US ERA ARCHIVE DOCUMENT

New HABs monitoring tools & approaches for developing and maintaining collaborative networks

USEPA Region 10 HABs workshop

Alan Wilson

Fisheries - Auburn University 29-20 March 2016

> Aquaculture pond Alabama, August 2008

Outline

- New HABs monitoring tools
 - CellScope
 - Phycocyanin
 - qPCR
 - Environmental Sample Processor (ESP)
 - Flow cytobots
 - Toxin test strips and tube kits
 - Real-time data
 - Forecasting websites







World Health Organization

Table 5.2 Guidelines for safe practice in managing bathing waters which may produce or contain cyanobacterial cells and/or toxins

Guidance level or situation	How guidance level derived	Health risks	Recommended action
Cyanobacterial scum formation in bathing areas	Inference from oral animal lethal poisonings Actual human illness case histories	Potential for acute poisoning Potential for long-term illness with some cyanobacterial species	Immediate action to prevent contact with scums; possible prohibition of swimming and other water-contact activities
		Short-term adverse health outcomes, e.g. skin irritations, gastrointestinal illness	Public health follow-up investigation Inform relevant authorities
100,000 cells cyanobacteria per ml or 50 µg chlorophyll a per litre with dominance of cyanobacteria	From provisional drinking water guideline for microcystin-LR, and data concerning other cyanotoxins	Potential for long-term illness with some cyanobacterial species Short-term adverse health outcomes, e.g. skin irritations, gastrointestinal illness	Watch for soums Restrict bathing and further investigate hazard Post on-site risk advisory signs Inform relevant health authorities
20,000 cells cyanobacteria per ml or 10 µg chlorophyll a per litre with dominance of cyanobacteria	From human bathing epidemiological study	Short-term adverse health outcomes, e.g. skin irritations, gastrointestinal illness, probably at low frequency	Post on-site risk advisory signs Inform relevant authorities

 $\frac{WHO\ Thresholds}{Cell\ density} \geq 20,000\ cells/ml$ $Chlorophyll \geq 10\ \mu g/L$ $Microcystin\ drinking\ water \geq 1\ \mu g/L$

 http://www.who.int/water_sanitation_health/resourcesqualit y/toxicyanbact/en/
 Chorus and Bartram 1999

- World Health Organization
- US Environmental Protection Agency



2015 Drinking Water Health Advisories for Two Cyanobacterial Toxins

Summary

EPA has issued 10-Day Drinking Water Health Advisories (HAs) for the cyanobacterial toxins microcystins and cylindrospermopsin.

EPA recommends HA levels at or below 0.3 micrograms per liter for microcystins and 0.7

(cyanobacterial toxins or "cyanotoxins") that are harmful to the environment, animals and human health. Winds and water currents can transport cyanobacterial blooms within proximity to drinking water intakes at treatment plants that, if not removed during treatment, can cause odor, taste and color problems in treated drinking water and

USEPA drinking water advisories microcystin

0.3 μg/L children 0-6yrs

 $1.6 \mu g/L > 6 yrs$

cylindrospermopsin

0.7 μg/L children 0-6yrs

 $3.0 \mu g/L > 6 yrs$

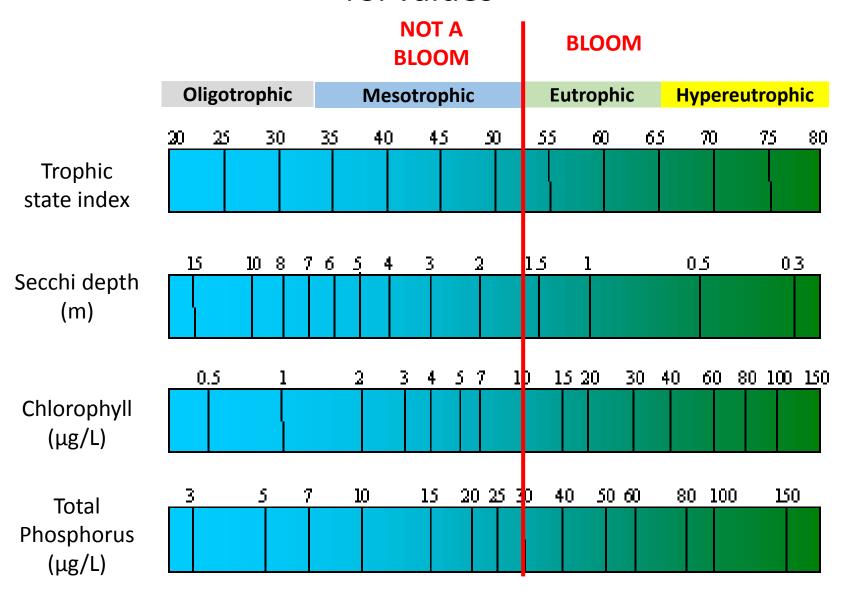
• https://www.epa.gov/nutrient-policy-data/drinking-water-health-advisory-documents

- World Health Organization
- US Environmental Protection Agency
- Carlson Trophic State Index (TSI)
 - widely used classification scheme
 - based on transparency (Secchi depth), chlorophyll a, total phosphorus to relate to algal biomass typically during the summer
 - scale from 0 110; increase of 10 TSI units = 2x algal biomass
 - TSI useful for comparing lakes within a region and for assessing changes in trophic status over time
 - http://aslo.net/lo/toc/vol 22/issue 2/0361.pdf

TSI values vs. water quality

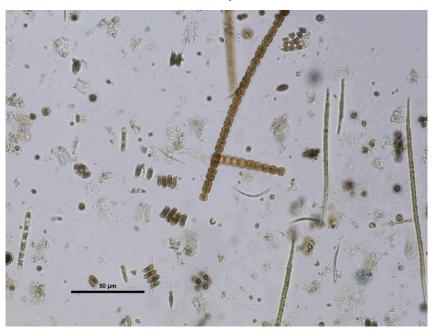
<40	Oligotrophic; clear water; high hypolimnetic O ₂ year-round but possible anoxia in the deeper hypolimnion part of year
40-50	Mesotrophic; moderately clear water; possible hypolimnetic anoxia in summer and/or under ice. Fully supportive of all swimmable /aesthetic uses; possible cold-water fishery
50-60	Mildly eutrophic; decreased secchi; anoxic hypolimnion; possible macrophyte "problems"; warm-water fishery; supportive of all swimmable /aesthetic uses but "threatened"
60-70	blue-green algal dominance with scums possible; extensive macrophyte problems; not supportive of all beneficial uses
>70	Heavy blooms and scums in summer likely; dense "weed" beds; hypereutrophic; possible fish kills; fewer plant beds due to high algae; not supportive of many beneficial uses

TSI values



Carlson 1977 Limnology and Oceanography

- Go sample!
 - Integrated and/or surface samples
 - Whole water samples
 - Secchi depth



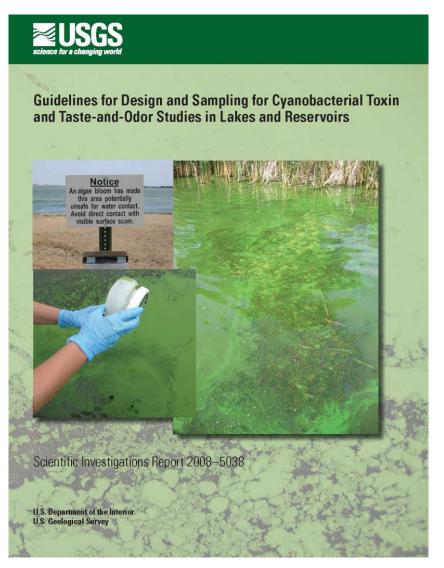


Table 10.1 Approaches to monitoring for cyanobacteria and analysis for cyanotoxins: requirements and options for their organisation

Monitoring type	Parameters/variables	Demands on equipment and skills	Who	Where
Basic		Minimal		
Site inspection for indicators of toxic cyanobacteria in waterbody	Transparency, discolouration, scum formation, detached mat accumulation	Secchi disc, regular site inspection by trained staff, skill requirement basic, training easily provided	Environmental or health officers, trained health staff or supervised local	Local
Background		Low to moderate		
Potential for cyanotoxin problems in waterbody	Total phosphorus, nitrate and ammonia, flow regime, thermal stratification, transparency	Photometer, boat, depth sampler, Secchi disc, submersible temperature/oxygen probe; skills basic but require specific training and supervision	Environmental officers or experts with limnological expertise	Local, regional
Cyanobacteria		Low to moderate		
In waterbody and drinking water	Dominant taxa (quantity): often determination to genus level only is sufficiently precise; quantification only as precise as needed for management	Microscope, photometer is useful; specific training and supervision is required, but quite easily achieved	Environmental or health officers (with occasional quality control by experts); consultants with limnological expertise	Local, regional
Toxicity assessment		Moderate		
In waterbody and drinking water	Toxicity	Demands on equipment are low, but rather high on skills	Toxicologists	Central
Toxin concentration		Moderate to high		
In waterbody and drinking water	Toxin concentration	New methods with lower financial demands presently in development for some cyanotoxins (e.g. immuno-assay); skill requirements vary widely from moderate to very high	Skilled analysts	Central

Lakeside monitoring approaches

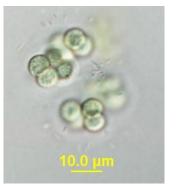
- Go sample!
 - Integrated and/or surface samples
 - Whole water samples
 - Secchi depth

- Important targets
 - Toxigenic cyanobacteria
 - Toxins (cell-bound and/or dissolved)
 - Poor transparency



How do we quantify cyanobacterial abundance?

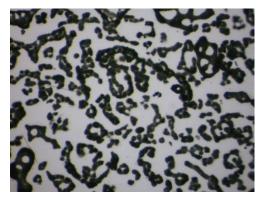
Chroococcus



Planktothrix



Microcystis



Anabaena



Merismopedia



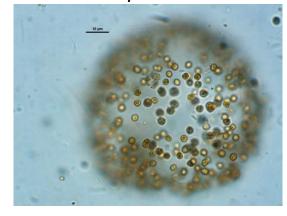
Cylindrospermopsis



Lyngbya



Coelosphaerium



Huge size range

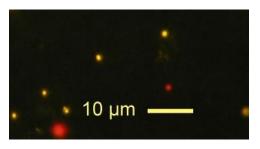
Algal group

Picoplankton Nanoplankton Microplankton Mesoplankton

Length (µm)

0.2-2.0 2.0-30 30-200 200-20,000 **100,000**x **difference**

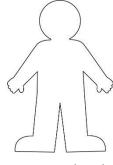
Kalff 2002 Limnology



Picoplankton



Mesoplankton

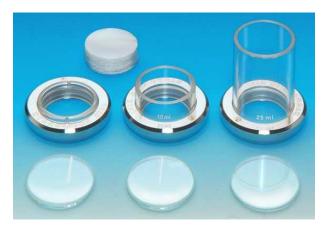


Human (2m)



Burj Khalifa in Dubai (830m)

Common techniques for enumerating phytoplankton



Hydrobios settling chambers



Field sonde Hydrolab minisonde



Quantitative PCR



Flow CAM imaging particle analyzer



Benchtop fluorometer Turner Designs Trilogy



High performance liquid chromatography

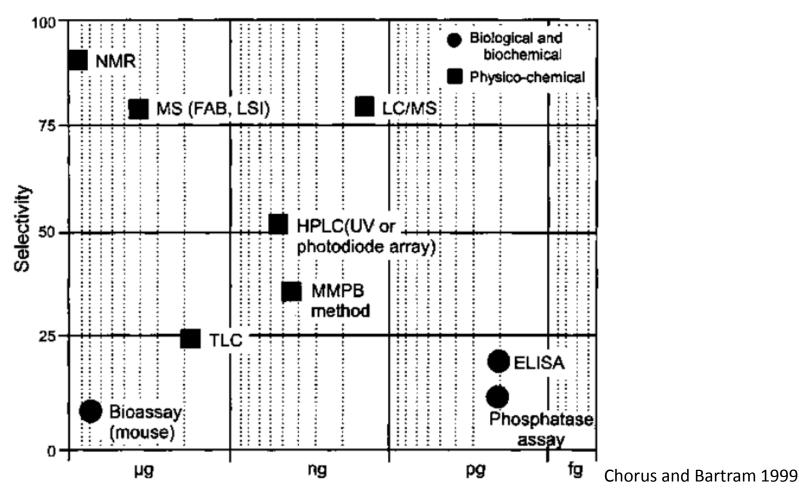
Common toxin analytical approaches

Table 3. Relative advantages and disadvantages of common analytical techniques utilized for analysis of cyanobacterial toxins and taste-and-odor compounds.

Analytical techniques	Advantages	Disadvantages	
	Bioassays		
Enzyme-Linked Immunosorbent Assay (ELISA)	Relatively easy to use	Data interpretation can be difficult	
Inhibition Assays	Cost per analyses lowest of all techniques	Inhibition assays and radioassays not always available	
Radioassays	Can be useful as screening tools	Bioassays frequently possess some reactivity towards compounds other than the intended target	
	Can indicate toxicity in some cases	Radioassays require permits to work with radioisotopes	
		Research objectives may require a chromatographic technique for compound specific quantitation	
	Gas Chromatography (GC)		
Flame Ionization Detector (GC/FID)	Compound specific	Toxins will most likely require derivitization	
Mass Spectrometry (GC/MS)	Cost per analyses intermediate	Not all compounds amenable to derivitization	
	Compound identification by GC/MS is superior to GC/FID	GC/FID may require further confirmation	
		Sample concentration techniques may be necessary	
	Liquid Chromatography (LC)		
Ultraviolet-Visible (LC/UV-Vis)	Derivitization typically not necessary	Matrix effects can be substantial	
Fluorescence (LC/Fluorescence)	Compound specific	Cost per sample most expensive	
Mass Spectrometry (LC/MS)	Greatest number of toxins are amenable to LC techniques	Spectroscopic techniques may require further confirmation	
Tandem Mass Spectrometry (LC/MS/MS)	Cost per analyte can be lowest in a multi-	Sample concentration techniques may be	
	analyte method	Graham et al. 200	8 USGS repor
Ion Trap Mass Spectrometry (LC/ITMS)	Compound identification is superior by LC/MS/MS or LC/ITMS	http://pubs.usgs.gov/sir/2008/5038/	•

Common toxin analytical approaches

Figure 13.1 Relationship between sensitivity and selectivity of analytical methods for microcystins (see text for explanation of methods)



Sensitivity

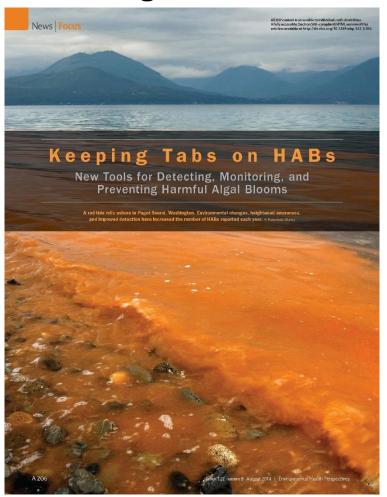
http://apps.who.int/iris/bitstream/10665/42827 /1/0419239308 eng.pdf?ua=1

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Outline

New HABs monitoring tools



Seltenrich 2014

http://ehp.niehs.nih.gov/wp-content/uploads/122/8/ehp.122-A206.pdf

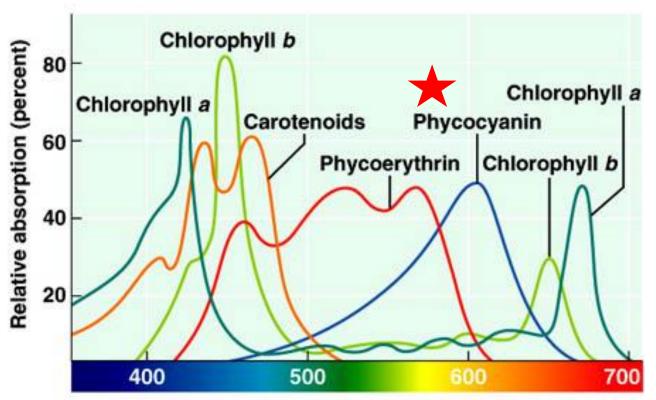
CellScope



• http://cellscope.berkeley.edu

Phycocyanin approaches

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Wavelength of light (nanometers)



Turner Designs Trilogy



Orange module 630nm excitation 660nm emission



Phycocyanin benchtop method development

- Field sampling
 - September 2012 16 ponds (pond survey)

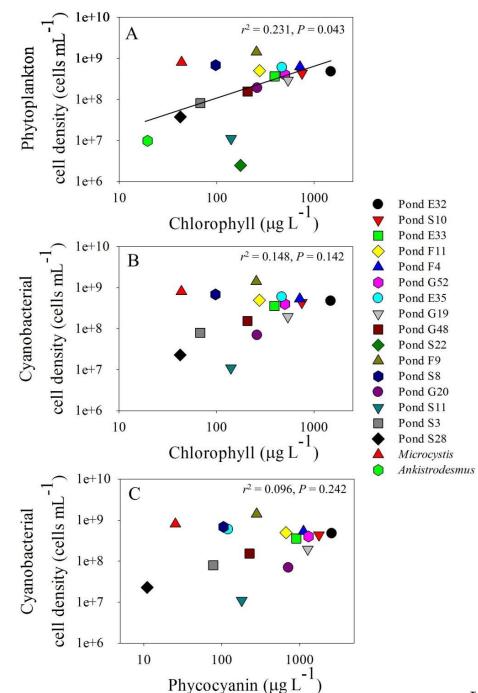
Auburn University pond facility





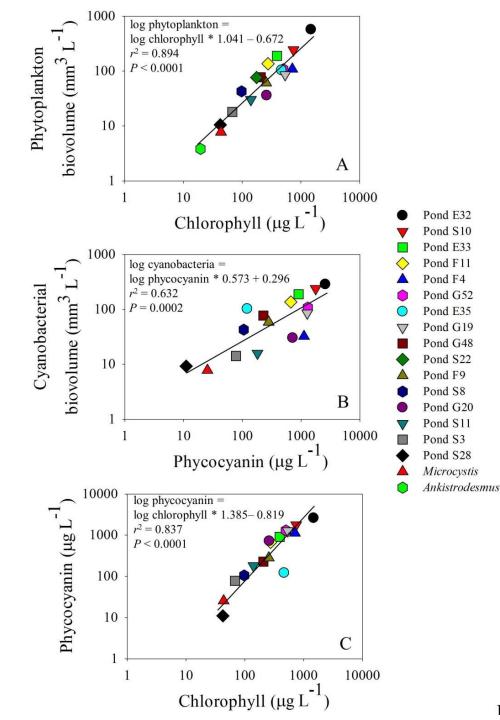
Pigments vs. cell densities

- Weak to no relationships between pigments and cell densities
 - Only statistically significant relationship was chlorophyll vs.
 phytoplankton



Pigments vs. biovolumes

- Strong relationships between pigments and biovolume or only pigments
 - Large range
 - Wide diversity of taxa
 - Chlorophyll good predictor of phycocyanin



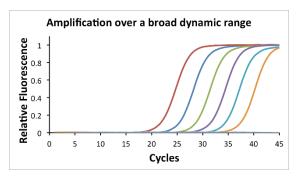
Kasinak et al. 2015 Journal of Plankton Research

http://plankt.oxfordjournals.org/content/37/1/248.abstract

qPCR assays

qPCR

 Quantifies number of gene copies associated with taxaand trait-specific primers and probes





Phytoxigene

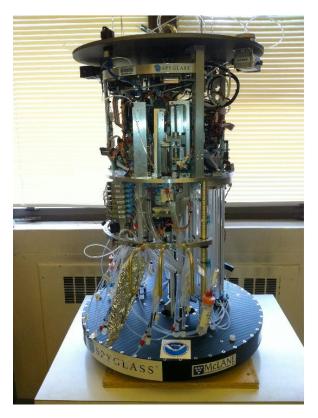
- CyanoDTec microcystin, nodularin, cylindrospermopsin, saxitoxin
- DinoDTec saxitoxin
- http://www.phytoxigene.com

Left: The team behind the new CyanoDTec test kit (R to L) on-site at one of Sydney's contaminated waterways: Professor Brett Neilan, UNSW, Mark Van Asten, Diagnostic Technology, and Dr Leonardo Pinheiro, NMI

https://www.youtube.com/watch?v=o2bYaY1TDLs

Environmental Sample Processor (ESP)

- Automated in situ collection and analyses for water quality and DNA analysis of biological community to identify toxigenic taxa
- Remotely shares data to improve forecasting models
- http://www.nwfsc.noaa.gov/resear ch/datatech/tech/esp.cfm



Flow cytobot

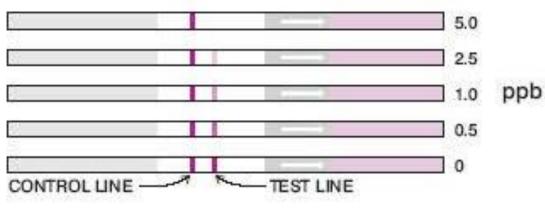
- Automated in situ collection and analyses to identify toxigenic phytoplankters using video, flow cytometric, and fluorescence technology
- Remotely shares data to improve forecasting models

 http://www.whoi.edu/main/im aging-flow-cytobot



Toxin test strips and tube kits

- Available for several compounds including microcystin, cylindrospermopsin, and anatoxin
- Abraxis
 - http://www.abraxiskits.com/products/algal-toxins/
- Beacon Analytical Systems
 - http://www.beaconkits.com/welcome/



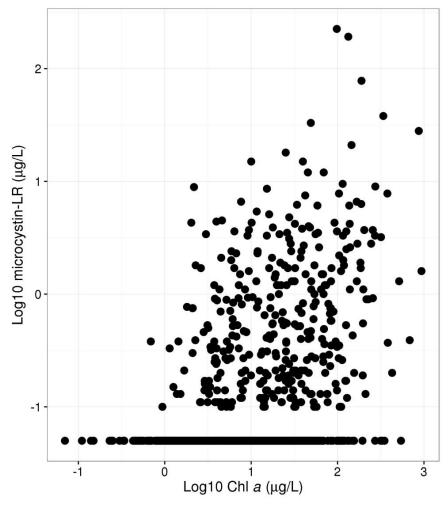




Beacon toxin tube kit $(0.3 - 5.0 \mu g/L)$

Toxin test strips and tube kits

- 2007 EPA National Lakes Assessment
- 1252 samples for microcystin
 - Range 0-225 μg/L
 - 68% undetectable
 - 16% 0.05-0.5 μg/L
 - 5% 0.5-1.0 μg/L
 - 9% 1.0-5.0 μg/L
 - 2% >5 μg/L
- http://www.epa.gov/national-aquaticresource-surveys/data-national-aquaticresource-surveys



Remote sensing

Satellite Comparison for cyano applications			
Satellite	Spatial	Temporal	Key Spectral
MERIS (2002- 12) OLCI Sentinel-3 2015	300 m	2 day	10 (5 on red edge)
MODIS high res Terra 1999; Aqua 2002	250/500 m OK	1-2 day good	4 (1 red, 1 NIRa)ginal
MODIS low res & SeaWiFS	1 km	1-2 day	7-8 (2 in red edge)
Landsat	30 m	8 or 16 day	4 (1 red, 1 NIR)
Sentinel-2 (2015)	20 m	10 day (5 day with 2 nd satellite in 2017)	5 (1 red; 2 NIR, 1 in red edge)
COASTALOCEANSCIENCE.NOAA.GOV Cyanos, May 2015 #			

http://www.sfei.org/sites/default/files/Rick%20Stum
 pf part2 RS basics.pdf
 Stumpf et al. 2015 talk

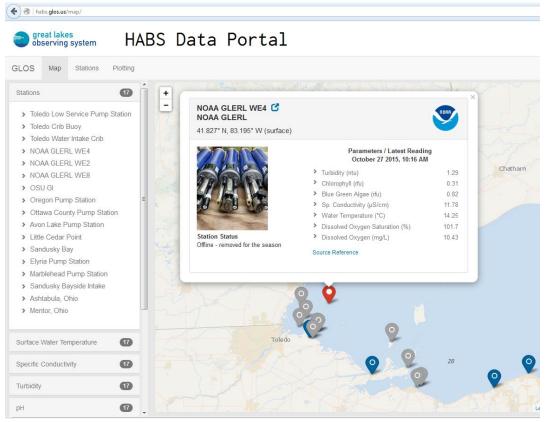
- NOAA Harmful Algal Bloom Operational Forecast System (HAB-OFS)
 - Gulf of Mexico Karenia brevis



- http://habsos.noaa.gov/
- http://tidesandcurrents.noaa.gov/hab/

- National Centers for Coastal Ocean Sciences (NCCOS) funding research in support of pilot regional HAB forecasts in...
 - Pacific Northwest Pseudo-nitzscha
 - Puget Sound Alexandrium
 - Gulf of Maine Alexandrium
 - Southern California Pseudo-nitzschia

- Great Lakes Observing System (GLOS)
 - Western Lake Erie



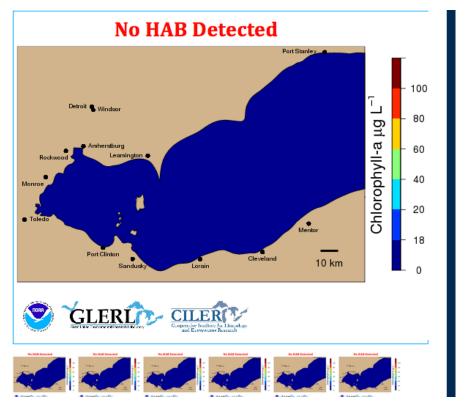
http://habs.glos.us/map/

- Great Lakes Environmental Research Lab (GLERL)
 - Western Lake Erie HABs tracker (forecast days 1-5)

Displacement arrows showing model-predicted movement of surface water from the initial position (from the satellite image below) to the final position.

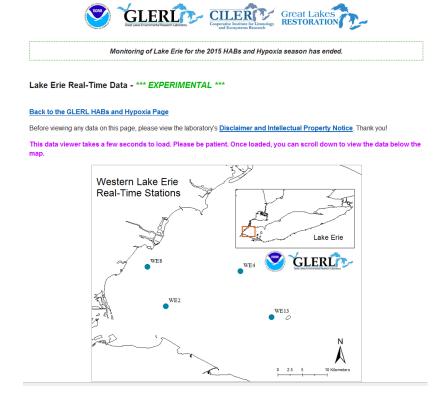
Click on the arrows below to advance the image from the nowcast (day 0) to forecast days 1 through 5. You may also click on the thumbnails below the larger image, representing days 0 through 5 respectively.





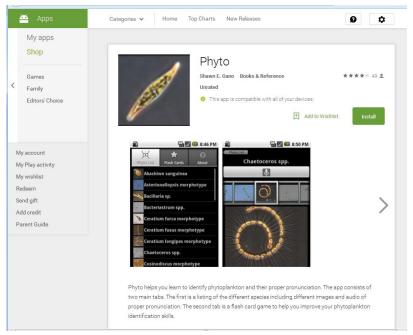
 http://www.glerl.noaa.gov/res/HABs and Hypoxia/habsTrac ker.html

- Great Lakes Environmental Research Lab (GLERL)
 - Western Lake Erie



 http://www.glerl.noaa.gov/res/HABs and Hypoxia/rtM onWLE.html

- Phytoplankton monitoring network
 - NOAA Citizen science outreach to educate public about harmful phytoplankton taxa in coastal areas and Great Lakes region



- https://play.google.com/store/apps/details?id=name.gano.phyto& hl=en
- https://www.nalms.org/media.acux/beb75c9c-f812-4753-b888-79864899c6d6

What about inland lakes?









- California Surface Water Ambient Monitoring Program (SWAMP)
 - Uses remote sensing surface water color satellite data to estimate and forecast HABs in fresh waterbodies



 http://www.sfei.org/news_items/noaa-remote-sensingtools-and-application-evaluating-cyanohabs-californialakes

 USEPA, NOAA, USGS, and NASA working together to develop an android HABs app

Cyanobacteria Assessment Network (CyAN) mobile app

Uses MODIS surface water color satellite data to

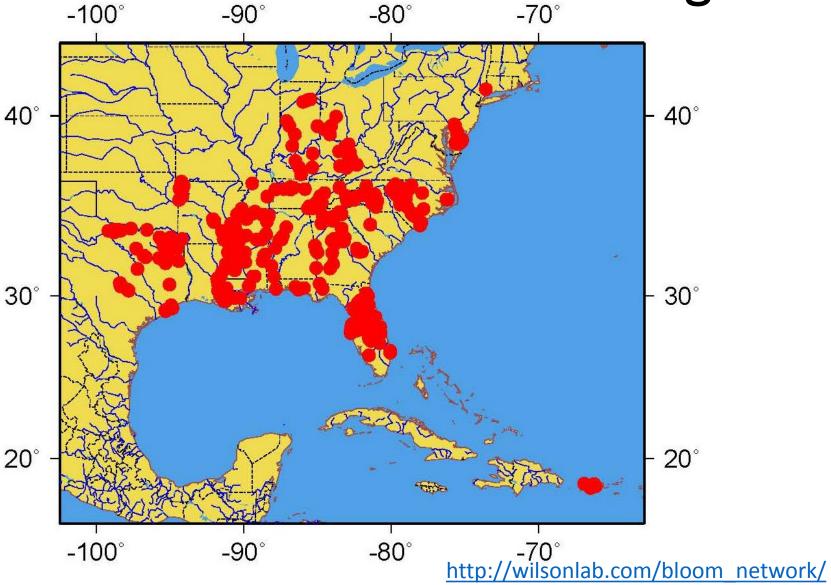
estimate and forecast HABs





Mattas-Curry et al. 2015 LakeLine

- https://developer.epa.gov/hab-challenge/
- https://www.nalms.org/media.acux/beb75c9c-f812-4753-b888-79864899c6d6



WilsonLab at Auburn University

Home

Lab members

Research interests

Projects

Publications

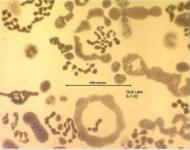
CV

Courses

Photos

Contact information







Models to forecast freshwater algal and cyanobacterial blooms

The following spreadsheet contains two models useful for water resource managers, lake owners, and researchers to forecast algal, cyanobacterial (blue-green algal), and toxic cyanobacterial blooms in lakes, ponds, and reservoirs. The models incorporate either Secchi depth (measured in meters) or commonly measured water quality parameters, such as chlorophyll a or total phosphorus concentrations, to predict algal blooms and their associated water quality risks. The current spreadsheet incorporates data from 103 waterbodies across Alabama that vary widely in morphology, mixing regime, flow, and nutrient concentrations sampled during the summers of 2008-2009. We are currently evaluating the utility of these models for sites throughout the Southeast. We will update the models, as well as provide alternative models specific for certain types of waterbodies, in the future. Please use the models and Let us know if they are useful for you and/or if you have any questions, comments, or concerns about the models.

Available forecasting models

- 1. General use Secchi depth model (ideal for homeowners and general public)
- 2. Complex water quality model (ideal for water quality managers, state agency scientists, and academics)

Website development and coding - Mark Bransby

School of Fisheries, 203 Swingle Hall, Auburn University, Auburn, AL 36849

Home Lab Research Projects Pubs CV Courses Photos Contact

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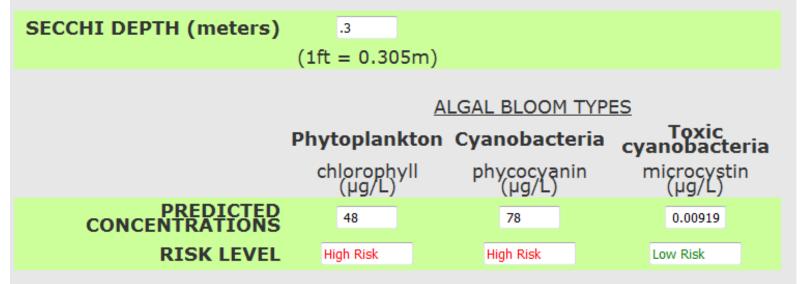
Web designer - Sarkis

SECCHI DEPTH FORECASTING MODEL

Secchi depth model to forecast freshwater algal and cyanobacterial blooms

How to use the model

Input Secchi depth (meters) in the first white box below. This value will be used to calculate the predicted concentrations of algae, cyanobacteria, or toxic cyanobacteria and associated risk level for your waterbody. Note that "risk" is a relative term. We used our knowledge of our dataset to relate risk with certain water quality parameters. If you are concerned about your waterbody, we encourage you to contact a local USDA extension agent.



Important note about the Secchi depth model

Please keep in mind that these models will be most relevant to systems sampled during warm summer months. Also, since Secchi depth is influenced by organic (phytoplankton) and inorganic (suspended sediment) particles, Secchi depths collected after rain events may provide spurious predictions.

http://wilsonlab.com/forecasting.html

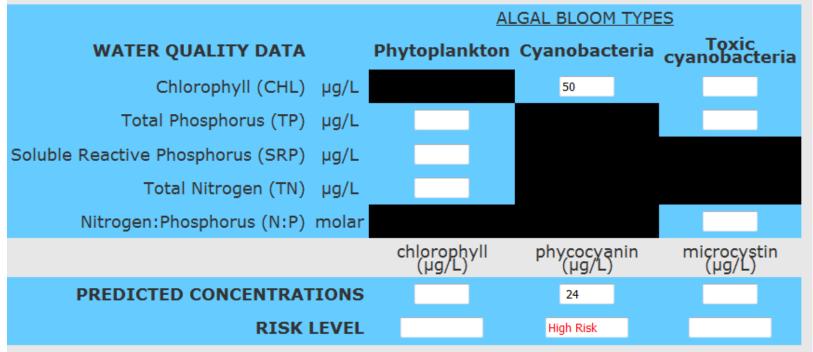
WATER QUALITY FORECASTING MODEL

Water quality model to forecast freshwater algal and cyanobacterial blooms

How to use the model

Input water quality parameters in the white cells below for the algal bloom type of interest (phytoplankton, cyanobacteria, or toxic cyanobacteria). These data will be used to calculate the predicted concentrations of algae, cyanobacteria, or toxic cyanobacteria and associated risk level for your waterbody. Note that "risk" is a relative term. We used our knowledge of our dataset to relate risk with certain water quality parameters. If you are concerned about your waterbody, we encourage you to contact a local USDA extension agent.

PHYTOPLANKTON AND CYANOBACTERIAL FORECASTING MODELS



http://wilsonlab.com/forecasting.html



Relevant publications and links

PAPERS

Carlson, R. E. 1977. A trophic state index for lakes. Limnology and Oceanography 22:361-369. http://aslo.net/lo/toc/vol 22/issue 2/0361.pdf

Chorus, I. and J. Bartram. 1999. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. http://www.who.int/water-sanitation-health/resourcesquality/toxicyanbact/en/

Doster, E., M. F. Chislock, J. F. Roberts, J. J. Kottwitz, and A. E. Wilson. 2013. Recognition of an important water quality issue at zoos: prevalence and potential threat of toxic cyanobacteria. Journal of Zoo and Wildlife Medicine 45:174-177. http://wilsonlab.com/publications/2014 JZWM Doster et al.pdf

Graham, J. L., K. Loftin, A. C. Keith, and M. T. Meyer. 2008. Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs: U.S. Geological Survey Scientific Investigations Report 2008-5038. http://pubs.usgs.gov/sir/2008/5038/pdf/SIR2008-5038.pdf

Kasinak, J.-M. E., B. M. Holt, M. F. Chislock, and A. E. Wilson. 2015. Benchtop fluorometry of phycocyanin as a rapid approach for estimating cyanobacterial biovolume. Journal of Plankton Research 37(1):248-257. http://plankt.oxfordjournals.org/content/37/1/248.abstract

Mattas-Curry, L., B. A. Schaeffer, R. B. Conmy, and D. J. Keith. 2015. A space satellite perspective to monitor water quality using your mobile phone. LakeLine 35(2):28-31 https://www.nalms.org/media.acux/beb75c9c-f812-4753-b888-79864899c6d6

Rosen, B. and A. St. Amand. 2015. Field and laboratory guide to freshwater cyanobacteria harmful algal blooms for Native American and Alaska Native communities. USGS Report 2015-1164. http://pubs.er.usgs.gov/publication/ofr20151164

Seltenrich, N. 2014. Keeping tabs on HABs: new tools for detecting, monitoring, and preventing harmful algal blooms. Environmental Health Perspectives 122(8):A206-A213 http://ehp.niehs.nih.gov/122-a206/

USEPA. 2015. Drinking water health advisories for two cyanobacterial toxins. https://www.epa.gov/nutrient-policy-data/drinking-water-health-advisory-documents

Relevant publications and links

TOOLS

CellScope http://cellscope.berkeley.edu

Cyanobacteria Assessment Network (CyAN) mobile app https://developer.epa.gov/hab-challenge/

Environmental Sample Processor http://www.nwfsc.noaa.gov/research/datatech/tech/esp.cfm

Flow Cytobot http://www.whoi.edu/main/imaging-flow-cytobot

Phyto cell phone app https://play.google.com/store/apps/details?id=name.gano.phyto&hl=en

Phytoxigene http://www.phytoxigene.com

Toxin strips http://www.abraxiskits.com/products/algal-toxins/

Toxin tube kits http://www.beaconkits.com/welcome/

REAL-TIME DATA AND FORECASTING

California Surface Water Ambient Monitoring Program (SWAMP) http://www.sfei.org/news_items/noaa-remote-sensing-tools-and-application-evaluating-cyanohabs-california-lakes

Great Lakes Environmental Research Lab (GLERL) http://www.glerl.noaa.gov/res/HABs_and_Hypoxia/habsTracker.html, http://www.glerl.noaa.gov/res/HABs_and_Hypoxia/rtMonWLE.html

Great Lakes Observing System (GLOS) http://habs.glos.us/map/

National Centers for Coastal Ocean Sciences (NCCOS) http://iodlabs.ucsd.edu/sgiddings/PNWTOX/infoModel.html, http://coastalscience.noaa.gov/projects/detail?key=148

NOAA Harmful Algal Bloom Operational Forecast System (HAB-OFS) http://habsos.noaa.gov/, http://habsos.noaa.gov/hab/

Phytoplankton monitoring network https://play.google.com/store/apps/details?id=name.gano.phyto&hl=en

Wilson, A. E. Cyanobacterial bloom forecasting website http://wilsonlab.com/forecasting.html