Evaluating the Efficacy of Harmful Algal Bloom Beach Re-opening Protocols in Two Putnam County Lakes.

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Abstract

Harmful algal blooms caused by cyanobacteria (CyanoHABs) are a public health concern because exposure to bloom material and associated cyanotoxins can cause adverse health effects in humans and animals (Wood 2016). CyanoHABs occur in New York State (NYS) waterbodies annually, disrupting local tourism and threatening ecosystem and human health. Over the last few decades, the frequency, intensity, and duration of these bloom events have increased worldwide due to anthropogenically-induced changes that have caused increases in climatic warming and nutrient inputs. Future trajectories suggest that these events will continue to intensify (Heisler et al. 2008; Paerl and Huisman 2009; O'Neil et al. 2012). Consistent with this, data from NYS Department of Health's (DOH) recreational beach program has demonstrated an increase in the number of waterbodies impacted by cyanobacterial blooms and total beach closure events within the last decade. For this study, NYSDOH partnered with Putnam County, a county in NYS that has consistently had multiple lakes with extended beach closures due to CyanoHABs, to evaluate the efficacy of current NYSDOH beach re-opening protocols. The current NYSDOH protocol for beach closures is based on the visual presence of an algal bloom. However, per NYSDOH protocol, beach re-openings can occur only after two criteria have been met: the bloom must no longer be present in the designated swim area for at least a day before collecting and submitting a beach water sample, and the sample must have total microcystin levels <4 µg/L based on measurements using the ELAP accredited EPA Method 546, an enzyme linked immunosorbent assay (ELISA). Putnam County Department of Health staff collected time series samples in two lakes (Lake Carmel and Lake Casse) during the summer seasons of 2019-2021 and designated samples as pre-bloom, bloom, or post-bloom. The time series samples were analyzed for total microcystin (EPA Method 546), a cyanobacteria specific 16S gene and the mcyE gene, the gene responsible for microcystin synthesis, via droplet digital PCR (ddPCR). Data from these two methods was compared to beach specific closure and re-opening data to evaluate current NYSDOH CyanoHAB beach re-opening protocols.

Introduction

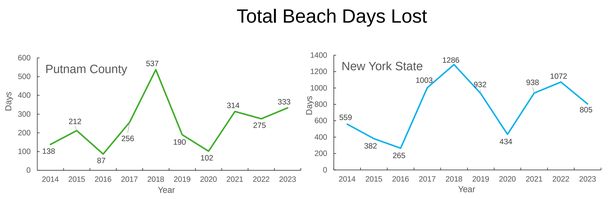

Figure 1: Beach Days Lost due to CyanoHABs: Putnam County vs New York State Total.

Cyanobacteria are photosynthetic organisms that are commonly associated with harmful algal blooms (HABs) in freshwater lakes. Cyanobacteria can produce numerous toxins that target different organs, including hepatotoxins (e.g., microcystin, cylindrospermopsin) and neurotoxins (e.g., saxitoxin, anatoxin-a). CyanoHABs disrupt tourism and pose significant health concerns due to elevated algal cell densities that can cause fish kills and allergic dermatitis, and produce toxins, foul odors, and tastes that can affect drinking water supplies. Anthropogenic changes are increasing the frequency and severity of these CyanoHABs. Changes include climatic warming and eutrophication due to increased nutrient load from sewage and fertilizer runoff. NYS Department of Health's recreational beach program has witnessed an increase in the number of waterbodies impacted by cyanobacterial blooms and total beach closure events over the last decade.

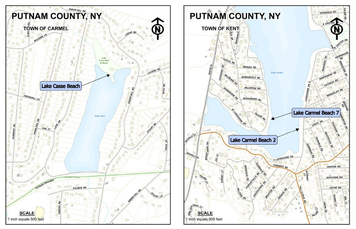

Figure 2: Sampling locations in Putnam County, NY where blooms are a consistent issue and result in a significant loss of recreational opportunities.

Current New York state protocols for beach closures are based on visual identification of a bloom. Beach re-opening protocols require beach operators to collect a re-opening sample at least a day after the bloom has dissipated. The beach is allowed to re-open if the sample is clear of visible algal load and the microcystin levels are below 4 µg/L as measured via EPA method 546.

We used Putnam Co. as a case study for assessing the efficacy of New York's beach re-opening protocols due to consistent beach closures within this county.

Methods

- Time Series Sampling:
  - Daily 30 mL samples were collected from three Putnam County beaches (Lake Carmel Beach 7, Lake Carmel Beach 2, Lake Casse) during the summers of 2019 through 2021.
  - Putnam County DOH staff designated samples as pre-bloom, bloom, and post-bloom accordingly.
  - Samples were stored at -20C until analysis.
- Samples were analyzed using ELISA and ddPCR.
- ELISA:
  - New York protocol follows EPA method 546 which analyzes for total microcystin concentration using an indirect competitive ELISA.
  - ADDA is used as the target analyte because of its presence in all congeners of microcystin.
  - ELISA was conducted on a Cyanotoxin Automated Assay System (CAAS) utilizing the ABRAXIS® Microcystins/Nodularins (ADDA; Cat# 520011) test kit.
- ddPCR:
  - Samples were bead-beated to induce cell lysis.
  - Samples were centrifuged and supernatant transferred to another tube to remove beads and debris.
  - Master mixes were prepared in a DNA-free PCR hood using mcyE/nda and 16S primers and probes (Al-Tebrineh et al. 2012) and ddPCR supermix for Probes (No dUTP).
  - Master mix and sample was combined to create 25µl reactions.
  - Droplets were generated using Bio-Rad's QX200 Droplet generator system and Droplet Generation Oil for Probes.
  - Samples were amplified in a thermocycler and analyzed using the Bio-Rad's QX200 Droplet Digital reader system using Droplet Reader Oil.
  - Samples were analyzed using QuantSoft software from Bio-Rad.

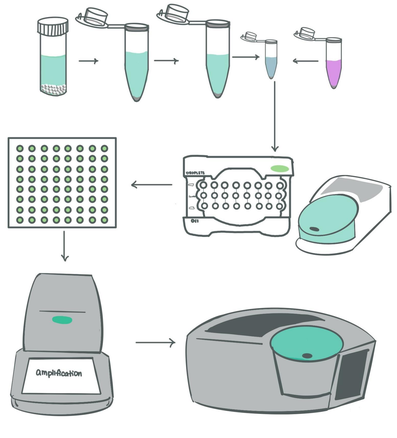

Figure 3: Droplet Digital PCR reaction procedure

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Results

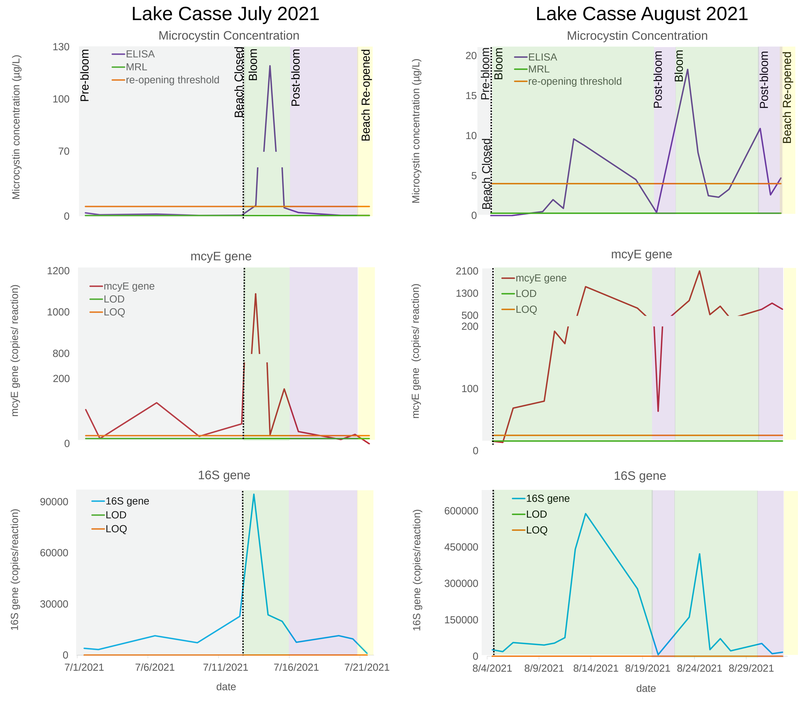

Figure 4: Microcystin concentrations (µg/L) compared to 16S and mcyE genes (copies per reaction) over the course of 2021 blooms in Lake Casse. Total microcystin re-opening threshold=4 µg/L. The minimum reporting level (MRL) for microcystin ELISA =0.3 µg/L. 16S gene: LOD= 8.3 copies per reaction; LOQ= 50 copies per reaction. mcyE gene: LOD=15.9 copies per reaction; LOQ=25 copies per reaction. Vertical dotted black line represents date of beach closure.

Microcystin concentrations vs beach closure/re-openings:

- ELISA data from 2019, 2020 and 2021 Lake Carmel Beaches 2 and 7 indicated that microcystin levels remained below the 4µg/L total microcystin re-opening threshold, but health and safety hazards beyond microcystin toxin were still present due to the visible algal load and the potential for other bloom-associated cyanotoxins.
- As per NYSDOH protocols, beach operators were following visual cues (increased algal load/turbidity) to close beaches, and this corresponded to microcystin concentrations that were below the re-opening threshold at the time of closure.
- In all cases, re-opening samples were appropriately collected at least a day after the bloom had dissipated and there was no longer a visible algal load, and this corresponded to microcystin concentrations that were below the re-opening threshold.

Microcystin concentrations (ELISA) vs ddPCR data:

- mcyE gene copies per reaction were above the limit of quantification (LOQ) 7-11 days prior to microcystin concentrations reaching the beach re-opening threshold (4µg/L).
- In general, both mcyE and 16S copies per reaction mimicked microcystin concentrations.
- On 7/14/21, mcyE and 16S copies per reaction plummeted but microcystin concentrations remained high, this is likely due to the release of toxins extracellularly as the cells lysed during bloom decline.

Conclusions

- Putnam County case study data indicates current NYSDOH beach re-opening protocols sufficiently protect swimmers from microcystin concentrations that exceed the US EPA's recommended magnitude for recreational waters.
- ddPCR was able to predict the occurrence of a toxic bloom at least a week prior to toxin concentrations reaching the beach water re-opening threshold.
- ddPCR can be used as a tool to screen lakes for toxin-producing algae.

Next Steps

- We are in the process of analyzing this sample set for additional cyanotoxins (anatoxin, saxitoxin, cylindrospermopsin), and cyanotoxin genes (anaC, sxtA, cyr) using ELISA and LC/MS/MS, and ddPCR, respectively.
- We are refining our sample extraction process, specifically, exploring whether filtration decreases the impact of ddPCR inhibitors.
- We hope to further confirm that current beach re-opening protocols are also protective of exposure to other toxins that can be produced by CyanoHABs by assessing additional lakes in NYS.

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