ABSTRACT

MANGOT, ALEXANDRE. Effects of Vertical Mixing on Phytoplankton Communities in a Shallow, Turbid Water Column Experiment. (Under the direction of Dr. Tarek N. Aziz).

Cyanobacteria harmful algal blooms (CyanoHABs) create issues in freshwater systems as they can impair water quality and release toxins (cyanotoxins) that can endanger human and animal health. This is particularly concerning in shallow, turbid lakes and reservoirs that are often used for drinking water and recreation, wherein CyanoHABs are increasing in frequency and severity during hot summer months. Artificial mixing systems have been proposed as solution to improve water quality and limit the growth of cyanobacteria. However, limited guidance on implementation of such systems and little is known on the effects of artificial mixing on phytoplankton communities and toxin-producing cyanobacteria taxa development. Water column reactors (WCR) were built and used in a controlled laboratory setting to study the effects of vertical mixing in a shallow, turbid water column on phytoplankton community dynamics. WCRs are 2 m tall cylindrical mesocosm that featured a light and a mixing system to imitate environmental conditions of shallow, turbid lakes and reservoirs. Natural plankton assemblages dominated by cyanobacteria were sampled in the summer from a shallow, turbid reservoir and were used to study the effects of mixing intensity and duration on phytoplankton growth, vertical distribution, and composition. Two experiments were performed with multiple treatments of mixing (no mixing, intermittent mixing, and continuous mixing). A semi-automatic particle identifier was used to perform community characterization. Changes in overall community structure in both experiments suggest that continuous mixing had an effect compared to no mixing, and that the intermittent mixing was largely indistinguishable from the unmixed system. Mixing reduced vertical variations of algal concentrations and little to no cyanotoxins were detected across treatments. Continuous mixing changed the community structure by reducing cyanobacteria relative abundance and limited

vertical migration of colonial cyanobacteria. Surface accumulations of buoyant cyanobacteria were observed in the no mixing and intermittent reactors and these accumulations reduced light availability in the column.

© Copyright 2018 by Alexandre Mangot

All Rights Reserved

Effects of Vertical Mixing on Phytoplankton Communities in a Shallow, Turbid Water Column Experiment

by
Alexandre Mangot

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Civil Engineering

Raleigh, North Carolina

2018

APPROVED BY:

_____	_____
Dr. Tarek N. Aziz Committee Chair	Dr. Daniel R. Obenour

_____
Dr. Astrid Schnetzer

DEDICATION

To my mother, Anne-Marie Mangot, and father, Noël E. Mangot, who have sacrificed a lot for me. Thank you.

BIOGRAPHY

Alexandre Mangot was born on May 10, 1992, in Paris, France. He attended SKEMA Bachelor for two years and transferred to North Carolina State University where he finished his BS in Civil and Environmental Engineering in 2015. Upon graduation, Alexandre started a Master of Science degree in Civil Engineering in 2016. Alexandre Mangot is a pianist and is passionate about music.

ACKNOWLEDGMENTS

I would like to thank my graduate advisor, Dr. Tarek N. Aziz, along with the rest of my committee, Dr. Astrid Schnetzer and Dr. Daniel R. Obenour. I would also like to thank Stefanie A. Hopkins for her constant support and advice, Jeremy Smithheart, Monica Camacho, Marco Valera, Daniel Wiltsie, Gabrielle Corradino, Darwin Rhoads, Mark Vander Borgh (DEQ), North Carolina State Center for Applied Aquatic Ecology (CAAE), Biological and Agricultural Engineering laboratory (BAE), University of South Carolina Baruch Marine Field Laboratory, North Carolina State Precision Instrument Machine Shop.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1. Introduction

The formation of harmful algal blooms (HABs) in freshwater systems is an increasingly common occurrence (Paerl and Huisman, 2008; Hudnell, 2008; Paerl and Paul, 2012). HABs are an overgrowth of algae leading to biomass accumulation in water bodies (Hallegraeff, 1993). They can deteriorate water quality, impact recreational use, and lead to increased water treatment costs (Codd, 2000; Hudnell, 2008). Of particular concern are HABs of toxin-producing cyanobacteria (CyanoHABs) that can endanger human and animal health (Hallegraeff, 1993; Paerl et al., 2001; Carmichael, 2001; Anderson, 2009; Visser et al., 2016). Cyanobacteria may also cause taste and odor issues in public water systems, creating challenges for water treatment (Chow et al, 1999; Paerl and Huisman, 2009; Graham et al., 2010; Visser et al., 2016).

Temperature, light, and nutrients are known drivers for the growth of phytoplankton (Eppley, 1972; Geider et al., 1998. Chapra, 2008) and have been linked to increases in magnitude and frequency of bloom events. Over the past two decades, CyanoHABs have been associated with the rise of global temperatures and eutrophication from watershed development (Wagner and Rita, 2009; Paerl and Huisman, 2009; Paerl and Otten, 2013; Visser et al., 2016). Moreover, CyanoHABs tend to occur during periods of low turbulent mixing, causing prolonged stratification in the water column (Butterwick et al., 2005; Joehnk et al., 2008; Huber et al., 2012). Some cyanobacterial species have evolved a competitive advantage; namely the ability to regulate their buoyancy using gas vesicles and migrate vertically to obtain optimal nutrient, light, and temperature conditions (Humphries and Lyne, 1988; Walsby, 1994; Walsby et al., 1997; Visser et al., 2005). These buoyant cyanobacteria can dominate the epilimnion, blocking access to light for other less- or non-motile phytoplankton (Walsby et al., 1997). Turbulent mixing affects vertical

distribution and may reduce the ability of buoyant cyanobacteria to dominate in the photic zone (Huffaker, 1958; Levin, 1974; Hassell et al., 1994; Kerr et al., 2002, Hulot and Huisman, 2004; Huisman et al., 2004). Redistribution of cyanobacteria, in turn, may create more favorable growth conditions for other phytoplankton in the water column (Ibelings, 1994; Walsby, 1994; Huisman et al., 2004). Chlorophytes and diatoms tend to dominate under well-mixed conditions whereas dinoflagellates and cyanobacteria dominate at low mixing intensities (Ibelings, 1994; Huisman et al., 1999; Huisman et al., 2004; Jäger et al., 2008).

Nutrient management, use of algaecides, and food web manipulation by introducing more zooplankton and filter-feeding shellfish are varying strategies that have been employed to reduce bloom formation (Ke et al., 2009; Jia et al., 2010; Barrington et al., 2013; Upadhyay et al., 2013). Engineering solutions have included the use of in-lake mixing technologies to increase the oxygen content of the water and control or reduce HAB frequency and intensity for decades (Oberholster et al., 2006; Hudnell et al., 2010; Upadhyah, 2013; Visser et al., 2016). Artificial mixing systems can limit temperature stratification and reduce the ability of buoyant cyanobacteria to stay in the upper layers of the water column (Huisman et al., 2004; Visser et al., 2016). There are a number of freshwater systems wherein artificial mixing has appeared to suppress the development of CyanoHABs (Wagner, 2015; Visser et al., 2016). However, the effects of lake mixing on HABs are not consistent (Oberholster et al., 2006; Visser et al., 2016). Past successful studies on the effects of lake mixing on phytoplankton communities have mainly focused on deep lake systems (Heo and Kim, 2004; Huisman et al., 2004). These select studies found that artificial mixing shifted the dominance from cyanobacteria to chlorophytes and diatoms but led to an increase in overall algal biomass. Field studies have suggested that artificial mixing will not suppress CyanoHABs in shallow reservoirs (<5 m depth) due to natural mixing from diurnal convection processes during

the summer (Oberholster et al., 2006; Visser et al., 2016). However, it is relatively common for artificial mixing systems to be deployed in shallow reservoirs. Moreover, several such systems have reported the successful suppression of CyanoHABs. There is limited research available on whether turbulence affects contribution of these systems on the development of potential toxin-producing taxa and the onset of toxin production (Hawkins and Griffiths, 1993).

Shallow and turbid freshwater bodies are often used as water supply reservoirs within the Piedmont region of North Carolina, USA, where the formation of CyanoHABs has been of growing concern (Paerl and Huisman, 2009; Wiltsie et al., 2018). However, there is little guidance for the implementation of artificial mixing systems in these shallow turbid reservoirs, where Secchi depths typically range from 0.04 m to 2.0 m and light extinction coefficients range from 0.2 m^{-1} to 5 m^{-1} year-round due to high non-algal turbidity (Weiss and Kuenzler, 1975; Smith, 1990; Mallin et al., 1994; Scheffer et al., 1992; Scheffer et al., 1997; Burkholder et al., 1998; Van den Berg et al., 1999; Oberholster et al., 2006; DEQ/DWR, 2014). Some experimental studies have looked at the effects of mixing on phytoplankton community changes in freshwater systems. Reynolds et al. (1984) found that mixing suppressed potentially toxic cyanobacteria and Jager et al. (2008) found that mixing led to less diversity in the community with the dominance by pennate diatoms, however, both were performed in a deep systems wherein there were substantial depths without light. Zhou et al. (2015) found that short-term mixing led to cyanobacteria dominance but over time this mixing shifted the community towards diatoms and chlorophytes; however, the water column depth in the study was 55 cm, therefore, having light penetrating through most of their column. Field mesocosm experiments have looked at the effects of turbulence induced by wind on the growth of cyanobacterial genus *Microcystis*, microcystin production in a light environment representative of deep water systems (Zhou et al., 2016a; Zhou et al., 2016b). The effects of

vertical mixing on phytoplankton community structure in shallow, turbid reservoirs are not consistent (Visser et al., 2016). As noted by Visser et al. (2016), background turbidity needs to be taken into account, as the importance of light limitation in relation to the depth of mixing is critical for the outcome of artificial mixing. A better understanding of the interplay of vertical mixing and algal bloom dynamics is necessary to develop and implement stable and cost-efficient solutions in shallow water supply reservoirs.

Laboratory mesocosm water column reactors (WCRs) can provide a controlled environment to study phytoplankton dynamics in response to changes in mixing, light, nutrients, and temperature. They allow for easy sampling access, isolation from various intrinsic uncontrollable factors (e.g. meteorology and natural predation), and can provide key insights into the role that mixing regimes play in algal growth and distribution. To date no known studies have used controlled laboratory experiments accounting for the light environment of shallow, turbid reservoirs under varying mixing intensity and duration with an aim to characterize the changes on freshwater phytoplankton communities including cyanobacterial species. This study details the design and operation of novel Water Column Reactors (WCRs) to study the effects of vertical mixing on phytoplankton growth, community structure and vertical distribution.

CHAPTER 2

2. Methods

2.1. Water Column Reactors

In total, three WCRs were constructed in the North Carolina State University Hydraulics Lab for the purpose of this study. The WCRs were 2 m tall, acrylic (6.35 mm thickness) columns with an inside diameter of 30 cm (Figure 1, S1). The internal volume of the WCRs was 134 L. 13 sampling ports of 9.53 mm (3/8 inch) diameter were evenly distributed along a vertical line at a 15 cm increment (Figure 1). Ball valves, equipped with compression fitting and a plastic grommet were located at each sampling port to aid sampling and avoid reactor leaks. Rigid 6.35mm (1/4 in. ID) polycarbonate plastic tubes attached to flexible polyurethane tubes by push-to-connect fittings and clamps were inserted in the ports for sampling (Figure S1, S9, S10). Individual sampling tubes were maintained for each port to avoid cross-contamination with depth. An enzyme detergent (Tergazyme 1%) was used to clean the sampling tubes after each sampling and the inside of the all reactors prior to an experiment. During a sampling event, a small amount of water from the WCR was allowed to flow through the sample tubes to rinse the ports. Mixing in the mixed WCRs was driven by a 24V DC motor (Uxcell 500 RPM) attached to a 9.53mm (3/8 inch) stainless steel vertical shaft. Three impellers, evenly spaced at 20 cm (8 in), were attached to the shaft centered in the WCRs (Figure S1, S4, S5). The motor was mounted directly at the top of the reactor. The top most impeller was located 15 cm (6 in) below the water surface. 3D printed, four-blade, axial impellers were built of white polylactic acid thermoplastic (PLA) to accomplish mixing. The

impellers were 7.5 cm in diameter, 1.5 cm in height, and blades are angled at a 45° angle (Figure SX).

A series of rhodamine dye tracer tests were conducted to determine the appropriate impeller speed necessary to emulate typical mixed conditions in a shallow reservoir. Details pertaining the dye testing calculations and experimental approach can be found in Table S1. Through experimental iteration we converged onto a diffusion coefficient in the range of 8 to 12 cm^2 s^{-1} achieved by operating the mixing system at 100 RPM. This level of mixing is close to turbulent diffusion coefficients found in shallow turbid lakes under mixed conditions (Jassby and Powell, 1975; Sheng et al., 1979; Malacic, 1991; Herb and Stefan, 2005). Temperature was maintained constant at 20.7 °C +/- 1.2 °C and was monitored using a temperature probe (DS18B20).

A light system including 12 fluorescent bulbs (Philips F17T8/TL841) was placed 15 cm above the water surface of each reactor (Figure 1). In total the WCR light system emitted an average light intensity of 75 W m^{-2} PAR (approximately 360 µE m^{-2} s^{-1}) on the surface and was monitored throughout the duration of this study. Irradiance spectra were measured using a radiometer (OceanOptics © Spectrometer USB4000-UV-VIS-ES) and were integrated in the Photosynthetic Active Radiation (PAR) range. The radiometer was attached vertically to a white PVC tube connected to hanging wires. This light sampling apparatus was designed to allow us to gently lower the radiometer in the WCRs close to the wall, away from the impellers, and was cleaned between each use. Timers were used to provide light at a 10:14-h light:dark cycle starting and stopping at 8am and 6pm, respectively. Lastly, as shown in Figure 1, the WCRs were wrapped in black plastic sheeting in order to block ambient light from the reactor side-walls (Mellard et al., 2012).

Figure 1. Sketch of the WCR (left), picture of the three WCRs during experiment B (center), and picture of the Mix reactor at the end of experiment A (right).

2.2. Field site and source water

Two experiments (Experiment A and B) were performed using natural phytoplankton assemblages collected from University Lake, Chapel Hill, North Carolina, USA, in the morning of June 26^{th,} 2017 (experiment A), and September 7th, 2017 (experiment B). University Lake is a 0.86 km^2 shallow, turbid reservoir used for drinking water and recreation. The mean depth is 3 m and typical Secchi depths of <1 m are found all year. Based on field sampling undertaken from 2015 to 2017, water temperature varies between 5 °C and 35 °C and cyanobacterial taxa tend to dominate University Lake with bloom forming during the summer.

Sub-surface water containing natural algal communities were collected in the morning at a depth of 0.5 m using 20 L carboys. In addition, algae from surface phytoplankton tows (20 µm mesh) were added to the whole lake water to increase initial population density within the reactors. Lake samples were also taken at the surface and at 0.5 m depth to characterize the natural algal

assemblage and conduct organic and inorganic nutrient analysis. An initial screening of the lake water and tow samples showed high numbers of copepods for experiment A, and therefore the inoculum was passed through a 100µm Nitex mesh to remove these grazers. Ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4) from field samples were measured at the North Carolina State University Biological and Agricultural Engineering Environmental Engineering Laboratory (BAE, NCSU). Algal assemblage analysis and chlorophyll-*a* (CHLA) measurements from the in-lake samples were performed at the North Carolina State University Center for Applied Aquatic Ecology (CAAE, NCSU).

2.3. Experimental design

Three treatments were tested including no mixing (NoMix), intermittent mixing (IntMix), and continuous mixing (Mix). The NoMix treatment represents the extreme case of a poorly mixed column, while intermittent mixing imitates the typical mixing schedule of shallow lakes, which become fully mixed at night due the cooling of the epilimnion. The full mixing treatment is representative of a water column being continuously mixed, which could be achieved using artificial mixing systems or high wind conditions in these shallow systems. The mixer of the IntMix reactor was set for the same mixing intensity as the Mix system (100 RPM) but was placed on a timer with a 12:12-h on:off cycle, starting and stopping at 8pm and 8am, respectively. The mixer timing of the IntMix WCR was based on the timing of typical diel destratification observed in University Lake.

Experiment A and B used two and three WCRs, respectively. Experiment A lasted 44 days and consisted of two WCRs (NoMix and a Mix), each filled with 10 L of lake water that was

diluted with deionized (DI) water to a final volume of 127 L. A third WCR was built after experiment A to add the IntMix treatment. Experiment B lasted 33 days and consisted of three treatments, Mix, IntMix, and NoMix, with 40 L of lake water in each reactor that was diluted to a final volume of 127 L.

Light extinction coefficients commonly found in regional shallow reservoirs vary between 0.2 m^{-1} and 5 m^{-1} (Smith, 1987; Scheffer et al., 1992; Scheffer et al., 1997; Van den Berg et al., 1999; Oberholster et al., 2006). In order to create a strong light gradient in the WCRs, typical of shallow reservoirs, a 10 L slurry of bentonite clay was prepared and added to all reactors of both experiments at a concentration of 300 mg L^{-1}. Preliminary testings in smaller column reactors as well as in the full-scale WCRs was performed to establish the correct dosing for artificial turbidity. The clay was gently added to DI water and the solution was vigorously mixed for 15 minutes to avoid clumping. The water was acclimated to room temperature for 24 hours prior to initiation of the experiments.

Sampling of the source water (University Lake) and the start of the experiments occurred on the same day (day 0). The combined lake water and tow samples were mixed together and were gently transferred to the WCRs in equal volume using a funnel and immersed silicone tubing. All treatments were amended with f/20 nutrient media without silica at the beginning of each experiment (Guillard & Ryther, 1962; Guillard 1975). Finally, we filled the remainder of the reactors with DI water to a final height of 1.9 m, representing a total volume of 127 L, which was maintained throughout the course of both experiments by periodic addition of DI water (without nutrients). The columns were thoroughly mixed manually after filling and the algal community was allowed to acclimate to the specific mixing and light conditions overnight and the first samples (day 1) were collected the next day.

Solid phase adsorption toxin tracking (SPATT) bags were placed in duplicate on the inside wall of each reactor at 50 cm, 95 cm, 190 cm (bottom; Figure S6, S7, S8) to screen for accumulated dissolved cyanotoxins (Kudela, 2011). The SPATT bags were filled with HP-20 resin (Sigma–Aldrich, St. Louis, MO, USA) and activated in 100% methanol for 30 min, then rinsed with three equivalent volumes of Milli-Q water (Millipore Corporation ®) and sonicated for 45 s at 50% amplitude with a sonic dismembrator (Fisher Scientific, Hampton, NH, USA, Model FB120) before deployment.

2.4. Reactor Sampling

The WCRs were sampled at 20 cm, 50 cm, 95 cm, and 140 cm depths during both experiments. 5 mL samples were collected for chlorophyll *in vivo* fluorescence analyses at 10 am every 1 to 5 days and measured immediately using a fluorometer (Turner Designs Trilogy; excitation: 441/82 nm, emission: 660-710 nm; module 7200-043; Table S2). Over time, changes in i*n vivo* fluorescence provided insights into the progress of the experiment and algal growth within the reactor. Experimental phases were described as the lag phase where little to no growth occured (LAG), followed by active growth during the exponential phase (EXP), then a stationary phase (STA) when net growth seized, and finally a decline phase (DEC) during which algal biomass decreased.

Grab samples (200 mL) were collected at 10 am every 3 to 11 days, throughout all experimental phases and were analyzed for CHLA, and dissolved inorganic nutrients (NH_4^+, PO_4, combined NO_2^- + NO_3^-; Baruch Marine Field Laboratory, USC; Table S2), as well as phytoplankton community composition. 15 mL of the 200 mL grab samples were preserved using

acid lugol solution (5%) for community analysis with the FlowCAM. CHLA extraction followed the EPA 445.0 method using 45 mm GF/F Whatmann filters, sonication, and 6 mL of 100% Acetone. Filtrate from CHLA extraction was kept at -20°C for future nutrients analysis. Surface samples were collected in experiment B at day 13 and 21 and were used to perform CHLA extraction and characterize the phytoplankton community composition. Due to the more frequent sampling of *in vivo* fluorescence, a linear regression was performed between *in vivo* fluorescence and extracted CHLA in order to extrapolate concentrations of CHLA based on *in vivo* fluorescence (Figure S3).

Light profiles were taken at 15 cm increments from the surface down to 170 cm depth within each reactor (Table S2). An additional sampling point was taken at 5 cm depth to increase the spatial resolution in the upper layer of the column. The water column was divided into three vertical segments to capture the non-homogeneous vertical distribution of algae - from 0 to 5 cm depth, 5 cm to 35 cm depth, and from 35cm to 170 cm. A regression fit to Beer-Lambert's law was used to estimate the light extinction coefficients (K_e) for the three segments (Eq. S3). The averaged light availability in the column was calculated by integrating each fitted light profiles over the whole water column depth and dividing by the total depth (190 cm).

After their retrieval, the SPATT bags were stored at -20ºC until extraction. Extraction followed previously established protocols (Kudela, 2011; Wiltsie et al., 2018) and extracts were tested using Enzyme-Linked Immunosorbent Assays (ELISA; Abraxis Inc.,Warminster, PA, USA) for microcystin-ADDA (Product #520011; sensitive to MCY-LR, -YR, -LF, -RR, LW, and nodularin; lower detection threshold = 0.10 µg L^{-1}) and cylindrospermopsin (Product #520040; sensitive to BMAA and other amino acids; limit of quantitation = 4 µg L^{-1}).

2.5. Community Characterization

Plankton community structure and abundance was determined using a digital flow cytometer (FlowCAM, Fluid Imaging Technologies) and VisualSpreadshseet®. Samples were passed through a 100-µm glass flow cell at an average rate of 0.14 mL min^{-1} using a 10x objective. Due to clogging of the flow cell associated with more suspended materials found in experiment B samples, pre-filtration was applied using a 60-µm mesh. Additionally, residual materials were diluted using MQ water (Millipore Corporation ®) and analyzed with light microscopy to verify that pre-filtration would not remove large plankton organisms and therefore bias community characterization. The FlowCAM was set on "AutoImage mode", a mode in which images of the particles in the field of view are captured at a user-defined time interval. The sample volume analyzed was collected, measured gravimetrically, and was corrected to account for particles passing outside the field of view of the camera. The FlowCAM allowed us to capture an image of each particle (unit) passing the instrument's laser and to record its size, diameter, fluorescence properties and other characteristics (Buskey et al, 2006; Álvarez, et al, 2011; Álvarez, et al, 2013). Colonies, chains, filaments, and individual cells of solitary forms were counted and analyzed as units for abundance measurements (henceforth referred to as unit density in Unit mL^{-1}). Algae with distinct morphological features (e.g., unit size, shape, and skeletal structures) could be readily identified based on previously established classification libraries using University Lake assemblage data (CAAE, NCSU). We validated the FlowCAM-based classification approach by community analysis based on light microscopy at the beginning of each experiment and for surface samples throughout experiment B. For each sample a total of 500 units were analyzed and sorted into taxonomic groups based on the lowest biological rank clearly defined (order, family, and

genus). The automated classifications were inspected and manually corrected whenever units were not assigned to the appropriate taxonomic groups (Bellinger and Sigee, 2015; Wehr et al., 2015). Overall, sixteen major planktonic groups were identified and they included members of the Oscillatoriaceae, Nostocales, Chroococales, genera *Aphanocapsa, Pediastrum, Ankistrodesmus*, *Staurastrum, Scenedesmus, Coelastrum, Pandorina, Tetraedron, Closteriopsis, Synedra* as well as pennate diatoms, cryptomonads, and ciliates (Table S3).

2.6. Statistical Analysis

Statistical analyses were performed on the defined taxonomic groups (order, family, and genus) using the PRIMER v7 statistical software package (Clarke and Gorley, 2015; PRIMER-E Ltd, Plymouth, UK). Community data (normalized unit densities) were square-root transformed and compared based on Bray–Curtis similarity indices. Two-way and three-way ANOSIM (analysis of similarity) tests were computed to examine the effects of treatment (NoMix, IntMix, Mix), time (lag, exponential, and stationary growth phases), and depth (surface, sub-surface). This resulted in the calculation of r values which represent a measure of distinction between groups. For instance, r values of 0 indicated that groups were similar, while an r value of 1, or close to 1, implied that groups were dissimilar (Clarke and Warwick, 2001). Similarity patterns over treatment and time were further illustrated using non-metric multidimensional scaling (nMDS) plots where more closely clustered data points represented higher similarity in community structure among varying assemblages. Stress values were calculated for MDS plots to reflect the level of distortion that results from representing similarity rankings between multiple samples in a two-dimensional space. It has been established that stress values of less than 0.2 are accurate

representations of similarity rankings (Clarke and Warwick, 2001). Additionally, one-way SIMPER (similarity percentages) tests were conducted to identify main phytoplankton taxa contributors to similarities and dissimilarities between treatments for which mixing was altered.

CHAPTER 3

3. Results

3.1. Nutrients

Dissolved inorganic nutrients decreased over time in all reactors of both experiments with no clear distinction across treatments (Figure 2; a, b, c, d). Initial NO_2^- + NO_3^- concentrations were lower on average in experiment A (793 µg L^{-1}) than in experiment B (1132 µg L^{-1}) at day 1 (Figure 2; a, b). Initial PO_4 concentrations were on average lower in experiment A (81 µg L^{-1}) than in experiment B (256 µg L^{-1}) at day 1 (Figure 2; c, d). A five times half-saturation constant of nitrogen (100 µg N L^{-1}) and phosphorus (25 µg P L^{-1}) were used as criteria to approximate nutrient limitation (when below values) for phytoplankton growth (Chapra, 2008). Nutrients were drawn down after day 44 and 21 in experiment A and B, respectively (Figure 2).

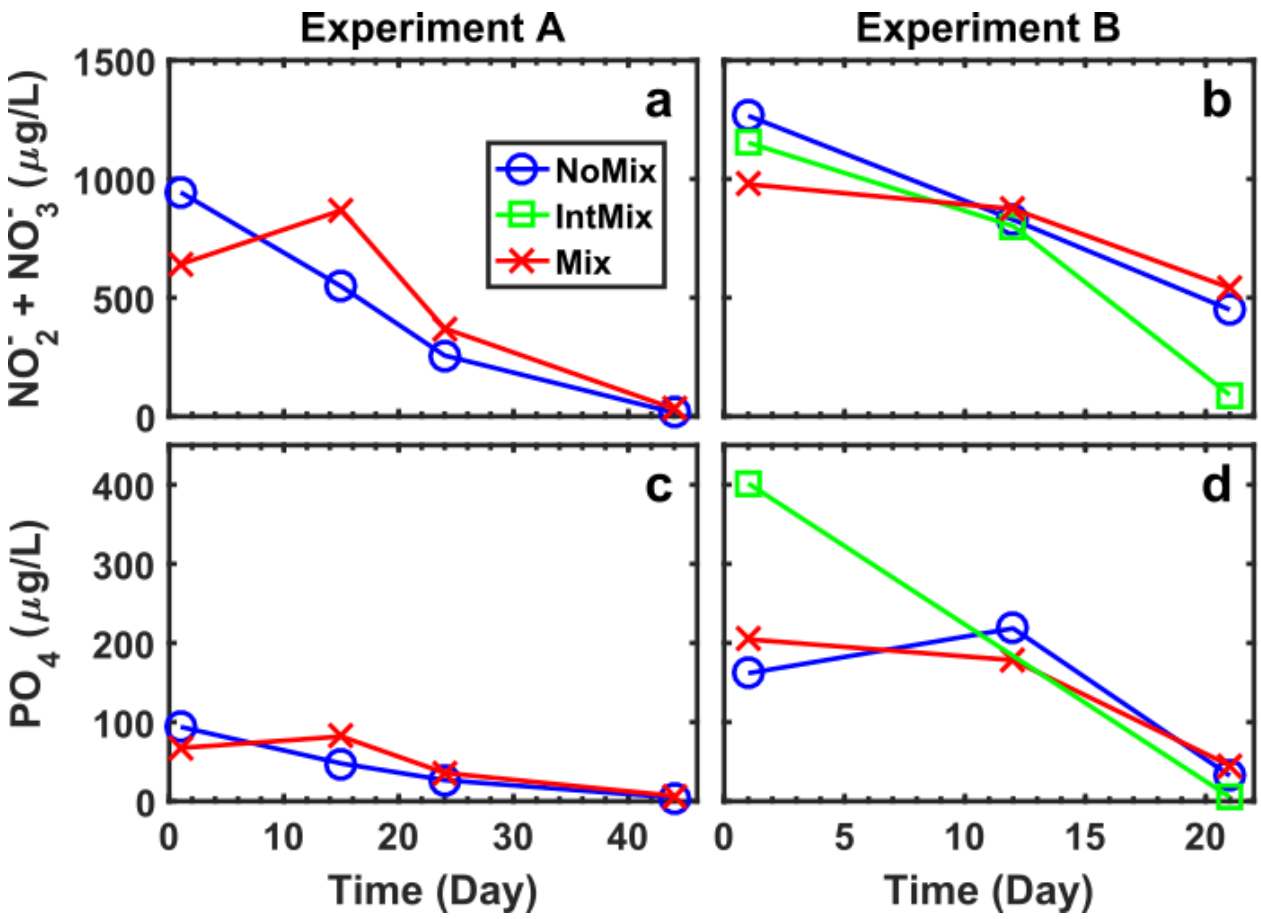

Figure 2. Concentrations of $NO_2^- + NO_3^-$ (a, b) and PO_4 (c, d) in the different treatments of both experiments. An outlier concentration of PO_4 in the IntMix reactor at day 12 of experiment B was removed (~1230 µg L^{-1}).

3.2. Chlorophyll-a and algal density

CHLA, and total unit density changes indicated the growth dynamics of the overall algal communities and vertical distributions across treatments (Fig 3 and 4). Varying growth stages completed within 44 days in experiment A, while they completed in 26 days in experiment B (Figure 3). Phytoplankton growth was minimal for several days (LAG) until exponential growth set in (EXP) followed by a period during which biomass started to decline (STA/DEC; Figure 3). In experiment A, exponential growth began around day 5 and subsided by day 30 (Figure 3; a).

Growth phases in experiment B were not as easily distinguishable as in experiment A. In experiment B, changes in CHLA suggested exponential growth during days 5 to 14, followed by general biomass decline (Figure 3; b). However, growth seemed to be sustained longer in the NoMix reactor (~day 21) (Figure 3; b). Increased mixing was linked to a faster decline in biomass (Mix first, IntMix second, then NoMix).

Initial CHLA concentrations and unit densities over all treatments were ~4 and ~7 times lower in experiment B compared to experiment A, respectively (Figure 3 and 4). Overall, higher column-integrated CHLA concentrations were observed in experiment A (peak at ~135 µg CHLA L^{-1}) than experiment B (peak at ~34 µg CHLA L^{-1}). In experiment A, CHLA within the NoMix reactor was higher than the Mix reactor up to day 17, and higher CHLA concentrations in the column were observed in the Mix reactor (peak at 135 µg CHLA L^{-1}) than in the NoMix reactor (peak at ~61 µg CHLA L^{-1}; Figure 3; a) after day 20. No surface accumulation of biomass was observed over time in any of the treatments during experiment A. A dense algal surface layer (matt) was formed by ~day 11 in the NoMix and IntMix treatments of experiment B (Figure 5). Higher CHLA concentrations in the NoMix reactor (peak at ~1537 µg CHLA L^{-1}) and in the IntMix (peak at ~833 µg CHLA L^{-1}) were observed relative to the Mix reactor (peak at ~42 µg CHLA L^{-1}; Figure 4). Surface accumulation was first established in the no mixing treatment and was more dense (181% more unit mL^{-1}) relative to the intermittent mixing treatment throughout the EXP phase. However, this shifted during STA/DEC stages, with denser (99% more unit mL^{-1}) surface accumulation in the intermittent mixing. A surface accumulation formed only much later in the Mix reactor (between day 26 and 32 after the STA/DEC phase), and when nutrient levels were low. Therefore, this surface accumulation may result from factors such as mineralization of nutrients from phytoplankton cells due to grazers, the accumulation of organisms at the surface

due to the mixing patterns and surface tension, and/or experimenting in an enclosed reactor system for a long period. Results for experiment B are consequently shown up to day 27.

In experiment A, unit densities increased over time and were similar in the Mix and NoMix reactor throughout (Figure 3; b). In experiment B, unit densities decreased over time in the IntMix and Mix reactor (Figure 4; d). However, the NoMix reactor showed a 42% increase in unit density from day 1 (LAG) to days 12/13 (EXP), after which densities decreased (STA/DEC; Figure 4; d). Differences between CHLA concentrations and unit density changes over time were observed across treatments in both experiments (Figure 3 and 4). Therefore, changes in CHLA content per phytoplankton biovolume over time were examined and no significant differences were observed across treatments of both experiments.

CHLA levels varied in their vertical distribution over time and among treatments (Figure 3 and 4). Upper layer values were represented as the mean of observations at 20 cm and 50 cm, where light intensities were high (26% of surface light on average), and the lower water column was defined as the mean of observations at 95 cm and 140 cm where light was low (4% of surface light in average). In experiment A, CHLA in the upper layer exceeded that within the lower column until day 25 in the NoMix treatment (Figure 3; a). In experiment B, CHLA levels were higher in the upper layer of the NoMix and IntMix reactors due to surface accumulation representing 48% and 37% of the column-average during the EXP phase, respectively (Figure 4; b). No significant vertical differences of CHLA concentrations were observed in the Mix reactor (Figure 4; b). A denser initial surface accumulation occurred in the NoMix reactor relative to the IntMix reactor throughout the exponential phase, and surface layer intensity leveled off between the NoMix and IntMix reactors by the end of experiment B (Figures 4 and 5). Formation of an algal surface layer was less intense in the Mix reactor (~35 µg CHLA L^{-1} during the EXP phase) compared to the

NoMix (~1537 µg CHLA L^{-1} during the EXP phase) and IntMix treatments (~833 µg CHLA L^{-1} during the EXP phase; Figures 4 and 5).

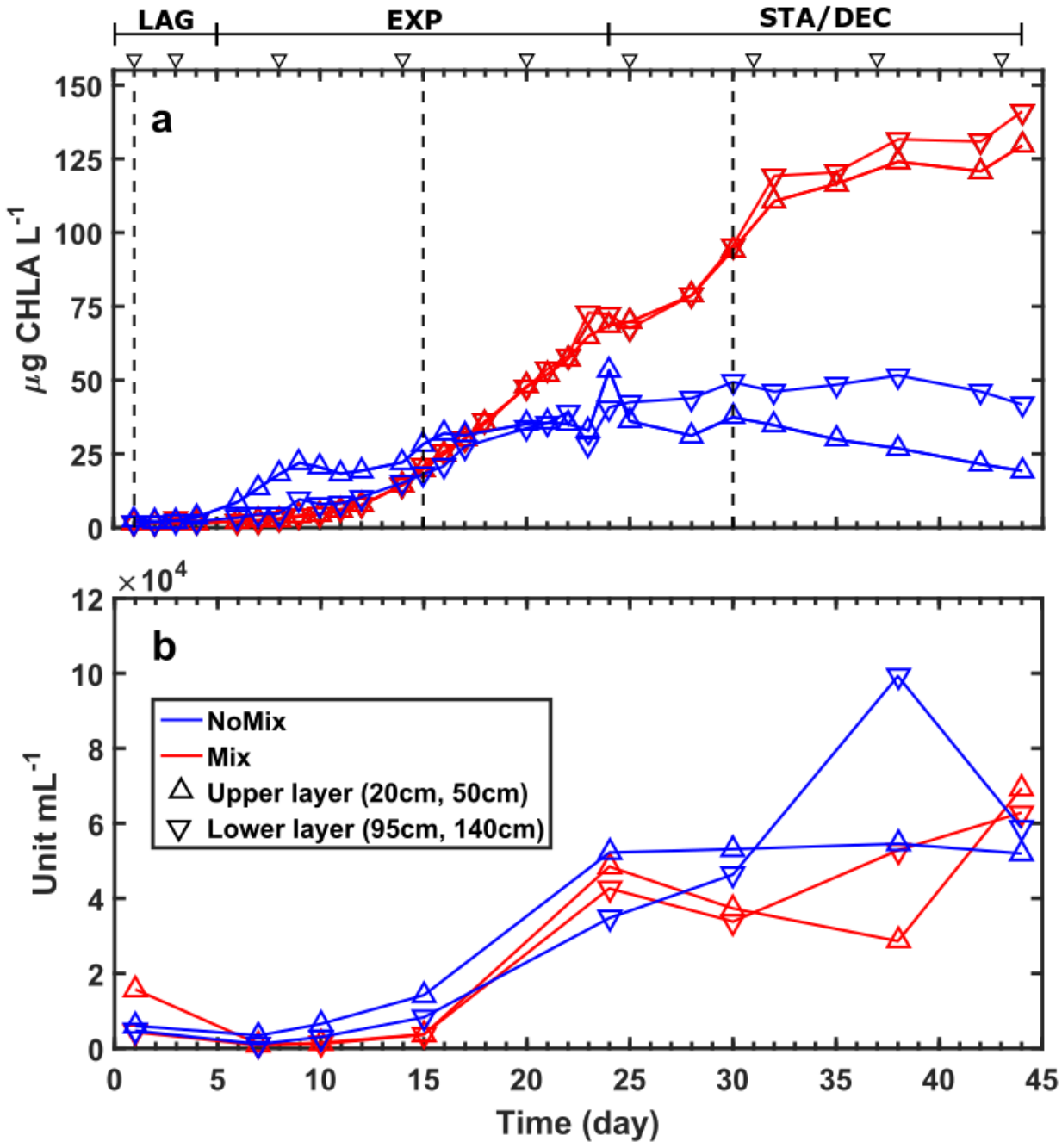

Figure 3. CHLA (a) and unit density (b) measurements for all treatments of experiment A in the upper (20 cm, 50 cm) and lower (95 cm, 140 cm) layer. Each data point represents the mean of triplicate measurements. Phytoplankton community composition was characterized at time points represented by a vertical dash line. Light profiles were measured at days shown by a downward triangle (∇). Estimation of growth phases, based on CHLA concentrations and unit density are as followed: LAG = onset of experiment; EXP = exponential growth; STA = stationary phase; DEC = decline/dying phase.

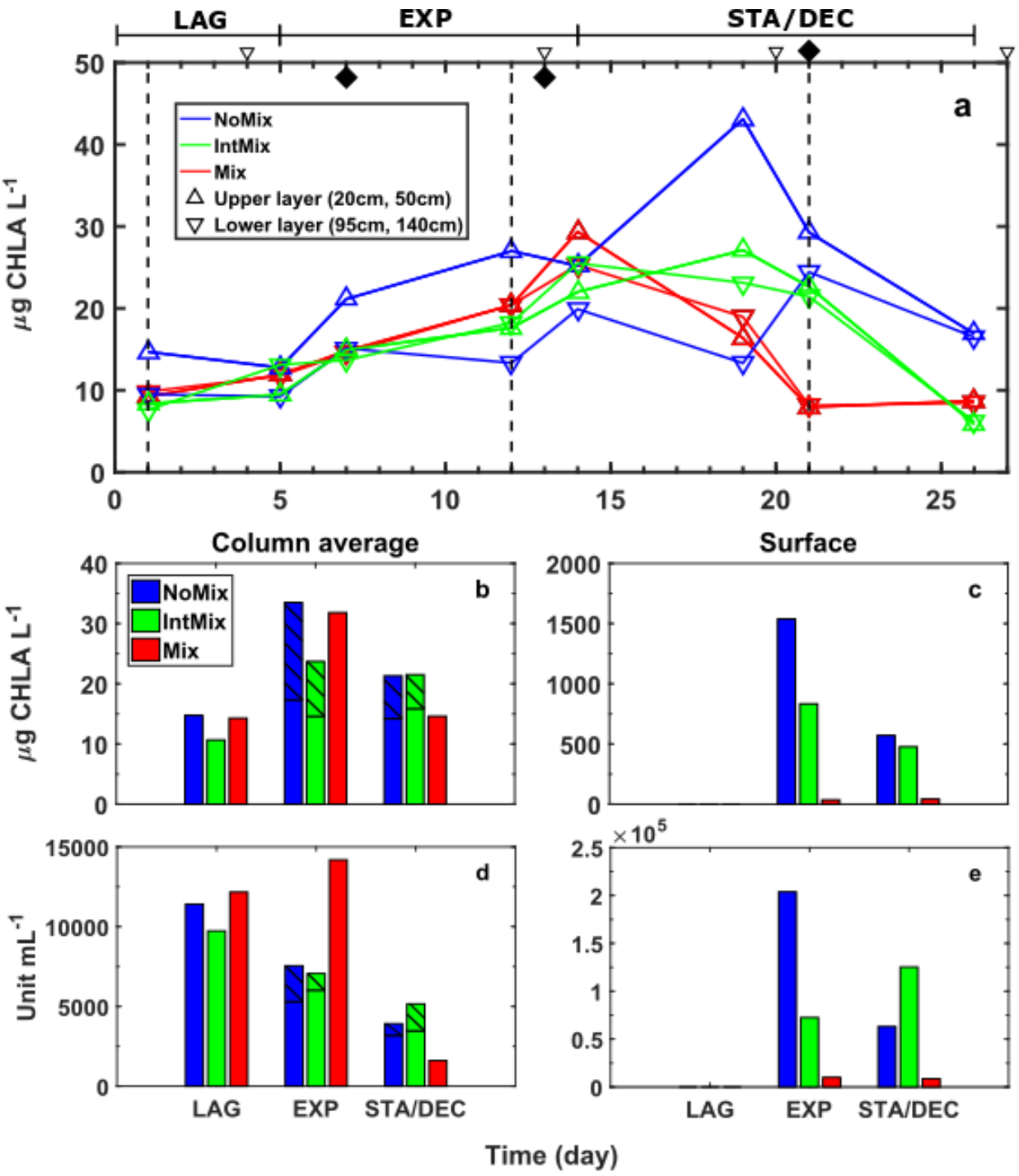

Figure 4. In vivo fluorescence (a), extracted CHLA (b, c), and unit density (d, e) observations over time for experiment B. Column-averages (b, d) were calculated from measurements at 0 cm, 20 cm, 50 cm, 95 cm, and 140 cm, and integrated over the whole water column depth (190 cm). Surface was sampled at time points shown by a black diamond (◆). Contribution of the surface matt (~2 cm thickness; c, e) to column averages for CHLA and unit densities were calculated at three different times and are indicated by a hatching in the bars. Plankton community composition was also characterized at time points represented by the vertical dashed lines. Based on CHLA concentrations and unit densities experimental phases were distinguished as followed: LAG = onset of experiment (day 1); EXP = exponential growth (day 12 and 13); STA/DEC = stationary, decline, and degradation phase (day 21). Light profiles were measured at days shown by a downward triangle (▽).

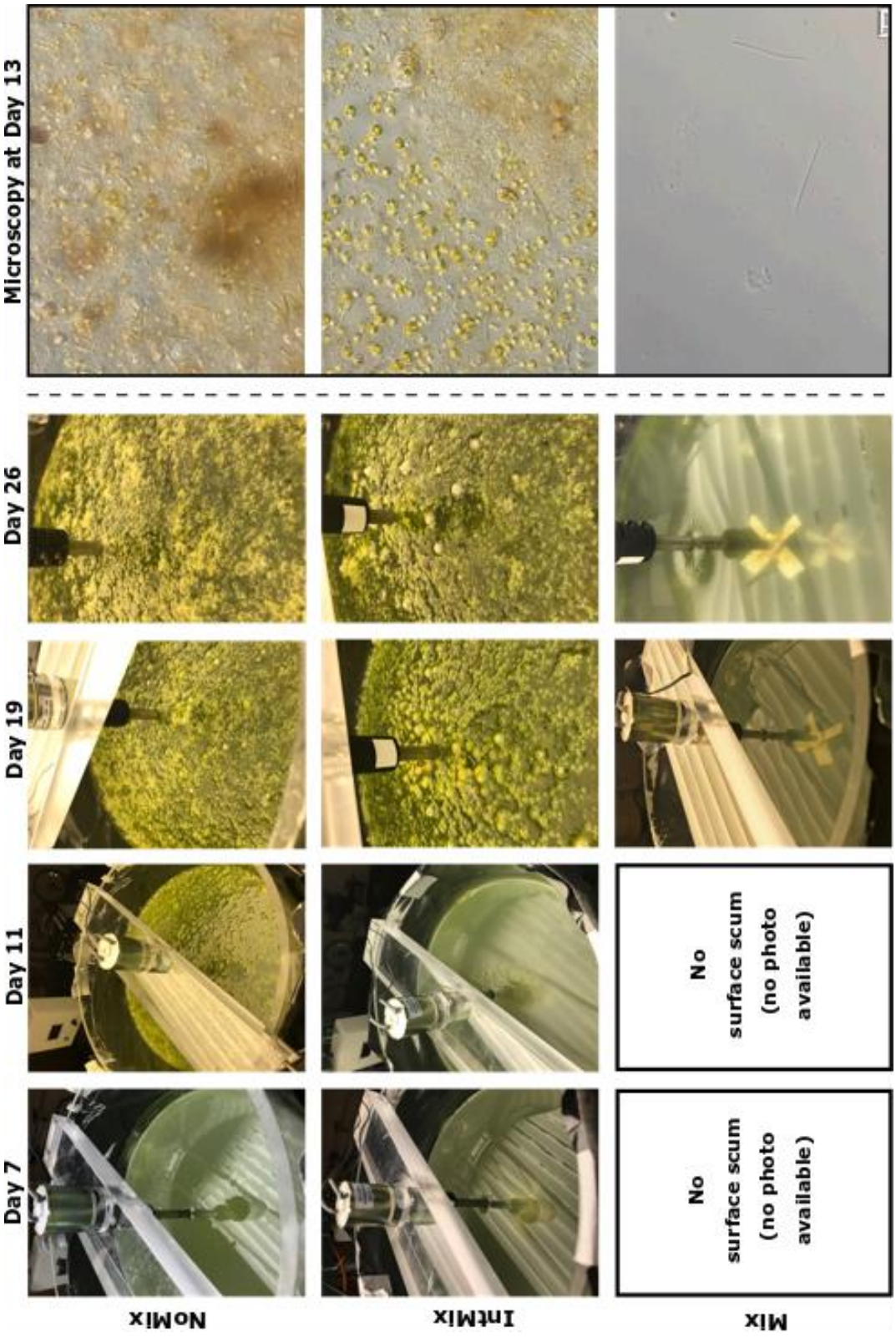

Figure 5. Pictures of the surface accumulations at multiple time points in all treatments of experiment B. Light microscopy pictures of the surface accumulation are shown for day 13 (EXP) for all treatments and were separated by a dash line from the rest of the pictures.

3.3. Light

The light environment changed in response to algal growth and vertical distribution of algal cells (Figure 6). Initial (LAG) biomass was low and light showed little differences between treatments, while overtime changes (STA) in light were a consequence of algal dynamics in relation to the different mixing treatments (Figure 6). Changes in fitted light extinction coefficients K_e from the surface to 5 cm were due to surface algal accumulations (Figure 6; e, f). The averaged light availability in the WCRs was representative of the effects of total column biomass production as higher algal concentrations would reduce light penetration through the water column (Figure 6; g, h).

Overall, 95% of the surface light was, on average, attenuated within the first 100 cm of the water column across all measured profiles of all treatments and experiments (Figure 6; a, b, c, d). In experiment A, light availability varied over time in both reactors and little differences were observed between the treatments (Figure 6; e, g). In experiment B, the Mix reactor had more light available throughout the water column (79% more than the NoMix reactor overall), followed by the IntMix reactor (32% more than the NoMix reactor overall), while the NoMix reactor showed the smallest amount of light penetration (Figure 6; h). Light in the column was reduced by the surface layer accumulation in the NoMix and IntMix reactors leading to higher K_e and lower light availability than in the Mix reactor of experiment B (Figure 6; f, h).

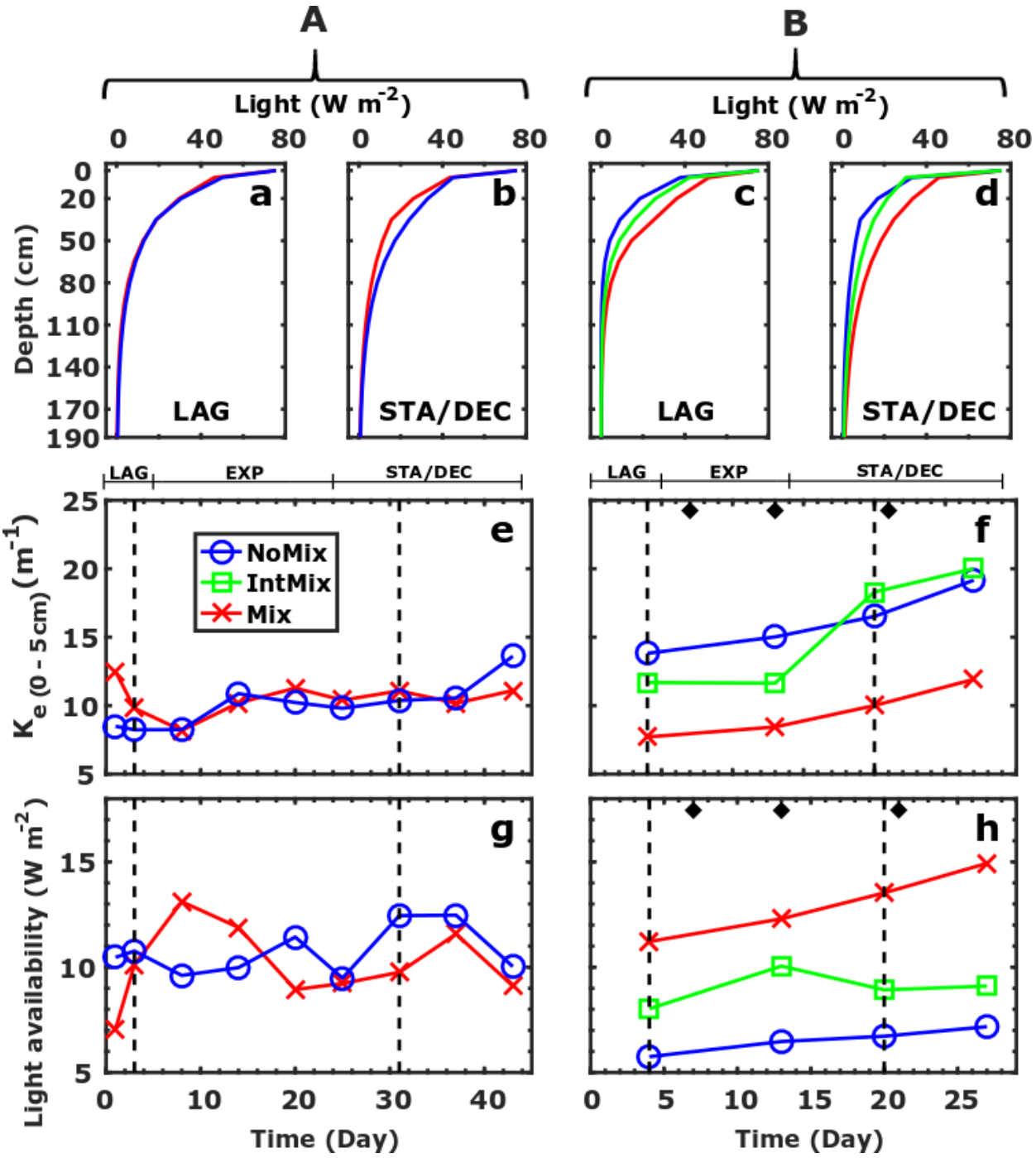

Figure 6. Example light profiles (a, b, c, d), light extinction coefficient (K_e) fitted from surface (0 cm) to 5 cm (e, f), and average light availability in the column (g, h) over time for the different treatments in experiment A (left) and B (right). The example profiles (a, b, c, d) are from the LAG and STA/DEC phases, as indicated by the dashed lines in the lower panels. Surface was sampled at time points shown by a black diamond (◆). Each line in a, b, c, and d represents Beer-Lambert's model fit for the observations (average r^2 = 0.99 for all light profiles). All fitted profiles were integrated over the whole water column depth (from surface to 190 cm; g, h).

3.4. Phytoplankton Dynamics

Cyanobacteria (Oscillatoriaceae, Nostocales, Chroococales, and *Aphanocapsa* spp.*)* dominated the plankton community at 93% and 87%, based on unit densities throughout experiment A and B, respectively (Figures 7 and 8). In experiment A, the Mix reactor had fewer cyanobacteria and cryptomonads but more chlorophytes (*Pediastrum* spp., *Ankistrodesmus* spp., *Staurastrum* spp., *Scenedesmus* spp., *Coelastrum* spp., *Pandorina* spp., *Tetraedron* spp., *Closteriopsis* spp.) and diatoms (*Synedra* spp. and pennate diatoms) than the NoMix reactor (Figure 7; a, c, e, g). Once growth set in, cyanobacterial abundance was lower in the Mix reactor relative to the NoMix treatment in experiment A (Figure 7; a). Cyanobacterial abundance increased over time in the NoMix treatment (Figure 6; a). In the Mix reactor of experiment A, cyanobacterial abundance decreased between the time point during the LAG phase and during the EXP phase and increased between the time points during the EXP and STA/DEC phases (Figure 7; a). Chlorophyte and diatom abundances increased in the NoMix and Mix reactors over time with higher abundances in the Mix treatment (Figure 7; c, e). Cryptomonad abundance increased over time in the Mix reactor, but seemingly only during the EXP phase in the NoMix reactor, with their final numbers having decline during STA/DEC phase (Figure 7; g). In experiment B, and based on unit densities, the Mix reactor had a higher number of cyanobacteria, chlorophytes, diatoms, and cryptomonads by the end of the EXP phase, and less algal abundances at the time point taken during STA/DEC phase, compared to the NoMix and IntMix treatments (Figure 7; b, d, f, h). The Mix reactor had more cyanobacterial units than in the NoMix and IntMix treatments during the EXP phase, after which abundances leveled off (Figure 7; b). Cyanobacteria at the surface represented 31% and 15% of the column average abundance of cyanobacteria in the NoMix reactor

and 10% and 23% of the column average abundance of cyanobacteria in the IntMix reactor throughout the EXP and STA/DEC phases, respectively (Figure 7; b). Cyanobacteria at the surface represent <6% of the column average cyanobacterial abundance in the Mix reactor over all time points (Figure 7, b). Chlorophyte abundance had increased by the end of the experiment in the NoMix and IntMix reactors, but decreased by the time a final sample was taken during STA/DEC phase for the Mix reactor (Figure 7; d). Diatoms were more abundant in the Mix reactor at the time points taken during the LAG and EXP phases, while they contributed more strongly to the final community in the IntMix relative to the NoMix and Mix reactor (Figure 7; f). Additionally, diatom abundance increased over time in the IntMix reactor, with ~68% of the diatoms located at the surface during the STA/DEC phase (Figure 7; f). Diatom abundance increased from the time point taken at the beginning of the experiment (LAG) to during the EXP phase in the NoMix and Mix treatments (Figure 7; f). Cryptomonad abundance decreased from LAG to EXP phase in the Mix reactor and increased in the NoMix and IntMix reactors, after which their abundance decreased (Figure 7; h). Cryptomonads at the surface represented 33% and 51% of the column average abundance of cryptomonads in the NoMix reactor and 24% and 18% of the column average abundance of cryptomonads in the IntMix reactor at the times EXP and STA/DEC phases were sampled, respectively (Figure 7, h).

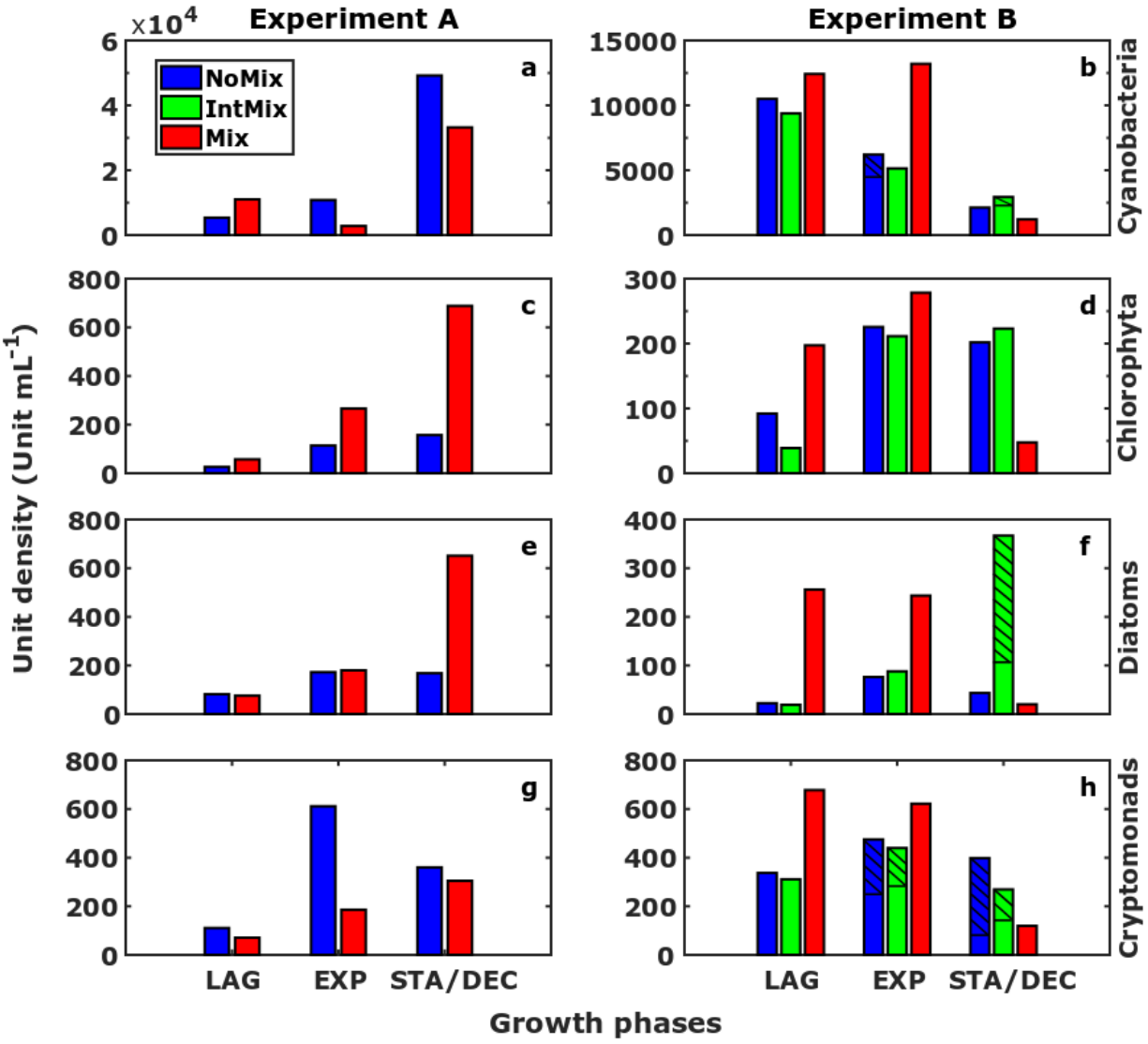

Figure 7. Absolute abundances based on unit counts over time in both experiments. Major taxonomic groups (cyanobacteria, chlorophytes, diatoms, and cryptomonads) are shown for experiment A (a, c, e, g) and B (b, d, f, h). Contribution of the surface accumulation to the column averages are represented by dashed lined and were not shown when <5% of the maximum value of all samples within a taxonomic group. Community characterization was performed at selected time points, as followed: LAG = onset of experiment; EXP = exponential growth; STA = stationary phase; DEC = decline and degradation phase. Observations were integrated over the whole water column depth (190 cm).

In both experiments, community structure based on Bray-Curtis similarities changed with treaments ($r = 0.124$ to $r = 0.605$; at $p = 0.0001$ to $p = 0.173$; two-way and three-way ANOSIM; Figure 8; Table S4) and over time ($r = 0.716$ to $r = 0.893$; at $p = 0.0001$ to $p = 0.002$; two-way and three-way ANOSIM; Figure 8; Table S4). No significant change was observed for community

structure when comparing depths in experiment A ($r = 0.033$; $p = 0.43$; two-way ANOSIM, Table S4; Figure 8). In experiment B, community structure changed when comparing the surface with the rest of the column ($r = 0.937$; $p = 0.0001$; three-way ANOSIM, Figure 8; Table S4). Based on these findings, assemblages from depths below the surface (20 cm, 50 cm, and 140 cm) were refered to as sub-surface and were treated as replicates for further statistical testing on community strucutre similaties. In experiment A, community structure in the NoMix and Mix treatments were distinct ($r = 0.605$; at $p < 0.006$; two-way ANOSIM, Table S4; Figure 8, a). Overall, cyanobacterial taxa Oscillatoriaceae, Nostocales, *Aphanocapsa* spp., and Chroococcales were linked to 25%, 19%, 15%, and 14% of variability in community structure of the Mix reactor, respectively, while in the NoMix treatment they contributed to 35%, 24%, 14%, and 10% of the total dissimilarity in community structure (one-way SIMPER, Table S4; Figure 8). *Aphanocapsa* spp., Oscillatoriaceae, and Nostocales were the main contributors to the dissimilarity between the Mix and NoMix treatments (one-way SIMPER). In experiment B, community structure in the NoMix and IntMix reactors were similar ($r = 0.124$; $p = 0.173$; three-way ANOSIM; Figure 8; Table S4) while both differed from assemblages in the Mix reactor ($r = 0.596$; at $p = 0.003$; three-way ANOSIM; Figure 8; Table S4). *Aphanocapsa* spp., Oscillatoriaceae, Nostocales, and Chroococcales were the main contributors to the dissimilarity between the Mix and both the NoMix and IntMix treatments (two-way SIMPER). Chroococcales, Oscillatoriaceae, *Aphanocapsa* spp., Nostocales, and cryptomonads were the main contributors to the dissimilarity between the surface and sub-surface assemblages (two-way SIMPER).

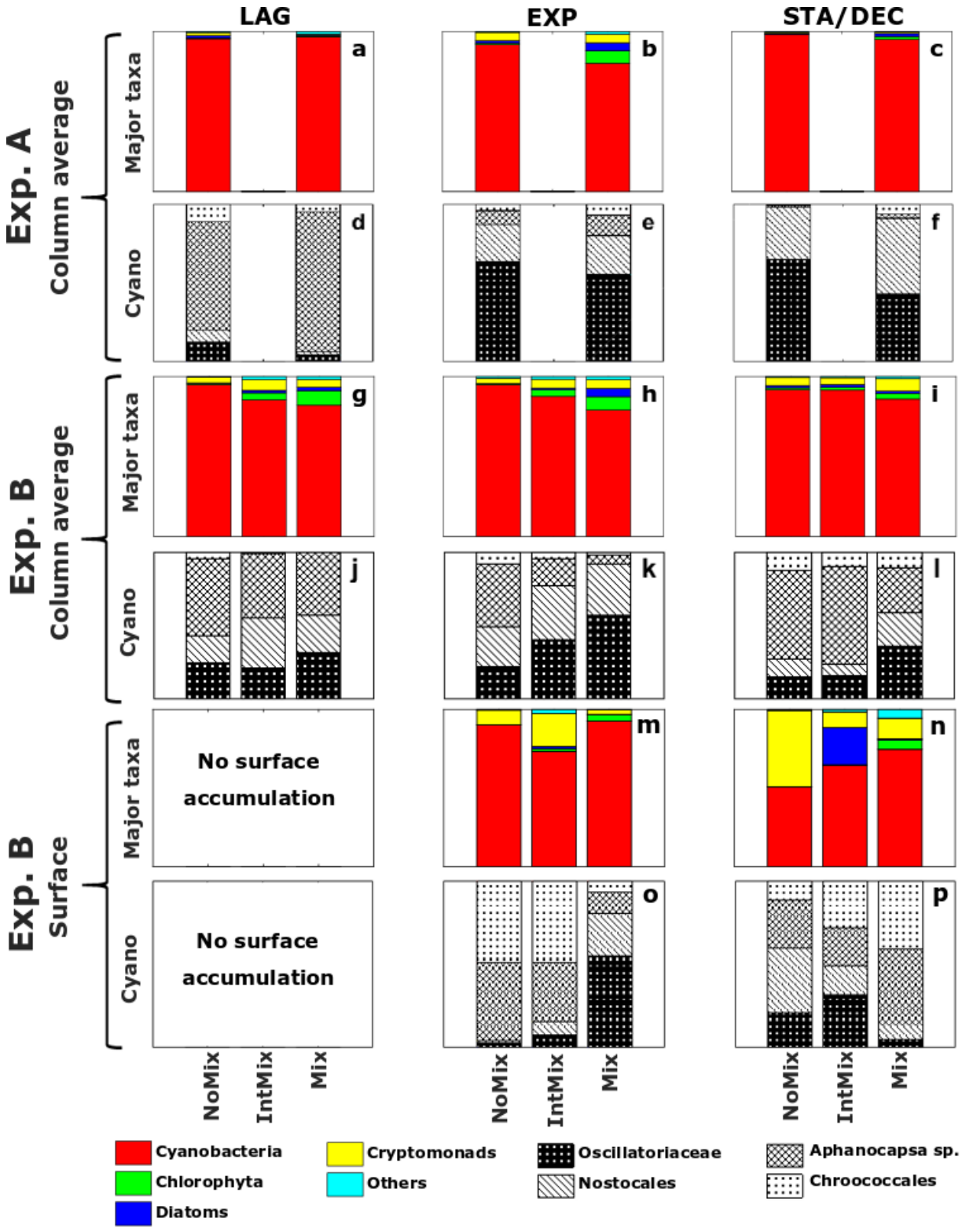

Figure 8. Relative abundances based on unit counts over time in both experiments. Major taxonomic groups are shown for experiment A (a, b, c) and B (g, h, i, m, n) as well as all cyanobacteria for experiment A (d, e, f) and experiment B (j, k, l, o, p). Data were separated for column averages (including surface accumulation) and surface accumulations in experiment B. Community characterization was performed at selected time points, as followed: LAG = onset of experiment; EXP = exponential growth; STA = stationary, decline, and degradation phase.

NMDS analysis applied to both experiments showed that community structure in the Mix was distinct from the NoMix in experiment A and from the NoMix and IntMix reactors in experiment B over time (Figure 9). In experiment A, community composition at the onset of the experiment (LAG) was distinct from assemblage makeup during active growth (EXP) and at the end of exponential growth (STA), when community structure also varied significantly between the Mix and NoMix reactors (Figure 9; a). In experiment B, community structure in the NoMix and IntMix reactors throughout the EXP and STA/DEC phases were distinct from that in the Mix (Figure 9; b). Community structure in the Mix reactor remained similar throughout experiment B (Figure 9; b). Assemblage composition at the surface (S) was distinct from that in the sub-surface (NS) for the NoMix and IntMix reactors (Figure 9, c).

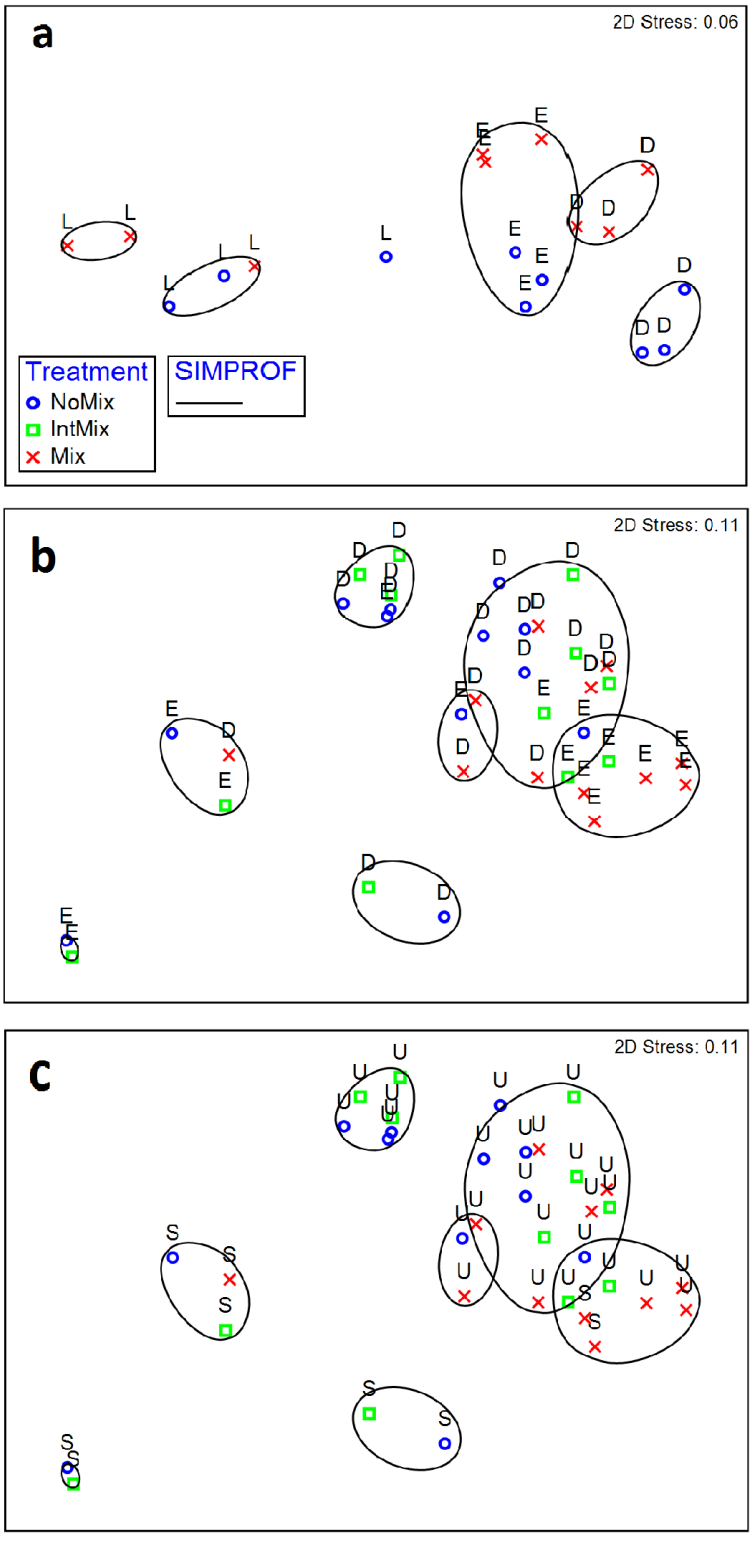

Figure 9. Non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarities for microbial communities superimposed with a CLUSTER analysis using the SIMPROF test for experiment A (a) and B (b, c). The lag (L), exponential (E), and stationary, decline, and degradation (D) growth phases were abbreviated. S = surface assemblages, U = sub-surface assemblages.

3.5. Cyanotoxins

Cumulative dissolved toxin analyses using SPATT bags found no detectable levels of cylindrospermopsin across any treatments in either experiment. However, cumulative dissolved microcystins were detected at all tested depths in the Mix reactor of experiment B. Concentrations of 1.45, 2.01, and 1.18 ng MCY $[g\ resin]^{-1}\ d^{-1}$ were measured at 50 cm, 95 cm, and 190 cm (bottom) depths, respectively.

CHAPTER 4

4. Discussion

4.1. Experimental approach

In this study we designed and implemented a series of water column reactors to study the effects of vertical mixing intensity and duration on phytoplankton community dynamics. Past experimental studies have looked at the effects of mixing in deep-lake environments where Secchi depth range from 1 to 10 m (Canfield and Bachmann, 1981; Reynolds et al., 1984; Chambers and Kaiff, 1985; Jager et al., 2008). To date, this is the first known suite of laboratory mesocosm experiments simulating environmental conditions of shallow and turbid water systems to examine the effect of varying mixing regimes.

Studying phytoplankton dynamics raises challenges as they are affected by biotic and abiotic factors that vary among water systems and seasons. In this study, source waters and algal communities were obtained in June 2017 and September 2017 for experiment A and B, respectively. The initial conditions of the two experiments were different in regard to initial community abundance and structure of the source water, as well as, background nutrients and may explain differences in overall phytoplankton dynamics between similar treatments of mixing across experiments. Pre-screening of the phytoplankton tow water for experiment A was performed to remove high number of copepods and may have reduced initial algal concentration and removed other large organisms or colonies. Cyanobacteria dominated the plankton composition in both experiments and relative abundances of cyanobacteria were different in the source water of each experiment (Table SX). Nutrient levels in both experiments were in sufficient

quantity to facilitate growth of plankton communities (Figures 2, 3, 4). Identical initial nutrient additions were performed on both experiments, but higher initial algal concentrations (37% based of unit densities and 547 % based on CHLA) were present in experiment B due to higher algal concentration in the source water as well as a smaller dilution factor of the lake water relative to experiment A. Low concentrations of dissolved inorganic nutrients were found throughout the stationary and decline growth phases in both experiments and nutrients may have been limiting growth. However for the majority of the experiment nutrient concentrations were well in excess of the concentrations measured in the source water (Figure 2). Limited conclusions can be made on the effects of nutrient limitations on observed changes in phytoplankton. All of the aforementioned differences may explain the faster growth cycle of phytoplankton in experiment B relative to experiment A. Experiment A and B were therefore studied separately and cross-comparisons between the two experiments were not the focus of this study.

4.2. Effects of vertical mixing

Vertical mixing affected phytoplankton distribution in relation to available light in both experiments. Plankton community characterization in experiment A may support competition theories for light by Klausmeier and Litchman (2001) and Huisman et al. (2004) suggesting that increased vertical mixing can provide a more suitable environment for non-motile chlorophytes and diatoms, therefore reducing cyanobacteria. In experiment A, even though all treatments were cyanobacteria dominated, continuous mixing increased the overall abundance of chlorophytes and diatoms and reduced that of cyanobacteria and cryptomonads relative to no mixing (Figure 7). In experiment B, continuous mixing increased overall algal abundances of all major taxa during

exponential growth compared to no mixing and intermittent mixing and this may be a direct consequence of higher light availability in the column due to less surface accumulation (Figures 6, 7). Small differences in light availability across treatment of mixing in experiment A may explain why no short-term increase in cyanobacterial abundance was observed in the continuous mixing reactor. Continuous mixing limited the formation of surface matts and provided higher light levels for the phytoplankton community of experiment B. Most filamentous and colonial taxa of cyanobacteria found in this study can regulate their buoyancy at different rates and produce toxins (Reynolds and Walsby, 1975; Thomas and Walsby, 1985; Reynolds et al., 1987). Community composition differences suggested that continuous mixing limited growth and vertical migration of colonial cyanobacteria in search for more light. Small differences between filamentous cyanobacteria (Oscillatoriaceae and Nostocales) and colonial cyanobacteria (*Aphanocapsa* spp. and Chroococcales) relative abundance was observed in experiment A. However, colonial cyanobacteria dominated the surface accumulation of the no mixing and intermittent mixing reactors in experiment B.

Throughout most of the experiments, light was the limiting factor for phytoplankton growth with a strong gradient in the upper water column (less than 5% of surface light below 1 m depth (Figure 6). Observed reductions in available light in Experiment A and B resulted from phytoplankton growth and vertical distribution. In experiment A, continuous mixing led to more overall algal concentrations in the column (>5000 unit mL^{-1} and >50 µg CHLA L^{-1}) after the exponential growth phase and explains why the averaged light available was lower than in the no mixing treatment. However, these differences in light availability in the column across treatments of mixing were not as strong as those noted in experiment B as no surface accumulations were observed in experiment A. Surface matt formation occurred in the no mixing and intermittent

mixing treatments in experiment B, therefore limiting light penetration through the surface layer. Continuous mixing in experiment B limited surface accumulation and explained higher light availability in the water column. We speculate that this increase in available light, in turn, led to higher algal concentrations in the water column. The intensity of the surface accumulation was stronger in the intermittent mixing treatment relative to the no mixing treatment throughout the stationary/decline growth phase as observed by higher algal concentrations and light extinction coefficient K_e in the surface layer. It is hypothesized that a local depletion of nutrients may have occurred in the no mixing treatment, which led to a decline of the surface accumulation intensity throughout the stationary/decline growth phase. Intermittent mixing may have been able to sustain more of a surface accumulation due to the daily redistribution of nutrients within the water column. Due to the upward migration of buoyant cyanobacteria, the surface K_e increased over time across all treatments of experiment B (Figure 6; f). This led in turn to higher light intensities in the lower layer as algal concentrations decreased below the surface accumulation, therefore increasing the averaged light availability in the column over time (Figure 6; h).

4.3. Implications for artificial mixing in shallow lakes

Artificial mixing systems have been implemented for several decades and their various effects on plankton community dynamics present challenges for researchers. Mixing intensities in this study were comparable to those found in shallow lakes occurring from wind and convection processes. The water column of the continuous mixing treatment was maintained well mixed and imitated the effect of artificial mixing systems in shallow lakes. However, mixing intensities imparted by artificial mixing systems vary spatially (Visser et al., 2016). The continuous mixing

treatment described in this study is therefore more representative of the water column in close proximity of artificial mixing systems or for whole-lake mixing systems. Temperature stratification is the strongest during daylight hours, and shallow lakes and reservoirs are often naturally mixed at night due to cooling of the epilimnion leading to convection processes and destratification of the water column (Tundisi and Tundisi, 2012). Implementing artificial mixing systems in shallow reservoirs and lakes that would be activated only during daylight hours and turned off at night, may therefore achieve continuous destratification of the water column. In our experiments, diurnal mixing processes in a lake without artificial mixing systems were simulated by the mixing schedule of the intermittent mixing reactor. This study indicated that continuous mixing reduced relative abundances of cyanobacteria within the plankton community and potentially reduced the ability of buoyant cyanobacteria to migrate to the surface. Temperature was maintained constant throughout this study. Further experimentations including temperature as a variable, and studying the interplay of vertical mixing, light, and temperature can be performed using controlled mesocosms such as the WCRs described here.

Application of artificial mixing systems needs to account for plankton community composition and water quality status specific to each lakes and reservoirs. Measurements such as CHLA and *in vivo* fluorescence are often used to study phytoplankton dynamics but must be approached with caution when predicting the effects of artificial mixing on phytoplankton abundance (Cullen, 1982). Characterization of phytoplankton communities is needed when studying phytoplankton competition and CyanoHABs. Community characterization in this study was performed using flow cytometry (FlowCAM). The FlowCAM provided a fast way to characterize and classify a large number of organisms and assign them to major taxonomic groups to genus level (Buskey et al, 2006; Álvarez, et al, 2011; Álvarez, et al, 2013). Differences in

CHLA compared to unit density changes were observed across treatments. As described by Chapra (2008) and Sadeghian et al. (2018), CHLA measurements are easier to obtain as opposed to algal density. CHLA/algal biomass ratios are often used in water quality models and are usually held constant for different species and/or taxonomic groups. Differences in phytoplankton dynamics between both experiments described in this study serve as evidence that the effects of mixing will differ depending on the phytoplankton community composition present in a water system.

4.4. WCR approach

The WCRs in this study allowed for a light-limited environment typical of shallow reservoirs as well as a controlled setting to limit variables of interests. We used natural plankton assemblages from a cyanobacteria-dominated shallow, turbid reservoir, in order to assess the ability of the WCRs to study phytoplankton communities for future experiments. Toxin-producing taxa were present in this study but dissolved toxin production based on SPATT measurements in the described experiments appeared negligible compared to previously reported natural concentrations for others NC reservoirs (Wiltsie et al., 2018). It should be noted that cyanotoxins may have been also present within cells, which would not be detected until they are released during lysis (Christoffersen, 1996; White and Fabbro, 2005). Water Column Reactors provide a unique tool for studying the effects of mixing on cyanotoxin production and can provide novel insights into the contribution of artificial mixing systems to cyanotoxin release in lakes and reservoirs. Modeling varying systems using the WCR approach can provide insights into the interplay of mixing, light, and nutrients as they drive phytoplankton and toxin dynamics.This first study using

WCRs showed the viability of this approach to gain predictive insights on mitigation strategies for CyanoHABs.

REFERENCES

Alcaraz, M., M. Estrada, and C. Marrase. "Interaction between turbulence and zooplankton in laboratory microcosms." Proceedings of the 21st EMBS. 1989.

Álvarez, Eva, et al. "How to effectively sample the plankton size spectrum? A case study using FlowCAM." Journal of Plankton Research 33.7 (2011): 1119-1133.

Álvarez, Eva, et al. "Routine determination of plankton community composition and size structure: a comparison between FlowCAM and light microscopy." Journal of plankton research 36.1 (2013): 170-184.

Anderson, Donald M. "Approaches to monitoring, control and management of harmful algal blooms (HABs)." *Ocean & coastal management* 52.7 (2009): 342-347.

Bakunin, Oleg G. Turbulence and diffusion: scaling versus equations. Springer Science & Business Media, 2008.

Barrington, Dani J., Elke S. Reichwaldt, and Anas Ghadouani. "The use of hydrogen peroxide to remove cyanobacteria and microcystins from waste stabilization ponds and hypereutrophic systems." Ecological Engineering 50 (2013): 86-94.

Bellinger, Edward G., and David C. Sigee. Freshwater algae: identification and use as bioindicators. John Wiley & Sons, 2015.

Burkholder, JoAnn M. A Study of Water Quality in Lake Raleigh. Water Resources Research Institute of the University of North Carolina, 1992.

Buskey, Edward J., and Cammie J. Hyatt. "Use of the FlowCAM for semi-automated recognition and enumeration of red tide cells (Karenia brevis) in natural plankton samples." Harmful Algae 5.6 (2006): 685-692.

Carmichael, Wayne W. "Health effects of toxin-producing cyanobacteria: "The CyanoHABs"." *Human and ecological risk assessment: An International Journal* 7.5 (2001): 1393-1407.

Chapra, Steven C. Surface water-quality modeling. Waveland press, 2008.

Christoffersen, Kirsten. "Ecological implications of cyanobacterial toxins in aquatic food webs." Phycologia 35.6S (1996): 42-50.

Clarke, K. Robert, and R. M. Warwick. "Change in marine communities." *An approach to statistical analysis and interpretation* (2001).

Clarke, K., and R. Gorley. "PRIMER v7: User manual/tutorial (p. 300)." Plymouth, UK: PRIMER-E Ltd (2015).

Codd, Geoffrey A. "Cyanobacterial toxins, the perception of water quality, and the prioritisation of eutrophication control." *Ecological engineering* 16.1 (2000): 51-60.

Cullen, John J. "The deep chlorophyll maximum: comparing vertical profiles of chlorophyll a." Canadian Journal of Fisheries and Aquatic Sciences 39.5 (1982): 791-803.

Einstein, Albert. Investigations on the Theory of the Brownian Movement. Courier Corporation, 1956.

Eppley, Richard W. "Temperature and phytoplankton growth in the sea." *Fish. bull* 70.4 (1972): 1063-1085.

Forsberg, B. R., and J. Shapiro. "Predicting the algal response to destratification." Restoration of lakes and Inland waters. EPA 440 (1980): 5-81.

Geider, Richard J., Hugh L. Maclntyre, and Todd M. Kana. "A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature." Limnology and oceanography 43.4 (1998): 679-694.

Guillard, Robert RL, and John H. Ryther. "Studies of marine planktonic diatoms: I. Cyclotella nana Hustedt, and Detonula confervacea (Cleve) Gran." Canadian journal of microbiology 8.2 (1962): 229-239.

Guillard, Robert RL. "Culture of phytoplankton for feeding marine invertebrates." Culture of marine invertebrate animals. Springer, Boston, MA, 1975. 29-60.

Hallegraeff, Gustaaf M. "A review of harmful algal blooms and their apparent global increase." *Phycologia* 32.2 (1993): 79-99.

Harris, Graham P. "The relationship between chlorophyll a fluorescence, diffuse attenuation changes and photosynthesis in natural phytoplankton populations." Journal of Plankton Research 2.2 (1980): 109-127.

Hassell, Michael P., Hugh N. Comins, and Robert M. May. "Species coexistence and self-organizing spatial dynamics." Nature 370.6487 (1994): 290.

Hawkins, P. R., and D. J. Griffiths. "Artificial destratification of a small tropical reservoir: effects upon the phytoplankton." *Hydrobiologia* 254.3 (1993): 169-181.

Herb, William R., and Heinz G. Stefan. "Dynamics of vertical mixing in a shallow lake with submersed macrophytes." Water Resources Research 41.2 (2005).

Hudnell, H. Kenneth, ed. *Cyanobacterial harmful algal blooms: state of the science and research needs*. Vol. 619. Springer Science & Business Media, 2008.

Hudnell, H. Kenneth, et al. "Freshwater harmful algal bloom (FHAB) suppression with solar powered circulation (SPC)." Harmful Algae 9.2 (2010): 208-217.

Huffaker, C. "Experimental studies on predation: dispersion factors and predator-prey oscillations." Hilgardia 27.14 (1958): 343-383.

Huisman, Jef, et al. "Changes in turbulent mixing shift competition for light between phytoplankton species." *Ecology*85.11 (2004): 2960-2970.

Hulot, Florence D., and Jef Huisman. "Allelopathic interactions between phytoplankton species: the roles of heterotrophic bacteria and mixing intensity." Limnology and Oceanography 49.4part2 (2004): 1424-1434.

Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsins—Draft; Report Prepared for Environmental Protection Agency; Environmental Protection Agency: Washington, DC, USA, 2016.

Ibelings, Bas W., BERND KROON, and Luuc R. Mur. "Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes." New Phytologist 128.3 (1994): 407-424.

Jäger, Christoph G., Sebastian Diehl, and Gertraud M. Schmidt. "Influence of water-column depth and mixing on phytoplankton biomass, community composition, and nutrients." Limnology and Oceanography 53.6 (2008): 2361-2373.

Jassby, Alan, and Thomas Powell. "Vertical patterns of eddy diffusion during stratification in Castle Lake, California." Limnology and oceanography 20.4 (1975): 530-543.

Jia, Yong, et al. "Inhibition of phytoplankton species by co-culture with a fungus." Ecological Engineering 36.10 (2010): 1389-1391.

Joehnk, Klaus D., et al. "Summer heatwaves promote blooms of harmful cyanobacteria." *Global change biology* 14.3 (2008): 495-512.

Ke, Zhi-Xin, Ping Xie, and Long-Gen Guo. "Impacts of two biomanipulation fishes stocked in a large pen on the plankton abundance and water quality during a period of phytoplankton seasonal succession." ecological engineering 35.11 (2009): 1610-1618.

Kerr, Benjamin, et al. "Local dispersal promotes biodiversity in a real-life game of rock–paper–scissors." Nature 418.6894 (2002): 171.

Klausmeier, Christopher A., et al. "Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton." *Nature* 429.6988 (2004): 171.

Kudela, Raphael M. "Characterization and deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater." Harmful Algae 11 (2011): 117-125.

Levin, Simon A. "Dispersion and population interactions." The American Naturalist 108.960 (1974): 207-228.

Malacic, Vlado. "Estimation of the vertical eddy diffusion-coefficient of heat in the gulf of trieste (northern adriatic)." Oceanologica Acta 14.1 (1991): 23-32.

Mallin, Michael A., Karen L. Stone, and M. Anne Pamperl. "Phytoplankton community assessments of seven southeast US cooling reservoirs." Water Research 28.3 (1994): 665-673.

Oberholster, P. J., A-M. Botha, and T. E. Cloete. "Toxic cyanobacterial blooms in a shallow, artificially mixed urban lake in Colorado, USA." Lakes & Reservoirs: Research & Management 11.2 (2006): 111-123.

Oviatt, Candace A. "Effects of different mixing schedules on phytoplankton, zooplankton and nutrients in marine microcosms." Marine Ecology Progress Series (1981): 57-67.

Paerl, Hans W., and Jef Huisman. "Blooms like it hot." *Science* 320.5872 (2008): 57-58.

Paerl, Hans W., and Jef Huisman. "Climate change: a catalyst for global expansion of harmful cyanobacterial blooms." *Environmental microbiology reports* 1.1 (2009): 27-37.

Paerl, Hans W., and Valerie J. Paul. "Climate change: links to global expansion of harmful cyanobacteria." *Water research* 46.5 (2012): 1349-1363.

Paerl, Hans W., et al. "Harmful freshwater algal blooms, with an emphasis on cyanobacteria." *The Scientific World Journal*1 (2001): 76-113.

Sadeghian, Amir, et al. "Improving in-lake water quality modeling using variable chlorophyll-a/algal biomass ratios." Environmental Modelling & Software 101 (2018): 73-85.

Scheffer, Marten, Marco R. de Redelijkheid, and Frank Noppert. "Distribution and dynamics of submerged vegetation in a chain of shallow eutrophic lakes." Aquatic Botany 42.3 (1992): 199-216.

Scheffer, Marten, et al. "On the dominance of filamentous cyanobacteria in shallow, turbid lakes." Ecology 78.1 (1997): 272-282.

Sheng, Y. Peter, and W. Lick. "The transport and resuspension of sediments in a shallow lake." Journal of Geophysical Research: Oceans 84.C4 (1979): 1809-1826.

Smith, Val H. "Effects of nutrients and non-algal turbidity on blue-green algal biomass in four North Carolina reservoirs." Lake and reservoir management 6.2 (1990): 125-131.

Reynolds, C. S., and A. E. Walsby. "Water-blooms." Biological reviews 50.4 (1975): 437-481.

Reynolds, Colin S., Rod L. Oliver, and Anthony E. Walsby. "Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments." *New Zealand journal of marine and freshwater research* 21.3 (1987): 379-390.

Ryther, John H. "Photosynthesis in the ocean as a function of light intensity." Limnology and Oceanography 1.1 (1956): 61-70.

Smith, Val H. "Effects of nutrients and non-algal turbidity on blue-green algal biomass in four North Carolina reservoirs." Lake and reservoir management 6.2 (1990): 125-131.

Thomas, R. H., and A. E. Walsby. "Buoyancy regulation in a strain of Microcystis." *Microbiology* 131.4 (1985): 799-809.

Tundisi, José Galizia, and Takako Matsumura Tundisi. *Limnology*. CRC Press, 2012.

United States, Department of Environmental Quality, Division of Water Resources, Intensive Survey Unit. "Lake & reservoir assessments cape fear river basin". 2014. https://files.nc.gov/ncdeq/Water%20Quality/Environmental%20Sciences/Reports/CapeFearRLakesAssess2013.pdf

Upadhyay, Sanjina, et al. "Mixing potential of a surface-mounted solar-powered water mixer (SWM) for controlling cyanobacterial blooms." Ecological engineering 61 (2013): 245-250.

Van den Berg, Marcel S., et al. "Dynamics and stability of Chara sp. and Potamogeton pectinatus in a shallow lake changing in eutrophication level." Shallow Lakes' 98. Springer, Dordrecht, 1999. 335-342.

Visser, Petra M., et al. "Artificial mixing to control cyanobacterial blooms: a review." Aquatic Ecology 50.3 (2016): 423-441.

Visser, Petra M., et al. "How rising CO 2 and global warming may stimulate harmful cyanobacterial blooms." Harmful Algae 54 (2016): 145-159.

Visser, Petra M., et al. "The ecophysiology of the harmful cyanobacterium Microcystis." Harmful cyanobacteria. Springer, Dordrecht, 2005. 109-142.

Wagner, Carola, and Rita Adrian. "Cyanobacteria dominance: quantifying the effects of climate change." Limnology and Oceanography 54.6part2 (2009): 2460-2468.

Walsby, Anthony E., et al. "The selective advantage of buoyancy provided by gas vesicles for planktonic cyanobacteria in the Baltic Sea." The New Phytologist 136.3 (1997): 407-417.

Wehr, John D., Robert G. Sheath, and J. Patrick Kociolek, eds. Freshwater algae of North America: ecology and classification. Elsevier, 2015.

Weiss, Charles M., and Edward J. Kuenzler. "Trophic state of North Carolina lakes." Annual report (1975).

White, Susan H., Leo Duivenvoorden, and Larelle D. Fabbro. "A decision-making framework for ecological impacts associated with the accumulation of cyanotoxins (cylindrospermopsin and microcystin)." Lakes & Reservoirs: Research & Management 10.1 (2005): 25-37.

Zhou, Jian, et al. "Effects of wind wave turbulence on the phytoplankton community composition in large, shallow Lake Taihu." Environmental Science and Pollution Research 22.16 (2015): 12737-12746.

Zhou, Jian, Boqiang Qin, and Xiaoxia Han. "Effects of the magnitude and persistence of turbulence on phytoplankton in Lake Taihu during a summer cyanobacterial bloom." Aquatic ecology 50.2 (2016): 197-208. (A).

Zhou, Jian, et al. "Turbulence increases the risk of microcystin exposure in a eutrophic lake (Lake Taihu) during cyanobacterial bloom periods." Harmful algae 55 (2016): 213-220. (B).

APPENDICES

Appendix A

Water Column Reactors (WCRs)

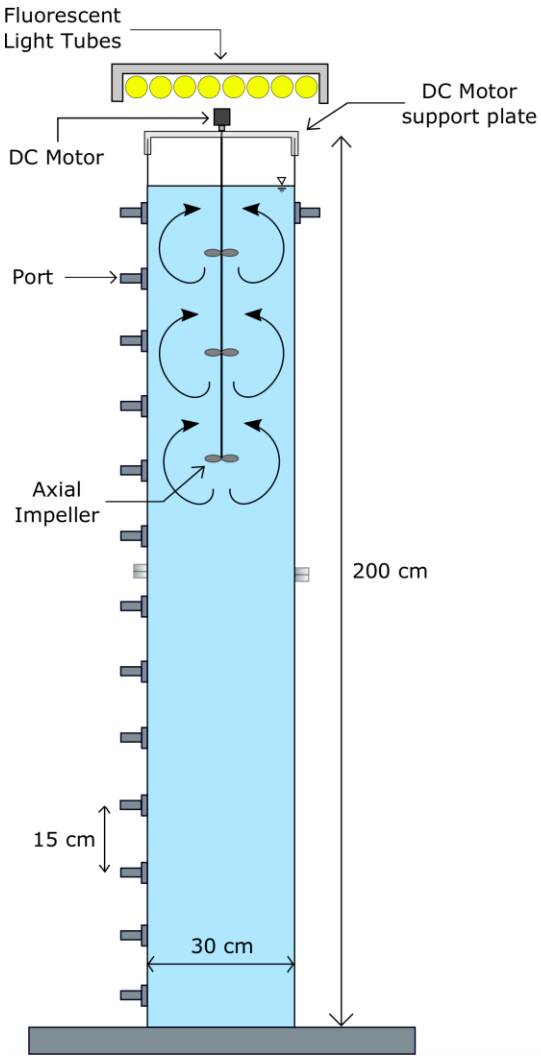

Figure A1. Side view of the Water Column Reactors (WCRs) with dimensions.

Characterizing Mixing

Tracer tests using rhodamine dye were conducted to characterize mixing intensity in the WCRs and approximate a typical range of diffusion coefficients found in shallow freshwater reservoirs. Suites of tracer experiments were performed under different mixing regimes and configurations using the mixing system previously described. *In vivo* fluorescence samples were taken from the WCR ports at discrete time points at three depths and were measured using a fluorometer (Turner Design, Trilogy). A second measurement approach using an *in vivo* fluorescence sonde (Hydrolab, MS5) was used. Both methods were compared to account for measurement and procedure errors. The sonde requires to be submerged in its environment at a specific depth, but is able to measure concentration at smaller time intervals (1 second). However, the sampling method is less intrusive and allows measurements at multiple depths within one test, but the time step is usually larger. Using the former approach, samples were taken at 50 cm, 110 cm, and 170 cm depths every 5 minutes. The sonde was placed at the bottom of the WCR (190 cm depth) and measured at a 1 second interval. Tap water was acclimated to room temperature and to the mixing intensity being tested. The concentration was calculated to be above the minimum detection limit of both instruments. In addition, the dye was gently injected at the surface at the center of the WCR using a syringe connected to a flexible tube. Two analysis approaches for measurement diffusion coefficients were compared. First, a 1D numerical diffusion model using a forward time centered space (FTCS) finite difference approach was developed. An optimization problem was set to fit a diffusion coefficient by minimizing the sum of square residuals between the model and observations.

$$\frac{\partial C}{\partial z} = D\frac{\partial^2 C}{\partial z^2} \qquad \text{(A1)}$$

D = diffusion coefficient

C = concentration of rhodamine dye

z = depth

The second method estimates the diffusion coefficient using a 1D random-walk model (Einstein, 1956; Bakunin, 2008) as described in Equation (X) below.

$$D = \frac{{z_{mix}}^2}{2t_{mix}} \qquad \text{(A2)}$$

t_{mix} = time to achieve steady-state concentration of rhodamine dye in the WCR

z_{mix} = depth of mixing (taken as the the total water depth in the water column = 190 cm)

Table A1. *Diffusion coefficient estimates from example tracer tests performed at 100 RPM in the WCR. Average value from each method along with standard deviation from the mean were calculated to approximate the range of diffusion coefficients achieved by the mixing system.*

	D ($cm^2\ s^{-1}$)	
Test #	**Fick's 2nd law**	**Random-walk**
1	8.9	8.0
2	8.9	9.1
3	13.5	13.2
4	7.4	10.3
Average	9.7	10.1
Standard deviation	2.7	2.2

Sampling

Table A2. *Sampling schedule of experiment A and B*

Time (Day)	Experiment A				Experiment B				
	In vivo fluorescence (5 mL)	Larger samples (200 mL)	Light profile	Bottom Plate	*In vivo* fluorescence (5 mL)	Larger samples (200 mL)	Light profile	Bottom Plate	Surface accumulation
1	x	x	x		x	x			
2	x								
3	x		x						
4	x						x		
5					x				
6	x								
7	x	x			x				
8	x		x						
9	x								
10	x	x							
11	x								
12	x				x	x			
13							x		x
14	x		x		x				
15	x	x							
16	x								
17	x								
18	x								
19					x				
20	x		x				x		
21	x				x	x			x
22	x								
23	x								
24	x	x							
25	x		x						
26					x				
27							x		
28	x				x				
29									
30	x	x							
31									
32	x						x		
33					x (END)	x		x	x
34									
35	x								
36									
37									
38	x	x							
39									
40									
41									
42	x								
43			x						
44	x	x							
45	(END)			x					

Preliminary experiment

Experiment 1 was performed in April 2017 for 13 days, and consisted of cultured assemblage of diatoms grown in an incubator at 22 °C (Percival Scientific). Experiment 1 was a preliminary test using one continuous mixing (Mix) reactor to gain insights on the behavior of phytoplankton the WCR. A 12:12-h light:dark cycle was used starting and stopping at 6am and 6pm, respectively. In addition, clay was not added to increase background turbidity.

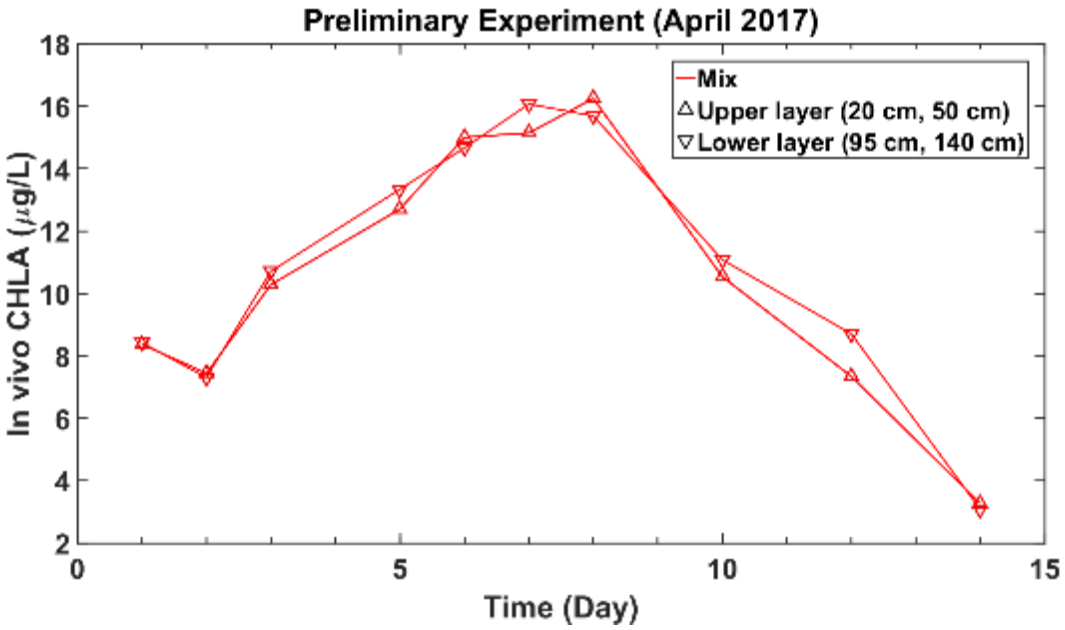

Figure A2. In vivo chlorophyll-a for the preliminary experiment performed in April 2017 using one Mix WCR.

In vivo fluorescence and extracted chlorophyll-a

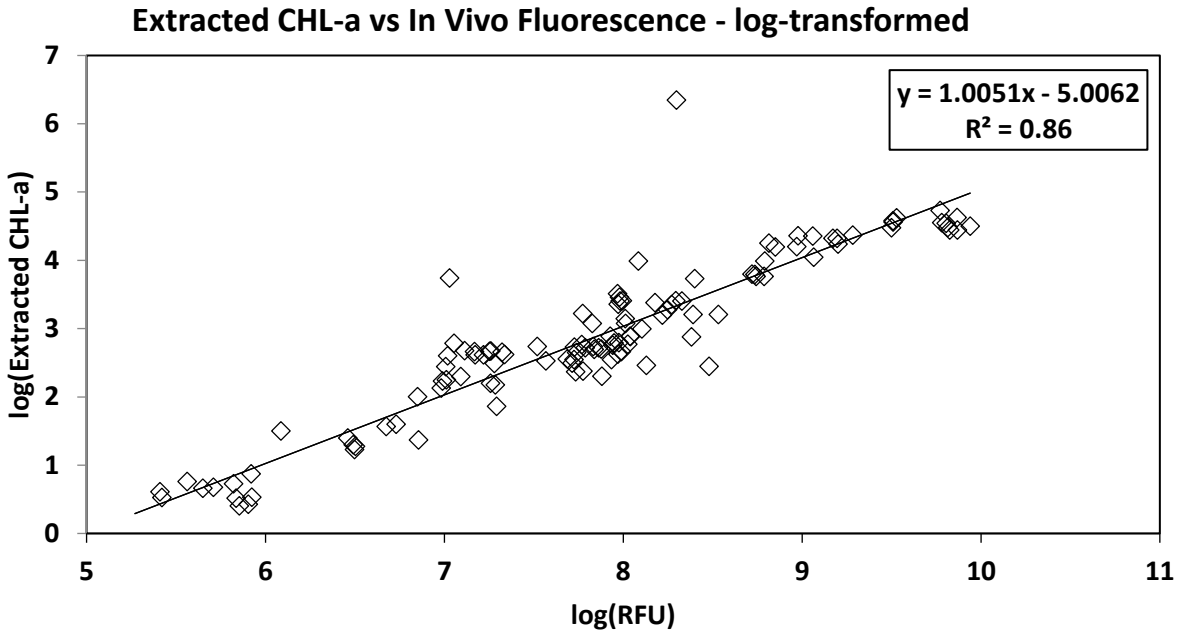

Figure A3. Linear regression using observations from the preliminary experiment, experiment A, and experiment B, in order to predict in vivo chlorophyll-a based on extracted chlorophyll-a concentrations. Extracted and in vivo fluorescence were log-transformed.

Modeling light

$$I(z) = I_0 e^{-K_e z} \qquad \text{(A3)}$$

I(z) = Light intensity in function of water column depth (W m^{-2})

I_0 = Average surface light intensity emitted by the light system (W m^{-2})

K_e = Light extinction coefficient (m^{-1})

z = water column depth (m)

Three K_e were fitted for each observed light profiles from 0 cm to 5 cm, 5 cm to 35 cm, and 35 cm to 170 cm depths. The fitted K_e from 35 cm to 170 cm was used to extrapolate the light intensities down to the bottom of the reactor at 190 cm.

Source water community

Table A3. *Unit density of the plankton taxa identified in experiment A and B in the source water.*

Unit density (Unit/mL)	Before dilution		After dilution		Normalized (%)	
Genera	Exp. A	Exp. B	Exp. A	Exp. B	Exp. A	Exp. B
Oscillatoriaceae	9726	8280	760	2588	304	140
Nostocales	3047	12457	238	3893	95	210
Aphanocapsa sp.	3554	12239	278	3825	111	207
Chroococcales	1055	436	82	136	33	7
Pediastrum sp.	0	36	0	11	0	1
Ankistrodesmus sp.	39	0	3	0	1	0
Staurastrum sp.	39	182	3	57	1	3
Scenedesmus sp.	39	0	3	0	1	0
Coelastrum sp.	0	36	0	11	0	1
Pandorina sp.	0	109	0	34	0	2
Tetraedron sp.	0	0	0	0	0	0
Closteriopsis sp.	156	0	12	0	5	0
Pennate diatom	234	0	18	0	7	0
Synedra sp.	625	0	49	0	20	0
Cryptomonad	469	1271	37	397	15	21
Ciliates	39	0	3	0	1	0
Unidentified organisms	234	617	18	193	7	10
TOTAL	19257	35663	1504	11145		

Statistical analysis

Table A4. *Statistical analysis results for community structure in both experiments.*

Experiment	Type	Factors crossed	Factor tested	Test	r	p
A	Three-way	Treatment X Time X Depth	Depth	Global	0.033	0.428
A	Two-way	Treatment X Time	Treatment	Pairwise - NoMix vs Mix	0.605	0.006
A	Two-way	Treatment X Time	Time	Pairwise - NoMix vs Mix	0.893	0.0001
B	Three-way	Treatment X Time X Depth	Treatment	Pairwise - IntMix vs Mix	0.596	0.003
B	Three-way	Treatment X Time X Depth	Treatment	Pairwise - IntMix vs NoMix	0.124	0.173
B	Three-way	Treatment X Time X Depth	Treatment	Pairwise - Mix vs NoMix	0.596	0.003
B	Three-way	Treatment X Time X Depth	Time	Pairwise - LAG vs EXP	0.716	0.002
B	Three-way	Treatment X Time X Depth	Time	Pairwise - LAG vs STA	0.877	0.001
B	Three-way	Treatment X Time X Depth	Time	Pairwise - EXP vs STA	0.733	0.0004
B	Three-way	Treatment X Time X Depth	Depth	Global	0.937	0.0001

Pictures

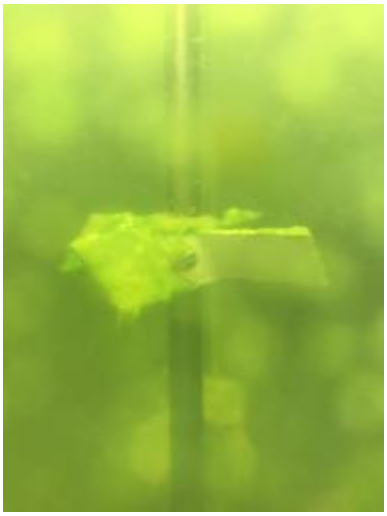

Figure A4. 10 Zoom on one axial impeller

Figure A5. 11 Three axial impellers attached to the stainless steel shaft

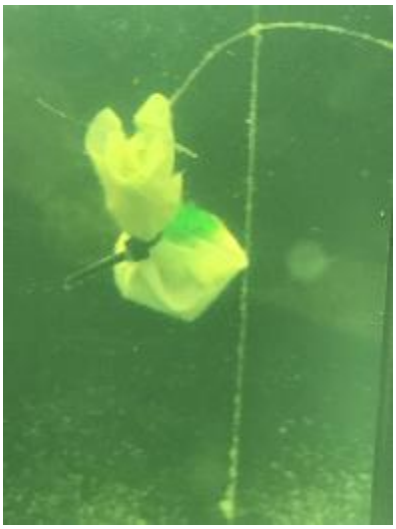

Figure A6. One SPATT bag

Figure A7. Two SPATT bags

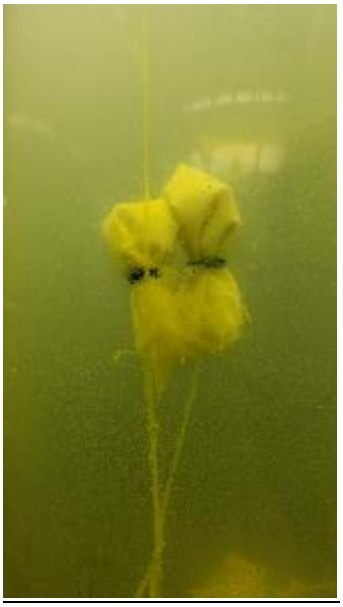

Figure A8. Two SPATT bags (2)

Figure A9. Sampling tube

Figure A10. Sampling tube through one of the WCR port