterium *Oscillatoria agardhii*. Journal of Plankton Research 7:487–495.

Reynolds, C. S. 2006. The ecology of phytoplankton. Cambridge University Press, Cambridge, UK.

Reynolds, C. S., R. L. Oliver, and A. E. Walsby. 1987. Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments. New Zealand Journal of Marine and Freshwater Research 21:379–390.

Rojo, C., and M. A. Cobelas. 1994. Population dynamics of *Limnothrix redekei*, *Oscillatoria lanceaeformis*, *Planktothrix agardhii* and *Pseudanabaena limnetica* (cyanobacteria) in a shallow hypertrophic lake (Spain). Hydrobiologia 275:165–171.

Rücker, J., C. Wiedner, and P. Zippel. 1997. Factors controlling the dominance of *Planktothrix agardhii* and *Limnothrix redekei* in eutrophic shallow lakes. Hydrobiologia 342:107–115.

Ryabov, A. B. 2012. Phytoplankton competition in deep biomass maximum. Theoretical Ecology 5:373–385.

Ryther, J. H. 1956. Photosynthesis in the ocean as a function of light intensity. Limnology and Oceanography 1:61–70.

Shade, A., C. C. Carey, E. Kara, S. Bertilsson, K. D. McMahon, and M. C. Smith. 2009. Can the black box be cracked? The augmentation of microbial ecology by high-resolution, automated sensing technologies. ISME Journal 3:881–888.

Søndergaard, M., J. P. Jensen, and E. Jeppesen. 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia 506:135–145.

Steinberg, C. E., and H. M. Hartmann. 1988. Planktonic bloom-forming Cyanobacteria and the eutrophication of lakes and rivers. Freshwater Biology 20:279–287.

Visser, P. M., J. M. H. Verspagen, G. Sandrini, L. J. Stal, H. C. P. Matthijs, T. W. Davis, H. W. Paerl, and J. Huisman. 2016. How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms. Harmful Algae 54:145–159.

Walsby, A. E., P. K. Hayes, and R. Boje. 1995. The gas vesicles, buoyancy and vertical distribution of cyanobacteria in the Baltic Sea. European Journal of Phycology 30:87–94.

Walsby, A. E., P. K. Hayes, R. Boje, and L. J. Stal. 1997. The selective advantage of buoyancy provided by gas vesicles for planktonic cyanobacteria in the Baltic Sea. New Phytologist 136:407–417.

Walsby, A. E., G. Ng, C. Dunn, and P. A. Davis. 2004. Comparison of the depth where *Planktothrix rubescens* stratifies and the depth where the daily insolation supports its neutral buoyancy. New Phytologist 162:133–145.

Walsby, A. E., and F. Schanz. 2002. Light-dependent growth rate determines changes in the population of *Planktothrix rubescens* over the annual cycle in Lake Zürich, Switzerland. New Phytologist 154:671–687.

Wetzel, R. G., and G. E. Likens. 1991. Limnological analyses. 2nd edition. Springer–Verlag, New York.

Wilhelm, S. W., G. S. Bullerjahn, M. L. Eldridge, J. M. Rinta-Kanto, L. Poorvin, and R. A. Bourbonniere. 2006. Seasonal hypoxia and the genetic diversity of prokaryote populations in the central basin hypolimnion of Lake Erie: evidence for abundant cyanobacteria and photosynthesis. Journal of Great Lakes Research 32:657–671.

Winslow, L., J. Read, R. Woolway, J. Brentrup, T. Leach, and J. Zwart. 2015. rLakeAnalyzer: package for the analysis of lake physics. R package version 1.7.6. R Project for Statistical Computing, Vienna, Austria. (Available from: http://CRAN.R-project.org/package=rLakeAnalyzer)

Zotina, T., O. Köster, and F. Jüttner. 2003. Photoheterotrophy and light-dependent uptake of organic nitrogenous compounds by *Planktothrix rubescens* under low irradiance. Freshwater Biology 48:1859–1868.