A Review of the Nanobubble Pilot Project in Constitution Gardens Lake

Prepared for:
National Park Service
U.S. Department of Interior
Washington D.C.

Prepared by:

Center for Coastal and Watershed Studies
Hood College
401 Rosemont Ave.
Frederick, MD 21701

Kevin Sellner, Senior Scholar
Drew Ferrier, Center Director and Professor of Biology

April 2020

HOOD COLLEGE
Center for Coastal and Watershed Studies

This report is CCWS Contribution #20-09 of Hood College's Center for Coastal and Watershed Studies, 401 Rosemont Avenue, Frederick, MD. The report should be cited as:

Sellner, K. and D. Ferrier. 2020. A Review of the Nanobubble Pilot Project in the Constitution Gardens Lake. April 2020. CCWS Contribution #20-09, Center for Coastal and Watershed Studies, Hood College, Frederick, MD. 10 pp.

Cover Photo: Blue Nano Technologies, LLC Generation Unit during treatment of Constitution Gardens, Washington D.C. , November 2019

Abstract

The Constitution Gardens Lake on the Washington, D.C. Mall is dominated by filamentous and planktonic algae and cyanobacteria for most of the summer months. In order to ascertain whether dispersal of ozone-rich nanobubbles into the lake would alter lake water quality and the distribution of algae and cyanobacteria in the system, the National Park Service and Blue Nano Technologies, LLC (BNT) agreed to conduct a pilot project in November, 2019. BNT staff initiated work on November 1, 2019 with ozone generated bubble dispersal continuing over an 8 d period. Water quality sampling was conducted before bubbling and additional discrete measurements of several parameters were made 1, 12, and 33 d after bubbling ceased. Other parameters were measured continuously during the period of bubble dispersal.

Several water quality parameters increased over the bubbling period, specifically dissolved oxygen (DO) and relative fluorescences of both chlorophyll (CHL) and phycocyanin (PC), the latter an accessory pigment of the cyanobacteria. The apparent increase in PC was likely not an increase in the cyanobacteria, however, as the ratio of PC/CHL remained constant over the entire period. This is indicative of the overlap in emission fluorescence from chlorophyll in the phycocyanin channel of the fluorometer. This interpretation was supported by Center for Coastal and Watershed Studies staff microscopy which found no detectable cyanobacteria populations immediately before or 24 days after bubbling.

DO reached approximately 20 mg/L on day 4 and then leveled off throughout the rest of the bubbling period. BOD_5 and COD increased to maximum concentrations 33 days post-bubbling. The Oxidation Reduction Potential (ORP) in the lake only increased slightly through time, at approximately 2.5 mV/d. Discrete measurements of nutrients over the 38 d period suggested that total nitrogen (TN) and total suspended solids (TSS) declined substantially, from approximately 56 and 1263 mg/L, respectively to approximately 6 and <37 mg/L in December, although the absence of post-treatment replicates prevent establishing statistically significant declines.

The reduction in TN was accompanied by an increase in nitrate+nitrite (NO_{23}), from 0.025 mg/L to 0.063 mg/L 12 d after treatment ceased. Ammonium declined from 2.79 to 0.178 mg/L in that same period, only to substantially increase in December to approximately 5.3 mg/L.

Total phosphorus (TP) had a similar 10-fold reduction, from 4.5 to 0.34 mg/L 12 d after treatment and then to 0.2 mg/L five weeks post-treatment. Ortho-phosphate phosphorus almost doubled 12 d after bubbling ceased, from 0.16 to 0.3 mg/L.

Overall, it is difficult to ascertain whether this nanobubble treatment of Constitution Lake meaningfully improves water quality or contributes to the decline in algae or cyanobacteria biomass. Although TN, TP, and TSS all declined, dissolved pools of nitrogen and phosphorus increased. Dissolved N and P compounds support algal and cyanobacterial growth, as evidenced, in part, by the increasing chlorophyll concentrations during the 8-day nanobubbling period.

Introduction

There are numerous mitigation methods that have been proposed to reduce blooms of algae and cyanobacteria that now afflict most areas of the world. Cyanobacteria (also referred to as blue-green algae) are increasing in most nutrient-rich freshwater areas of our region, from planktonic taxa in lakes within Frederick and Montgomery Counties in Maryland, to the attached bottom cyanobacteria of the non-tidal Potomac River, each threatening human, domestic animal, and wildlife health. Constitution Gardens Lake on the Washington Mall is no exception. It has experienced these blooms for multiple years, with several methods attempted for their control but usually ineffective to reduce or eliminate these accumulations of surface scum.

Recent technological advances in nanobubble (bubbles typically about 100 nm in diameter) formation and their use in increasing dissolved oxygen concentrations in/near bottom sediments and the water column have been promoted as another strategy that might also reduce bloom biomass during their use. There are several short summaries of the effectiveness of this size nanobubble in water quality improvements in a few Asian water bodies (e.g., AKC 2018) as well as several ponds in the southeastern US (e.g., BNT 2019), with reductions in phytoplankton chlorophyll and increasing water clarity as the primary results. However, none of these reports have employed experimental controls, been replicated, or undergone peer review. Likewise, nanobubble control of cyanobacteria populations/blooms remains undocumented at this time.

The National Park Service (NPS) administers and manages the Washington, D.C. Mall lakes, ponds, and fountains and, in this role, invited BNT to intervene in Constitution Gardens Lake to reduce cyanobacteria accumulations. A pilot study was to be initiated in late summer during high cyanobacteria scum formation, but unfortunately due to paperwork delays, the study could not be initiated until November 1, 2019 when lake cyanobacteria were drastically reduced or absent. This report provides a summary of possible nanobubble-induced changes in lake water quality but considering 1) fall temperatures were already limiting cyanobacteria growth and 2) there was no nanobubble-free lake to serve as a control, the results can only suggest some speculative conclusions on the efficacy of nanobubbles in lake improvements.

Methods

BNT staff set up their nanobubble generating unit and dispersal hoses at the Constitution Gardens Lake at the end of October 2019. The bubble generator dispersed ozone-rich nanobubbles into the lake from November 1 until November 9, 2019. Lake water was drawn from a depth 14” off-bottom into the nanobubble generator, passed over the ceramic ozone dispersing frit array, and shear-induced nanobubble formation was created as the flow of water carried the nanobubbles out into the lake through PVC hoses.

Prior to bubble generation and 1, 12, and 33 d after bubbling ceased, grab samples were collected at 4 stations on the lake’s periphery, at northwest, northeast, southeast, and southwest corners of the lake. Pre-treatment samples were collected by BNT at each station from near-bottom, mid-depth, and near-surface and then the three depths were composited. However, post-treatment samples on days 1, 12, and 33 were taken by BNT at the same four station but all were mistakenly composited to produce a single sample (i.e., there was no replication for post-treatment grab samples on each sampling date.) BNT was responsible for the analyses of these samples. All samples were sent to Florida laboratories for measurements of nutrients (total nitrogen, TN; total Kjeldahl nitrogen, TKN; nitrate, nitrite, ammonium; total phosphorus, TP; ortho-phosphate), total organic carbon (TOC), total suspended solids (TSS), BOD_5 and COD, and phytoplankton pigments chlorophyll and phycocyanin.

Additionally, during nanobubbling a YSI EXO2 sensor package was used to make hourly measurements of dissolved oxygen (DO), air and water temperature, oxidation reduction potential (ORP), factory-calibrated chlorophyll and phycocyanin levels, and pH. Aperiodic measurements of turbidity were also made with the same system. For this report, sensor-derived pigment levels are reported as relative fluorescence units (RFU) as professional experience often finds dramatic differences between factory-assigned fluorometer pigment levels and those detected in laboratory analyses.

Results and Discussion

Hourly Distribution of Lake Water Quality

The introduction of ozone-enriched nanobubbles increased DO in the lake, from initial concentrations approximating 6.6-8.1 mg/L to approximately 20 mg/L by day 4 of bubbling (Fig. 1). The large increase in DO was, however, only minimally complemented by an increase in ORP (Fig. 2). This is surprising as ozone should result in substantial increases in free radicals of oxygen (reactive oxygen species, ROS). The free radicals of oxygen are important as it is these small molecules that should be most effective in reductions of cyanobacteria since this group of organisms do not have the enzymes to protect themselves from the strong oxidation posed by such radicals (see Matthijs et al. 2016). As well, non-cyanobacteria (eucaryotes) that make up the remainder of the lake's phytoplankton (mostly chlorophytes, CCWS unpublished data) have the enzymes required for protection and hence this may explain why chlorophyll increased through time (Fig. 3) by using the nitrogen and phosphorus possibly released from the breakdown of TN and TP pools during bubbling (discussed below in Long-term Water Quality Changes section).

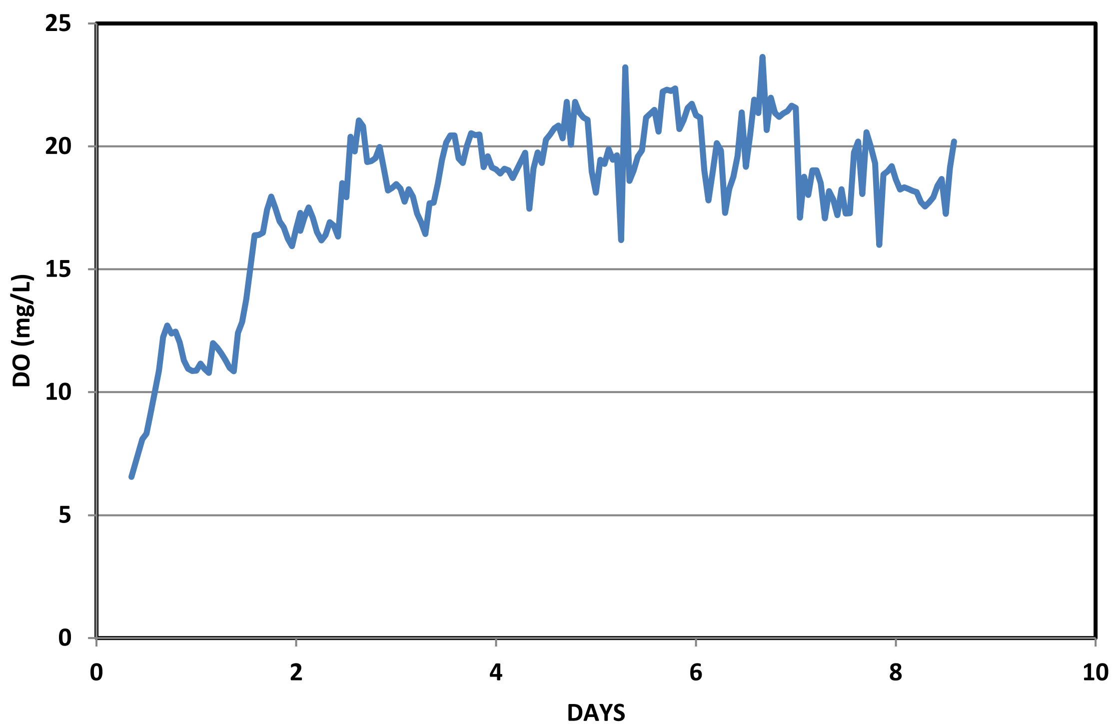

Figure 1: Changes in DO (mg/L) over the 8 d nanobubbling period in Constitution Gardens Lake, 2019

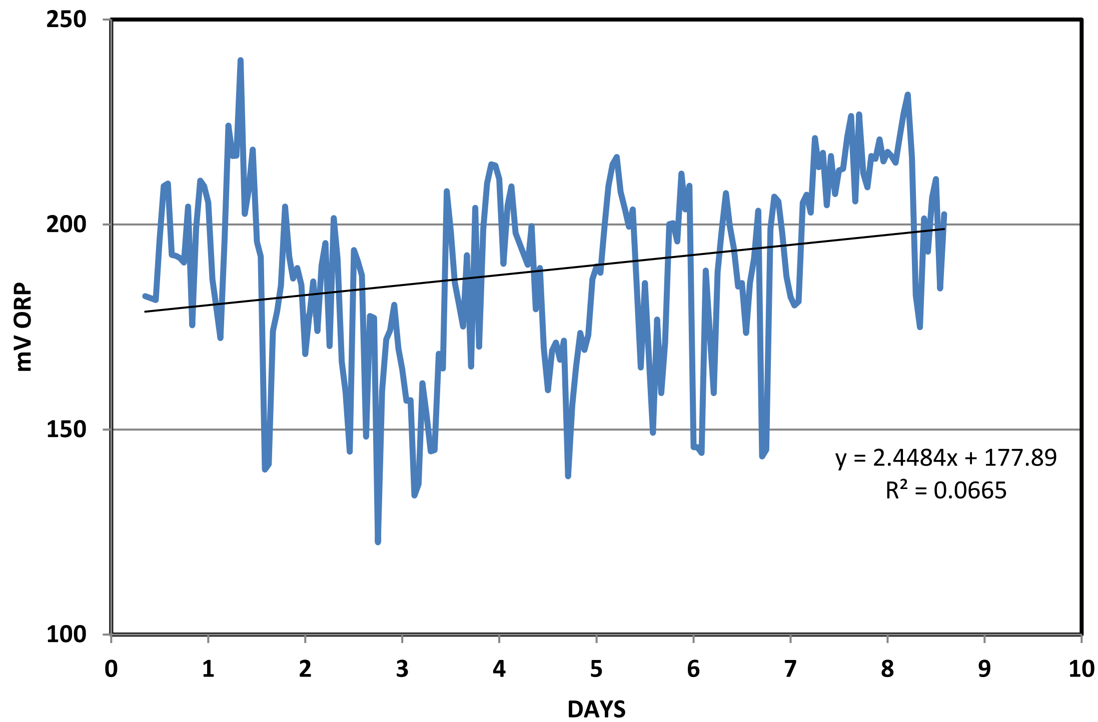

Figure 2: Changes in ORP (mV) over the 8 d nanobubbling period in Constitution Gardens Lake, 2019.

Phycocyanin fluorescence (PC RFU) measured hourly with the YSI EXO2 sensor system (data not shown) followed an identical pattern noted for CHL RFU. This constant relationship can be ascertained by examining the PC RFU/CHL RFU ratio which, over the 8 day period, remained constant at 0.07±0.00, suggesting that the contribution PC RFU readings were not actually from cyanobacteria but were an artifact of light emitted in exciting chlorophyll within eukaryote cells and detected on the PC emission channel of the fluorometer. CCWS staff have found similar PC artifacts with their fluorometers using a chlorophyll-rich cultured eukaryote, *Chlorella sp.,* that contains no phycocyanin.

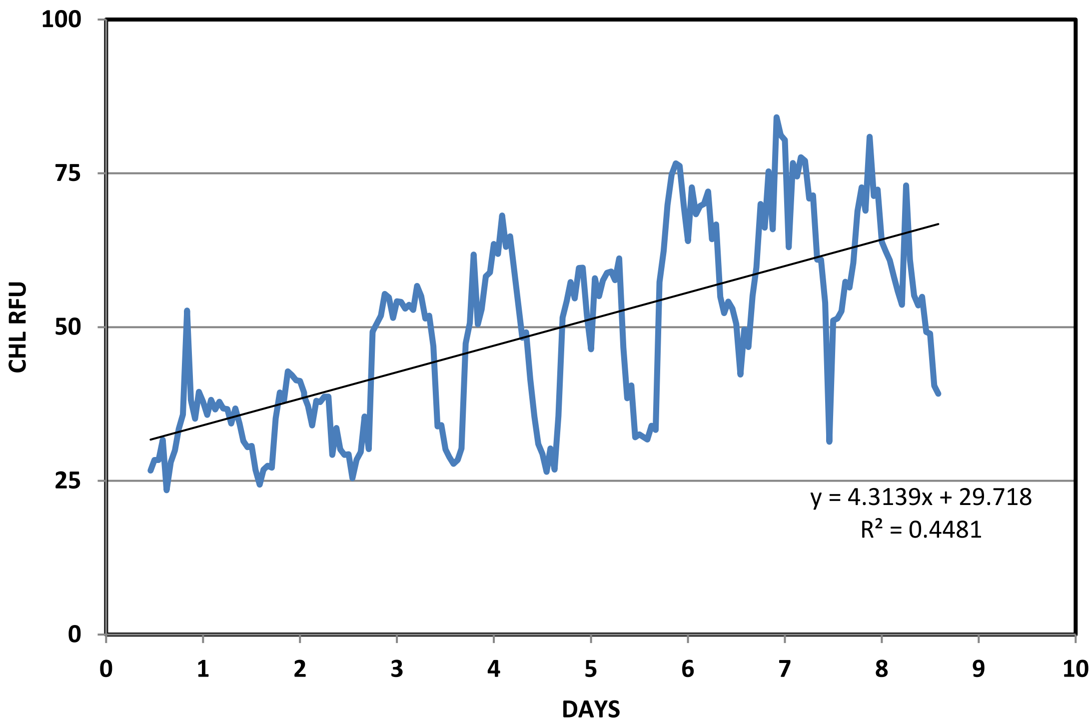

Figure 3: Change in CHL RFU during the 8 d nanobubbling period in Constitution Gardens Lake, 2019.

Long-term Water Quality Changes
Laboratory analyzed samples from pre-bubbling and 1, 12, and 33 days after bubbling revealed several longer term patterns potentially attributed to the generation of persistent nanobubbles following 8 days of treatment (Table 1). Pre-treatment levels of TN and TP concentrations were high, approximating 56.1 and 4.54 mg/L, respectively, (Table 1), indicative of eutrophic conditions. Other water quality parameters were also elevated (Table 1), including TSS (1263 mg/L), ammonium (2.79 mg/L), and TOC (24.5 mg/L). Average (±se) chlorophyll and phycocyanin content approximated 27.0±3.9 and 14.3±3.3 µg/L, respectively.

Table 1. Constitution Gardens Lake water quality through time, November 1 – December 12, 2019. Nanobubbles were dispersed for 9 days, November 1-9 with discrete samples collected 1, 12, and 33 d after bubbling ceased. Concentrations (mg/L for all nutrients, RFU or µg/L for pigments) for 11/1/19 represent the mean ± se (standard error) of samples from 4 stations around the lake. Concentrations for the 3 later dates represent levels for a composite sample from the 4 stations around the lake.

DATE	TN	NH_4^+	NO_{23}	TP	Ortho-P	TSS	TOC	CHL	PC	PC/CHL
11/1/19	56.1	2.79	0.025	4.54	0.17	1263	24.5	27.0	14.3	0.52
Pre-Trt	±20.1	±0.23	±0.005	±1.84	±0.13	±168	±1.9	±3.9	±3.3	
11/10/19	5.8	0.53	0.058	0.34	0.01*	36.7	22.1	75.2	8.9	0.11
11/21/19	6.2	0.18	0.625	0.33	0.3	28.5	23.8	28.3	13.7	0.48
12/12/19	6.8	5.28**	0.455	0.20	0.1	39.3	25.1	17.2	41.7	2.42

*Estimated
** Increased ammonium levels on 12/12/20 may be attributable to Canada geese use of the lake during early December (Personal communication, from NPS via NBT)

Nanobubbling appears to have substantially reduced TN and TP concentrations up to 33 days after bubbling had ceased although whether the reductions are significant cannot be determined due to a change in compositing methods from pre- to post-treatment sampling that prevented a statistical comparison of concentrations observed. However, the magnitudes of the declines suggest appreciable nanobubble effects on TN (56 to approximately 6 mg/L), ammonium (2.79 to 0.18 mg/L on 11/21), TP (4.54 to 0.20 mg/L), and TSS (1263 to ~24 mg/L), whereas changes in TOC and the pigments were equivocal. The large declines in TN and ammonium were countered by increases in dissolved nitrogen as nitrate+nitrite (0.025 to 0.455 mg/L) which could be a result of oxidation and breakdown of organic nitrogen in the TN pool or nitrification (oxidation of ammonium to the oxidized nitrogen compounds). A similar pattern, an increase in dissolved ortho-phosphate from the breakdown of TP, could not be ascertained due to loss of the 1-day post-treatment sample and the absence of replication.

BOD_5 (5-day biological oxygen demand) and COD (chemical oxygen demand) increased over the entire period (Fig. 4) indicating increasing quantities of oxidizable organic matter through time even though TOC concentrations (23.9±0.6 mg/L) remained remarkably stable.

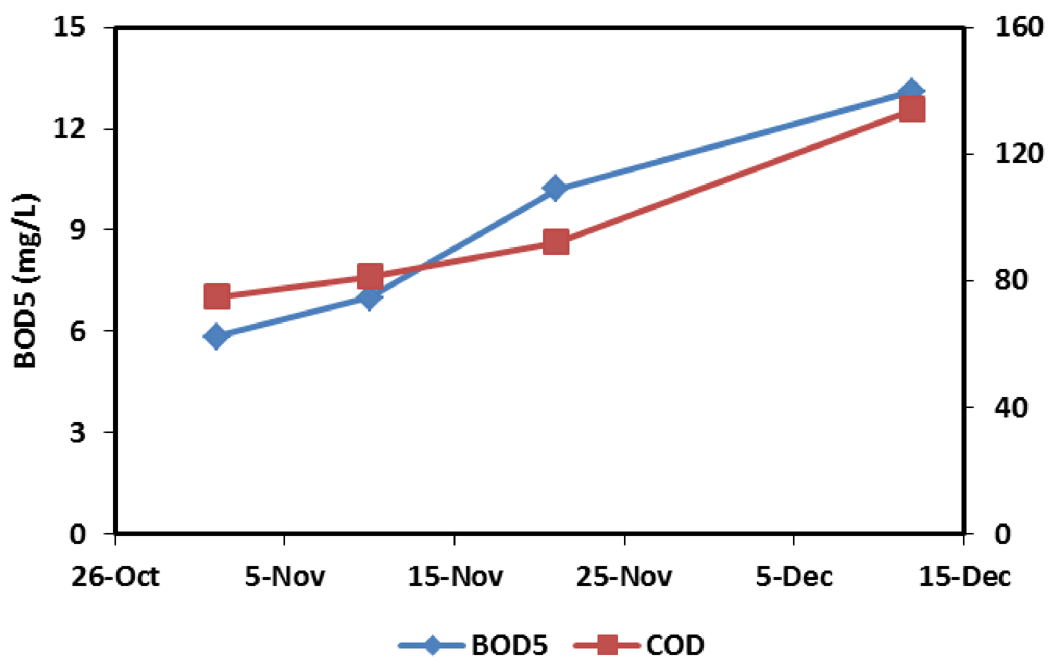

Figure 4: Pre- to post-bubbling changes in BOD5 and COD in Constitution Gardens Lake in November-December, 2019.

A comparison of results from hourly YSI EXO2 sensor measurements and laboratory-analyzed grab samples poses some interpretation problems and warrants discussion here for future use of the two approaches. First, static measurements of CHL RFU and PC RFU at 4 stations around the lake approximately 0.5 h before nanobubbling initiation resulted in mean concentrations of 80.7±11.9 CHL RFU and 9.2±1.6 PC RFU (YSI would contend that these are actual pigment concentrations, i.e., µg/L). Thirty minutes later with bubbling commencing, CHL RFU and PC RFU were noted at 26.7 CHL RFU and 2.21 PC RFU, respectively. It is unlikely that phytoplankton pigment fluorescences or cell biomass declined 3-4 fold in one-half hour. Might this represent algae and cyanobacteria patchiness across the lake as the range in the 4 station values was large, from 47-101? Another explanation is that the 4 measured concentrations are each composites pooled across three depths, immediately above the bottom, mid-depth, and near surface while the lower fluorescences found for the continuous sensor data were from an intake at one specific depth. An additional explanation might be that the near-bottom collections at the 4 stations resuspended bottom pigment-rich settled algae and cyanobacteria leading to the higher composite pigment levels; no such resuspension would occur with the ozone generator intake. For future work, 1) sensor and grab samples for laboratory determination of pigment levels should always occur simultaneously at the intake. And 2) prior to, during, and at several times post-bubbling, similar simultaneous pigment measurements (sensor and lab) should be made at multiple locations within a water body with sample number dependent on the visible heterogeneity in surface color or turbidity as well as water body size: the more 'patchy' the distributions of color or 'scum' and the larger the water body, the more dual sampling should occur. This might enable quantified assessments of lake- or pond-wide effects of nanobubbling the system. Without adequate spatial coverage of sensor and laboratory-derived pigment concentrations, detecting nanobubble technology efficacy for algae or cyanobacteria control will be difficult.

Second, diel periodicity in intracellular pigment fluorescence is well known (e.g., Owens et al. 1980, Brand 1982) and evident in the daily up-and-down patterns of CHL RFU obvious in Figure 3. Hence, comparing changes in fluorescence between successive days should only be done at the same hour to normalize these diel changes in intracellular pigment fluorescence (i.e., 10 AM of one day with 10 AM of another day).

Third, absolute concentrations of pigments determined in laboratory analyses reveal very different patterns than noted with sensor measurements in the lake. For example, as noted above, PC RFU/CHL RFU from the YSI EXO2 sensor approximated 0.07 throughout the bubbling period whereas the ratio of actual pigments for the 1.5 month period was widely variable, from 0.11 one day post-bubbling (close to the 0.07 fluorescence ratio) to 0.52 and 2.42 prior to and 1.5 months after bubbling, respectively (Table 1, last column). These later higher ratios imply substantial amounts of cyanobacteria, unlikely but possible at the colder November (57^{o}F) and December temperatures (44^{o}F); further, CCWS December samples contained no visible cyanobacteria and only chlorophytes (green algae; CCWS unpublished data). It is worth noting, however, that the laboratory-derived chlorophyll concentration of 75.2 µg/L one day post-bubbling (Table 1) would be the approximate pigment concentration derived from the chlorophyll fluorescence regression in Figure 3 for day 10, i.e., 72.8 µg/L. One possible remedy for deciphering whether the longer-term changes in laboratory-determined pigment concentrations (CHL and PC columns, Table 1) can be explained is to determine species composition and abundances via microscopy. This would entail examining a small sub-sample of the grab sample used for pigment analysis under a microscope, an added technique to be considered in future projects to verify cyanobacteria susceptibility.

In contrast to what one would expect with air bubbles visible with the naked eye, 100 nm bubbles sink to the bottom and hence aerate bottom sediments, turning anaerobic muds to aerobic habitat (AKC Unknown, 2013, 2017). That shift in distribution reportedly allows oxygen release for weeks to months, thereby providing fairly long-term re-aeration capacity to nanobubbled systems. This suggestion might be supported in the lake, at least for the 1.5 months of the study, in that December DO concentrations averaged 11.2 mg/L for 5 of 6 stations routinely sampled in a CCWS project, a dramatic increase from the very low levels (5.3 mg/L) seen on October 29th (Sellner and Ferrier 2020) as well as during the 8-day nanobubbling period when levels exceeded 20 mg/L mid-way through the treatment (Fig. 1).

However, with a December water temperature of 44°F, about 12.5 mg DO/L would be expected at full saturation so whether nanobubbling or normal winter re-oxygenation was responsible for the high concentration cannot be determined.

Summary

It appears that dispersing 100 nm ozone-rich bubbles into Constitution Gardens Lake had several positive effects, including rapid increases in concentrations of dissolved oxygen (to >20 mg/L) and lower levels of TN, ammonium, TP, and TSS. Pools of dissolved nitrate+nitrite increased, however, providing potentially rapidly assimilable nitrogen for algae and cyanobacteria re-growth in the lake. Supporting this idea is a slow increase in chlorophyll over the bubbling period suggesting uptake of available nitrogen as well as dissolved inorganic phosphorus (ortho-phosphate). Pools of the latter macronutrient may have declined during the bubbling period (although there is only an estimate of the ortho-P levels 1 day after bubbling) but they climbed again after 12 days post-bubbling and still remained at eutrophic concentrations (≥0.1 mg/L, EPA) in December, 33 days after bubbling. If that pattern of increasing dissolved nitrogen and phosphorus from nanobubble dispersal is a consistent outcome in all bubbled water bodies, those nutrient pools would likely support post-treatment phytoplankton re-growth.

Unfortunately assessment of nanobubble effects on algae, and particularly cyanobacteria, is not possible. First, cyanobacteria levels are expected to be low and falling in November, as summer bloom-forming cyanobacteria die-off with cold temperatures. Hence, we cannot determine whether low PC RFU concentrations were from nanobubbling or merely a drop in seasonal temperatures. Second, and in further support of this inability to assess impacts on cyanobacteria, is that discrete measurements of extracted phycocyanin were highly variable through the study period while YSI sensor measurements indicated no change in PC over what would be expected from the increases in chlorophyll (the constant 0.07 PC RFU/CHL RFU). Again this provides no systematic evidence that cyanobacteria are inhibited by nanobubbling. Further experimentation needs to be undertaken to ascertain cyanobacteria susceptibility to this new technology. This work should be initiated under replicated, closely controlled, laboratory studies before being scaled up to a whole lake case study such as the one undertaken here.

In summary, it is our professional opinion that the current demonstration study undertaken by BNT at Constitution Gardens Lake cannot address the efficacy of nanobubble technology for the short- or long-term control of harmful and nuisance algae in the Lake.

Given the short-comings we have noted in the current nanobubble study, if NPS and BNT agree to continue to treat Constitution Garden Lake using nanobubble technology, we recommend that, at a minimum, a 2020 NPS-BNT Summer Nanobubbling Project be undertaken with the following considerations in mind:

1. CCWS staff will notify BNT of microscopists/firms and toxin analytical laboratories for aperiodic cyanobacteria identification and enumeration of taxa (species) and measurement of toxins and toxin concentration, respectively.
2. A month prior to setting up BNT nanobubbling gear at Constitution Gardens Lake, BNT and CCWS staff should establish pre- and post-bubbling sample collection (stations, replicates, depths, discrete vs. composite samples, an intake location and depth, bottom sediments) and analytical protocols for the entire project, with NPS staff providing final review. Sellner (2019) has previously provided a lengthy SOP (Standard Operating Procedures) to BNT and this could be reviewed and portions adopted.

3. Due to the likely aeration of bottom sediments in the lake from nanobubbling, some type of bottom sediment measurements should be done before and after bubbling to document that lake benefit. The bottom sediment measurements might minimally include eH (using the ORP probe) and coring and subsequent laboratory measures of sediment organic carbon.
4. Prior to bubbling initiation, BNT staff should a) calibrate any sensors and cross-check fluorescence measurements with simultaneous measurements of extracted pigments, b) ensure adequate collection supplies, c) establish agreements with analytical laboratories for receipt and analyses of specific water quality parameters, and agree to d) provide 200 mL subsamples to CCWS for microscopy. Finally, BNT should deliver all data to CCWS during the course of the experiment, with all data transferred within 1 month of the completion of the project.
5. A day or two prior to any bubbling, multiple (approximately 10) discrete grab samples will be collected from around the lake with simultaneous sensor measurements of water quality parameters, sub-samples for pigment fluorescence and extraction/determination, phytoplankton analyses via microscopy, toxins, and if agreed, bottom sediment samples. This should be coordinated with CCWS staff so BNT and CCWS methods are simultaneously applied. Portions of this approach should be repeated immediately at the end of bubbling, 2 weeks thereafter, and a month later. Monthly CCWS monitoring will continue its routine data collection into December 2020.
6. CCWS will analyze all data received and combine BNT data with CCWS collected data to generate a report in January-February, 2021.

References

AKC (Ansai Kantetsu Co.). Unknown. Example of water quality improvement at Hakkeijima Island by introduction of micro-nanobubble generator employing ultrafine pores. Kanagawa, Japan. 9 pp.

AKC (Ansai Kantetsu Co.). 2013. Reduction of Sludge – Lake Hyōko – Japan. Project Overview. Kanagawa, Japan. 4 pp.

AKC (Ansai Kantetsu Co.). 2017. Remediation of Lake Suwa – Japan. Project Overview. Kanagawa, Japan. 3 pp.

AKC (Ansai Kantetsu Co.). 2018. Project to eliminate blue-green algae at the outer moat of the Imperial Palace Hibiya Moat. Project Report, October 2018. Kanagawa, Japan. 6 pp.

BNT (Blue Nano Technologies, LLC). 2019. Project to Beta-Test and Validate the Anzai Nano-Bubble Oxygen/Ozone Technology's Ability to Remediate Blue-Green Algae in Ponds and Lakes. 12 pp.

Brand, L.E. 1982. Persistent diel rhythms in the chlorophyll fluorescence of marine phytoplankton species. Mar. Biol. 69: 253-262.

Matthijs, H.C.P., D. Jančula, P.M. Visser, and B. Maršálek. 2016. Existing and emerging cyanocidal compounds: New perspectives for cyanobacterial bloom mitigation. Aquat. Ecol. 50: 443-460.

Owens, T.G., P.G. Falkowski, and T.E. Whitledge. 1980. Diel periodicity in cellular chlorophyll content in marine diatoms. Mar. Biol. 59: 71-77.

Sellner, K.G. 2019. Methods for Quantitative Assessment of Nanobubble Treatment Efficacy. Draft#1. October 27, 2019, Frederick, MD. 3 pp.

Sellner, K.G. and D. Ferrier. 2020. Constitution Gardens Lake Water Quality, Algae, and Cyanobacteria. Quarterly Report Prepared for the National Park Service by Hood College for October-December 2019. Center for Coastal and Watershed Studies, Hood College, Frederick, MD. 2 pp.