A persistent benthic *Lyngbya wollei* bloom in Lake Wateree SC: analytical methods, toxin, and impact

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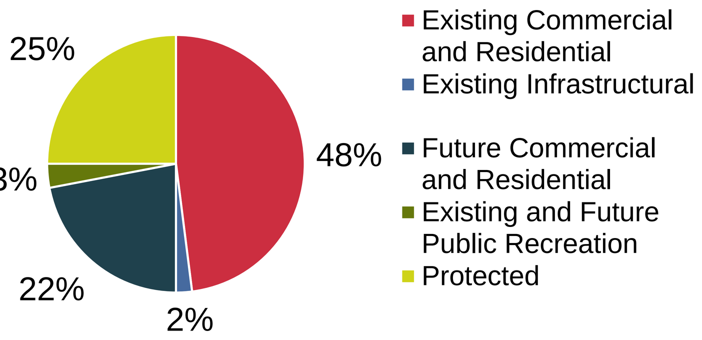
Summary

Lake Wateree SC is a man-made impoundment at the end of the Catawba River. The Catawba watershed is a heavily developed region stretching from the Appalachians of North Carolina to below the fall line on the South Carolina coastal plain. There has been a persistent infestation of *Lyngbya wollei* in this lake for approximately ten years and it has grown to encompass approximately 140 km of shoreline. The Center for Oceans and Human Health at the University of South Carolina has been working to determine the molecular identity of harmful algal bloom toxins entering Lake Wateree as a result of this bloom and identify the cause of toxin production. Toxin production may be a result of chemical signaling between different microbial populations in the lake or provoked by nutrient availability. Methods for detection and quantification of toxin in natural environments are discussed. The current work details the amount of toxin entering the lake on a yearly basis from the bloom and attempts to determine its trends over time.

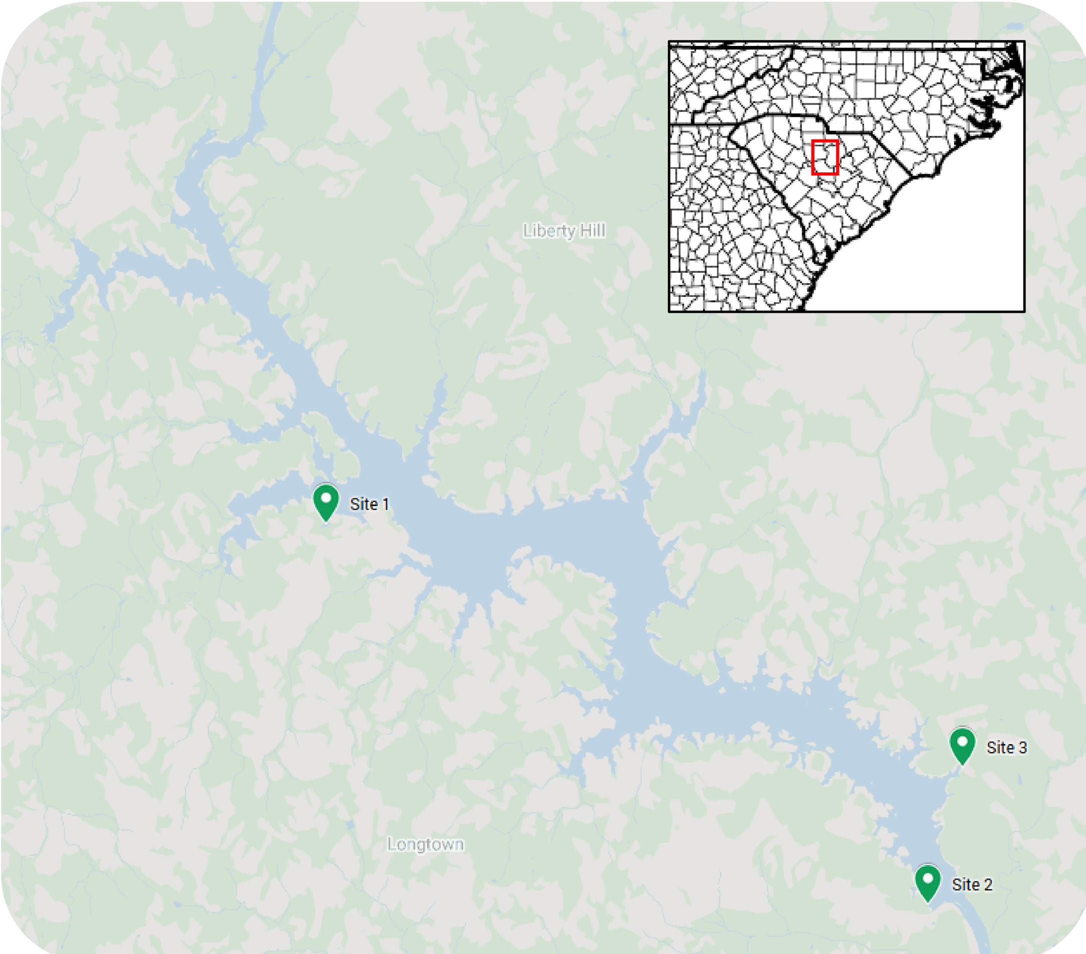
Lake Wateree Shoreline Usage



Source: Catawba-Wateree Comprehensive Relicensing Agreement, Duke Energy 2013

Lake Wateree

Lake Wateree is the final lake in a series of impoundments on the Catawba River. Covering over 21 square miles and with 181 miles of shoreline, the lake is a center of residential, commercial, and recreational activity for the surrounding counties. The residence time of the lake can vary from many months during dry times, to only a few days during rain events.

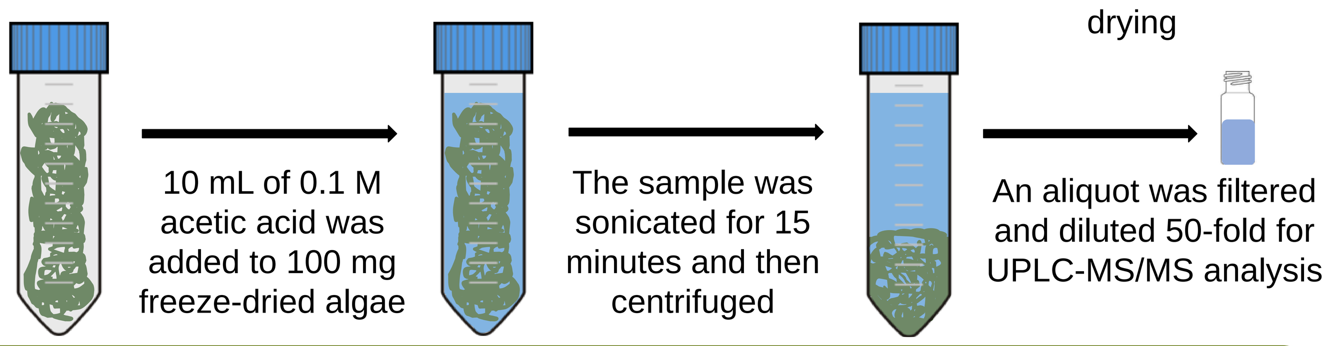


Above: Aerial photograph of a *Lyngbya wollei* bloom in the northern cove near the causeway of Lake Wateree State Park. *L. wollei* is seen at the surface during periods of high activity. Color variation in the bloom indicates natural variation and presence of pine pollen and plants like duckweed.

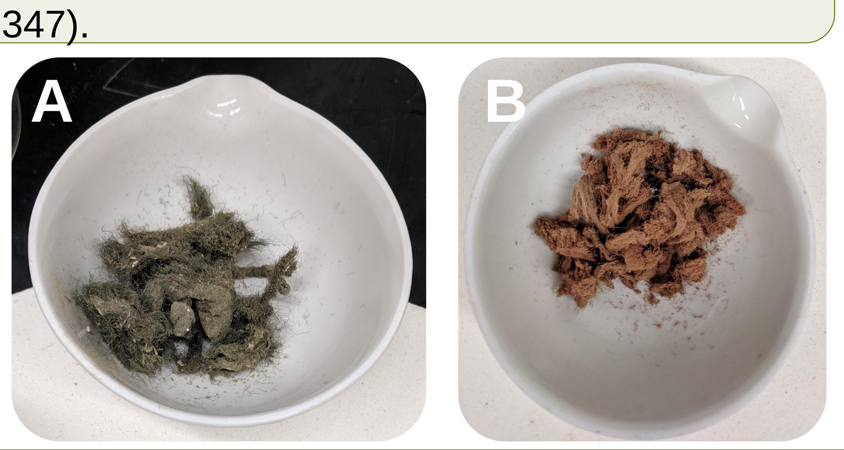
Left: Map of Lake Wateree, showing the three sites regularly sampled for *L. wollei*. Inset shows location in South Carolina.

Sampling, Extraction, and Carbon Analysis

Live *L. wollei* floating at the surface → *L. wollei* collected in a net from the water → Freeze-dried *L. wollei*, 82% mass loss from drying

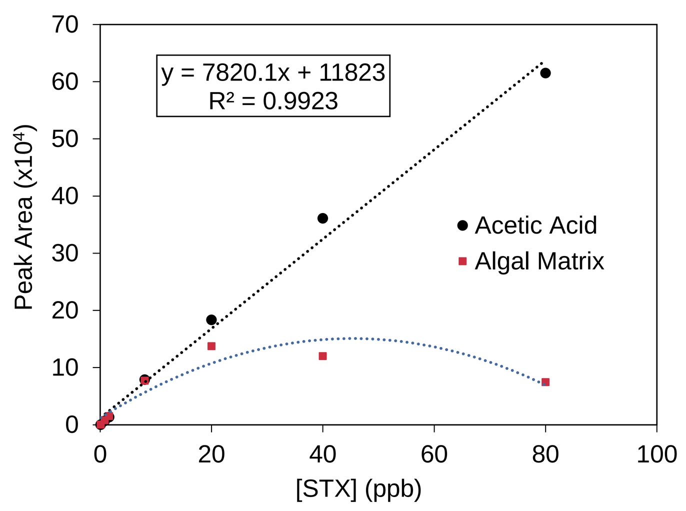


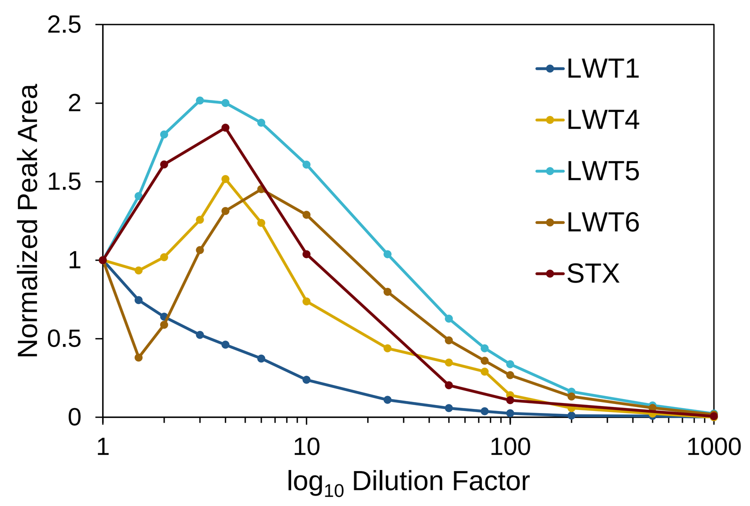
Three sampling locations were chosen based on 10-year history of blooms (Site 1) and proximity to drinking water intakes (Sites 2 & 3), shown in the map in the center. These sites were sampled approximately every two weeks for 16 months. Sampling and extraction procedure is described in the above figure. In addition to toxin quantification, each individual sample was analyzed by solid state total carbon analysis to determine the percent carbon content and incombustible inorganic material. Average carbon content was 28.1 ± 0.7%, and inorganic material was 27.6 ± 1.3%, both at the 95% confidence level (n=347).



Right: Freeze-dried *L. wollei* before (A) and after (B) combustion in a 850°C furnace for 30 minutes. The algae contains a large amount of inorganic content and continues to maintain its shape even after combustion.

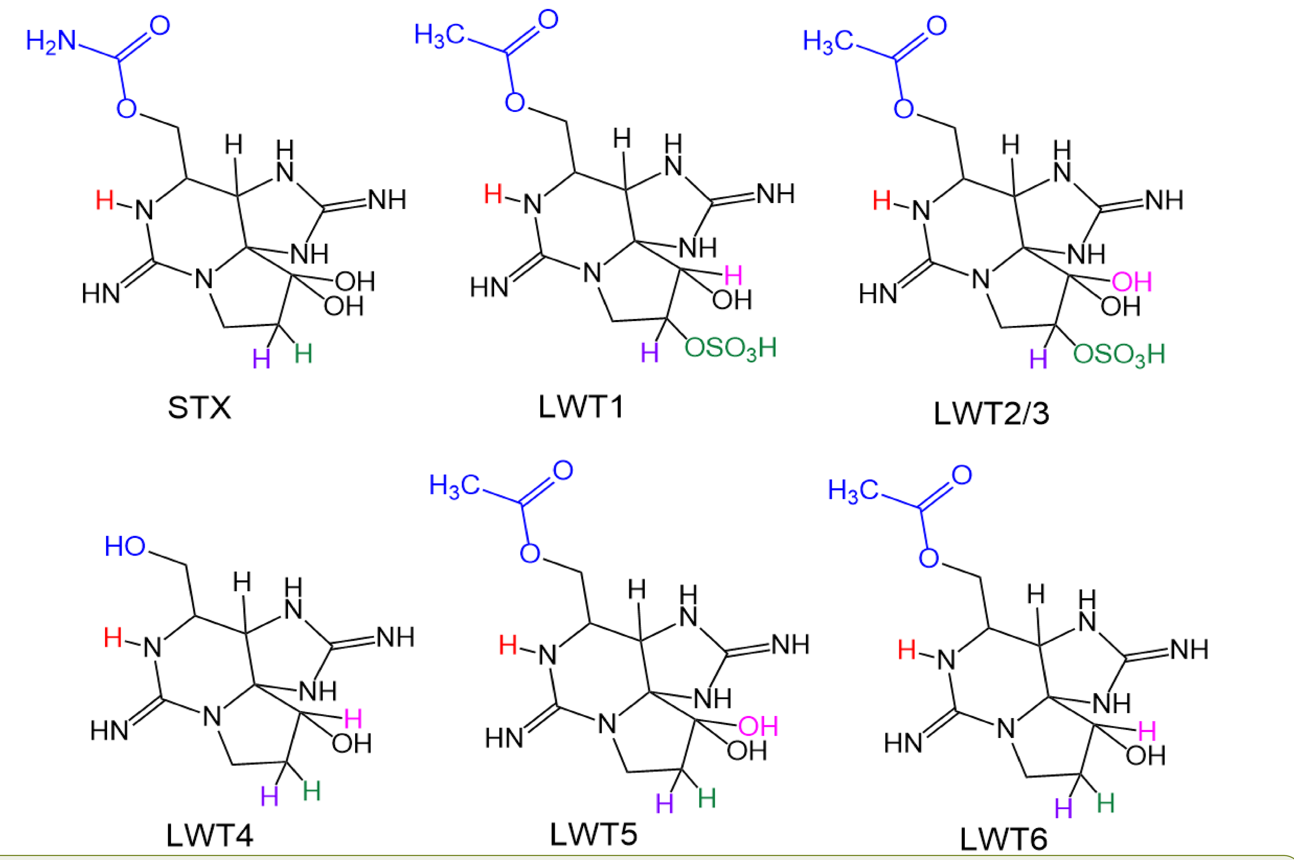
Resolving Ion Suppression





All toxins were quantified using Ultra Performance Liquid Chromatography (UPLC) with a triple quadrupole mass spectrometer. Since analytical standards are not available for most of the LWTs, saxitoxin was used as a surrogate external calibration standard and all toxins were quantified against saxitoxin response. When saxitoxin calibration curves were prepared in an extract of algae, the resulting regression fit was non-linear (above left). The same curve prepared in neat acetic acid produced a linear regression. Dilution of the extract samples resulted in an increase in signal for saxitoxin and all LWTs except LWT 1 (above right). Maximum analytical signal was reached at a dilution factor of between 4 and 10, depending on the toxin. A dilution factor of 50 was chosen for all samples in order to ensure that changes in the matrix would not mask toxin signal.

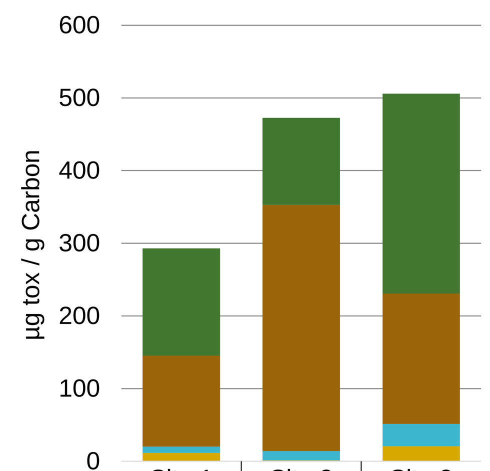
Saxitoxin and LWTs



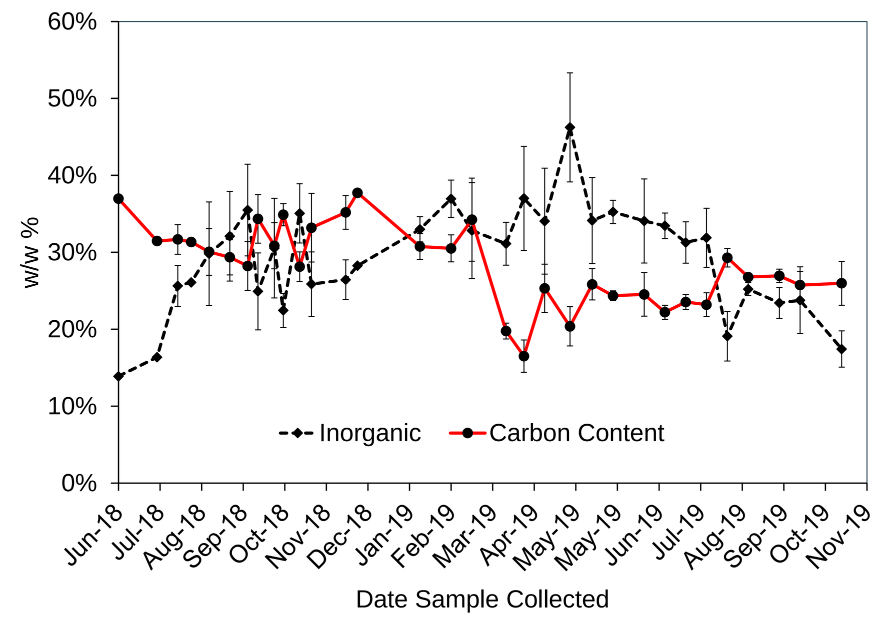
Lyngbya wollei toxins (LWTs) are part of the saxitoxin (STX) family of neurotoxins. Toxicity ranges on the mouse assay from LWT5, which is 13% as toxic as STX, to LWTs 1, 4, and 6 which do not produce a response. LWT 2 & 3 are structural isomers of each other. Saxitoxin was used as a calibration standard, and all LWTs except 2/3 were quantified by mass spectrometry.

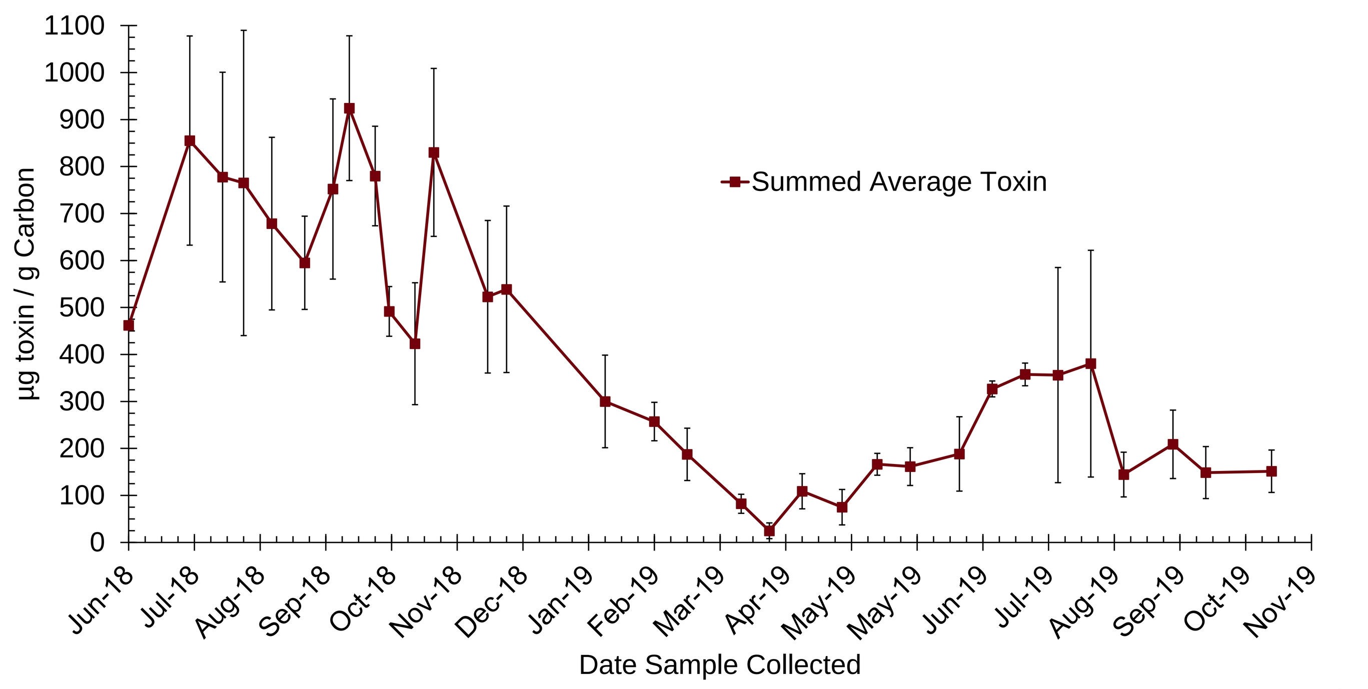
Results

Carbon and inorganic content data was collected for each data point, then all three sites were averaged and plotted over time (below right). LWTs 1, 4, 5, & 6 were quantified, summed, and averaged across three sites to show the temporal variation in toxin (bottom). Toxin profiles and average summed toxin are plotted for each location, demonstrating that LWTs 5 & 6 are the most predominant (below left). Error bars for these graphs demonstrate the large natural variations in the samples.

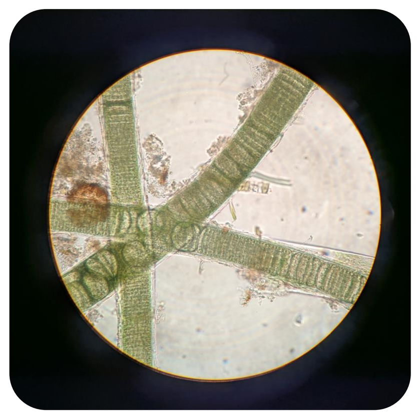


	Site 1	Site 2	Site 3
LWT6	147.5	120.0	275.2
LWT5	125.2	338.7	179.3
LWT4	8.6	12.7	30.9
LWT1	11.5	1.3	20.6





Analytical Method Results



- The natural matrix sampled causes ion suppression in the mass spectrometer, leading to a loss of signal. Dilution is necessary to remove this interference.
- Algae grab samples are highly variable and contain varying amounts of inorganic content. Measuring the carbon content of each individual sample is crucial to properly normalize the data.

Acknowledgements

Thanks to Ashley Womer, Emily Sellers, and Samantha J. McClain for carbon analysis. We also thank Lake Wateree Association and WaterWatch for their help in sampling the lake. This research was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number P01ES028942. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Environmental Trends and Impact

- Carbon and inorganic content remain mostly constant over time. Although the trend is consistent, the variability is still high.
- Toxin reaches a low point in the spring, before growth starts for the next season.
- This low point in toxin concentration may be the ideal time to treat these blooms, as the impact of the release of toxin upon cell death would be the lowest.