

**Running head:** Thermocline deepening affects phytoplankton  
**Experimental thermocline deepening alters vertical distribution and community structure  
of phytoplankton in a four-year whole-reservoir manipulation**

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## Abstract

1. Freshwater phytoplankton communities are currently experiencing multiple global change stressors, including increasing frequency and intensity of storms. An important mechanism by which storms affect lake and reservoir phytoplankton is by altering the water column's thermal structure (e.g., changes to thermocline depth). However, little is known about the effects of intermittent thermocline deepening on phytoplankton community vertical distribution and composition or the consistency of phytoplankton responses to varying frequency of these disturbances over multiple years.
2. We conducted whole-ecosystem thermocline deepening manipulations in a small reservoir. We used an epilimnetic mixing system to experimentally deepen the thermocline in two summers, simulating potential responses to storms, and did not manipulate thermocline depth in two succeeding summers. We collected weekly depth profiles of water temperature, light, nutrients, and phytoplankton biomass as well as discrete samples to assess phytoplankton community composition. We then used time-series analysis and multivariate ordination to assess the effects of intermittent thermocline deepening due to both our experimental manipulations and naturally-occurring storms on phytoplankton community structure.
3. We observed inter-annual and intra-annual variability in phytoplankton community response to thermocline deepening. We found that peak phytoplankton biomass was significantly deeper in years with a higher frequency of thermocline deepening events (i.e., years with both manipulations and natural storms) due to weaker thermal stratification and deeper depth distributions of soluble reactive phosphorus. Furthermore,

we found that the depth of peak phytoplankton biomass was linked to phytoplankton community composition, with certain taxa being associated with deep or shallow biomass peaks, often according to functional traits such as optimal growth temperature, mixotrophy, and low-light tolerance.

4. Our results demonstrate that abrupt thermocline deepening due to water column mixing affects both phytoplankton depth distribution and community structure via alteration of physical and chemical gradients. In addition, our work supports previous research that phytoplankton depth distribution and community composition interact at inter-annual and intra-annual timescales.
5. Variability in the inter-annual and intra-annual responses of phytoplankton to abrupt thermocline deepening indicates that antecedent conditions and the seasonal timing of surface water mixing may mediate these responses. Our findings emphasize that phytoplankton depth distributions are sensitive to global change stressors and effects on depth distributions should be taken into account when predicting phytoplankton responses to increased storms under global change.

## **Introduction**

Phytoplankton in lakes and reservoirs are ecologically important organisms that are currently experiencing multiple global change stressors (Winder & Sommer, 2012). These stressors include nutrient (Smith, 2003) and sediment pollution (Donohue & Garcia Molinos, 2009), increased frequency and intensity of storms (Kirchmeier-Young & Zhang, 2020), and increased water temperatures (O'Reilly *et al.*, 2015) that result in altered thermal stratification

(Woolway *et al.*, 2019; Dokulil *et al.*, 2021). Phytoplankton responses to these global change stressors range from changes in total biomass (Ho, Michalak & Pahlevan, 2019) to altered phenology and seasonal succession (Henson *et al.*, 2018) and changes in the presence and relative abundance (Carey *et al.*, 2012; Winder & Sommer, 2012) or spatial distribution (Stockwell *et al.*, 2020) of phytoplankton taxa. Because of the fundamental role phytoplankton play in freshwater ecosystem function, changes in the composition and distribution of phytoplankton communities can alter nutrient cycling (Cottingham *et al.*, 2015), increase or decrease ecosystem productivity and dissolved oxygen levels (Diaz, 2001), and affect food quantity and quality for higher trophic levels (Danielsdottir, Brett & Arhonditsis, 2007). Global change stressors may also increase the prevalence of algal and cyanobacterial blooms (Ho & Michalak, 2020), which can have a variety of undesirable impacts including release of toxins (Chorus & Welker, 2021), unsightly surface scums, and taste and odor problems (Watson *et al.*, 2016).

One important mechanism by which global change affects freshwater phytoplankton is via alteration of thermocline depth (Gray *et al.*, 2019), which can occur either gradually due to changing air temperatures (Kraemer *et al.*, 2015; Flaim *et al.*, 2016) or abruptly due to storms (Jennings *et al.*, 2012; Klug *et al.*, 2012; Ren *et al.*, 2020; Stockwell *et al.*, 2020). Alteration of thermocline depth can affect the vertical distribution and composition of the phytoplankton community during the summer stratified period (Garneau *et al.*, 2013; Jobin & Beisner, 2014), as phytoplankton biomass in many stratified lakes and reservoirs is shown to vary across depth, with peak biomass concentration often occurring well below the surface (Hamilton, Brien & McBride, 2010; Cullen, 2015; Latasa *et al.*, 2017; Leach *et al.*, 2018; Lofton *et al.*, 2020). These biomass peaks form in response to vertical environmental gradients (e.g., of water temperature

and light) within the water column (Longhi & Beisner, 2009; Cullen, 2015; Albers *et al.*, 2018; Lofton *et al.*, 2020; Reinl, Sterner & Austin, 2020), and may be especially sensitive to global-change induced alteration of thermal stratification (Carey *et al.*, 2012; Winder & Sommer, 2012).

Changes in thermocline depth may affect both the depth and width of phytoplankton biomass peaks (Fig. 1; Beisner & Longhi, 2013; Jobin & Beisner, 2014; Leach *et al.*, 2018; Lofton *et al.*, 2020), as well as phytoplankton community composition (Lydersen & Andersen, 2007; Jobin & Beisner, 2014; Stockwell *et al.*, 2020). Deepening thermoclines can increase the proportion of the water column where light availability and water temperature are suitable for phytoplankton growth (Huisman *et al.*, 2004) or entrain nutrients from below the thermocline into the photic zone (Stockwell *et al.*, 2020). As a result, thermocline deepening could either lead to: 1) a deeper phytoplankton biomass peak as phytoplankton shift to access entrained nutrients (Fig. 1B; e.g., Garneau *et al.*, 2013), or 2) a wider, more diffuse peak as some phytoplankton shift their depth to maximize entrained nutrient availability while others remain at shallow depths to maximize light availability (Fig. 1C; e.g., Jobin & Beisner, 2014). Additionally, a thermocline that is shallower than the photic zone depth might favor taxa that are tolerant of high ultraviolet radiation and grow well at warm temperatures, while a deeper thermocline might favor taxa that are low-light tolerant or mixotrophic (Klausmeier & Litchman, 2001; Diehl *et al.*, 2002; Huisman *et al.*, 2004).

Gradual changes in thermocline depth due to air temperature warming and abrupt changes in thermocline depth due to storms likely evoke different phytoplankton responses at different time scales. Whole-ecosystem studies examining the response of phytoplankton to gradual thermocline deepening over multiple years, as might occur due to warming air temperatures, found that thermocline deepening affected the relative abundance of phytoplankton

taxa and phytoplankton vertical distributions (Lydersen & Andersen, 2007; Cantin *et al.*, 2011; Jobin & Beisner, 2014). Specifically, thermocline deepening increased species richness, decreased the abundance of chlorophytes and diatoms, and increased the abundance of mixotrophic dinoflagellates in an oligotrophic Norwegian lake over three years, although total phytoplankton biomass did not change (Lydersen & Andersen, 2007). Conversely, thermocline deepening led to increased chlorophyte abundance and total biomass (Cantin *et al.*, 2011) and shallower, wider biomass peaks of phytoplankton (Jobin & Beisner, 2014) in different summers during a multi-year whole-ecosystem experiment in an oligotrophic lake in Québec, Canada. These contradictory findings highlight the possibility for multiple mechanisms of thermocline deepening effects on phytoplankton community structure.

Variability in observed whole-ecosystem effects of a single abrupt thermocline deepening event on phytoplankton at daily to seasonal timescales (Rinke *et al.*, 2009; Garneau *et al.*, 2013; Wu *et al.*, 2015; Planas & Paquet, 2016; Kasprzak *et al.*, 2017) underscores the need for improved understanding of the potential cumulative effects of an increased frequency and intensity of multiple abrupt thermocline deepening events, especially at the inter-annual scale. Previous studies looking at single thermocline deepening events have observed conflicting results. Several studies which examined the effect of a single storm event on phytoplankton vertical distributions at the whole-ecosystem scale reported homogenization of biomass across the epilimnion (warm surface waters) after a storm due to internal seiches, upwelling, and surface water mixing (Rinke *et al.*, 2009; Wu *et al.*, 2015; Planas & Paquet, 2016; Kasprzak *et al.*, 2017; represented in Fig. 1C). In contrast, another study did not report homogenization but instead a deepened biomass maximum after a storm (Garneau *et al.*, 2013; represented in Fig. 1B). Finally, abrupt experimental thermocline deepening caused increases in small, silica-

138 containing flagellates and decreases in colonial, filamentous phytoplankton taxa but inconsistent  
139 total biomass responses during a single summer (Lofton *et al.*, 2019). These varying responses to  
140 single events highlight the pressing need to understand the *cumulative* response of phytoplankton  
141 to an increased frequency and intensity of intermittent, abrupt thermocline deepening, such as  
142 might occur due to an increasing frequency of storm events, over multiple years due to global  
143 change.

144         The relationship between the frequency of abrupt thermocline disturbance and  
145 phytoplankton community structure over multiple summers has important implications for the  
146 predictability of phytoplankton community response to increased storms. Some research suggests  
147 that an increased frequency and intensity of thermocline deepening disturbance could result in  
148 greater rates of change in the presence and relative abundance of phytoplankton taxa (Pannard,  
149 Bormans & Lagadeuc, 2008). Alternatively, as the frequency of thermocline deepening events  
150 increases, the phytoplankton community could shift to include more taxa that are well-adapted to  
151 mixed conditions (Winder & Sommer, 2012; Stockwell *et al.*, 2020). Examining the effects of  
152 thermocline deepening events at varying frequencies over multiple years will enhance our ability  
153 to predict phytoplankton community responses to future increases in storm frequency.

154         We conducted a whole-ecosystem manipulation in which we experimentally deepened  
155 the thermocline of a small, eutrophic reservoir and examined the responses of phytoplankton  
156 depth distribution and community structure over four years. Our study addressed two research  
157 questions: 1) How do phytoplankton depth distribution and community structure change in  
158 response to an increased frequency of thermocline deepening events? and 2) What are the  
159 duration and consistency of these responses at intra-annual and inter-annual scales? We  
160 performed five thermocline deepening manipulations over two summers, and then did not

manipulate the thermocline in two reference summers. We monitored phytoplankton depth distribution and community structure weekly. We also measured a suite of physicochemical variables to assess responses to changes in gradients of light, temperature, and nutrients associated with thermocline deepening on weekly to inter-annual timescales.

## Methods

### *Study site*

We conducted two summers of thermocline deepening manipulations (2016–2017), followed by two summers with no manipulations (2018–2019) in Falling Creek Reservoir (FCR), a small drinking water supply reservoir located in Vinton, VA, USA (37°18'12" N, 79°50'14" W; Fig. 2). FCR is owned and operated by the Western Virginia Water Authority (WVWA), has a maximum depth of 9.3 m (Fig. 2), and is thermally stratified from approximately April to October each year (Carey *et al.*, 2021c), with ice cover and inverse stratification occurring intermittently during the winter months in most years (Carey, 2021). From May to September in 2016–2019, FCR's trophic state indicated mesotrophic to eutrophic conditions (following Carlson & Simpson, 1996): mean total phosphorus (TP) across the water column was  $19 \pm 11 \mu\text{g L}^{-1}$  (1 S.D.), mean total nitrogen was  $343 \pm 242 \mu\text{g L}^{-1}$  (Carey *et al.*, 2020a), and mean Secchi depth was  $2.0 \pm 0.7 \text{ m}$  (Carey *et al.*, 2020b). Over the course of the study, water residence time in FCR had a median of 174 days (Carey *et al.*, 2021b; see Text S1), and the reservoir was managed to have a constant water level.



### *Thermocline deepening manipulations*

An engineered bubble-plume epilimnetic mixing (EM) system was deployed in FCR for water quality management (Visser *et al.*, 2016). The EM is installed at a depth of 5 m and extends throughout the lacustrine region of the reservoir (Fig. 2). The system comprises an onshore air compressor coupled to a diffuser line of porous hose which can inject bubbles of atmospheric air into the water column at rates up to 25 standard cubic feet per minute (SCFM; see Lofton *et al.* 2019 for an in-depth description of the EM system).

In 2016 and 2017, we conducted a total of five discrete, short (24–72-hour) manipulations in FCR to test operation of the EM in collaboration with the WVWA. Manipulations occurred in late May, late June, and late July of 2016 and in late May and early July of 2017 and had varied timing, intensity, and duration (Table 1) to provide the WVWA with information on the effects of EM operation under a range of conditions. These short, intense thermocline deepening manipulations are well-suited to simulate the abrupt changes in thermocline depth that can accompany storms (e.g., Kasprzak *et al.*, 2017) and contrast with previous empirical work examining phytoplankton response to thermocline deepening via gradual mixing (Lydersen & Andersen, 2007; Cantin *et al.*, 2011; Jobin & Beisner, 2014). EM system testing was halted after two years and so the thermocline formed in the absence of manipulations during the summers of 2018 and 2019.

### *Field sampling*

To identify naturally-occurring storm events that might result in thermocline deepening, we measured wind speed (05103-L Wind Monitor, R.M. Young, Traverse City, MI, USA) and

precipitation (TE525WS-L Rain Gauge, Texas Electronics, Dallas, TX, USA) at one-minute temporal resolution throughout the study period with a meteorological station (CR3000 Micrologger, Campbell Scientific, Logan, UT, USA) deployed on the dam of FCR (Fig. 2; Carey *et al.*, 2021a).

To assess the effect of thermocline deepening on phytoplankton, we collected weekly depth profiles of phytoplankton biomass and grab samples for microscope identification of phytoplankton taxa from May to September in 2016–2019. All sampling was conducted at the deepest site of the reservoir (Fig. 2). Biomass depth profiles (~10 cm resolution) were collected using a FluoroProbe (bbe Moldaenke, Schwentinental, Germany; Catherine *et al.*, 2012; Carey *et al.*, 2021c). FluoroProbes report total biomass as the summation of biomass across four spectral groups (green algae, brown algae, cyanobacteria, and cryptophytes; Beutler *et al.*, 2002). For this study, we focused on total biomass profiles (vs. individual spectral groups) to represent the entire community's depth distribution patterns. We chose to sample phytoplankton community composition at the depth of peak phytoplankton biomass according to total biomass profiles. Depth samples were collected using a 4-L van Dorn sampler (Wildco, Yulee, FL, USA) at the depth of peak biomass estimated from FluoroProbe depth profiles. Samples were immediately preserved in opaque 250 mL high-density polyethylene (HDPE) bottles by adding ~1% Lugol's iodine by volume for subsequent microscope analysis.

We also collected a suite of physicochemical variables each week to assess the effect of thermocline deepening over the four-year study. We obtained ~0.1 m-resolution depth profiles of water temperature (Carey *et al.*, 2020b, 2021c e) and photosynthetically active radiation (Carey *et al.*, 2020b, 2021c) and measured Secchi depth (Carey *et al.*, 2020b). We also collected 1-2 m-resolution depth profiles of water chemistry, including dissolved organic carbon (DOC), nitrate

(NO<sub>3</sub>), ammonium (NH<sub>4</sub>), and soluble reactive phosphorus (SRP; Carey *et al.*, 2020a). Detailed field sampling methods for physicochemical variables can be found in Text S2.

### *Laboratory analyses*

Phytoplankton grab samples were enumerated on a Nikon Eclipse Ci microscope (Nikon, Minato City, Tokyo, Japan). Before counting, samples were permanently mounted on slides following Crumpton (1987). Samples were then enumerated at 400× until at least 300 natural units (either single cells or colonies) had been counted (Acker, 2002; Brierley *et al.*, 2007). Phytoplankton were identified to genus and the first ten natural units of each genus were measured and used to calculate biovolume following Hillebrand *et al.* (1999). All phytoplankton microscopy was conducted by M.E.L.

All chemistry samples were analyzed following standard methods within six months of collection (Carey *et al.*, 2020a; Text S3). All data associated with this study are published in the Environmental Data Initiative repository (Carey *et al.*, 2020a b, 2021a, b, c, d e; Carey, 2021; Lofton *et al.*, 2021).

### *Calculation of phytoplankton community and distribution metrics*

We calculated multiple metrics to describe both phytoplankton biomass depth distributions and community composition at the depth of maximum biomass. First, we used the fluorescence-based depth profiles to calculate the depth of maximum phytoplankton biomass, the magnitude of biomass at that depth, and width of the biomass peak (peak width) across depth (following Leach *et al.*, 2018; Lofton *et al.*, 2020). We determined the closest depth above and below the depth of maximum biomass where phytoplankton biomass concentration was less than

or equal to the median concentration across the water column. The difference between these two depths was assigned as the peak width (see Fig. S1 for a visual explanation of peak width calculation).

Second, we used phytoplankton count data to assess community composition at the depth of peak biomass. We determined genus richness and calculated the relative abundance and Shannon diversity of phytoplankton groups (diatoms, chlorophytes, chrysophytes, cryptophytes, cyanobacteria, desmids, dinoflagellates, euglenoids, raphids) at the depth of maximum biomass on each sampling day. To assess potential changes in both the relative abundance and the presence/absence of genera over time, we also calculated Bray-Curtis and Jaccard dissimilarity between samples each week within each year using the *vegdist* function of the *vegan* package (Oksanen *et al.*, 2020).

#### *Calculation of physicochemical metrics*

We analyzed field data to calculate metrics describing the temperature, light, nutrient, and carbon conditions in the reservoir following previous studies (Jobin & Beisner, 2014; Cullen, 2015; Leach *et al.*, 2018; Lofton *et al.*, 2020). Thermocline depth, Schmidt stability, and buoyancy frequency were calculated using the R package *rLakeAnalyzer* (Winslow *et al.*, 2019). In addition, we determined the water temperature at the depth of peak phytoplankton biomass and phytoplankton depth sample on each sampling day. Photic zone depth was determined from PAR depth profiles as the depth where 1% of incident surface light was available, and the coefficient of light attenuation ( $K_d$ ) was calculated as the slope of the best fit line of the natural logarithm of percent surface light plotted against depth (Wetzel & Likens, 1991). We also determined the percent of surface light that was available at the depth of peak biomass and

phytoplankton depth sample on each sampling day. To characterize carbon and nutrient conditions, we determined the concentration of DOC, dissolved inorganic nitrogen (DIN, calculated as the sum of  $\text{NO}_3$  and  $\text{NH}_4$ ), and SRP at the sampling depth closest to the biomass peak and phytoplankton depth sample on each sampling day, as well as the coefficient of variation of carbon and nutrients across the photic zone. We also determined the magnitude of maximum observed carbon and nutrient concentrations across the photic zone on each sampling day, as well as the depth at which each concentration's maximum occurred.

#### *Assessing effects of naturally-occurring intense storms*

To determine whether our thermocline deepening manipulations approximated naturally-occurring storms, we calculated daily sums of precipitation and daily mean wind speed during the four-year study period to identify intense storms. Intense storms were defined as when both daily mean wind and summed precipitation were in the top 5% of all observation days (total  $n = 572$ ) across the study period (following Dousek *et al.*, 2021). Following this, we identified two intense storm events during our four-year study period: one on 5 May 2016 and one on 8 June 2019. We then examined thermocline depths immediately before and after these naturally-occurring intense storm events (within  $\pm 1$  week) and compared them to changes in thermocline depth due to our manipulations.

Across the four years, manipulation summers experienced four (2016) or two (2017) thermocline deepening events (natural + experimental summed), whereas the reference summers experienced zero (2018) or one (2019) thermocline deepening events, representing a substantial difference in thermocline deepening frequency between manipulation and reference summers (Table 1).

299 *Assessing effects of increased thermocline deepening frequency*

300         We used Anderson-Darling tests (Razali & Wah, 2011) to assess the effects of increased  
301 thermocline deepening frequency, including both natural and experimental thermocline  
302 deepening events, on physicochemical and phytoplankton community structure metrics at the  
303 inter-annual scale. Anderson-Darling tests can be used to assess significant differences between  
304 distributions of a variable and give more weight to distribution tails or extreme values,  
305 permitting assessment of whether manipulations shifted both the mean and range of ecosystem  
306 variables (Razali & Wah, 2011). We considered a variable to be responsive to an increased  
307 frequency of thermocline deepening if variable distributions between summers with  
308 manipulations and reference summers were significantly different. All Anderson-Darling test p-  
309 values were Holm-Bonferroni corrected for multiple comparisons (Holm, 1979).

310  
311 *Analysis of phytoplankton depth distributions*

312         We used autoregressive integrated moving average (ARIMA) models to assess how an  
313 increased frequency of thermocline deepening events affected phytoplankton depth distributions.  
314 ARIMA models are a well-established method for identifying the most important predictors of a  
315 response variable over time while accounting for autocorrelation (Hyndman & Athanasopoulos,  
316 2018). We developed best-fit ARIMA models using physicochemical metrics to predict  
317 phytoplankton peak depth, peak width, and the magnitude of biomass at the peak depth over  
318 time. We fit models to the full time-series of depth distribution profiles (2016–2019) as well as  
319 to manipulation summers (2016-2017) and reference summers (2018-2019) separately, and then  
320 compared predictors of phytoplankton depth distributions between manipulation and reference  
321 summers and the full time-series. We did not fit ARIMA models to phytoplankton vertical

distribution time series for each individual summer or before and after thermocline deepening events or intense storms within a year because each summer's time series had <20 observations.

We developed a model selection algorithm based on the *auto.arima* function in the *forecast* package in R (Hyndman & Khandakar, 2008; Hyndman *et al.*, 2021). Our algorithm compared models fit by *auto.arima* using all possible combinations of predictors to a global model using all predictors and a null model using no predictors. The algorithm then selected the model with the lowest corrected Akaike Information Criterion (AICc) as well as all models within 2 units of the lowest AICc value (Burnham & Anderson, 2002). The models had a maximum of one autoregressive term (AR(1)), following results of partial autocorrelation functions (Hyndman & Athanasopoulos, 2018) calculated for each depth distribution metric (Fig. S2).

We considered the water temperature, light, and carbon and nutrient concentration metrics described above as potential predictors of phytoplankton peak depth, peak width, and the magnitude of biomass at the peak depth. We conducted pairwise Spearman correlations among all potential predictors; if two potential predictors had a pairwise Spearman's  $\rho \geq 0.7$ , we selected the predictor that was most strongly correlated with the corresponding response variable (Table S1). The distributions of all predictors and response variables were checked for skewness, log-transformed if appropriate, and standardized to Z-scores before fitting ARIMA models.

#### *Analysis of phytoplankton community data*

To relate phytoplankton community structure to physicochemical and phytoplankton depth distribution metrics, we conducted a non-metric multidimensional scaling analysis (NMDS) and post-hoc tests on ordination output. We used NMDS because this ordination

technique does not assume a multivariate normal distribution of community data and does not constrain ordination results based on environmental gradients (McCune & Grace, 2002), which was appropriate given the non-normal distribution of our data and our goal of assessing community responses to thermocline deepening. We used the *metaMDS* function in the *vegan* package to perform NMDS analyses across all summers (2016–2019) as well as for each summer individually. This function provides the benefit of including several random starts of the NMDS ordination process to ensure a stable result (Oksanen *et al.*, 2020). We assessed the stress of each ordination result to determine the minimum number of axes needed to adequately explain variability in community composition data (McCune & Grace, 2002).

We subsequently conducted several post-hoc analyses of our phytoplankton community data and ordination output. We used analysis of similarities (ANOSIM) on NMDS ordination output to assess phytoplankton community differences among summers and months, between manipulation and reference summers, and before and after thermocline deepening events and intense natural storm events within a summer. ANOSIM is an appropriate post-hoc test for NMDS ordinations because both operate using ranked dissimilarities (McCune & Grace, 2002). ANOSIM tests report an R statistic, where values approaching 0 indicate similar groups, values approaching 1 indicate dissimilar groups, and values less than 0 indicate greater dissimilarity within a group than among groups (McCune & Grace, 2002). We considered month of year to be a proxy for change in the phytoplankton community due to seasonal succession, and so conducting ANOSIM tests on both month of year and the periods before and after thermocline deepening events within a summer allowed us to compare the relative importance of seasonal succession and thermocline deepening manipulations at the intra-annual scale. Although an intense storm occurred on 5 May 2016, no ANOSIM test was conducted for phytoplankton



communities before and after this storm event due to insufficient pre-storm data (n = 1 pre-storm sampling point).

We also examined associations between phytoplankton genera and manipulation vs. reference summers, periods before and after thermocline deepening events or intense storms within a summer, and each summer and month using indicator species analysis (*multipatt* function in the *indicspecies* package; De Cáceres, Jansen & Dell, 2020). We selected the *multipatt* method for indicator species analysis because it permits species to be significantly associated with more than one group (e.g., a genus can be associated with both July and August communities; see De Cáceres, Legendre & Moretti, 2010).

Finally, we evaluated associations between physicochemical variables, depth distribution metrics, and phytoplankton community structure ordination output using the *envfit* and *ordisurf* functions in the *vegan* package. Briefly, the *envfit* function fits linear models between vectors of environmental variables and ordination scores and determines which fits are significant, and the *ordisurf* function fits a smoothed surface of an environmental variable to ordination scores using a generalized additive model (GAM; Oksanen *et al.*, 2020).

All analyses were conducted in the R statistical environment (v. 4.0.3; R Core Team 2020). All analysis code is available on GitHub (<https://github.com/melofton/FCR-phytos>) and published via Zenodo (DOI: 10.5281/zenodo.5146268).

## Results

### *Thermocline deepening manipulations were representative of intense storms*

Comparison of our thermocline deepening manipulations with two naturally-occurring intense storms during the study period revealed that our manipulations reasonably approximated storm-induced thermocline deepening. A storm on 5 May 2016 deepened the thermocline from 3.1 to 4.2 m (change of 1.1 m), and a storm on 8 June 2019 led to a 0.3 m deepening of the thermocline (Table S2; Fig. S3; Fig. S4), while changes in thermocline depth due to our manipulations ranged from 0.5 to 3.1 m (Table S2; Fig. S3).

### *Increased frequency of thermocline deepening altered physicochemical variables*

Multiple thermocline deepening manipulations in 2016 and 2017 significantly altered the summer thermal structure of FCR compared to reference summers, despite the occurrence of an intense storm in 2019 (Table 2; Fig. 3). The manipulations deepened the thermocline by over a meter on average (Fig. 3A, B; Fig. 4), as mean thermocline depth in manipulation summers was  $3.9 \pm 1.1$  m (1 S.D.), vs.  $2.8 \pm 0.4$  m in reference summers (adj. Anderson-Darling  $p = 0.0004$ ). In addition to being deeper, thermocline depth was also more variable in manipulation summers (Fig. 3A, B).

Thermocline deepening decreased thermal stratification (Table 2; Fig. 3), with a mean Schmidt stability of  $25.1 \pm 8.4$  J m<sup>-2</sup> in manipulation summers vs.  $36.9 \pm 6.7$  J m<sup>-2</sup> in reference summers (adj. Anderson-Darling  $p < 0.0001$ ; Fig. 3C, D). Buoyancy frequency was also significantly lower in manipulation summers ( $6.1 \times 10^{-3} \pm 1.8 \times 10^{-3}$  s<sup>-1</sup>) vs. reference summers ( $9.3 \times 10^{-3} \pm 2.0 \times 10^{-3}$  s<sup>-1</sup>; adj. Anderson-Darling  $p < 0.0001$ ; Fig. 3E, F).

SRP depth distributions across the photic zone were different in manipulation and reference summers (Table 2; Fig. 3). Thermocline deepening manipulations increased the depth of maximum SRP concentration in the photic zone by over a meter, and the depth of maximum SRP was also more variable in manipulation summers (Fig. 3G, H; Fig. 4). The mean summer depth of maximum SRP concentration within the photic zone was  $1.2 \pm 0.7$  m during reference summers and  $2.4 \pm 1.7$  m in manipulation summers (adj. Anderson-Darling  $p = 0.0008$ ). The magnitude of maximum summer SRP concentration in the photic zone ranged from 7 to 20  $\mu\text{g L}^{-1}$  across all summers (Fig. 4), but was not different between manipulation and reference summers (Table S3).

Several metrics of DOC in the photic zone were also different between manipulation and reference years (Table 2). However, this was primarily due to lower DOC concentrations in 2016 compared to all other years. As such, we considered evidence of a manipulation effect on DOC to be inconclusive. No other metrics of water temperature, light, nutrients, or carbon were significantly different between manipulation and reference years (Table S3).

#### *Deeper maximum phytoplankton biomass in manipulation years*

Phytoplankton depth distributions were altered by an increased frequency of thermocline deepening (Table 2; Fig. 3I, J) in support of the hypothesis presented in Fig. 1B. Peak biomass depth ranged from 0.31 – 7.25 m in 2016–2019, and the mean depth of maximum phytoplankton biomass was significantly deeper in manipulation summers ( $4.5 \pm 1.3$  m) than in reference summers ( $3.1 \pm 1.3$  m; adj. Anderson-Darling  $p = 0.003$ ). Across all years, the median depth of maximum summer phytoplankton biomass was below the thermocline and above the depth of 1% available surface light (photic zone depth; Fig. 4).

Increased thermocline deepening frequency did not affect the width of the biomass peak or the magnitude of biomass at the peak biomass depth (Table S3). High-biomass events (i.e., blooms, defined as more than two standard deviations greater than the mean biomass for 2016–2019) occurred in 2017 and 2019 (Fig. S5), indicating that manipulations neither consistently caused nor prevented bloom formation.

#### *Predictors of depth distributions differed between manipulation and reference summers*

Physicochemical predictors of phytoplankton depth distributions differed between manipulation and reference summers (Table 4; Table S9). Overall, metrics of thermal stratification tended to be more important in best-fit ARIMA models for manipulation summers, while metrics characterizing the light environment tended to be more important in models fit for reference summers. Nutrient conditions were important in all summers.

The best-fit ARIMA model for peak depth across all summers and during manipulation summers included Schmidt stability, thermocline depth, and the depth of maximum SRP in the photic zone (Table 4), all of which were significantly different between manipulation and reference summers (Table 2; Fig. 3). As Schmidt stability and thermocline depth increased, peak depth decreased, and as the depth of maximum SRP in the photic zone increased, peak depth increased. In general, manipulation summers were associated with mean lower Schmidt stability, deeper thermoclines, deeper maximum SRP, and deeper peak biomass (Table 2, Fig. 3), so the unexpected inverse relationship between thermocline depth and peak depth was likely driven by a consistent intra-annual pattern of shallower peak depths in the early fall as the thermocline deepened seasonally (Fig. 3). During reference summers,  $K_d$  was the strongest predictor of peak

depth, with peak depth tending to be shallower when the light attenuation rate was high (Table 4).

Despite no significant response of peak width or maximum biomass at the depth of peak biomass to thermocline deepening, the predictors of these two variables significantly differed between manipulation and reference summers. During manipulation years, the strongest predictor of peak width was buoyancy frequency. Peak width tended to decrease as buoyancy frequency increased (Table 4), so that peaks were narrowest in strongly stratified conditions. In reference summers, the strongest predictor of peak width was  $K_d$ . Peak width tended to decrease as  $K_d$  increased, so that peaks were narrowest when light attenuation rates were high. During manipulation summers, the strongest predictor of maximum biomass at the depth of peak biomass was the AR(1) term, indicating strong autocorrelation. In reference summers, maximum biomass was most strongly predicted by a positive relationship with water temperature at the depth of biomass maximum. The relation of biomass to SRP concentration was equivocal, with biomass exhibiting a positive relation with SRP concentration in manipulation summers and a negative relation with SRP concentration in reference summers and across the full time series. We note that estimated coefficients for SRP concentration in maximum biomass models had high standard errors and did not differ greatly from zero (Table 4).

#### *Increased thermocline deepening affects inter-annual phytoplankton community composition*

Despite no significant change in maximum biomass, phytoplankton community composition was significantly different between manipulation and reference summers (Table 3; Fig. 5; Fig. 6). We found that phytoplankton community composition at the inter-annual scale was sufficiently explained by three NMDS ordination axes. According to post-hoc analysis of

NMDS ordination output, occurrence of experimental thermocline deepening events (along with year and month of year) strongly predicted phytoplankton community structure in the first and second dimensions of the 2016–2019 NMDS ordination (Fig. 6A). Experimental thermocline deepening within a summer was also a strong predictor (along with month of year and peak depth) of phytoplankton community structure in the second and third dimensions of the NMDS output (Fig. 6B). A post-hoc ANOSIM test indicated that phytoplankton communities were significantly different between manipulation and reference summers (ANOSIM  $R = 0.15$ ,  $p = 0.0002$ ; Table 3). Additionally, pairwise ANOSIM comparisons of individual summers indicated that phytoplankton communities in the two manipulation summers were not different (ANOSIM  $R = 0.04$ ,  $p = 0.18$ ), but that all other pairwise summer comparisons were significantly different (Table S4).

Although total genera richness did not vary between manipulation and reference summers (adj. Anderson-Darling  $p = 1.0$ ; Table S3), a post-hoc indicator species analysis revealed that seven genera were associated with reference summer conditions, three of which were desmids (*Staurostrum* and *Staurodesmus* in 2018 and *Spondylosium* in 2019). Anderson-Darling tests also indicated that desmids were more abundant in reference summers (adj. Anderson-Darling  $p = 0.03$ ; Table 2). Other taxa associated with reference summer communities included cryptophyte taxa (*Rhodomonas*) and green algae taxa (*Oocystis*, *Monomastix*, *Selenastrum*). No taxa were significantly associated with manipulation summer communities. From 2016–2019, genus richness in phytoplankton samples from the depth of maximum biomass ranged from 7 to 18 genera, and a total of 65 genera were observed from 2016–2019 (Table S5; see Text S4 for further description of phytoplankton community structure).

#### *Thermocline deepening affects intra-annual phytoplankton community composition*

Phytoplankton communities before and after each thermocline deepening event were different in 2016, but not 2017 (Table 3). Additionally, phytoplankton communities before and after the intense naturally-occurring storm in 2019 were substantially different (Table 3; Fig. 6). We found that phytoplankton community composition at the intra-annual scale was sufficiently explained by two NMDS ordination axes for each year. Pairwise ANOSIMs of phytoplankton communities after each deepening event in 2016 indicated that phytoplankton communities after the second and third deepening events in late June and July were different from early-season (May-early June) phytoplankton communities (Text S2; Table S6; Fig. 6). Across all summers and within each summer, phytoplankton communities were substantially different from month to month (Table 3; Text S3), as expected due to seasonal succession. However, months within manipulation summers were more dissimilar (2016  $R = 0.50$  and 2017  $R = 0.47$ ) than months within reference summers (2018  $R = 0.24$  and 2019  $R = 0.33$ ), indicating higher community turnover during reference summers.

Indicator analysis revealed that several taxa were associated with post-storm and post-thermocline-deepening communities. A colonial, filamentous cyanobacterium (*Dolichospermum*), a single-celled, flagellated mixotroph (*Rhodomonas*), and a centric diatom (*Cyclotella*) were associated with post-storm communities in 2019, as well as following thermocline-deepening manipulations in 2016. Congruence between taxa associated with late summer communities and post-storm or post-thermocline-deepening communities (e.g., *Dolichospermum*, *Trachelomonas*, *Cyclotella*; Text S3) emphasizes the difficulty of disentangling the effects of thermocline deepening or intense storms from seasonal succession (Text S5; Table S7, S8).

Both across summers and within each summer, water temperature was the physicochemical variable most strongly associated with phytoplankton community composition in NMDS ordination results (Fig. 6; Fig. 7; see Text S6 for further discussion of physicochemical variables associated with phytoplankton community structure within and among summers).

*Phytoplankton community structure differed between deep and shallow biomass peaks*

Different peak biomass depths were associated with different phytoplankton communities at the inter-annual and intra-annual scale (Fig. 7). Aggregated across 2016–2019, median peak depth was 3.6 m, and the inter-quartile range of peak depths was 3.1 to 4.9 m. Phytoplankton communities that occurred above and below the 3.6 m median were substantially different during 2016–2019 (ANOSIM  $R = 0.28$ ,  $p = 0.0001$ ; Fig. 7A, B), and phytoplankton communities that were associated with deeper peaks were also associated with deepening in the first and second dimensions of the inter-annual NMDS (Fig. 6A). According to indicator species analysis, the top two dominant genera from 2016–2019 (*Cryptomonas* and *Dolichospermum*) were associated with deep and shallow peaks, respectively (Fig. 7). In addition to *Cryptomonas*, one green algae genus (*Elakatothrix*) was associated with peaks deeper than 3.6 m, while a diverse suite of genera other than *Dolichospermum*, including green algae (nanoplankton  $< 5 \mu\text{m}$  GALD, *Selenastrum*), dinoflagellate (*Parvodinium*), diatom (*Nitzschia*), and cryptophyte (*Rhodomonas*) taxa were associated with peaks shallower than 3.6 m. Intra-annually, phytoplankton communities also differed in biomass peaks above and below 3.6 m in 2017 and 2019 (see Text S7 for a description of intra-annual differences in phytoplankton community structure between deep and shallow peaks).



## Discussion

Our four-year whole-ecosystem manipulation revealed that an increased frequency of thermocline deepening events led to deeper phytoplankton biomass peaks at the inter-annual scale, and this effect was mediated by changes in thermal stratification and the depth of maximum SRP concentration in the photic zone. Responses of phytoplankton depth distributions to increased thermocline deepening frequency were consistent across summers, and desmids were consistently associated with reference summer conditions. However, other responses of phytoplankton to experimental thermocline deepening and intense storms, such as drivers of phytoplankton community composition, the magnitude of maximum biomass, and bloom occurrence, varied at both the inter-annual and intra-annual scale. Our results indicate that antecedent conditions (*sensu* Perga *et al.*, 2018) and the seasonal timing of thermocline deepening may mediate the effect of abrupt thermocline deepening on phytoplankton community composition and distribution. Moreover, our finding that different taxa were associated with deep and shallow biomass peaks suggests that phytoplankton depth distributions and community composition are linked at both inter-annual and intra-annual scales. Below, we discuss our results in the context of predicting phytoplankton responses to the increased frequency of thermocline deepening anticipated under global change.

*Question 1: How do phytoplankton depth distribution and community structure change in response to an increased frequency of thermocline deepening events?*

Our findings indicate that an increased frequency of thermocline deepening events affected phytoplankton depth distributions via alteration and increased variability of both physical and chemical gradients in the water column at the inter-annual scale. Thermocline

deepening manipulations weakened stratification and increased variability in thermocline depth, increased the depth of maximum SRP and variability of SRP depth distributions in the photic zone, and deepened phytoplankton biomass peaks, supporting the hypothesis shown in Fig. 1B. These results were complemented by ARIMA models indicating that decreases in stratification and increases in the depth of maximum SRP mediated the deepening of phytoplankton biomass peaks in manipulation years. The finding that thermal stratification drives biomass peak depth aligns with previous research suggesting that storms affect phytoplankton community structure via altered thermal stratification (Stockwell *et al.*, 2020). Alteration of nutrient depth distributions in response to changes in thermocline depth also aligns with expectations from previous work on storms (e.g., Jennings *et al.*, 2012), but to our knowledge, the effects of these alterations in nutrient depth distributions on phytoplankton depth distributions at the whole-ecosystem scale has not been previously reported.

An increased frequency of thermocline deepening events also altered the relative importance of physicochemical drivers of phytoplankton depth distributions at the inter-annual scale. Specifically, more frequent thermocline deepening disrupted the ability of phytoplankton to respond to depth gradients of light. In reference years, light attenuation was strongly associated with both shallower and narrower biomass peaks, supporting previous findings that light attenuation is the most important driver of deep chlorophyll maximum depth across a broad variety of lake types under stratified conditions (Leach *et al.*, 2018). However, in manipulation years, the relative importance of available light as a driver of biomass peak depth and width decreased, while the relative importance of thermal stratification increased. Strong thermal stratification was associated with shallower, narrower peaks in FCR, similar to results from a survey of phytoplankton depth distributions in 51 lakes in Québec, Canada (Lofton *et al.*, 2020).

The increased importance of thermal stratification as a driver of phytoplankton depth distributions in manipulation years indicates that a thermally-stratified water column may be a prerequisite for phytoplankton to optimize their depth distribution in response to gradients in light, supporting previous research regarding the importance of thermal stratification for formation of deep chlorophyll maxima (Cullen, 2015 and references therein). Finally, our finding that maximum biomass was positively associated with warm temperatures at the peak biomass depth in reference years and by thermal stratification in manipulation years supports previous work suggesting that strong thermal stratification, in addition to warm temperatures, is needed for phytoplankton blooms to occur (Carey *et al.*, 2012; Winder & Sommer, 2012).

Our study indicates that the depth distribution and community composition of phytoplankton are related, which may be linked to phytoplankton functional traits (*sensu* Litchman, Klausmeier & Schofield, 2007). Different phytoplankton taxa were associated with deep and shallow biomass peaks, and some of these associations may have been driven by trait-based responses to physicochemical conditions. For example, the filamentous cyanobacterium *Dolichospermum* was associated with shallow peaks across years, likely because that taxon is capable of both buoyancy regulation (Walsby, 1994) and nitrogen fixation (Wood *et al.*, 2010), so it does not sink out of the water column and is not dependent on entrainment of nitrogen from deeper water for growth. By contrast, the cryptophyte *Cryptomonas* was associated with deeper peaks, possibly due to its low light tolerance (deNoyelles Jr *et al.*, 2016) and ability to metabolize organic matter settling on the thermocline via mixotrophy (Mitra *et al.*, 2016).

In other cases, the reasons for association of certain phytoplankton taxa with deep or shallow peaks were less clear. For example, *Rhodomonas* is functionally similar to *Cryptomonas* (Mitra *et al.*, 2016), but was associated with shallow peaks in 2016–2019. It is possible that this

non-intuitive association of *Rhodomonas* with shallow peaks is due to interspecific interactions of phytoplankton within the biomass peak. For example, mixotrophic *Rhodomonas* might metabolize organic carbon released via leaching or decomposition of *Dolichospermum* filaments accumulated in shallow peaks (Kritzberg *et al.*, 2004; Ye *et al.*, 2015). Recent research indicates that mixotrophy likely plays an important role in lake plankton dynamics (Gonçalves Leles *et al.*, 2018; Beisner, Grossart & Gasol, 2019), and may be important for determining community composition at the phytoplankton biomass peak in FCR.

*Question 2: What are the duration and consistency of phytoplankton responses to thermocline deepening at intra-annual and inter-annual scales?*

We found that phytoplankton community responses to experimental thermocline deepening and intense storm events were variable at both intra-annual and inter-annual scales. Our results indicate that the antecedent conditions and seasonal timing of thermocline deepening events may be as important as their frequency in determining phytoplankton community responses, contradicting expectations from previous research that an increased frequency of thermocline deepening events would either cause increased change in phytoplankton community structure (Pannard *et al.*, 2008) or lead to alteration of phytoplankton community structure in favor of mixing-tolerant taxa (Winder & Sommer, 2012; Stockwell *et al.*, 2020).

At the inter-annual scale, phytoplankton community composition responses were inconsistent. Specifically, different environmental drivers were associated with phytoplankton community composition at the depth of peak biomass in each year, maximum biomass was not different between manipulation and reference years, and blooms occurred in both a manipulation and a reference year and their timing was not associated with either thermocline deepening

manipulations or storms. This lack of consistency in thermocline deepening responses across years suggests that the effects of abrupt thermocline deepening on phytoplankton community composition may be mediated by other factors. For example, antecedent conditions including winter ice cover or variability in seasonal catchment conditions affecting in-lake characteristics such as water residence time could influence phytoplankton responses to thermocline deepening (Chase, 2003; Perga *et al.*, 2018; Stockwell *et al.*, 2020; Thayne *et al.*, 2021).

At the intra-annual scale, variability in phytoplankton responses to thermocline deepening in 2016 and 2017 and an intense storm in 2019 indicates that both the timing and frequency of thermocline disturbance within the summer stratified period are important. Our findings indicate that disruption of established thermal stratification in summer (mid-June to mid-September) could cause greater disruption to phytoplankton communities than in late spring (May to early June) when stratification is weaker. Differences in phytoplankton community composition among 2016 mixing periods were primarily due to communities after the third mixing event in late July being substantially different from communities earlier in the summer. In addition, phytoplankton communities were substantially different before and after an intense storm in mid-June 2019. The observed stronger response of phytoplankton community composition to thermocline deepening when thermal stratification is already established supports the framework proposed by Stockwell *et al.* (2020), which predicts the response of phytoplankton to storm events is mediated by their functional traits. While spring and early fall plankton communities in temperate lakes might be well-adapted to mixing and changes in stratification, summer communities are likely not (de Senerpont Domis *et al.*, 2013), and so a mid-summer thermocline deepening event could lead to greater change in community structure. Alternatively, differences in phytoplankton community structure after an intense storm in 2019

may be due to storm effects that we could not simulate via thermocline deepening manipulations, which are discussed further below.

The relative consistency in seasonal succession among all years, demonstrated by the fact that June and September communities did not significantly vary among years, indicates that intermittent, abrupt thermocline deepening does not completely disrupt seasonal succession. The maintenance of seasonal succession dynamics despite our experimental manipulations and intense storms signals the potential importance of multiple mechanisms, including littoral propagule seed banks, overland dispersal, and top-down control by zooplankton grazing, in maintaining phytoplankton seasonal succession (Sommer *et al.*, 2012; Padisák, Vasas & Borics, 2016; Cottingham *et al.*, 2021).

#### *Limitations and future opportunities*

While our study provides insight into the inter-annual and intra-annual responses of phytoplankton communities to intermittent, abrupt thermocline deepening, some limitations must be considered. There are several differences between the effects of our experimental thermocline deepening manipulations and storm effects on lake ecosystems. First, we were unable to simulate storm effects such as increased inflow and nutrient loading or changes in epilimnetic water temperature (e.g., Klug *et al.*, 2012; Stockwell *et al.*, 2020; Doubek *et al.*, 2021). Second, FCR's engineered mixing system is installed at a depth of 5 m, or approximately the middle of the water column, so mixing action is initiated from the middle of the water column rather than the surface, as would occur due to precipitation and wind action from a storm. However, examination of two naturally-occurring intense storm events confirmed that our thermocline deepening manipulations were a reasonable representation of thermocline deepening due to natural storms,

and that both storm-driven mixing and epilimnetic mixing manipulations caused turnover in phytoplankton community composition at the depth of peak biomass at the intra-annual scale.

There are also limitations conferred by our sampling program and experimental design. First, phytoplankton depth distributions were likely influenced by factors in addition to thermocline deepening, including inter-annual differences in water residence time, precipitation, and other factors contributing to natural year-to-year variability in phytoplankton communities, which can be especially pronounced in reservoirs (Hayes *et al.* 2017). Second, our weekly field sampling protocol precluded analysis of patterns of phytoplankton community dynamics at sub-weekly timescales, which may be particularly important for understanding short-term phytoplankton response to storms (Stockwell *et al.*, 2020). In particular, the importance of microscale turbulence (Li *et al.*, 2018; Wu *et al.*, 2019) and convective processes (Bouffard & Wüest, 2019) in movement of phytoplankton cells and formation of surface blooms at short timescales is well-documented, though not quantified in this study. However, the fact that we still observed consistent differences in phytoplankton depth distributions between manipulation and reference years supports the strength of our results and approach. Third, an alternative experimental design, such as alternating summers with and without experimental manipulations, might have permitted more robust comparison of manipulation and reference summers. However, our experimental design was limited by drinking water quality management requirements, and still enabled assessment of increased thermocline deepening frequency over multiple sequential summers.

Our findings indicate that examination of the effects of antecedent conditions and seasonal timing of storms on phytoplankton responses and consideration of inter-specific interactions in biomass peaks could be promising avenues for future research. Explicit

consideration of how precipitation, water residence time, phytoplankton propagule seed banks, pre-storm thermal stratification strength, and other antecedent conditions affect phytoplankton community response to abrupt thermocline deepening could enhance our understanding of future phytoplankton response to increased frequency and intensity of storms (e.g., Cottingham *et al.*, 2021; Thayne *et al.*, 2021). In addition, our finding that some phytoplankton taxa are associated with peak depths that do not align with theoretical expectations based on functional traits signals that further attention may be needed on the role of complementary co-occurrence of phytoplankton taxa (e.g., Posch *et al.*, 2015) in the formation of phytoplankton depth distributions.

## *Conclusions*

Our work demonstrates that an increased frequency of thermocline deepening events has the potential to alter both phytoplankton depth distributions (following Fig. 1B) and community structure via alteration of both physical and chemical environmental conditions at the inter-annual scale. Furthermore, our findings support previous research suggesting that phytoplankton depth distribution and community composition are linked at both the inter-annual and intra-annual scale. Finally, we show that responses of phytoplankton community composition to intermittent, abrupt thermocline deepening are not consistent summer to summer or among thermocline deepening events or storms within a summer, indicating that antecedent conditions likely play an important role in mediating phytoplankton responses to increased frequency and intensity of storms under global change. Overall, our findings emphasize that both the spatial distribution and composition of freshwater phytoplankton communities are sensitive to



thermocline deepening at multiple scales, and so both distribution and composition must be considered when predicting phytoplankton responses to storms under global change.

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## Data availability statement

All data associated with this study are published in the Environmental Data Initiative repository and are cited in the text (Carey *et al.*, 2020a b, 2021a b c d e; Carey, 2021; Lofton *et al.* 2021).

All analysis code is available on GitHub (<https://github.com/melofton/FCR-phytos>) and published via Zenodo (DOI: 10.5281/zenodo.5146268).

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1051

**Table 1:** Schedule of thermocline deepening events and field sampling from 2016–2019. †SCFM  
= standard cubic feet per minute.

Year	Thermocline deepening events				Sampling season	
	Date	Mixer operation	Intensity (SCFM †)	Duration (hrs)	Start date	End date
2016	May 29	continuous	25	6	May 2	Sept. 20
	June 27-28	continuous	15	24		
	July 25-27	continuous	7.5-25	56		
2017	May 30	continuous	15	24	May 15	Sept. 4
	July 10-12	intermittent (8 hrs per day)	15	72		
2018	--	--	--	--	May 7	Sept. 10
2019	--	--	--	--	May 6	Sept. 11

1061 **Table 2:** Results of significant Anderson-Darling (A-D) tests to determine effect of thermocline deepening on physical, chemical, and biological  
1062 variables at the inter-annual scale. All p-values were Holm-Bonferroni corrected for multiple comparisons; non-significant variables are  
1063 presented in Table S3. PZ = photic zone; CV = coefficient of variation; SRP = soluble reactive phosphorus; DOC = dissolved organic carbon; BV  
1064 = biovolume; RA = relative abundance

Driver	Mean $\pm$ 1 S.D. in manipulation years	Mean $\pm$ 1 S.D. in reference years	adj. A-D <i>p</i> -value	Mean $\pm$ 1 S.D. in 2016	Mean $\pm$ 1 S.D. in 2017	Mean $\pm$ 1 S.D. in 2018	Mean $\pm$ 1 S.D. in 2019
Thermocline depth (m)	3.9 $\pm$ 1.1	2.8 $\pm$ 0.4	3.2 $\times$ 10 <sup>-4</sup>	4.1 $\pm$ 1.1	3.7 $\pm$ 1	2.9 $\pm$ 0.4	2.8 $\pm$ 0.4
Schmidt stability (J m <sup>-2</sup> )	25.1 $\pm$ 8.4	36.8 $\pm$ 6.6	2.5 $\times$ 10 <sup>-5</sup>	24.6 $\pm$ 8.7	25.8 $\pm$ 8.3	37.6 $\pm$ 6.3	36 $\pm$ 7.1
Buoyancy frequency (s <sup>-1</sup> )	0.006 $\pm$ 0.0018	0.0093 $\pm$ 0.0021	3.2 $\times$ 10 <sup>-6</sup>	0.0063 $\pm$ 0.0018	0.0056 $\pm$ 0.002	0.0098 $\pm$ 0.002	0.0087 $\pm$ 0.002
Depth of max. SRP in PZ (m)	2.4 $\pm$ 1.9	1.2 $\pm$ 0.7	7.0 $\times$ 10 <sup>-4</sup>	3 $\pm$ 1.9	1.8 $\pm$ 1.8	0.9 $\pm$ 0.8	1.5 $\pm$ 0.4
Mean PZ DOC (mg L <sup>-1</sup> )	2.6 $\pm$ 1	4 $\pm$ 1.4	1.6 $\times$ 10 <sup>-4</sup>	2.1 $\pm$ 0.7	3.2 $\pm$ 1.1	4.6 $\pm$ 1.1	3.5 $\pm$ 1.4
Max. PZ DOC (mg L <sup>-1</sup> )	3.2 $\pm$ 1.4	4.7 $\pm$ 1.6	8.0 $\times$ 10 <sup>-4</sup>	2.7 $\pm$ 1.4	3.7 $\pm$ 1.2	5.5 $\pm$ 1.8	4.2 $\pm$ 1.2
DOC at peak depth (mg L <sup>-1</sup> )	2.5 $\pm$ 1.3	3.7 $\pm$ 1.5	1.6 $\times$ 10 <sup>-3</sup>	2.2 $\pm$ 1.3	2.9 $\pm$ 1.2	4.3 $\pm$ 0.9	3.2 $\pm$ 1.8
DOC at depth sample (mg L <sup>-1</sup> )	2.5 $\pm$ 1.3	3.7 $\pm$ 1.4	5.9 $\times$ 10 <sup>-4</sup>	2.2 $\pm$ 1.3	2.9 $\pm$ 1.3	4.3 $\pm$ 0.9	3.3 $\pm$ 1.7
Biomass peak depth (m)	4.5 $\pm$ 1.3	3.1 $\pm$ 1.3	2.3 $\times$ 10 <sup>-3</sup>	4.7 $\pm$ 1	4.1 $\pm$ 1.6	2.9 $\pm$ 1.1	3.2 $\pm$ 1.5
BV Desmids (μm <sup>3</sup> mL <sup>-1</sup> )	13000 $\pm$ 37000	130000 $\pm$ 180000	0.02	3800 $\pm$ 11000	26000 $\pm$ 55000	220000 $\pm$ 220000	48000 $\pm$ 90000
RA Desmids	0.01 $\pm$ 0.02	0.08 $\pm$ 0.11	0.02	0 $\pm$ 0.01	0.02 $\pm$ 0.02	0.15 $\pm$ 0.12	0.03 $\pm$ 0.06

1065



**Table 3:** Analysis of similarities (ANOSIM) results conducted on non-metric multidimensional scaling (NMDS) ordination output. Phytoplankton community data were assessed for differences based on grouping by year, month, two thermocline deepening indicator variables, and one peak depth indicator variable. Pairwise ANOSIM results for year, months within a year, and month of year across years are presented in Tables S3-S6. R = ANOSIM R, where values approaching 0 indicate similar groups, values approaching 1 indicate dissimilar groups, and values less than 0 indicate greater dissimilarity within a group than among groups. P = ANOSIM p-value. † indicates the inter-annual thermocline deepening regime and was coded as 0 for manipulation years and 1 for reference years; ‡ indicates the intra-annual thermocline deepening regime and was coded as 0 for reference years or pre-thermocline deepening during manipulation years, 1 for after the first thermocline deepening event within a manipulation year, 2 after the second thermocline deepening event, and 3 after the third thermocline deepening event; § assigned as a 0-1 indicator variable, where 0 was shallow peaks < 3.6 m (median peak depth across 2016–2019) and 1 was deep peaks ≥ 3.6 m. ¶ Although an intense storm occurred on 5 May 2016, no ANOSIM test was conducted due to insufficient pre-storm data (n = 1 pre-storm sampling point). Significant results are italicized in bold.

NMDS input data	Year		Month		DEEP1 <sup>†</sup>		DEEP2 <sup>‡</sup>		Peak biomass depth <sup>§</sup>		Pre- and post- intense storms <sup>¶</sup>	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
2016–2019	<b><i>0.20</i></b>	<b><i>1×10<sup>-4</sup></i></b>	<b><i>0.19</i></b>	<b><i>1×10<sup>-4</sup></i></b>	<b><i>0.15</i></b>	<b><i>2×10<sup>-4</sup></i></b>	0.06	0.19	<b><i>0.28</i></b>	<b><i>1×10<sup>-4</sup></i></b>	--	--
2016	--	--	<b><i>0.50</i></b>	<b><i>1×10<sup>-4</sup></i></b>	--	--	<b><i>0.30</i></b>	<b><i>9×10<sup>-3</sup></i></b>	0.03	0.32	--	--
2017	--	--	<b><i>0.47</i></b>	<b><i>1×10<sup>-3</sup></i></b>	--	--	-0.05	0.58	<b><i>0.46</i></b>	<b><i>0.01</i></b>	--	--
2018	--	--	<b><i>0.24</i></b>	<b><i>0.04</i></b>	--	--	--	--	-0.02	0.47	--	--
2019	--	--	<b><i>0.33</i></b>	<b><i>0.01</i></b>	--	--	--	--	<b><i>0.78</i></b>	<b><i>6×10<sup>-4</sup></i></b>	<b><i>0.98</i></b>	<b><i>3×10<sup>-4</sup></i></b>

**Table 4:** Best-fit autoregressive integrated moving average (ARIMA) models predicting depth of peak phytoplankton biomass, peak width, and the magnitude of maximum biomass across all summers (2016–2019), as well as manipulation (abbreviated as “man”; 2016-2017) and reference (“ref”; 2018-2019) summers. ARIMA models that were within 2 corrected Akaike Information Criterion (AICc) units of the best-fit models for each response variable and time period are reported in Table S9. Note that AICc values in this table cannot be compared as models were fit to different datasets. SRP = soluble reactive phosphorus;  $K_d$  = light attenuation coefficient; DIN = dissolved inorganic nitrogen; DOC = dissolved organic carbon; CV = coefficient of variation; AR(1) = an autoregressive term with a time lag of one; MA(1) = a first-order moving average term. Model order is specified as (p,d,q) where p is the order of the autoregressive term, d is the order of the integration term, and q is the order of the moving average term.

Depth distribution metric	Years	ARIMA model structure		Predictor variables						AICc	RMSE
		Order	Terms	Thermal environment		Light environment	Chemical environment				
			MA(1)	Thermocline depth (m)	Schmidt stability ( $J\ m^{-2}$ )	$K_d$	Depth of max. SRP in photic zone (m)	Depth of max. DIN in photic zone (m)	Depth of max. DOC in photic zone (m)		
Peak depth (m)	all	(0,1,1)	$-0.90 \pm 0.06$	$-0.42 \pm 0.13$	$-0.62 \pm 0.16$	--	$0.15 \pm 0.11$	$-0.02 \pm 0.12$	$0.03 \pm 0.12$	124.08	0.74
	man	(0,0,0)	--	$-0.44 \pm 0.18$	$-0.57 \pm 0.18$	--	$0.31 \pm 0.17$	$-0.09 \pm 0.18$	$-0.09 \pm 0.18$	65.85	0.74
	ref	(0,0,0)	--	--	$-0.16 \pm 0.20$	$-0.50 \pm 0.20$	$0.11 \pm 0.19$	--	$0.20 \pm 0.18$	70.49	0.82
				Buoyancy frequency ( $s^{-1}$ )		$K_d$	CV of SRP in photic zone	CV of DIN in photic zone	CV of DOC in photic zone		
Peak width (m)	all	(0,0,0)	--	--	$-0.31 \pm 0.11$	$-0.13 \pm 0.11$	--	--	$-0.11 \pm 0.11$	191.64	0.93
	man	(0,0,0)	--	--	$-0.26 \pm 0.15$	--	$0.18 \pm 0.15$	--	--	102.35	0.91
	ref	(0,0,0)	--	--	$-0.22 \pm 0.17$	$-0.34 \pm 0.18$	--	--	$-0.16 \pm 0.18$	95.16	0.93
			AR(1)	Water temperature at the depth of peak biomass ( $^{\circ}C$ )	Buoyancy frequency ( $s^{-1}$ )	% of surface light at depth of peak biomass	SRP concentration at depth of peak biomass ( $\mu g\ L^{-1}$ )	DIN concentration at depth of peak biomass ( $\mu g\ L^{-1}$ )	DOC concentration at depth of peak biomass ( $mg\ L^{-1}$ )		
Maximum biomass ( $\mu g\ L^{-1}$ )	all	(1,0,0)	$0.55 \pm 0.11$	$0.64 \pm 0.17$	--	$-0.42 \pm 0.14$	$-0.09 \pm 0.11$	--	--	145.30	0.70
	man	(1,0,0)	$0.65 \pm 0.13$	--	$0.23 \pm 0.14$	$-0.22 \pm 0.16$	$0.21 \pm 0.13$	--	--	81.11	0.64
	ref	(0,0,0)	--	$1.1 \pm 0.17$	--	$-0.75 \pm 0.15$	$-0.24 \pm 0.15$	--	--	64.09	0.67

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## Figure captions

**Figure 1:** Conceptual figure illustrating hypothesized relationships between thermocline depth and peak phytoplankton biomass in the water column. (A) In an unmanipulated scenario, phytoplankton peak biomass occurs at a depth which optimizes light, temperature, and nutrients (optimal zone). In response to storm-induced thermocline deepening, B and C present two alternative hypotheses of phytoplankton responses. In (B), thermocline deepening shifts the location of the optimal zone for phytoplankton growth downwards as phytoplankton access nutrients that are entrained across the thermocline. In (C), thermocline deepening homogenizes biomass across depth as some phytoplankton shift deeper to access entrained nutrients while others remain at shallow depths to maximize light availability, resulting in a broader optimal zone for phytoplankton. Gray dashed lines represent the thermocline.

**Figure 2:** A map of Falling Creek Reservoir (FCR), located in Vinton, Virginia, USA. The EM system line shows the extent of the epilimnetic mixing diffuser line within the reservoir that was used to implement the thermocline deepening manipulations.

**Figure 3:** Variables that were determined to be different between manipulated (2016, 2017) and reference (2018, 2019) summers according to Anderson-Darling tests. (A, B) Thermocline depth; (C, D) Schmidt stability; (E, F) Buoyancy frequency; (G, H) Depth of maximum soluble reactive phosphorus (SRP); (I, J) Depth of peak phytoplankton biomass. Manipulation summers are shown in coral/red and reference summers are shown in teal/blue. Note the reversed y-axes in panels (A, B, G, H, I, and J) so that the water surface is at the top of the y axis. Panel G is shown without lines for improved legibility due to discrete sampling depths of SRP.

**Figure 4:** Distributions of the photic zone depth, thermocline, depth of peak biomass, and depth of maximum dissolved organic carbon (DOC), dissolved inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP) in the photic zone during reference summers (A; 2018-2019) and manipulation summers (B; 2016-2017), as well as the magnitude of phytoplankton biomass, DOC, DIN, and SRP at the depth of the maximum for each of these variables in (C) reference and (D) manipulation summers. Variables that were significantly different between manipulation and reference summers according to Anderson-Darling tests are marked with (\*). The color gradients behind panels (A) and (B) are for illustrative purposes to connect the results presented here to hypotheses presented in conceptual Figure 1.

**Figure 5:** Time series of relative abundance of phytoplankton divisions and total biovolume for (A, B) manipulation summers and (C, D) reference summers. Thermocline deepening manipulations in 2016 and 2017 are denoted with dashed black vertical lines, and naturally-occurring intense storms in 2016 and 2019 are denoted with solid black vertical lines.

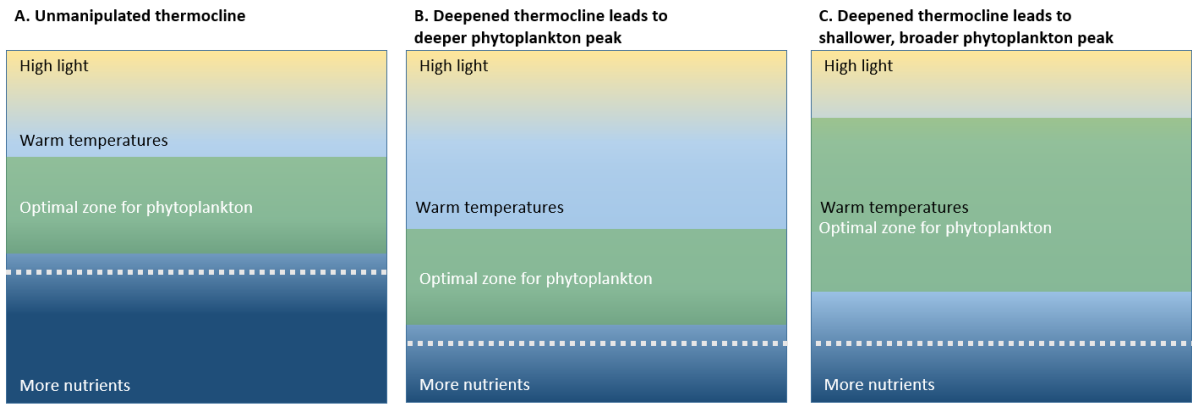
**Figure 6:** (A) First and second and (B) second and third axes of non-parametric multidimensional scaling (NMDS) ordination for phytoplankton communities across all summers (2016–2019), as well as NMDS ordination results for individual summers: (C) 2016, (D) 2017, (E) 2018, and (F) 2019. Gray and black arrows in (A) and (B) are fitted vectors of physicochemical variables plotted at a  $p < 0.01$  significance level, with variables that differed in manipulation vs. reference summers in black. DEEP1 indicates the inter-annual manipulation regime, coded as 0 for reference summers and 1 for manipulation summers. DEEP2 indicates the intra-annual mixing regime, coded as 0 for reference years or pre-thermocline deepening during manipulation summers, 1 for after the first thermocline deepening event within a manipulation summer, 2 after the second thermocline deepening event, and 3 after the third thermocline

1132 deepening event. Other vector label abbreviations: Biom = magnitude of biomass in the  
1133 phytoplankton biomass peak; Mo = month; N2 = buoyancy frequency; PD = phytoplankton  
1134 biomass peak depth; T = water temperature at the depth of the phytoplankton grab sample (peak  
1135 biomass depth); Yr = year. The month color legend in (E) applies to all sub-plots.

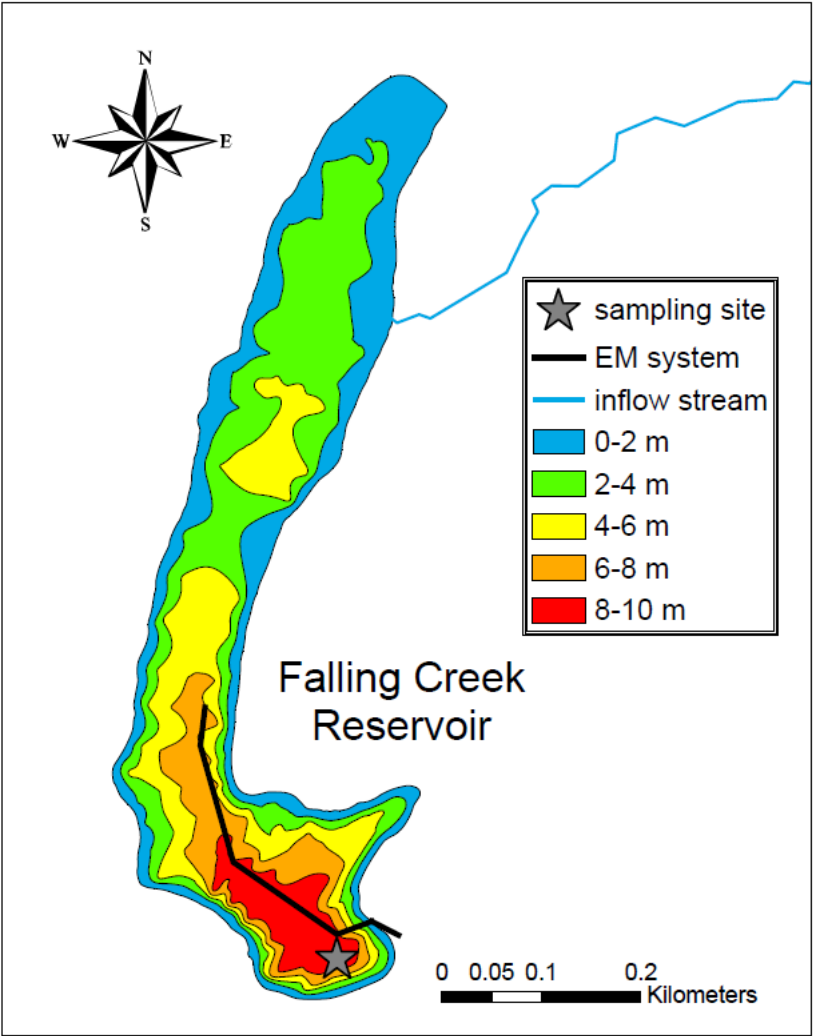
1136 **Figure 7:** Non-parametric multidimensional scaling (NMDS) ordinations overlain with the  
1137 smooth surface plane for biomass peak depth for all summers (A,B), 2016 (C), 2017 (D), 2018  
1138 (E), and 2019 (F). Plane contours are shown for median peak depth from 2016 to 2019 (3.6 m,  
1139 plotted in gray) as well as the inter-quartile range of peak depth (first quartile = 3.1 m, plotted in  
1140 light gray; third quartile = 4.9 m, plotted in dark gray). Genera shown in text were associated  
1141 with either shallow (<3.6 m) or deep (>3.6 m) peak depth in indicator species analysis. Genus  
1142 abbreviations: Aph = *Aphanocapsa*; Ast = *Asterionella*; Chl = small green algae nanoplankton (<  
1143 5  $\mu$ m greatest axial linear dimension); Cry = *Cryptomonas*; Cyc = *Cyclotella*; Dic =  
1144 *Dictyosphaerium*; Dol = *Dolichospermum*; Ela = *Elaktothrix*; Eug = *Euglena*; Nit = *Nitzschia*; Par  
1145 = *Parvodinium*; Rho = *Rhodomonas*; Sel = *Selenastrum*; Spo = *Spondylosium*. Black arrows in  
1146 (C-F) show fitted vectors of physicochemical variables (significance level for vector plotting is  $p$   
1147 < 0.01). Arrows that overlap (C) are labeled with a single label for legibility. DEEP2 indicates  
1148 the intra-annual mixing regime, coded as 0 for reference years or pre-thermocline deepening  
1149 during manipulation summers, 1 for after the first thermocline deepening event within a  
1150 manipulation summer, 2 after the second thermocline deepening event, and 3 after the third  
1151 thermocline deepening event. Other vector label abbreviations: Biom = magnitude of biomass in  
1152 the phytoplankton biomass peak; DOC = concentration of DOC at the depth of peak biomass;  
1153 Mo = month; N2 = buoyancy frequency; PD = depth of peak biomass; SRP = concentration of  
1154 SRP at the depth of peak biomass; T = water temperature at the depth of peak biomass.

**Figures**

**Figure 1**

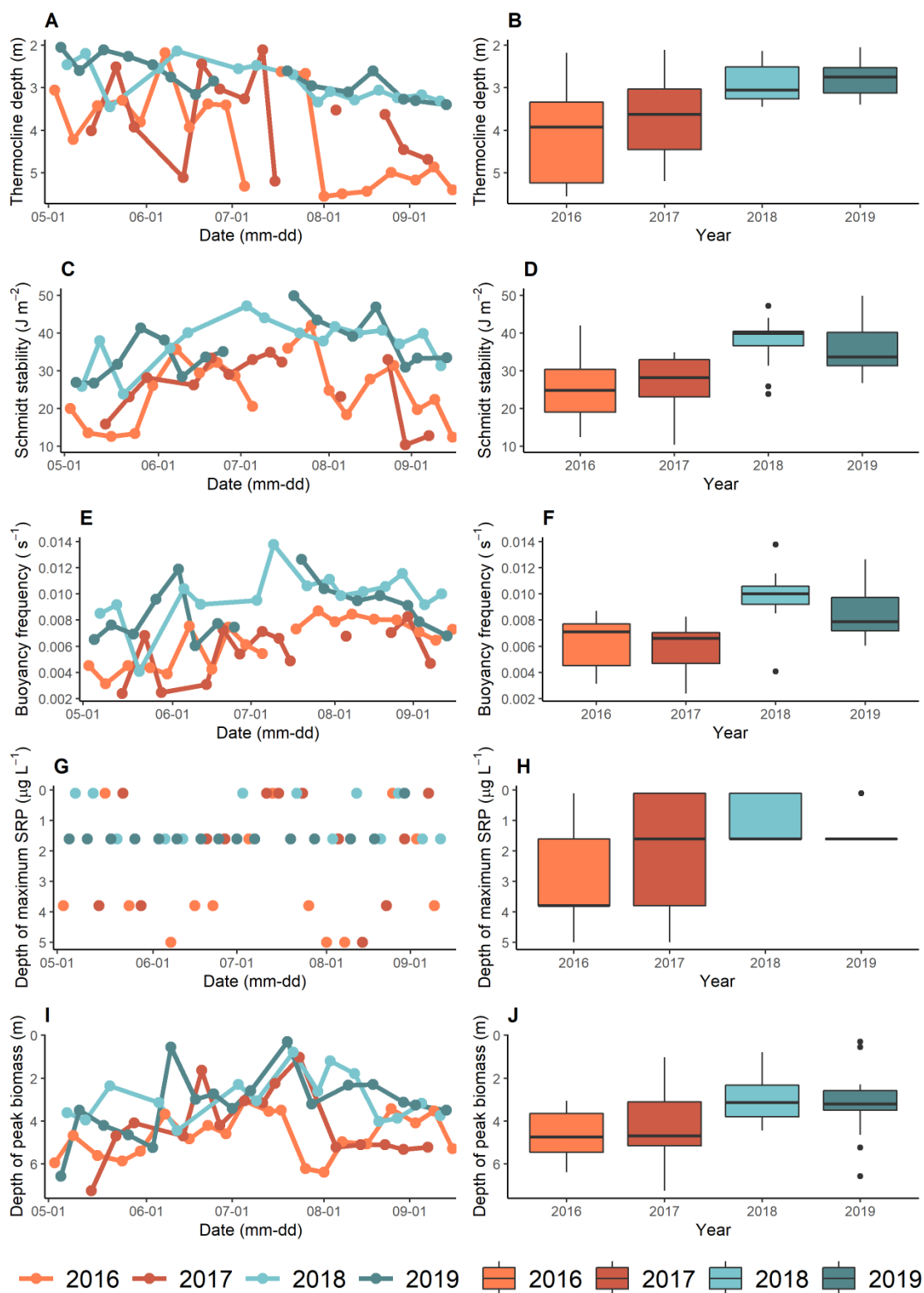


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1184 Figure 3

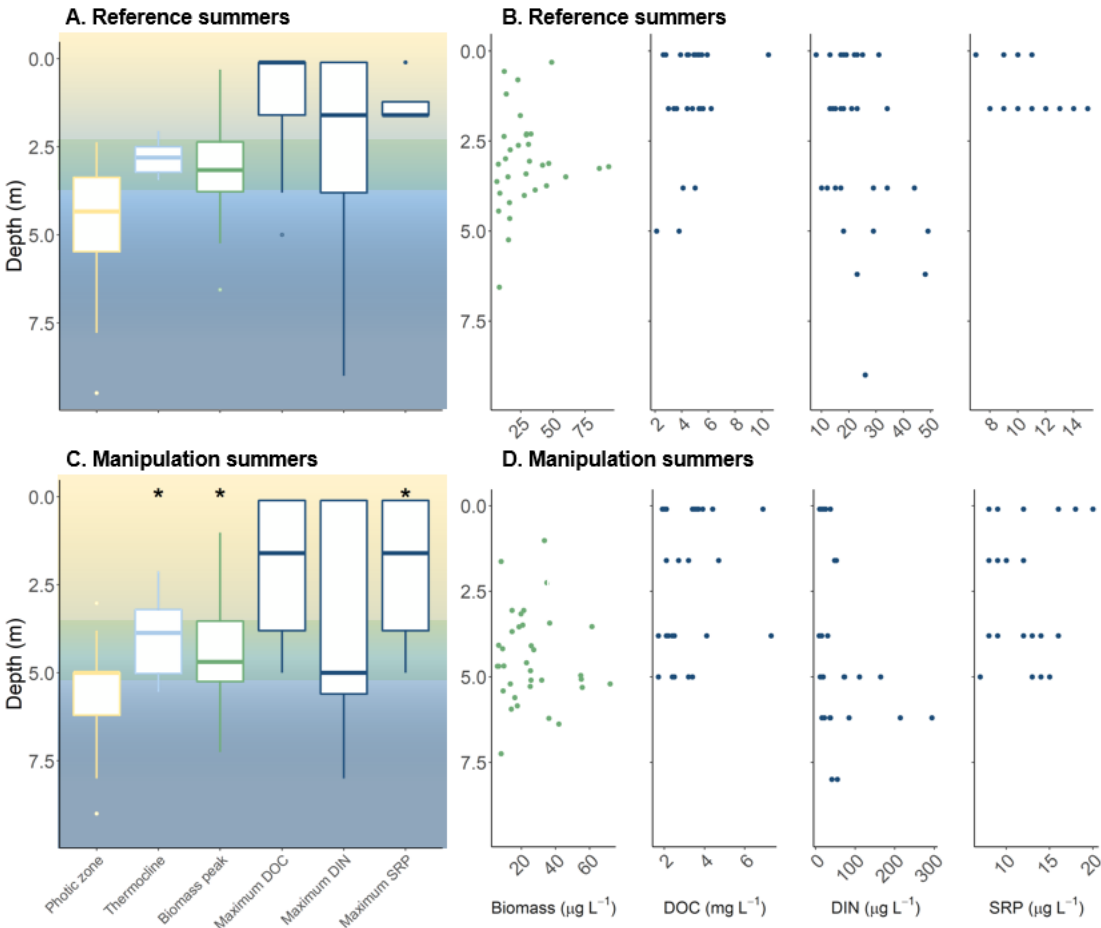


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1187     Figure 4



1197 Figure 5

