Notable FlowCam Studies on Cyanobacteria

Cyanobacterial blooms in the central basin of Lake Erie: Potentials for cyanotoxins and environmental drivers

Introduction | "Lake Erie western basin cyanobacterial blooms are a yearly summer occurrence; however, blooms have also been reported in the offshore waters of the central basin (CB), and very little is known about what drives these blooms or their potential for cyanobacterial toxins... The purpose of this research was to: 1) identify and quantify the early summer central basin cyanobacterial blooms, 2) determine likely environmental drivers of the central basin blooms... and 3) determine potential for cyanobacterial toxins of the blooms."

FlowCam Method | "Water for phytoplankton enumeration was poured into 0.5-L or 1.0-L glass bottles, preserved with Lugol's solution (1%), and kept dark... Phytoplankton from the Lugol's concentrated samples were identified and quantified with a FlowCam...FlowCam areal measurements were used as surrogates for cell counts... Biomass was determined by a FlowCam in the units of areal based diameter (μm2/mL)."

Results & Conclusions | "Field sampling from 2013 to 2017 identified *Dolichospermum* as the dominant cyanobacterium in the central basin during June and July... The two largest *Dolichospermum* blooms occurred in the past five years, and there has been an increase of *Microcystis* biomass in the central basin during the last ten years. *Dolichospermum* blooms occurred in the central basin of Lake Erie before the onset of the western basin *Microcystis* blooms indicating that separate environmental factors affect the central basin bloom dynamics.... The sxtA gene was present when *Dolichospermum* dominated the cyanobacterial community, and future research is needed to determine if *Dolichospermum* blooms are producing saxitoxins in the central basin."

Interactions between nitrogen form, loading rate, and light intensity on *Microcystis* and *Planktothrix* growth and microcystin production

Introduction | "The toxin-producing, bloom-forming cyanobacterial genera *Microcystis* and *Planktothrix* require fixed nitrogen (N), such as nitrate, ammonium, or organic N (e.g., urea) for growth and production of microcystins (MC)... This study examined how three forms of N (nitrate, ammonium, and urea) interacted with N loading rate...and light intensity to stimulate *Microcystis* and *Planktothrix* growth and MC production using nutrient enrichment experiments."

FlowCam Method | "Phytoplankton samples were analyzed with a FlowCam...The 100x [sic] objective was used to image particles greater than 10 μm in diameter. The 100x [sic] objective had a flow rate of 0.150 mL/min...The 200x [sic] objective was used to image particles 3–10 μm in diameter (i.e., single cells). The 200x [sic] objective had a flow rate of 0.020 mL/min...The sample volume analyzed and length of image collection time was dependent on the density of cells. Images were first classified using the Auto Classification function of VisualSpreadsheet and then manually classified to sort unclassified or wrongly classified phytoplankton... Area measurements were converted to biovolume by multiplying colony area by average cell diameter (e.g., for *Microcystis*), assuming a cylinder (e.g., for *Planktothrix*), or assuming a sphere (e.g., single cells)."

Results & Conclusions | "This study showed that cyanobacterial blooms could reach higher biomass and produce more MC as a response to both low continuous and large pulse N loading. There was also a significant effect found of N speciation, light intensity, and their interaction on the stimulation of cyanobacteria growth and mcy gene expression. Thus, all forms of N and the interaction between N and water turbidity (light intensity) must be accounted for in management scenarios."

Impacts of the 2014 severe drought on the Microcystis bloom in San Francisco Estuary

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Introduction | "Although some data are available on the variation of *Microcystis* blooms with wet and dry conditions in San Francisco Estuary (SFE), no data are available on the impact of severe drought conditions on the amplitude, toxin content, duration or causal factors associated with *Microcystis* blooms… The purpose of this study was to characterize the amplitude, species composition and toxin concentration of the *Microcystis* bloom in SFE and its association with environmental conditions during the severe drought of 2014."

FlowCam Method | "A field sampling program was conducted between July and December 2014…The biovolume of *Microcystis* colonies was computed using area-based diameter with a FlowCam digital imaging flow cytometer…Cell abundance estimates based on FlowCam measurements were closely correlated with those determined by microscopic analyses (r = 0.88, p < 0.01) …= 0.88, p < 0.01). Whole water samples collected from 0.3 m depth were used to determine phytoplankton and cyanobacteria biovolume and taxonomic composition (>10 mm size fraction) in sub-surface water. These samples were kept at 4 C and processed live within 1 to 3 h with a FlowCam. The FlowCam was fitted with a fluorescence trigger to isolate live phytoplankton from detritus."

Results & Conclusions | "*Microcystis* biomass in 2014 reached record levels and greatly expanded the range of bloom conditions in SFE…The 2014 severe drought study suggested anticipated future increases in the frequency and intensity of drought in SFE will lead to an increase in the magnitude, duration, diversity and toxic potential of *Microcystis* blooms in SFE."

Vertical distribution of buoyant *Microcystis* bloom in a Langrangian particle tracking model for short-term forecasts in Lake Erie

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Introduction | "Existing forecast models give the present location and extent of cyanobacterial harmful algal blooms (CHABS) from satellite imagery, then predict two-dimensional (surface) CHAB movement in response to meteorology…In this study, we simulated vertical distribution of buoyant *Microcystis* colonies, and 3-D advection, using a Lagrangian particle model forced by currents and turbulent diffusivity from the Finite Volume Community Ocean Model."

FlowCam Method | "We measured *Microcystis* colony diameter of Lugol preserved samples collected from western Lake Erie in the summers of 2012, 2013, and 2014. In 2012 and 2013, colony diameters were measured by microscopy. In 2014, we used the FlowCam. The FlowCam captures images of individual colonies… *Wang et al.* [2015] showed that counts and colony diameters of *Microcystis* given by FlowCam and microscopic image analysis diameters were nearly identical…Samples were diluted in 0.2 mm filtered algal culture media [e.g., *Vanderploeg, et al.*, 2001] and injected into the FlowCam with a 60 mL syringe, which was constantly turned over so as to prevent the buoyant colonies from aggregating in the syringe. The image analysis algorithm was calibrated to identify the colony outline including the mucilage."

Results & Conclusions | "The 3-D model is initialized with a better estimate of total biomass than the 2-D model because an estimate of the surface mixed layer depth for buoyant *Microcystis* colonies is used to assign the depth over which the satellite-derived surface concentration is applied. In addition, the 3-D model is able to simulate changing surface concentration in response to changing mixed layer depth. Finally, the 3-D model produced different final CHAB spatial distribution than the 2-D model, which likely results from the more accurate vertical distribution within a complex 3-D flow field."

High-resolution imaging particle analysis of freshwater cyanobacterial blooms

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Introduction | "Here, we report on the development of a FlowCam-based method for providing high taxonomic resolution and accurate cell count data in a timely manner (e.g. 50 samples enumerated per week) to the Alberta Health Services cyanobacterial monitoring network of 50-60 lake beaches across the province … We compared total cyanobacterial and finer taxonomic cell counts generated through the use of a FlowCam vs. a standard light microscopy method."

FlowCam Method | "All taxonomic analyses using both the FlowCam and light microscope were performed by the same taxonomist (M.D. Graham)." First, Graham performed an "initial [sample] inspection using standard light microscopy… to qualitatively determine the general taxonomic composition [and maximum particle size to inform flow cell and objective selection]… Then, samples were taxonomically enumerated [using] a modified Utermöhl technique … [The FlowCam's] 50-μm deep flow cell with the x20 objective maximize[d] taxonomic resolution [but] was only feasible [for] cells less than 50 μm in diameter [and] require[d] longer run times…

"We did not dilute or size fractionate via sieving dense samples to avoid both time consuming additional processing of samples and potential introduction of handling error... [W]e occasionally observed cyanobacterial cells adhering to ... the flow cell [which we remedied using a Lugol's solution]... Thereafter, each Lugol's preserved sample passed through a 50-µm deep flow cell at a flow rate of 0.02 mL min-1 and analyzed using the x20 objective... Concordance between light microscopy- and FlowCam-based cyanobacterial cell counts was determined using correlation and correspondence analyses."

Results & Conclusions | "In general, use of the FlowCam equipped with a x20 objective [and 50 µm flow cell] enabled taxonomic identification to the genus- and often species-level, thereby equating approximately to light microscopy performed using a x63 objective... [The FlowCam with a x20 objective] also enabled detection of smaller picocyanobacteria (2-5 µm diameters).

"Total [FlowCam] cyanobacterial cell counts for live and preserved water samples... showed significant positive correlation.... Lugol's preserved samples tended to produce higher estimates of cyanobacterial abundance [relative to live samples].

"The x20 FlowCam objective "resulted in cell density estimates... four times higher than the ×10 objective...

"Better image resolution... helped maximize both detection and taxonomical identification of cyanobacteria... [H]igher total cell counts and greater species richness were mainly attributable to detection of... smaller genera measuring less than 5 µm in cell diameter, such as *Cyanodictyon*, *Merismopedia*, and *Synechococcus*.

"Total cyanobacterial cell counts based on light micrscopy vs. the FlowCam showed significant positive correlation... [as did] total cell counts for major cyanobacterial taxa (*Anabaena spp.*, *Microcystis spp.*, and *Aphanizomenon spp.*)... [and] *Microcystis*." By comparison, "total cell counts for *Aphanizomenon* were often underestimated [with the] FlowCam [Figure 5]... [T]otal cyanobacterial and species-level cell counts performed using either FlowCam or light microscopy showed strong agreement, attesting to the reliability of cyanobacterial enumerations derived from the relatively less time-consuming digital flow cytometry approach.

"Unfortunately, accurate species-level identification... using AutoClassification in VisualSpreadsheet... remains a work in progress because of several challenging factors, including their relatively small cell sizes, polymorphism, and lack of taxonomically diagnostic sexual reproductive structures... [I]nterference from multiple co-occurring cyanobacteria within a sample can also hamper detection and enumeration of certain taxa... [W]e strongly believe that complementary use of light microscopy is essential to QA/QC assurance when using a FlowCam for... enumeration of cyanobacterial cells within a water sample...Nevertheless,... FlowCam

greatly relieves operator fatigue,... facilitating turnaround times and greater throughput of sample enumerations [e.g. 50 samples per week vs. 15]... Most importantly, our comparative investigation... highlights that use of a FlowCam can facilitate intensive lake monitoring by providing the data to end users in an equally reliable, yet more timely manner."
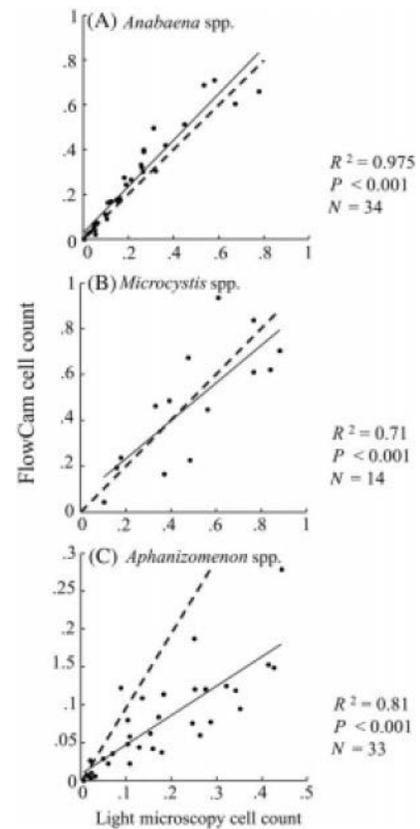
Fig. 5. Comparisons of total *Anabaena* spp. (**A**), *Microcystis* spp. (**B**), and *Aphanizomenon* spp. (**C**) densities (X 105 cells/mL) obtained with light microscopy (*x*-axis) vs. the density obtained with FlowCam (*y*-axis). All samples were preserved with 5% Lugol's solution. Solid line represents the best-line line while the dashed line depicts the 1 : 1 relationship.
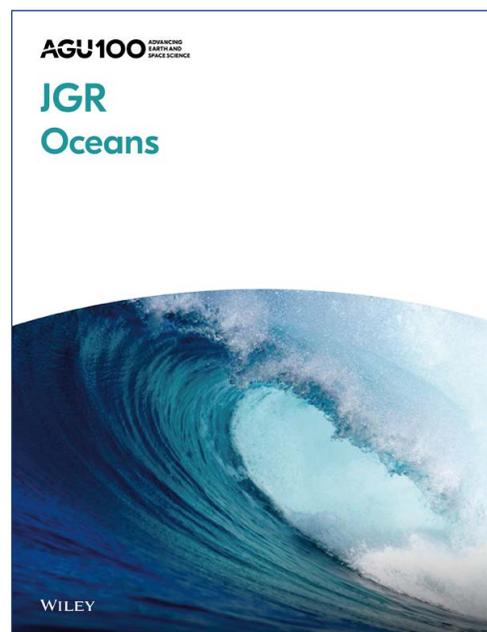
Species diversity of resident green algae slows the establishment and proliferation of the cyanobacterium *Microcystis aeruginosa*

Introduction | "There have been increased efforts to predict when and why blooms occur, as well as to identify the ecological factors that limit their formation...Here we report the results of a laboratory experiment in which we examined how the establishment and proliferation of *Microcystis aeruginosa*, a common HAB species, was influenced by competition with resident green algae."

FlowCam Method | "Biovolumes for each species were determined for each of the algal stocks by imaging ≥70 individual cells using a FlowCam, and then using the area by diameter method to estimate cell volumes..."

Results & Conclusions | "We found that competition with resident green algae did prevent *Microcystis* from dominating the communities despite *Microcystis* being typically thought of as a strong competitor. However, we found that the diversity of the resident community was only important in the high (nitrogen) & (phosphorus) environment. In treatments where we manipulated (nitrogen) or (phosphorous) to be scarce...all species of green algae strongly competed with *Microcystis* so there was no added effect of a diverse community of green algae."