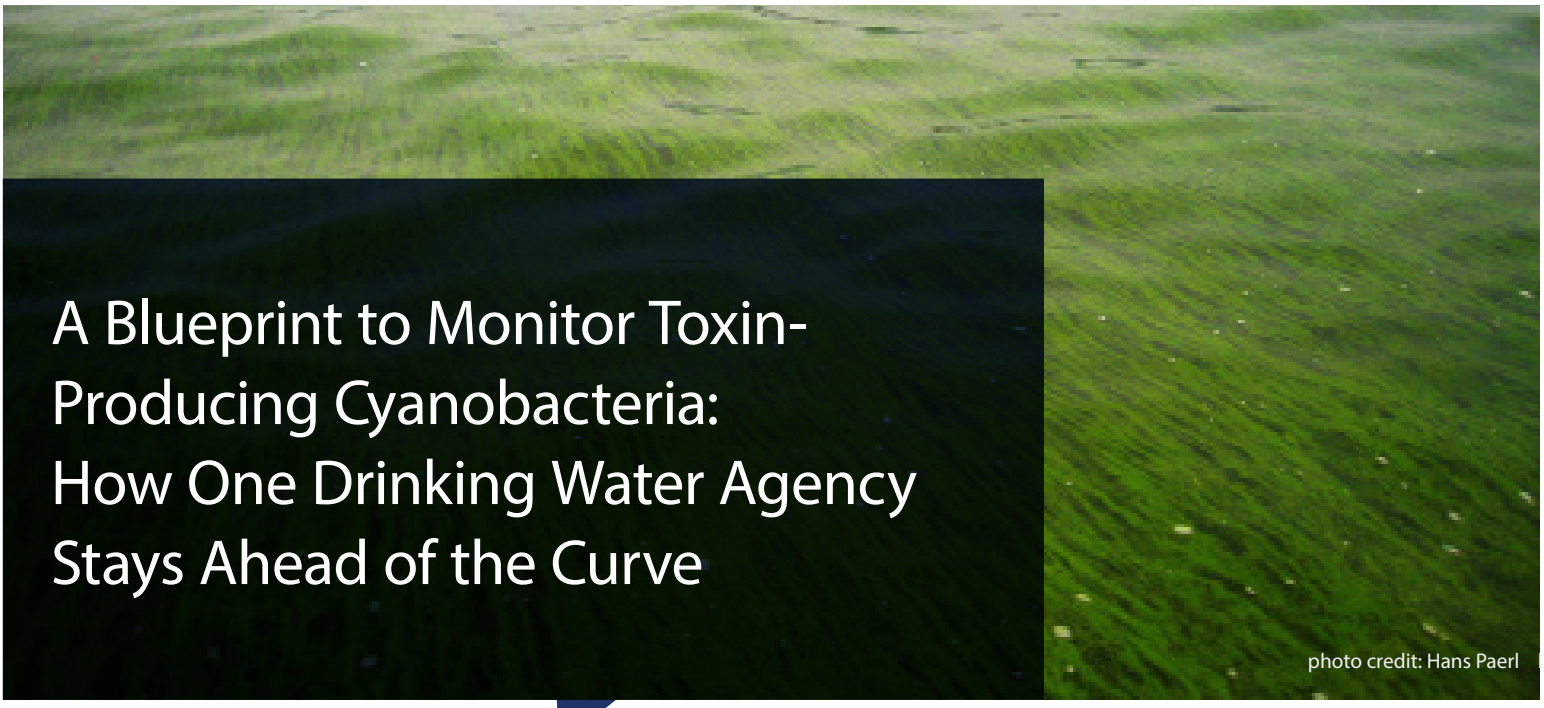

A Blueprint to Monitor Toxin-Producing Cyanobacteria: How One Drinking Water Agency Stays Ahead of the Curve

photo credit: Hans Paerl

"***We got a positive for microcystin.***"

Most drinking water agencies enter high alert mode upon receiving such news.

Hunter Adams, Water Laboratory Supervisor for the City of Wichita Falls, TX was able to respond calmly when he received that dreaded phone call in July 2018.

What follows is an example of how one drinking water agency has incorporated a FlowCam as part of an integrated solution with other tests.

BACKGROUND

Climate conditions are conducive to both harmful algae blooms (HABs) as well as taste and odor events with increasing frequency and intensity. As a result, EPA regulations are moving toward requiring cyanobacteria monitoring. Proactive drinking water agencies are seeking a streamlined approach to monitor cyanobacteria and nuisance algae. Unfortunately, there is no single method that answers all the fundamental questions needed to make treatment decisions and ensure a safe water supply:

1. What is the abundance of cyanobacteria?
2. Is the species capable of producing toxins?
3. Which species are present in the reservoir?
4. What is the concentration of cyanotoxins?
5. How do I know if we have a problem?

When Hunter Adams, Water Laboratory Supervisor for the City of Wichita Falls, TX learned that there was an impending water crisis, he responded as if it were a crime scene, asking who, what, where, how and when - to identify the source of the problem. Those questions are answered through their monitoring strategies.

"There are multiple pieces of the puzzle you have understand to see the big picture. When we have a cyanobacteria spike, we can treat our reservoir while contamination levels are still at barely detectable limits, likely preventing large-scale *Microcystis* outbreaks and cyanotoxin issues. Our customers are safe and should remain confident in our ability to provide clean drinking water. We are prepared." says Adams.

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CASE STUDY CONTINUED ON REVERSE

FROM HOURS TO MINUTES

Adams incorporated the FlowCam with three other methods - qPCR, LC-MS/MS & GC/MS - in an integrated strategy to monitor two lakes and one holding reservoir. The methods build upon one another. Some are used multiple times per week for triage, while other methods, such as LC-MS/MS are used only on an as-needed basis due to the high cost per sample.

The FlowCam is used to run samples three times per week in the summer and once each week in the winter. The FlowCam – an imaging flow cytometer – is used to identify and enumerate filter-clogging algae, taste and odor algae, and cyanobacteria. The FlowCam requires an upfront capital investment, but there is no cost per sample and no limit to the number of samples that can be analyzed.

"It used to take us 3-4 hours to do algae counts in the summer. Now it takes 15 minutes. We use the FlowCam because it's quick and easy."

—Hunter Adams, Water Laboratory Supervisor

"It used to take us 3-4 hours to do algae counts in the summer. Now it takes 15 minutes. We use the FlowCam because it's quick and easy and shows you what's there. If you can see what you have in a 3-minute test, then you have what you need. You're wasting time and money if you test for toxins in the winter with no toxin-producing organisms in the sample."

Elevated FlowCam counts for filter-clogging algae or taste and odor producers are used to trigger immediate treatment. *Anabaena* concentrations of 100-200 chains/mL indicate an oncoming taste and odor event. *Microcystis* concentrations of 500 colonies/mL trigger immediate qPCR tests. Other agencies calculate cells/mL rather than colonies/mL with their FlowCam; Adams' multi-year history of FlowCam data and understanding of his reservoir's ecology enables him to accurately leverage a simplified data set.

Every region is different, where other agencies may find that different counts have different effects. It's not a cookie cutter situation, especially with limited recommendations and guidance coming from the EPA.

qPCR Testing

Once per week a qPCR test is used to confirm if cyanobacteria in the samples have toxin-producing genes. Having *Microcystis* in a sample doesn't mean that you will have the neurotoxin, microcystin. If the toxin-producing genes are not present, or not activated, then microcystin will not be present. In fact,

Wichita Falls sees *Microcystis*, *Anabaena*, and *Oscillatoria* in their reservoirs, and all three can produce microcystin. The FlowCam sample in July yielded 400 chains/mL of *Oscillatoria*, justifying an immediate qPCR test. The qPCR test that followed FlowCam analysis showed that microcystin-producing genes were present. qPCR costs $50 per sample and takes 40-60 minutes to yield results.

PCR is a positive/negative test, while qPCR is quantitative-PCR. qPCR establishes a calibration curve to quantify results. Adams' qPCR results in July 2018 showed a detection with the PCR curve, but the cycle time was later than their lowest calibration standard. This showed that microcystin-producing genes were present at >20 copies/µL, but at a rate lower than their lowest calibration level. Despite the low levels, Adams played it safe and confirmed toxicity levels with an LC-MS/MS test.

AN INTEGRATED APPROACH

If the qPCR results are positive for the presence of toxin-producing genes, then it's time to do a toxin test. Adams' sample tested positive using an LC-MS/MS (Liquid Chromatography – tandem Mass Spectrometry) toxin test for the microcystin. The concentration of the neurotoxin in both lake and tap water samples was below the lowest calibration standard, tagging 0.015 ug/L.

LC-MS/MS analysis is currently conducted by a 3rd party for Wichita Falls with a standard turnaround time of two weeks (or 5 days at double the price). Microcystin & nodularin are both analyzed by EPA 544, and cylindrospermopsin and anatoxin are both analyzed by EPA 545. The battery of toxin tests for 3 samples (raw surface water and two plant samples) costs around $900.

There have only been two events that warranted a toxin test, and therefore it has been cost effective to outsource sample analysis. FlowCam data and qPCR test results are used in combination to justify or forgo a toxin test.

There is more than one type of toxin test available. The ELISA, a well-known method, is a lot cheaper than LC-MS/MS. However, ELISA results are not always reliable. When it comes to public safety, reliability is critical. As such, Adams' team uses mass spectrometry because LC-MS/MS results are more consistent and reliable.

AN EXPANDED TOOL KIT

GC-MS (Gas Chromatography – Mass Spectrometry) analyses are also part of the tool kit utilized by the City of Wichita Falls to monitor taste and odor taxa. They run tests three days each week to monitor MIB and geosmin, common taste and odor compounds in drinking water. If FlowCam counts exceed 200 chains/mL for *Anabaena*, this may indicate a taste and odor event is on the horizon because geosmin has been seen to increase with high counts for *Anabaena (Fig. 1 & 2)*. Wichita Falls treats their water with PAC (powder activated carbon) to remove geosmin and MIB. Treatment with PAC is initiated when GC-MS results confirm the taste and odor compounds are present.

"Using the FlowCam as part of our integrated strategy has prevented larger outbreaks, compared to previous years, and has all but eliminated taste and odor customer complaints."

—Hunter Adams, Water Laboratory Supervisor

Outsourcing sample analysis costs around $200/sample for MIB/geosmin. The fastest turnaround time for sample results is one hour per sample. In a normal run, results for the entire sample set are available by the beginning of the next work day.

This strategy has prevented larger outbreaks, compared to previous years, and has all but eliminated taste and odor customer complaints. It's been 672 days since Wichita Falls' last taste and odor outbreak. Promoting that number helps the city and customers appreciate what their water provider is doing for them.

Wichita Falls faces incredibly challenging ecological conditions in Texas that are conducive to significant cyanobacteria blooms and taste and odor events. Adams and his team have put the best technologies to work, keeping their water clean and safe, and their consumers happy. FlowCam is part of an integrated, economical approach that incorporates many data points and several testing strategies while offering an intelligent combination of resource utilization. The bottom line, no single test will provide the answers. A comprehensive analysis is necessary.

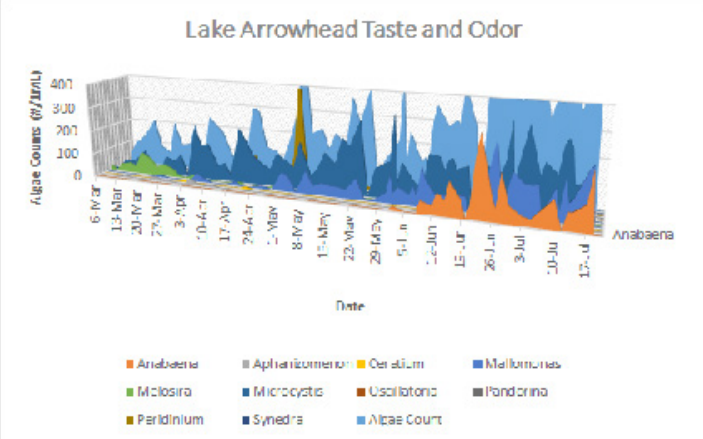

Figure 1: Algae counts measured by FlowCam saw elevated levels of *Anabaena* in June. A spike in *Anabaena is used as a predictive indicator of an* impending taste and odor event, as it often correlates with a spike in geosmin (Fig. 2)

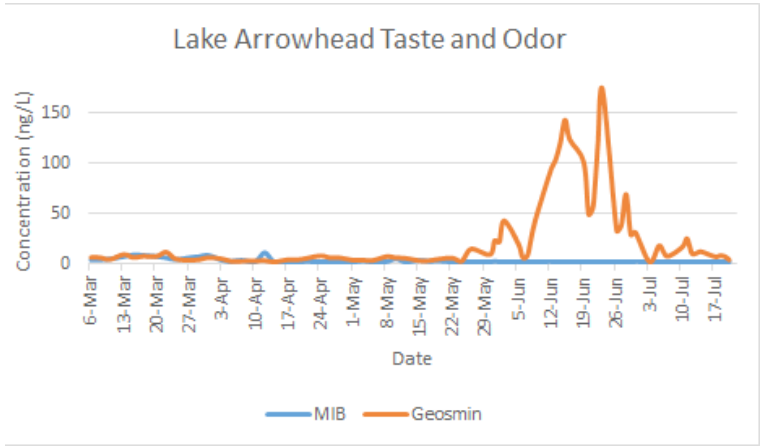

Figure 2: GC-MS test results confirm elevated levels of geosmin in June, which correlates with the spike of *Anabaena (Fig. 1)*