Title: Comparative Analysis of *Microcystis* Buoyancy in Western Lake Erie and Saginaw Bay of Lake Huron

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Highlights:

- *Microcystis* colonies in western Lake Erie were mostly buoyant
- *Microcystis* colonies in Saginaw Bay were mostly sinking
- Buoyant and sinking velocities were weakly correlated with colony size
- Apparent colony density of small colonies was more variable and responsive to light

Abstract

Microcystis is the predominant genus of harmful cyanobacterium in both Lake Erie and Saginaw Bay of Lake Huron and has the capacity to regulate the buoyancy of its colonies, sinking under certain conditions while floating towards the surface in others. Understanding the factors that control buoyancy is critical for interpretation of remote sensing data, modeling and forecasting harmful algal blooms within these two systems. To determine if *Microcystis* colony buoyancy in the two lakes responds similarly to diurnal light cycles, colony buoyant velocity (floating/sinking terminal velocity in a quiescent water column) and size were measured after manipulating the intensity of sunlight. Overall, there were more positively buoyant (floating) colonies in Lake Erie while most of the colonies in Saginaw Bay were negatively buoyant (sinking). In Lake Erie the colonies became less buoyant at increased light intensities and were less buoyant in the afternoon than in the morning. In both lakes, apparent colony density was more variable among small colonies (< 200 μm), whereas larger colonies showed a diminished response of density to light intensity and duration. These findings suggest that colony density becomes less plastic as colonies increase in size, leading to a weak relationship between size and velocity. These relationships may ultimately affect how the bloom is transported throughout each system and will help explain observed differences in vertical distribution and movement of *Microcystis* in the two lakes.

Keywords: *Microcystis*, buoyancy, vertical migration, Lake Erie, Saginaw Bay

1. Introduction

Harmful algal blooms (HABs) are a globally widespread problem in nearshore marine and freshwater ecosystems (Harke et al., 2016; Paerl and Otten, 2013). HABs can cause major disruptions in ecological communities and pose health risks to both animals and humans (Hilborn and Fournie, 2008; Paerl et al., 2001). In the Laurentian Great Lakes of North America, HABs occur as a symptom of eutrophication with likely promotion from invasive dreissenid mussels in western Lake Erie (Vanderploeg et al. 2001, 2002; Stumpf et al., 2012; Watson et al., 2016), Saginaw Bay of Lake Huron (Vanderploeg et al. 2001, 2002; Fishman et al., 2010), and several other locations (Bartlett et al., 2018; Carmichael and Boyer, 2016). In western Lake Erie and Saginaw Bay, HABs are often composed of cyanobacteria of the genus *Microcystis*, which frequently dominates warm, shallow, eutrophic lakes. *Microcystis* is capable of producing toxins called microcystins (Harke et al., 2016; Jöhnk et al., 2008; Paerl et al., 2011) that can affect drinking water quality or lead to recreational contact advisories (www.epa.gov/cyanohabs). Like other phytoplankton, the population dynamics of *Microcystis* are driven principally by light intensity, nutrient availability, predation, and temperature (Harke et al., 2016; Litchman and Klausmeier, 2008). Much of the research on HABs in the Great Lakes has focused on growth and toxicity in response to nutrients (Bertani et al., 2016; Chaffin et al., 2011; Michalak et al., 2013) and trophic interactions, particularly by introduced dreissenid mussels (Vanderploeg et al. 2001, 2002, 2009; Johengen et al., 2013; Tang et al., 2014). However, unlike most eukaryotic phytoplankton in the Great Lakes, *Microcystis* exhibits buoyant vertical migration, which allows the genus to exploit gradients in nutrients, light, and consumer abundance that occur with depth in lakes (Reynolds et al., 1987).

Although the capacity of phytoplankton to adjust their buoyancy is not unique to cyanobacteria, only cyanobacteria possess gas vesicles which are important to driving buoyancy, and *Microcystis* shows particularly flexible buoyancy regulation. *Microcystis* can form large colonies (i.e. > 1 mm), consisting of ellipsoid cells (~ 5 µm) in a mucilaginous aggregation, which can achieve buoyant velocities greater than one meter per hour (Nakamura et al., 1993; Wallace and Hamilton, 1999). Buoyancy of *Microcystis* is governed by the size of colonies and their density relative to water (Li et al., 2016; Nakamura et al., 1993; Wu et al., 2020a). Colony size is highly variable (Wu et al., 2020a) and the buoyant velocity (or sinking velocity) for a colony of any size is also influenced by the colony's shape and compactness (Li et al., 2016; Wu et al., 2020a; Zhu et al., 2014a). The density of a colony is determined as a result of the balance between intracellular gas vesicles, which decrease cell density compared to water, and macromolecules and intracellular carbohydrate stores, which increase density of the cell (Konopka et al., 1987; Kromkamp and Mur, 1984; Oliver, 1994; Thomas and Walsby, 1985). Ultimately, the extent of vertical migration by *Microcystis* is governed by changes in colony density and size in combination with the turbulent environment in the water (Hozumi et al., 2019; Wallace et al., 2000; Wallace and Hamilton, 1999).

Buoyancy regulation and vertical migration in *Microcystis* are likely adaptations to exploit vertical gradients of light, nutrients, and consumer abundance (Bormans et al., 1999; Reynolds et al., 1987) and may contribute to the competitive advantage of *Microcystis* over eukaryotic phytoplankton in eutrophic conditions (Huisman et al., 2004; Xiao et al., 2018). For example, *Microcystis* biomass can accumulate near the surface under quiescent conditions, forming 'scums' early in the morning and sinking when light intensity increases later in the day (Ibelings et al., 1991b; Reynolds, 1973; Reynolds et al., 1987; Zhu et al., 2014b). This behavior is thought to be an adaptation to obtain sufficient light for photosynthesis, while avoiding damaging effects of high light intensity and short wavelengths at the surface, particularly during mid-day (Reynolds and Walsby, 1975). This pattern is supported by both in

situ observations (Ibelings et al., 1991b; Visser et al., 1996) and laboratory experiments (Ibelings et al., 1991a; Visser et al., 1997; Wu et al., 2020b; Xiao et al., 2018) and has been included in mechanistic models (Aparicio Medrano et al., 2013; Chien et al., 2013; Visser et al., 1997; Wallace et al., 2000). The response of *Microcystis* buoyancy to light is a function of both intensity and duration (Wallace and Hamilton, 1999) and is mediated by rapid changes in carbohydrate stores (Konopka et al., 1987; Wallace and Hamilton, 1999; Xiao et al., 2012). Buoyancy regulation has also been proposed as an adaptation for cyanobacteria, including *Microcystis*, to exploit higher inorganic nutrient concentrations in the metalimnion or near the sediments (Fogg and Walsby, 1971). Several laboratory studies have shown that nitrogen (N) or phosphorus (P) limitation can diminish the response of *Microcystis* buoyancy to light (Brookes and Ganf, 2001; Chu et al., 2007; Konopka et al., 1987). While the response of buoyancy to nutrient limitation appears consistent with an adaptation to acquire nutrients at depth, Bormans et al. (1999) argued that the tendency to sink under nutrient stress could be due to low growth rates and stresses that occur under nutrient limitation. Similar to the effects of nutrient limitation, low temperature also decreases buoyancy and increases the tendency for colonies to sink (Thomas and Walsby, 1986; Visser et al., 1995; You et al., 2018).

In addition to the ecological significance of buoyancy regulation and vertical migration by *Microcystis*, these phenomena also have important implications for remote sensing, modeling and forecasting of HABs in lakes. Ocean color remote sensing is used to monitor HABs, but assessing total bloom biomass from remote sensing is complicated by the varying vertical distribution of colonies as a function of buoyancy and turbulence (Wynne et al., 2011). Rowe et al. (2016) described a model to predict *Microcystis* vertical distribution as a function of colony size distribution, buoyant velocity, and turbulent diffusivity, and showed improvement in forecasts of bloom trajectory when a model of colony vertical distribution was linked to a three-dimensional hydrodynamic forecast. The model of Rowe et al. (2016), and similar models of phytoplankton vertical distribution (Ross and Sharples, 2004), require

input of empirically-measured buoyant velocity. While models have been developed to predict changes in density as a function of light intensity and duration due to changing carbohydrate content (Aparicio Medrano et al., 2013; Ibelings et al., 1991b; Okada and Aiba, 1983, 1986; Visser et al., 1997; Wallace et al., 2000), they still cannot predict all variables that control buoyant velocity and vertical distribution, including gas vesicle volume fraction, the influence of nutrient limitation, and colony size distribution.

To date, the vertical migration of buoyant *Microcystis* colonies in the Great Lakes has been studied under a limited set of environmental conditions in western Lake Erie, including both experimental methods and vertical profiles collected in situ (Bosse et al., 2019; Kramer, 2018; Rowe et al., 2016). The aim of the present study was to measure buoyant velocities of colonies as a function of light intensity, time of day, and colony size in western Lake Erie and Saginaw Bay, which have different nutrient loading concentrations and stoichiometry (Johengen et al. 2013). Intact lake water samples were incubated under a range of light intensities in outdoor incubations and microscopic cinematography (micro-cinematography) was used to empirically measure both the size of colonies and their terminal floating or sinking velocity in a quiescent water column (buoyant velocity). We hypothesized that increasing light intensity would cause *Microcystis* colonies to become less buoyant, prolonged exposure to high light intensities would cause colonies to become negatively buoyant, and buoyant velocity would increase with colony size.

2. Methods

2.1 Experimental design overview

Short-term incubation experiments were performed with water samples collected from Lake Erie and Saginaw Bay. These experiments were designed to maintain the temperature and other conditions found in the lakes, while manipulating the intensity of sunlight to mimic different depths within the water column. The vertical velocities of colonies were empirically measured after the samples acclimated to these different levels of light intensity and this was repeated in the morning and afternoon to examine the effect of exposure history on buoyancy. The complete details of the experimental setup and analyses are described in the following sections.

2.2 Sample collection and processing

Samples of whole lake water were collected during the 2016 and 2019 summer bloom seasons in western Lake Erie and during the 2019 season in Saginaw Bay. In 2016, samples were collected at five stations in western Lake Erie between August 29 and October 11 (See Table 1, Gill et al., 2017). During 2016, the stations were selected based on highest abundance of phytoplankton (much of which is *Microcystis)*, as quantified by chlorophyll concentration measured using a FluoroProbe fluorometer (bbe Moldaenke). In 2019 the sampling design was adapted to contrast the two lakes and so samples were collected at one station in western Lake Erie (WE2) and one station in Saginaw Bay (SB14), between July 10 and August 12.

For all experiments, water was collected in the late morning or early afternoon from three discrete depths: surface, 1m below surface, and 1m above the lake bottom. Those volumes were then immediately combined to provide an integrated water-column sample. During 2016 the water samples were collected using 5L Niskin bottles and in 2019 the water was collected via peristaltic pump. Water samples were stored in polyethylene bottles inside insulated coolers at ambient temperature for transport back to the NOAA Great Lakes Environmental Research Laboratory in Ann Arbor, MI. Experiments were set up around 16:00 local time, which was 3 to 5 hours after collection. As described in the section below, measurement of colony velocities began the day following collection.

At each site, water temperature was recorded from either a buoy thermistor (averaged over 24 hours) or, for stations where no buoy was present, a temperature sensor deployed during collection (CIGLR and NOAA GLERL, 2019). Photosynthetically active radiation (PAR) was measured throughout the water column using a submersible instrument (Biospherical Instruments Inc., QSP-2300) and the light extinction coefficient (m^{-1}) was estimated by regressing the natural log of PAR against depth (m) (Weiskerger et al., 2018). More detailed descriptions of the collection methods, locations, and environmental context have been published previously ([dataset] Burtner et al., 2020; [dataset] CIGLR and NOAA GLERL, 2019). Environmental data for each sampling event is provided in Table 1 and Supplementary Material Table S1.

2.3 Experimental setup

In order to simulate light intensity expected at different depths in the water column, microcosms (acid-cleaned 2-liter borosilicate bottles) were filled with lake water and assigned to different light treatments: ambient light intensity, 30% of ambient intensity, and 10% of ambient intensity. Bottles designated for the ambient light treatment were not covered, but polyester neutral light density films (LEE Filters, Hampshire, UK) were applied to others to achieve light levels of 30% (only in 2019) and 10% of ambient (2016 and 2019). The bottles were then submerged upside down in an outdoor incubation tank (0.5 m^3) filled with water, which was exposed to sunlight through the open top. Water within the tank was continuously recirculated via pumps and the water was maintained at lake temperature using a thermostatic water bath (Cole Parmer, Polystat). Temperature within the incubation tank was recorded at 10-minute intervals during the 2019 experiments using a temperature logger (RBR Ltd.) and it was found to remain within 2ºC of the target temperature. To quantify incident light exposure within the tank, PAR was continuously recorded using an underwater spherical sensor (LI-COR, LI-192 Underwater Quantum Sensor) and the mean of the 6-h period before sampling was used to represent light exposure history.

2.4 *Microcystis* velocity and size measurements

Microcystis size and velocity were empirically measured during the day following collection, with the morning measurements made approximately 16 hours after the start of the incubation in the outdoor tanks. Sub-samples were collected for micro-cinematography in the morning (09:00 local time) and afternoon (15:00). At each time point, the bottles were gently inverted several times to mix the contents before pouring an aliquot of each sample into a 25 mL cuvette. The cuvette (10mm × 10mm inner dimension × 305mm tall, Friedrich & Dimmock, Inc.) was housed inside a thermal water jacket (140mm × 140mm inner dimension × 400mm tall) that was placed inside a temperature-controlled room matching ambient lake temperature at time of collection. A transparent ruler with mm graduations was

placed inside the thermal water jacket to allow for calibration between each set of recordings. The micro-cinematography apparatus was located inside the temperature-controlled room and consisted of a camera mounted on a 3D positioning frame that was controlled from the outside of the room via joystick, allowing for the user to focus and move the camera system along the cuvette (Bundy et al., 1998; Gill et al., 2017 their Fig 2.5). In contrast to the Schlieren system used by Bundy et al. (1998) and Rowe et al. (2016) the present study used a shadowgraph optics system (Rasenat et al., 1989; Trainoff and Cannell, 2002) with a laser light source (400-710nm, Stocker Yale Canada Inc., LASIRIS^{TM}) and a digital video camera (Basler acA1300 – 60g mNIR) with image capturing software (Contemplas, GmbH, Germany). The shadowgraph system allowed visualization of *Microcystis* colonies as small as 80 µm in diameter. We verified accurate measurement of buoyant velocities in the system by measuring the known buoyant velocities of 50 and 100 µm polystyrene microspheres (Gill et al., 2017).

A minimum of ten video segments were captured at each timepoint, taken at discrete points along the length of the cuvette. Following video recording, *Microcystis* colonies were identified by morphology and buoyant velocities were estimated using the motion analysis software Vicon Motus (Contemplas, GmbH, Germany). Simultaneously with acquiring the buoyant velocities, we assigned identities to colonies in calibrated image stills from Vicon Motus and were then imported into ImagePro Insight (Media Cybernetics) to estimate colony size by equivalent spherical diameter (ESD). ESD of an irregularly shaped object is the diameter of a sphere of equivalent volume. In this case, the ESD was estimated by converting the projected area of a *Microcystis* colony image to a diameter of a circle of equivalent area. Sizes were then paired with each colony's corresponding velocity data. Velocity and size data were acquired for a minimum of 20 (2016) or 26 (2019) colonies from each combination of experiment, light intensity and time of day. In total, size and velocity were estimated for 729 individual colonies in

2016 and 1,522 colonies in 2019. Hereafter we refer to the estimated velocity and estimated colony size as velocity and size, respectively.

The relationship between colony size and velocity (either positive or negative) is expected to follow Stokes' law (Reynolds, 1973), where the force of gravity on a colony balances with the fluid drag force when the colony reaches its terminal velocity.

$$V = \frac{g\,\Delta\rho\,D^2}{18\,\varphi\,\mu} \qquad \text{(Equation 1)}$$

In Equation 1, V is the colony velocity (m s^{-1}), g is the gravitational acceleration constant (9.81 m s^{-2}), μ is the viscosity of water (Pascal-second), D is the diameter of the colony (m), φ is the shape coefficient, and $\Delta\rho$ is the difference in density between the water and the colony (ρ_{water} – $\rho_{colony,}$ kg m^{-3}) (Reynolds et al., 1987). The density of water at 22.5ºC, the approximate average temperatures across experiments, was estimated at 997.6557 kg/m^3 using the package 'marelac' in the R statistical programming platform. If g, ρ_{water}, and μ are known, and φ is set to 1, Equation 1 can be solved to estimate apparent colony density, an estimated parameter, using empirical buoyant or sinking velocity observations and size measurements from a colony (Li et al., 2016; Reynolds et al., 1987).

$$\rho_{colony} = \rho_{water} - \frac{18\,\varphi\,\mu\,V}{g\,D^2} \qquad \text{(Equation 2)}$$

In Equation 2, ρ_{water} is the density of freshwater and ρ_{colony} is apparent colony density (kg/m^3). Because this method assumes that the shape factor is constant, apparent colony density represents not only the density of a colony but also potential differences in shape or structure that influence velocity.

2.5 Statistical analyses

The effects of treatments on observed velocity and apparent colony density were analyzed by fitting hierarchical linear models separately for each lake and year. The models included population-level effects of light intensity (10%, 30% and ambient) and time of day (morning, afternoon) in complete factorial and with their interaction. We distinguished between large and small colonies using the approximate median size of 200 µm equivalent spherical diameter. To evaluate whether our findings were sensitive to the size used for distinguishing large and small colonies, we repeated our analyses using a size cutoff of 150 µm or 250 µm (Supplementary Materials Figures S1-S2). Each model also included group-level effects, allowing the intercept and the population-level effects to vary among stations and dates. The 'leave one out' information criterion was used to compare models with and without each interaction or grouping term (Gelman et al., 2013). When those terms did not improve the information criterion they were omitted.

All statistical analyses were performed within the R statistical platform. Models were fitted in a Bayesian framework using a Gaussian error distribution and priors with a mean of zero and standard deviation of 5. The package 'brms' (Bürkner, 2017) was used to compile and execute the Hamiltonian Markov Chain Monte Carlo algorithm in the 'stan' language (Carpenter et al., 2017). Each model was fitted in four separate Monte Carlo chains, each with 1,000 warm up iterations and 1,000 sampling iterations. Adequate mixing of the chains was confirmed using the r-hat statistic, which was less than 1.01 in all cases. We assessed the fit of each model containing a continuous predictor using a Bayesian R-squared (Gelman et al., 2018) and checked for influential observations using leave-one-out cross-validation. The effects were tabulated directly from the pooled posterior distributions. The mean posterior value was used as the estimate and the 2.5% and 97.5% quantiles as the credible interval (CI). Select contrasts within each model were assessed using an evidence ratio (ER), or the ratio of the number of posterior samples consistent with the hypothesis to the number of posterior samples

opposing the hypothesis. While an arbitrary cutoff was not used for evidence ratios (i.e. a threshold p-value, Wasserstein et al., 2019), contrasts with ER below 5 were interpreted as having little support. Estimates of mean velocities and densities from the models are listed in Supplementary Material Table S2.

3. Results

3.1 Effects of light intensity and time of day on observed velocities in Saginaw Bay

Figure 1 (A, B) shows that the mean observed velocity of colonies from Saginaw Bay was negative for each experiment and ranged from -30 to -71 μm s^{-1} among the treatment combinations. The light treatments behaved similarly in morning and afternoon and so the final model for Saginaw Bay did not include an interaction between light intensity and time of day. In the July experiment the mean observed colony velocity was similar across all treatment combinations and differed by only 13 μm s^{-1}. Compared to the experiment performed in July, colonies were more responsive to light intensity and time of day in August. For the experiment performed in August, observed velocities were similar in the 10% and ambient light intensity treatments, but the 30% light intensity had velocities that were less negative (ER= 51). In the August experiment the observed velocities were also less negative in the morning than afternoon (ER= 17).

3.2 Effects of light intensity and time of day on observed velocities in western Lake Erie

Figure 1 (C, D) shows that *Microcystis* colonies from western Lake Erie in 2019 were more often positively buoyant compared to Saginaw Bay and that their observed velocity was more responsive to light intensity and duration. This pattern was apparent in the morning of each experiment, but the difference in observed velocities between the morning 10% and ambient light intensities were greater in July (59-149 μm s^{-1}, ER>4,000) than in August (0.8 - 25 μm s^{-1}, ER= 22). Unlike the monotonic effect of light intensity observed in the morning, in both experiments the afternoon 10% light intensity treatment showed positive buoyancy whereas the 30% and ambient treatments exhibited negative buoyancy (ER> 1,300). Within each light intensity treatment, the colonies were consistently less buoyant in afternoon compared to the morning.

Figure 1 (E) shows that the *Microcystis* colonies from western Lake Erie were both more buoyant and more variable in 2016 than in 2019. The model for the 2016 experiments did not include an interaction between light intensity and time of day. In 2016, colonies were more positively buoyant at 10% light intensity than at ambient light intensity (ER = 147). While these effects of light intensity and time of day were largely consistent among the experiments (Figure 1 E), the mean velocities in each combination of treatments varied among locations and dates. In each experiment the mean velocities were positive for both light intensity treatments during the morning and the 10% treatment in the afternoon. In the afternoon the ambient light treatment's CI overlapped zero for five out of seven experiments.

3.3 Relationships between observed buoyant velocity, size, and apparent colony density

Previous studies have shown that colony size is a particularly influential determinant of buoyant velocity in natural populations of *Microcystis* (Nakamura et al., 1993; Reynolds, 1973). For spherical particles following Stokes' Law, the relationship between size and velocity follows a power function, which means that buoyant or sinking velocities should increase proportionally to the square of colony diameter (Reynolds, 1987). Given this expected relationship between size and velocity, a regression of log velocity versus log size should have a positive slope equal to 2 for spherical colonies of uniform density. Figure 2 shows that in Saginaw Bay the relationship between observed velocity and estimated colony size, was negative and the slope for both positively buoyant (mean slope = -0.73, CI -0.18 to -1.31) and sinking colonies (mean slope= -0.45, CI -0.18 to -0.70) was low and the fit of the prescribed relationship was weak for both sinking and floating colonies (R-squared = 0.03 and 0.06, respectively). The experiments from 2019 in Lake Erie showed weak positive relationships between estimated colony size and observed buoyant velocity (mean slope = 0.41, CI 0.08 to 0.24, R-squared = 0.29) and between estimated size and observed sinking velocity (mean slope= 0.42, CI 0.20 to 0.64, R-squared = 0.08, Figure 2). Figure 3 shows that apparent colony density was not correlated with estimated size, but the variance in apparent colony density decreased dramatically with estimated colony size in both lakes. This pattern was also evident in each experiment and within light intensity treatments.

3.4 Response of colony density to treatments in Saginaw Bay

Figure 4 shows that the mean apparent colony density in Saginaw Bay experiments was greater than that of water, consistent with the observation that those colonies tended to sink. In the July experiment from Saginaw Bay the mean apparent colony density was similar between large (>200 µm ESD) and small colonies, but in August the apparent colony density was consistently greater for small colonies than large colonies (ER> 4000).

3.5 Response of colony density in western Lake Erie

Unlike colonies from Saginaw Bay, large and small *Microcystis* colonies from western Lake Erie showed different responses to light intensity and time of day. Figure 4 (C, D) shows that small colonies were more responsive to light intensity in western Lake Erie. In morning, the difference in apparent colony density between the 10% and ambient light intensity treatments was 1.97 (July) and 0.36 kg m^{-3} (August) for large colonies, but the difference was 4.75 (July) and 2.32 kg m^{-3} (August) in the smaller colonies. The interaction among estimated colony size, light intensity treatment, and time of day also means that the difference in apparent colony density between large and small colonies depends on both the light intensity and duration. Under 10% ambient light intensity, small colonies had lower apparent colony density than large colonies in morning (ER >4000) and in the afternoon (ER= 500 in July, 5.5 in August). Under 30% light intensity, small colonies were less dense than large colonies in morning (ER=1330 in July, 2.4 in August) and more dense in the afternoon (ER= 2.2, 16.5). Those patterns were similar in the ambient light treatment where small colonies were less dense in the morning (ER= 50 in July, 2.5 in August) and more dense in the afternoon (ER= 7.9, 30).

4. Discussion

4.1 Effects of light intensity and time of day on buoyancy

Consistent with previous studies on buoyant movement by *Microcystis* colonies, this study provides support for the hypothesis that increased light intensity makes colonies less buoyant. All of the experiments from western Lake Erie were consistent with the observations of buoyant regulation from natural systems (Aparicio Medrano et al., 2013; Reynolds, 1973; Zhu et al., 2014b) and experiments (Konopka et al., 1987; Li et al., 2016; Thomas and Walsby, 1985). These short-term changes in buoyant velocities can likely be attributed to the accumulation of carbohydrates under higher light intensities,

384 contributing to a lower observed positively buoyant velocity or negative velocities (Kromkamp and Mur,
385 1984; Visser et al., 1997). However, the two experiments from Saginaw Bay do not support the
386 hypothesis that increasing light intensity causes *Microcystis* colonies to become less buoyant and,
387 moreover, do not suggest that increasing light intensity makes colonies sink more quickly.

388 ## 4.2 Relationship between buoyant observed velocity and size

389 Figure 2 shows that the slope of log observed colony velocity versus log size is smaller than
390 previous studies. Nakamura et al (1993) proposed that a slope of 1.5 is appropriate owing to the
391 presence of voids within 'floc-type' colonies. The slopes estimated for both western Lake Erie and
392 Saginaw Bay were less than 1, suggesting that the colonies likely have voids and structure that influence
393 their movement. The same regression model was fitted to 15 published datasets from other studies
394 (Supplementary Material Tables S3 and S4), which had a population-level mean slope of 1.03 (CI 0.77 to
395 1.29) for buoyant colonies and a mean slope of 0.77 for sinking colonies (CI 0.43 to 1.17). Moreover, the
396 slopes for those individual studies were higher than those observed in either western Lake Erie or
397 Saginaw Bay. The contradictory relationship between colony size and velocity in Saginaw Bay and the
398 weak relationship found in western Lake Erie both suggest that the density of the colonies may not be
399 independent of their size. A similar pattern was reported previously (Li et al., 2016) and could reflect
400 differences in colony density with size or differences in structure and shape of large versus small
401 colonies (Zhang et al., 2007; Zhu et al., 2014a). Regarding colony structure and shape, general
402 differences in colony morphology were observed between larger and smaller colonies. The larger
403 colonies observed often had large voids in their colony shape, whereas smaller colonies consisted of
404 cells more densely packed aggregates of cells (Figure 5).

405

406

4.3 Colony density and size

Apparent colony densities were not correlated with colony size (Figure 3). The decreased variance in density and size among large *Microcystis* colonies may be explained by the observation that small colonies are mostly packed aggregates of cells, whereas larger colonies tend to have voids among their mucilage and cells (Figure 5, Nakamura et al., 1993). Thus, larger colonies should have a colony density that is more similar to the density of water. A study by Li et al. (2016) found that density of buoyant *Microcystis wesenbergi* and *M. ichthyoblabe* colonies increased linearly with colony size, but that density of buoyant *M. aeruginosa* colonies was independent of size. The weak relationship between colony density and colony size was attributed to the increased fraction of intercellular space in larger colonies and also the influence of a relatively dense extracellular matrix (i.e., mucilage) in larger colonies. Li et al. (2016) hypothesized that decreasing colony density and increasing colony size are separate and non-exclusive means for *Microcystis* to regulate buoyancy and migrate vertically within the water column. While Figure 3 does not show that colony density changes monotonically with colony size, it does suggest that the apparent density of small versus large colonies could show differential responses to light intensity and time of day. The model by Visser et al (1997) predicts that while all colony sizes change their density in response to light intensity and duration, smaller colonies should exhibit more variable densities in response to light than large colonies. This prediction is partially supported by the patterns shown in Figure 4, although in the present study the largest colonies (> 200 µm ESD) exhibited little variation in apparent colony density.

4.4 Response of apparent colony density to light

The absence of discernible effects of light intensity on apparent colony density in Saginaw Bay is expected given the lack of consistent effects of light intensity on velocity (Figures 2, 4). Similarly, the greater mean apparent colony density in small colonies is expected from the relationship shown in Figure 2. However, colonies from western Lake Erie did vary apparent colony density in response to light

intensity and time of day, particularly among the smaller colonies. The pattern of greater density change in smaller colonies is consistent with the prediction from some models (Visser et al., 1997; Wallace and Hamilton, 1999) and has been suggested as a mechanism by which small colonies can avoid the damaging effect of high light intensities, whereas larger colonies are able to achieve sufficient velocities for migration using smaller changes in colony density (Xiao et al., 2012). A complementary explanation for this pattern is that larger colonies tend to have voids between the cells and mucilage (Li et al., 2016; Nakamura et al., 1993), which serves to minimize the effect of changes in cell density on colony density. While small versus large colonies showed different responses in density, the net effect in terms of vertical migration and vertical distribution of biomass depends on the combined effect of density and colony size (and also shape). Importantly, vertical divergence of colony sizes within the water column (Aparicio Medrano et al., 2013; Zhu et al., 2014b) can potentially lead to different colony density in small versus large colonies at the same time of day (Chien et al., 2013).

4.5 Differences in buoyancy behavior between Saginaw Bay and western Lake Erie

The experiments found dramatic differences in buoyancy behavior between the two lakes (Figures 1, 4). Although there has not been a systematic comparison of buoyancy characteristics in populations from different lakes, several environmental factors are known to play a role in regulating buoyancy in *Microcystis*. As described previously, light intensity and duration are predominant drivers of changes in buoyancy. While the lakes are at similar latitude and receive comparable incident radiation, the attenuation of light within the water was more rapid in western Lake Erie than in Saginaw Bay (Table 1). Phytoplankton living in a turbulent mixed layer experience a light exposure averaged over the mixed layer. For the mean PAR attenuation in Saginaw Bay (0.75 m^{-1}, Table 1), PAR averaged over a 5-m water column would be 26% of the surface value, compared to 11% for the same water column depth with the mean PAR attenuation for Lake Erie (1.9 m^{-1}, Table 1). Thus, the *Microcystis* colonies that were sampled from Saginaw Bay may have been acclimated to a greater light level than those sampled in Lake Erie.

Another important determinant of buoyant behavior is N and P limitation, which acts secondarily or interactively with light intensity (Brookes and Ganf, 2001; Chu et al., 2007; Konopka et al., 1987). Total P concentrations were consistently greater in the experiments from western Lake Erie than those from Saginaw Bay (Supplementary Material Table S1), while dissolved inorganic N was similar or greater in Saginaw Bay. The dissolved inorganic N:P ratio was several-fold higher in Saginaw Bay than western Lake Erie (Supplementary Material Table S1) and the seston C:P and N:P ratios in Saginaw Bay (Figure 6) suggest potential for strong P limitation (Guildford and Hecky, 2000; Healey and Hendzel, 1979). The lower P concentrations in Saginaw Bay could be responsible for decreased buoyancy, although further experimentation would be required to directly test that hypothesis. Temperature is also known to be important for buoyancy, with warmer temperatures favoring increased buoyancy (Thomas and Walsby, 1986; You et al., 2018). The lake water and incubation temperatures varied little between Saginaw Bay and western Lake Erie in 2019, but in 2016 two experiments were performed when the water temperature was less than 20ºC (Table 1). In those experiments (October 3 and 11, Fig 1) the colonies remained buoyant under each combination of light intensity and time of day, which suggests that temperature was still adequate for this behavior.

Another explanation for the difference in buoyancy behavior between the two lakes, and among different sizes of colonies within each lake, is physiological variation among *Microcystis* strains. The capacity to regulate buoyancy varies among *Microcystis* strains (Li et al., 2016; Xiao et al., 2012) and has a genetic basis (*gvp* gene) required for gas vesicle formation (Mlouka et al., 2004). Different strains can have different gas vesicle production characteristics that vary with light intensity; for example, strains that may not float at typical low lab culture light intensities may become buoyant at high light intensities, while floating strains may sink at high light intensities (Xiao et al. 2012). Because these blooms are not composed of a single strain (Meyer et al., 2017), further research will be necessary to determine whether those populations have different buoyancy responses.

4.6 Conclusions

The results of this study show that overall, *Microcystis* colonies from western Lake Erie were more positively buoyant than those from Saginaw Bay and increased in density in response to light, similar to previous experiments and models. The lack of a response to light in Saginaw Bay might be attributable to higher light intensities or strong P-limitation in that environment. These differences between *Microcystis* populations in the two lakes will likely affect how HABs are transported throughout each system by influencing their likelihood to manifest as floating scums on the surface of the lake, or settling to the bottom, during periods of calm weather. These differences also have important implications for the ability to monitor total HAB biomass from ocean color remote sensing methods, which can only detect surface concentrations. An unexpected finding from these experiments is that buoyant velocities were weakly correlated with colony size and that this was explained by greater plasticity of colony density in small colonies despite the same environmental conditions. This pattern is likely explained by the observation that smaller colonies tend to be more geometrically compact whereas larger colonies develop more complex shapes that include intercellular spaces.

Acknowledgments

This work was supported by the Great Lakes Restoration Initiative (GLRI) and the Cooperative Science and Monitoring Initiative (CSMI) through awards to NOAA GLERL. Support for P.A.D., S.H., and C.M.G. was provided through the NOAA Cooperative Agreement with the Cooperative Institute for Great Lakes Research (CIGLR) at the University of Michigan (NA17OAR4320152). We gratefully thank Tonghui Ming who aided in sample collection and buoyancy measurements in 2016, Russ Miller for help with improving the function of the motor-drive and joystick for camera positioning, David Fanslow for help with fabricating the sample column, and Timothy Maguire for his assistance within the R statistical

platform. This work is listed under GLERL contribution number: XXXX and CIGLR contribution number: XXXX (*Will request number at proof correction step*).

Data availability statement

The complete dataset and annotated R code are provided as supplementary materials.

Author contributions

H.A.V., M.D.R, and C.M.G. designed the experiments. H.A.V. and J.R.S. designed and configured the optics setup. P.A.D. and S.B.H. performed the experiments and analyses. All authors contributed to analyzing the data and writing the manuscript and have approved the final article.

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728 **Figures and Tables - Titles (bold) / Captions**

Collection date	Station	Station depth (m)	Surface water Temperature (°C)	Station PAR extinction coefficient (m^{-1})	PAR (µmol m^{-2} s^{-1})	
					Morning	Afternoon
8/29/16	WE4	8.7	25.7	1.22	197	865
9/6/16	WE8	5.1	24.8	2.54	182	1298
9/12/16	WE9	2.4	23.3	3.32	180	1474
9/19/16	WE13	8.9	23.0	1.39	86	1366
9/28/16	WE6	2.3	18.3	1.64	15	240
10/3/16	WE13	2.8	18.9	3.52	81	949
10/11/16	WE8	5.0	17.3	1.44	69	956
7/29/19	WE2	5.6	25.7	2.66	95	1933
8/12/19	WE2	5.8	24.8	1.69	39	1380
7/10/19	SB14	4.3	23.5	0.57	178	2186
8/6/19	SB14	4.3	24.2	0.92	154	2012

729 Table 1. **Summary of temperature and light data for experiments.** Station SB14 is in Saginaw Bay of
730 Lake Huron and stations WE2, WE4, WE8, WE9, and WE13 are in western Lake Erie. PAR is the mean of
731 measurements recorded over the 6 hours prior to each set of buoyancy measurements. Additional
732 chemical and biological parameters are provided in Appendix Table A1.

Figure 1. **Observed colony buoyant velocities by treatment.** Colony buoyant velocities in response to light intensity treatments during the morning and afternoon, for experiments performed in Saginaw Bay in 2019 (A,B), western Lake Erie in 2019 (C,D) and western Lake Erie in 2016 (E). Values above zero on the vertical axis indicate positively buoyant (floating) colonies and values below zero indicate negatively buoyant (sinking) colonies. Each color point represents a measurement for a single colony, the black markers and whiskers represent the mean and credible interval from the hierarchical linear model.

Figure 2. **Observed colony velocity as a function of colony size.** Log-log plot of colony buoyant and sinking velocities as a function of colony size, represented by (equivalent spherical diameter, ESD), for 2019 Saginaw Bay (A,B) and western Lake Erie (C,D) samples. Negative velocities for sinking colonies were converted to their absolute values to display the relationship on a log scale. Each regression model included a group-level intercept for each sampling date, but including group-level effects on the slope did not improve the information criterion and thus those terms were not included.

Figure 3. **Estimated apparent colony density as a function of colony size**. Size is represented by (equivalent spherical diameter, ESD) in 2019 Saginaw Bay (A) and western Lake Erie (B).

Figure 4. **Estimated apparent colony density by treatment.** Apparent colony density according to light intensity treatment, time of day, and colony size for experiments performed in Saginaw Bay (A,B) and Lake Erie (C,D). Points are the mean of the posterior draws from each model and the error bars denote the CI. The final model for colony density in Saginaw Bay did not include any interactions, but the model for western Lake Erie was improved by the inclusion of the interactions among light intensity treatment, time of day, and colony size. Horizontal lines represent the density of freshwater at the temperature for each experiment

754 Figure 5. ***Microcystis*** **colony microcinematography captures.** Microcinematography image capture of

755 *Microcystis* (circled in red) collected on July 30, 2019 from Lake Erie (A) and August 7, 2019 from

756 Saginaw Bay (B). Smaller colonies tend to have a more compact geometry whereas larger colonies

757 reveal more complex shapes with intercellular voids as they increase in size.

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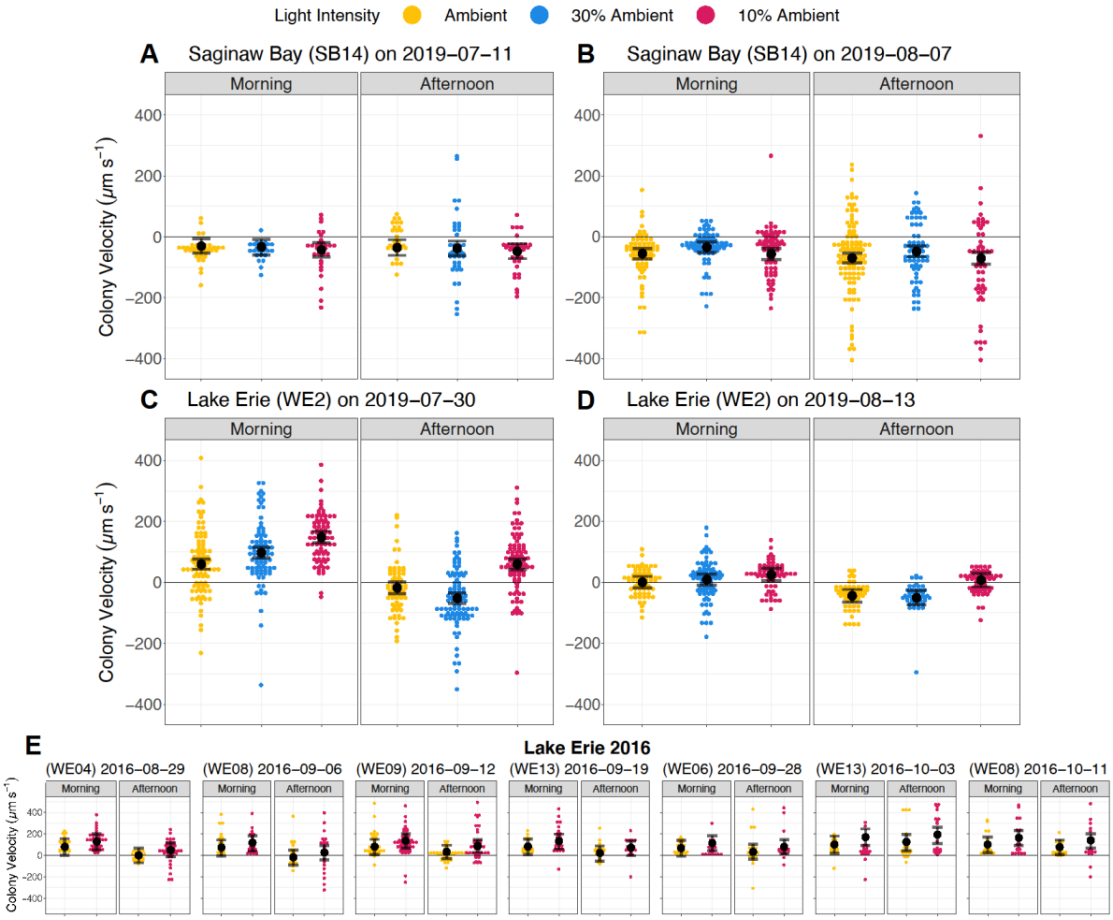
Figure 6. **Seston N:P and C:P stoichiometry in Lake Erie and Saginaw Bay.** Plot of seston N:P (A) and C:P (B) stoichiometry for samples collected from western Lake Erie (n=9) and Saginaw Bay (n=2). Data from Lake Erie are depicted as a box-whisker chart where the horizontal line denotes, the median, the ends of the box are the 25^{th} and 75^{th} percentiles, the whiskers are the 5^{th} and 95^{th} percentiles, and points represent outliers more than twice the interquartile distance from the median. Data from Saginaw Bay are illustrated as discrete measurements. The inset dashed lines are indicators of P limitation proposed by Healey and Hendzel 1979.

783 Figure1



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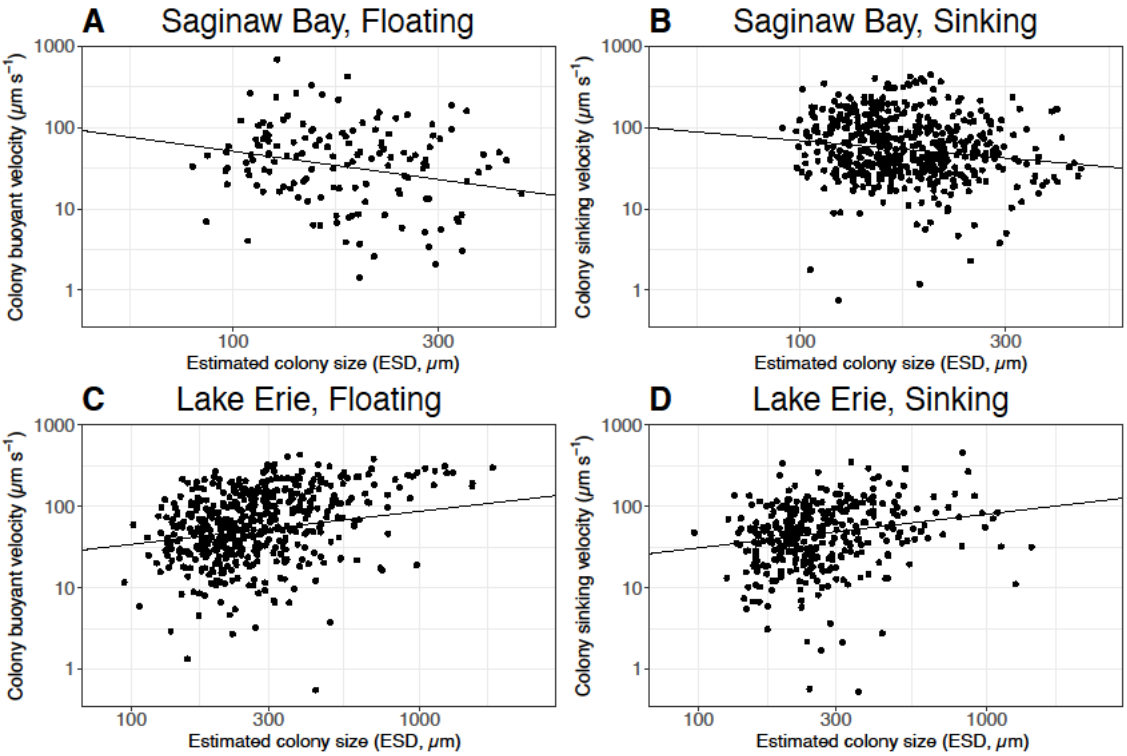
794 Figure2



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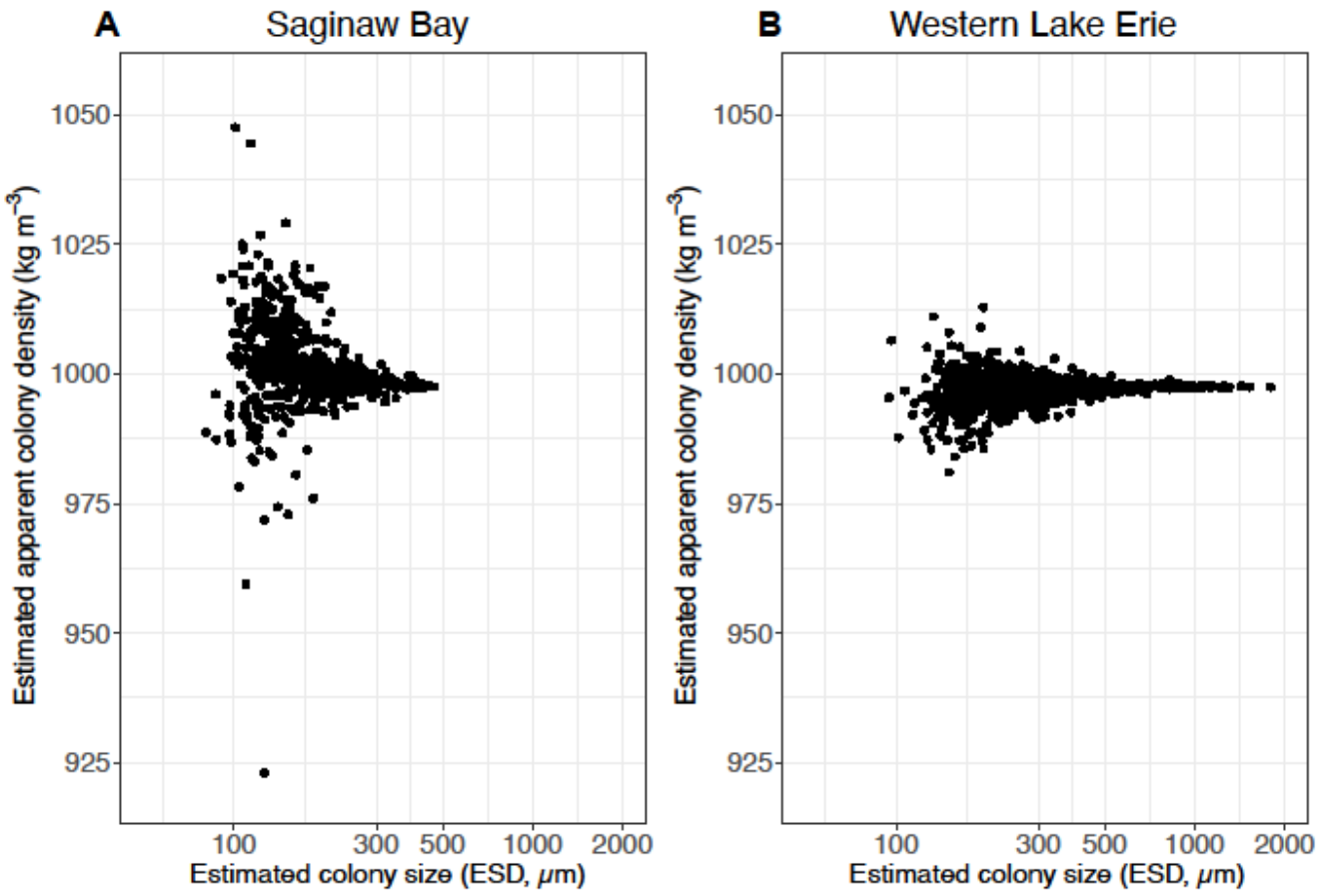

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820 Figure4

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832 Figure5

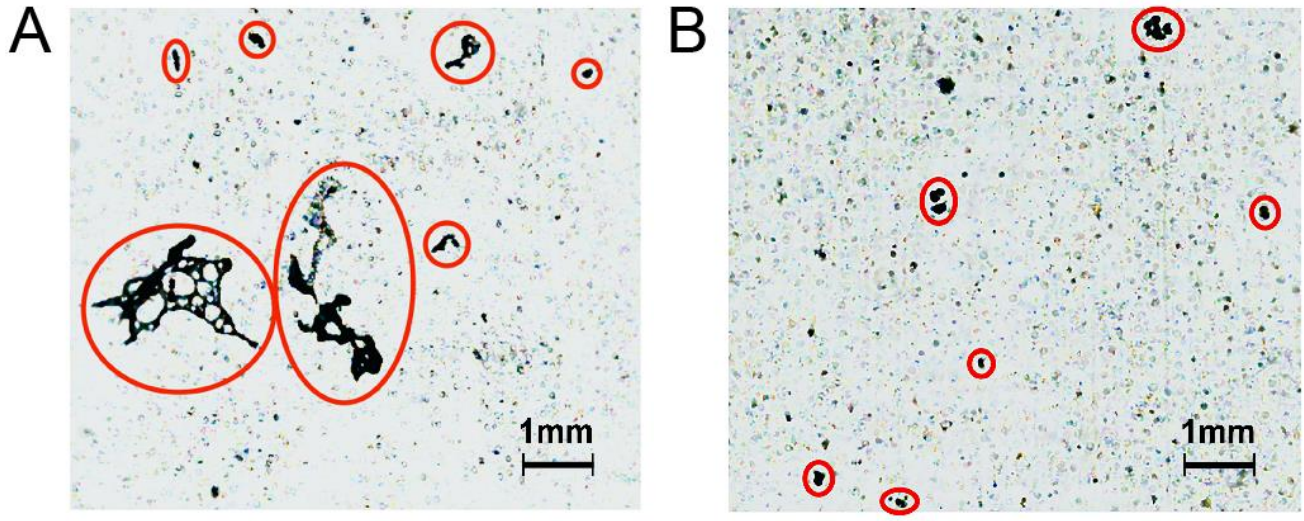

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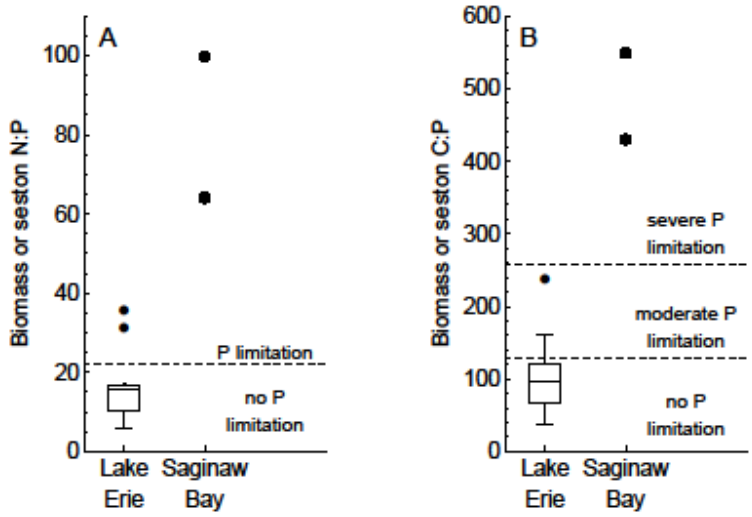
Figure6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Highlights:

- *Microcystis* colonies in western Lake Erie were mostly buoyant
- *Microcystis* colonies in Saginaw Bay were mostly sinking
- Buoyant and sinking velocities were weakly correlated with colony size
- Apparent colony density of small colonies was more variable and responsive to light