New HAB Monitoring and Assessment Techniques and Tools

Meredith Howard

Southern California Coastal Water Research Project

Measurements to Meet Monitoring Objectives

- Which potential toxin producing organisms/species are in the waterbody?
  - Microscopy, molecular tools (DNA Barcoding, qPCR), imaging instruments
- Which toxins are present?
  - Toxin detection in the field and lab
  - Passive samplers
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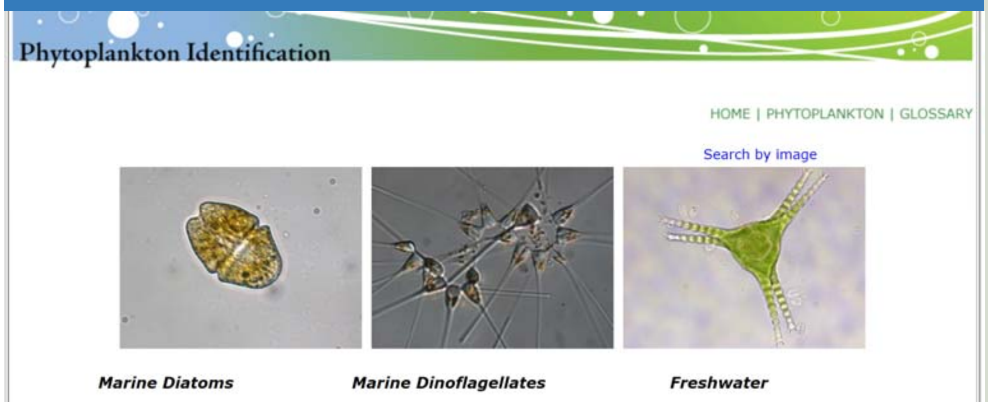
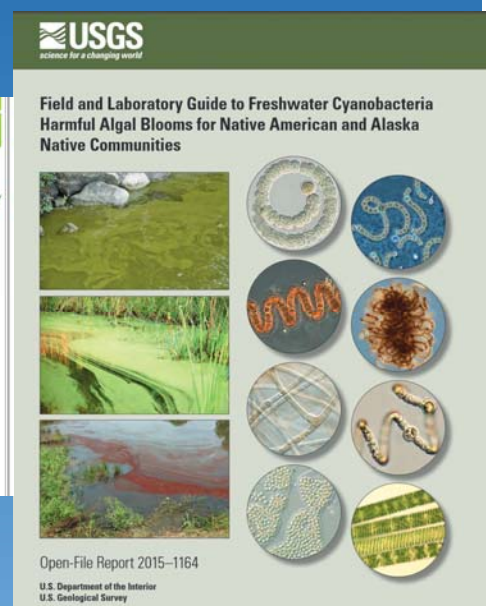
Microscopy

- Traditional ‘gold standard’ method for quantification
- Cell counts are time consuming and expensive
- Cryptic or rare species can be missed

- Alternative to formal cell counts: Relative abundance



Phytoplankton ID Gallery
http://oceandatacenter.ucsc.edu/home/
Kudela Lab, UC Santa Cruz



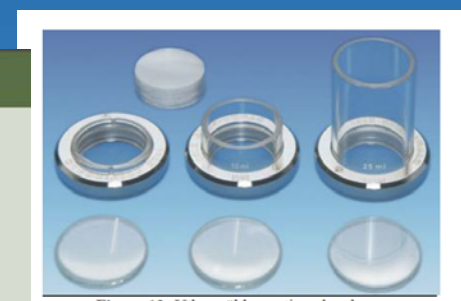
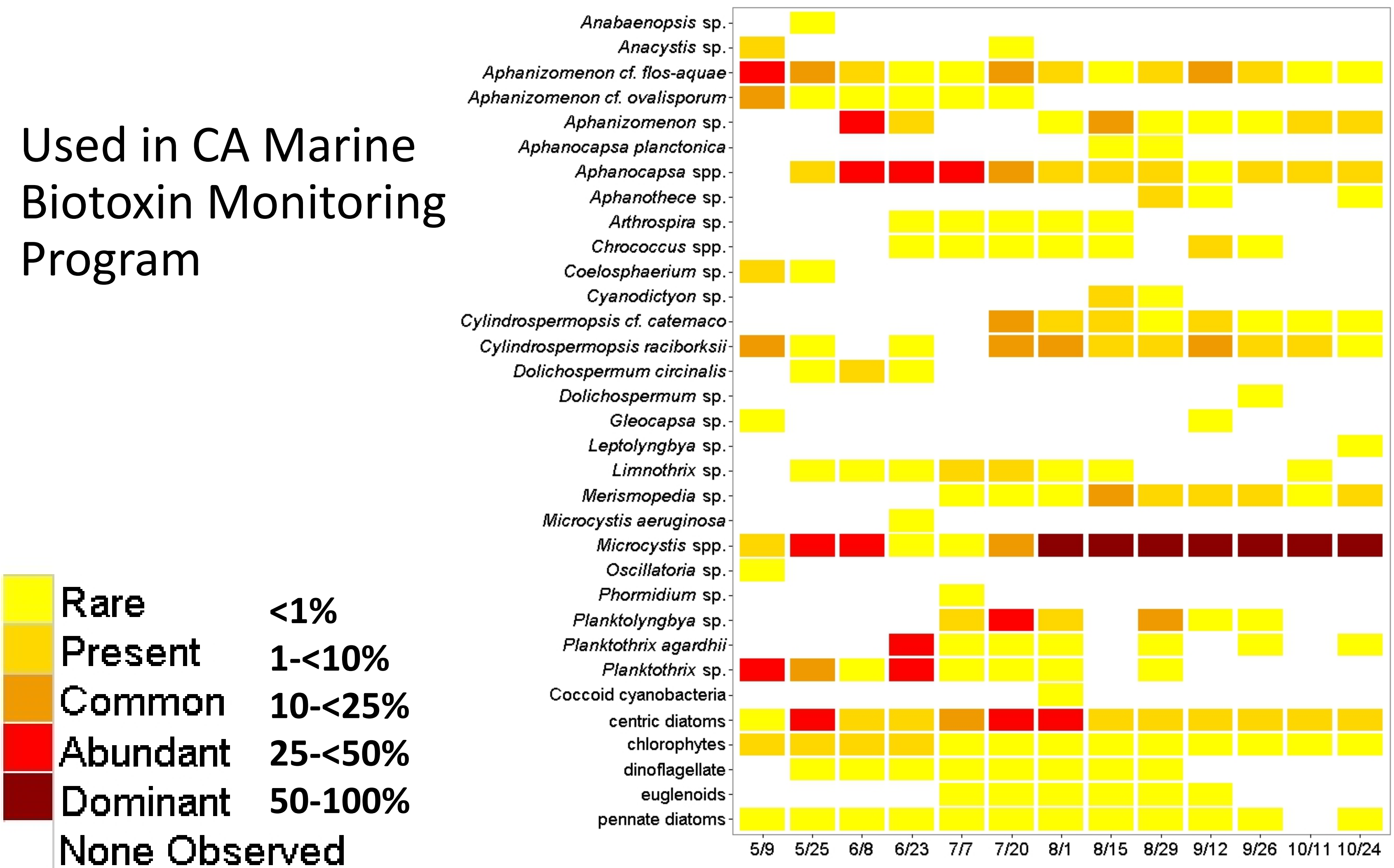


Figure 12: Uthermöhl counting chamber


Tatters et al., 2017

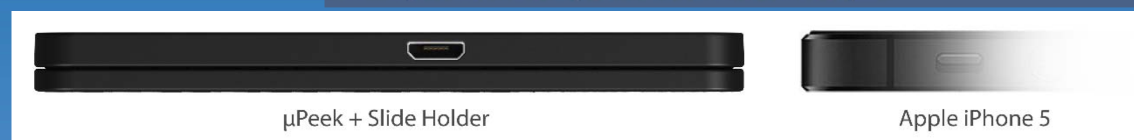
Relative Abundance

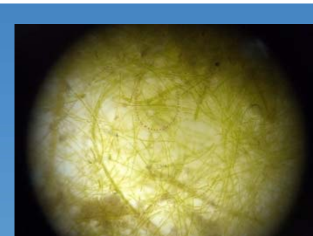
Used in CA Marine Biotoxin Monitoring Program

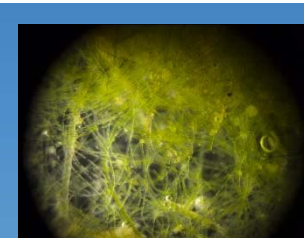


Field Microscopes

Nostoc - 200x – iPhone Field Scope







SCRONA

BF brightfield microscopy | DF darkfield microscopy | UV fluorescence microscopy[3]

FlowCAM

- Semi-automated imaging flow cytometer
- Identification and biovolume estimation

Imaging FlowCytobot (IFCB): *in situ* Automated Microscope

- *In situ* submersible flow cytometer that generates images of particles
  - Images classified to identification, abundance, biovolume

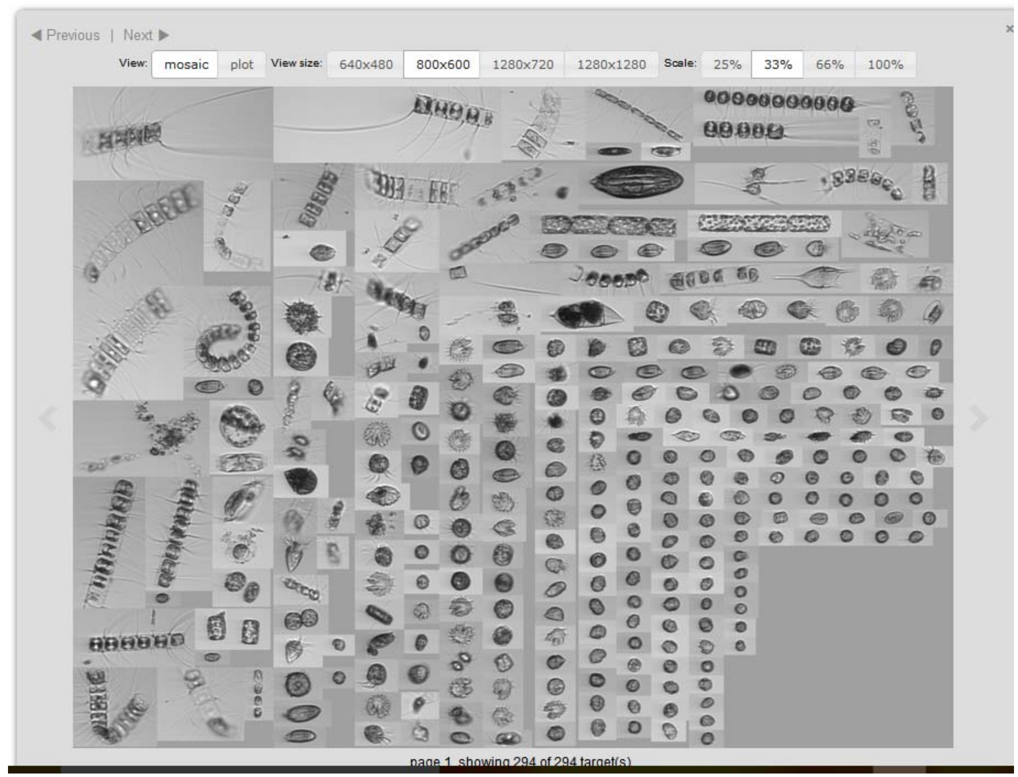


Image courtesy of Raphael Kudela, Santa Cruz Wharf
http://oceandatacenter.ucsc.edu/home/

Quantitative Polymerase Chain Reaction (qPCR)

- Identify and quantify taxa
  - Rapid, sensitive and specific approach for taxa identification
- Detection and quantification of toxin producing genes

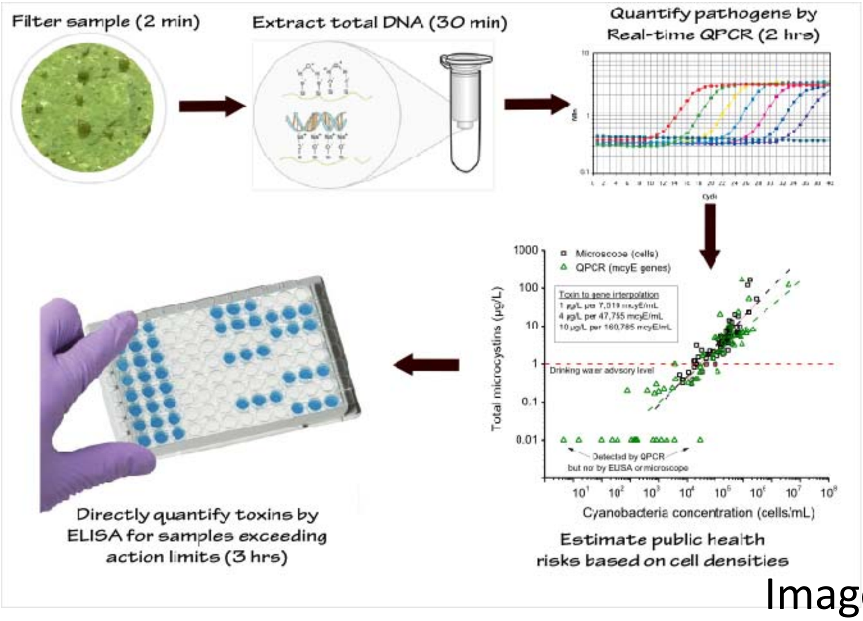
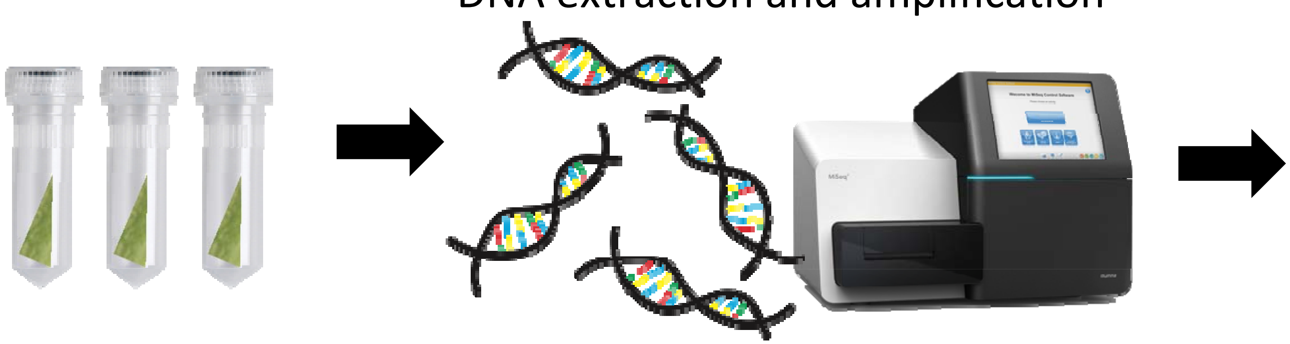


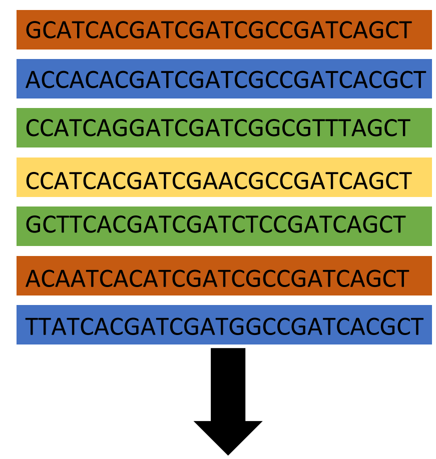
Image courtesy of Bend Genetics

DNA Barcoding:
DNA sequencing to identify algal species

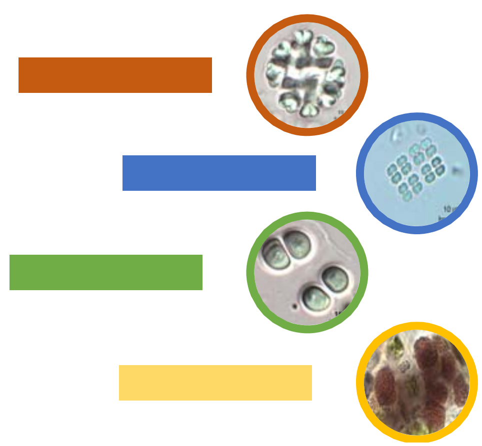
DNA extraction and amplification



Compare to reference library



Taxonomy assignments



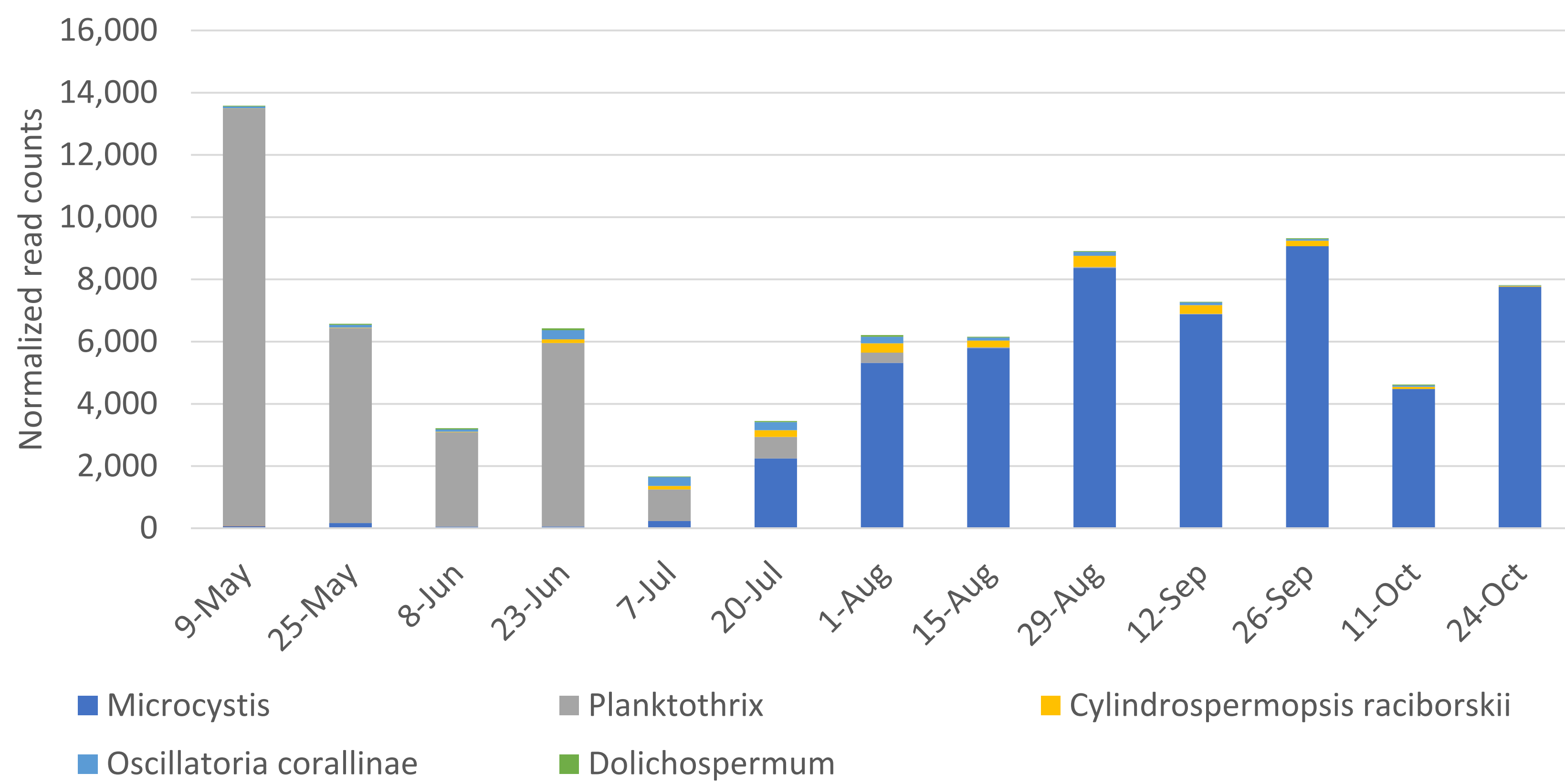
Advantages:

- Faster than traditional microscopy methods
- Can identify rare and cryptic species

Disadvantages:

- Requires a reference library

Potential toxin producers from Lake Elsinore



Theroux and Howard, unpublished data

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Field Tests for Cyanotoxins

- Envirologix QualiTube
  - Microcystins

- Beacon Analytical Systems Tube Kit
  - Microcystins

- Abraxis strip tests (‘dipsticks’)
  - Anatoxin-a, cylindrospermopsin, microcystins

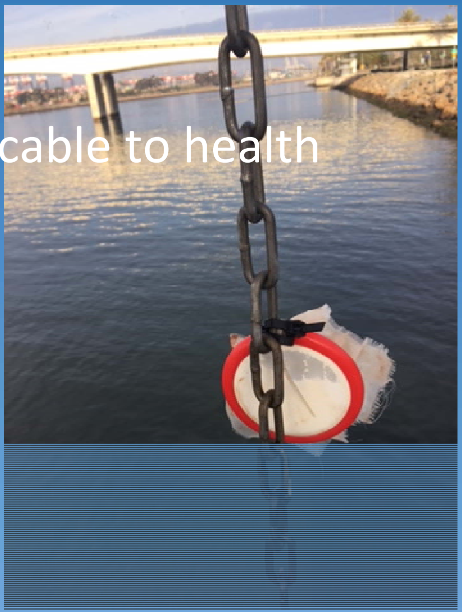
Monitoring Tool: Solid Phase Adsorption Toxin Tracking (SPATT)

Passive sampler that is time-integrative

- Applicable in all waterbody types (marine, brackish, freshwater)
- Provides continuous toxin detection to capture ephemeral events that discrete samples can miss
- Low cost, simple and easy to deploy/recover

Disadvantage:

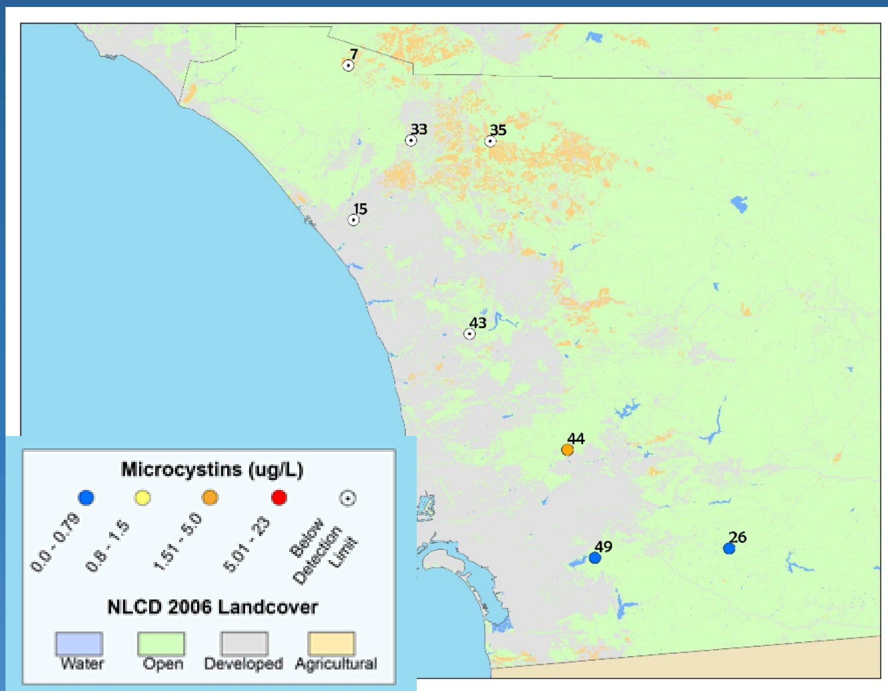
- SPATT will not provide a concentration of toxin that is applicable to health advisory thresholds (ng/g)
- Only measures dissolved toxins not total toxins



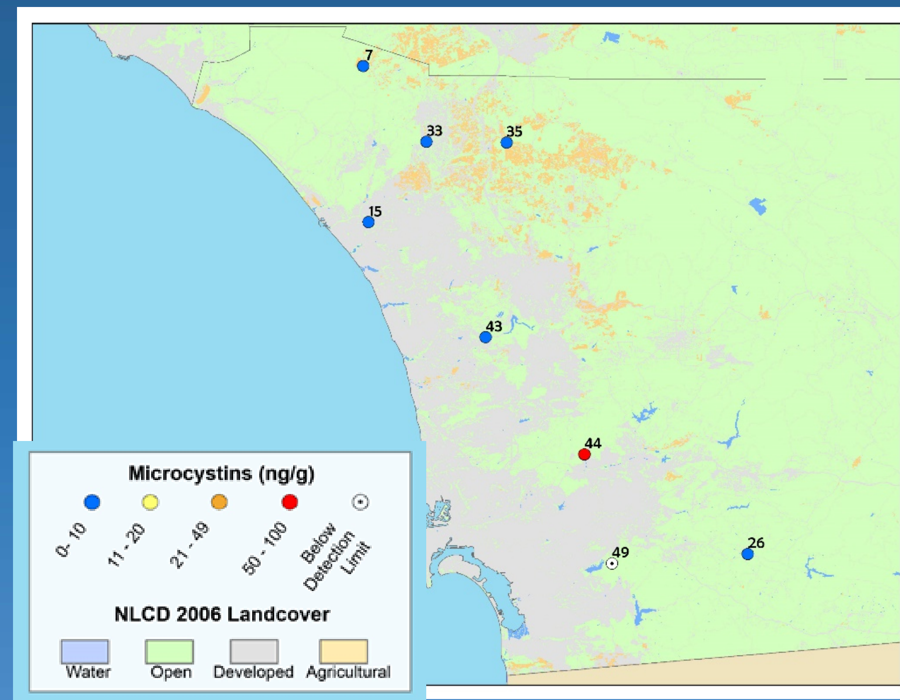
MacKenzie, 2010, Lane et al., 2010, Kudela, 2011

Microcystin Prevalence Underestimated From Grab Samples By ~50%

Grab Samples



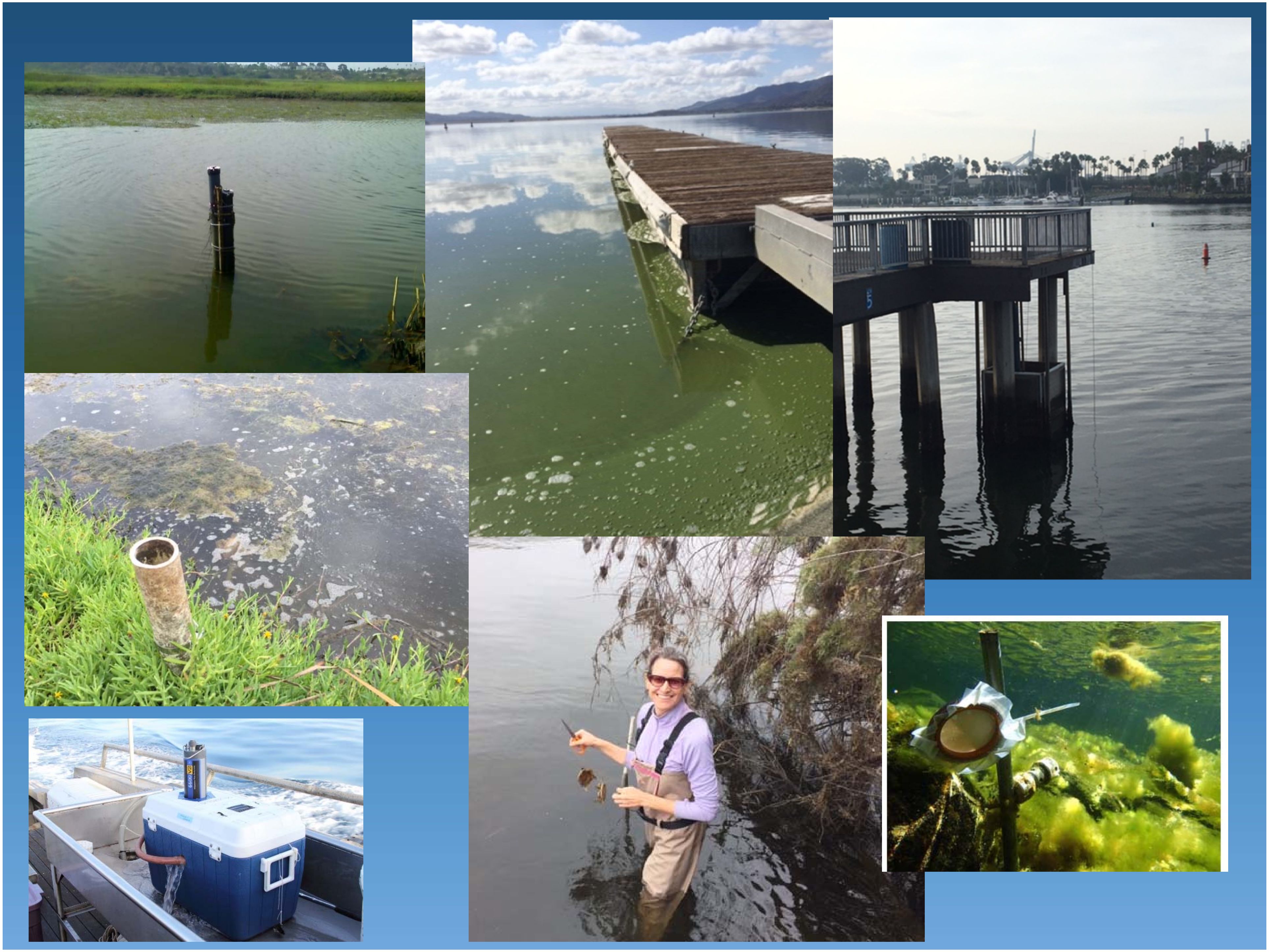
SPATT Samples



% of Toxic Sites: Depressional Wetlands

Grab Samples	**29%**
SPATT Samples	**83%**

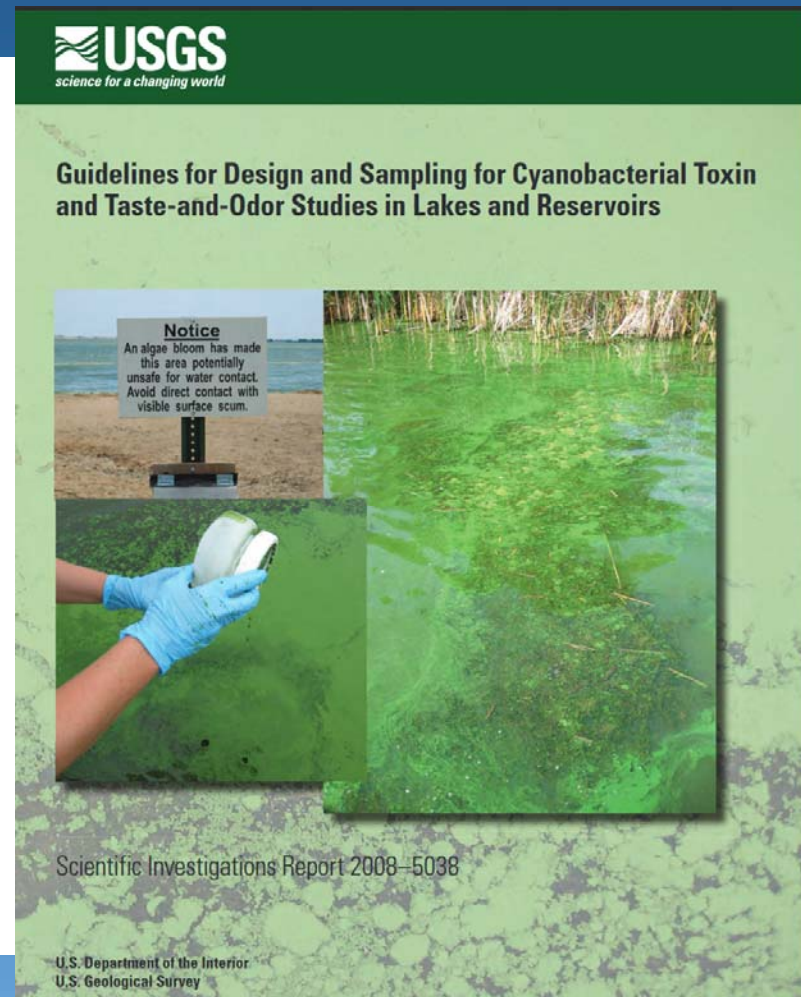
Howard et al., submitted



Common Toxin Analytical Techniques

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-analyte method	Sample concentration techniques may be necessary
Ion Trap Mass Spectrometry (LC/ITMS)	Compound identification is superior by LC/MS/MS or LC/ITMS	



Graham et al. 2008 USGS report

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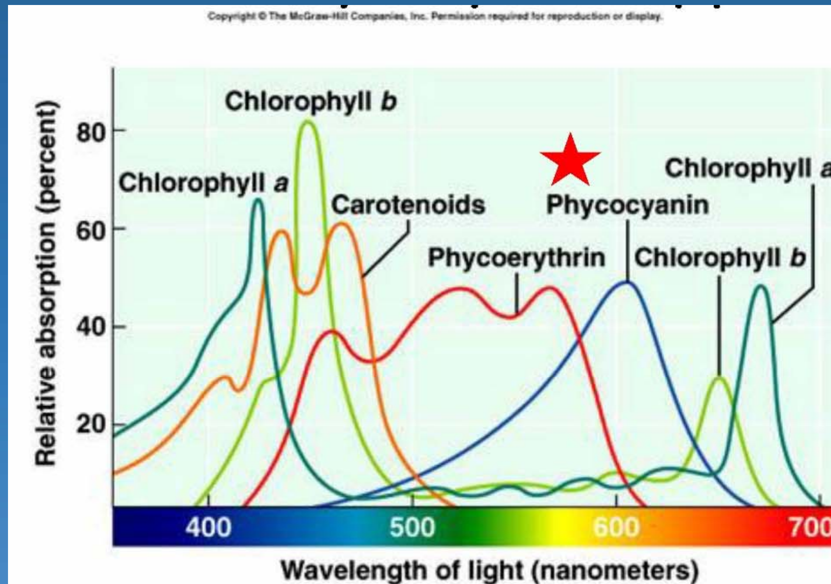
Common techniques for chlorophyll and phycocyanin quantification



Turner Designs Trilogy

Orange module
630nm excitation
660nm emission

Field sonde
Hydrolab minisonde

ALLIANCE FOR COASTAL TECHNOLOGIES

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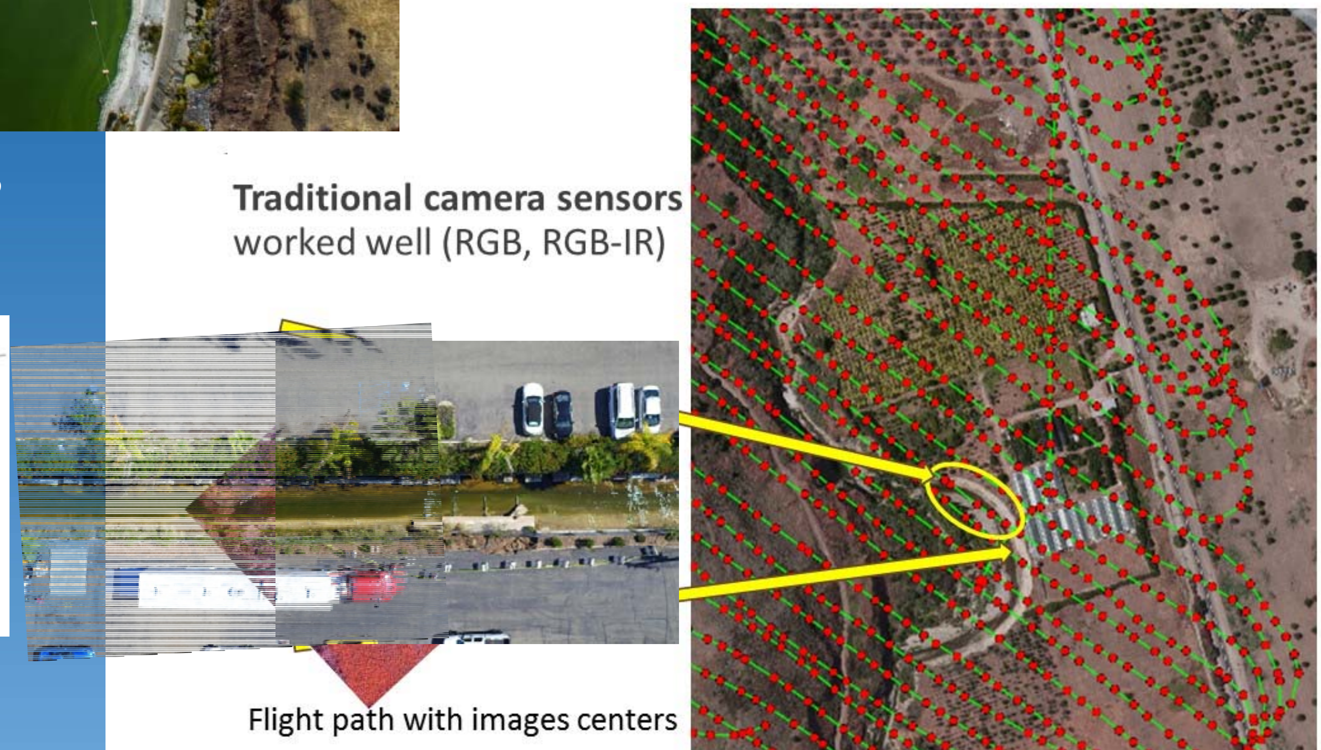
Aerial Cameras and Drones

Iron Gate Reservoir, October 2016
Photo courtesy of Susan Fricke

Traditional camera sensors worked well (RGB, RGB-IR)



Flight path with images centers

Steinberg, unpublished data

The data provide a three-dimensional map of the various water quality parameters.

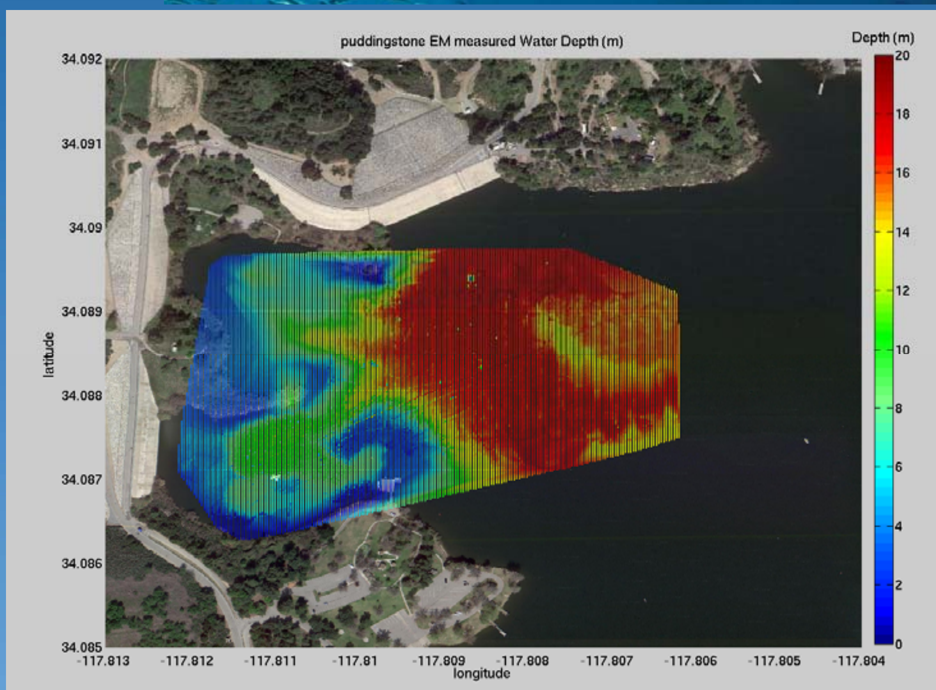
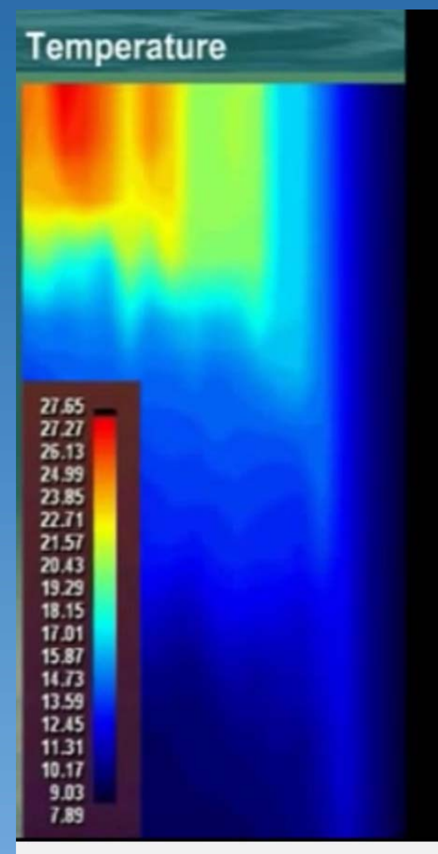


Figure courtesy of Stephanie Kemna, D. Caron & RESL at University of Southern CA



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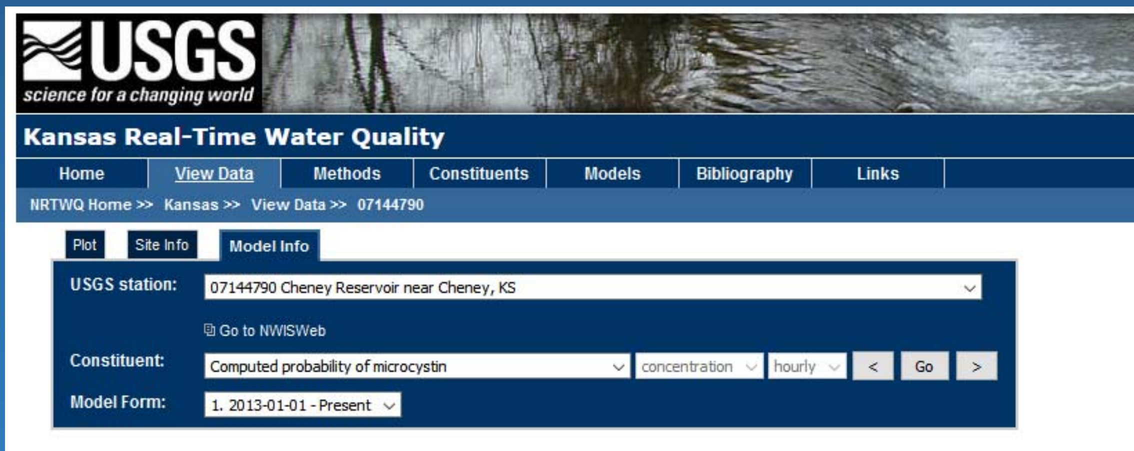
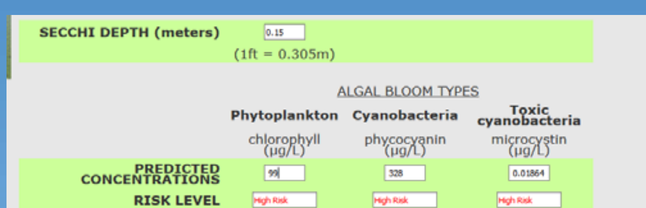
Models to Forecast Freshwater Blooms and Risk Levels

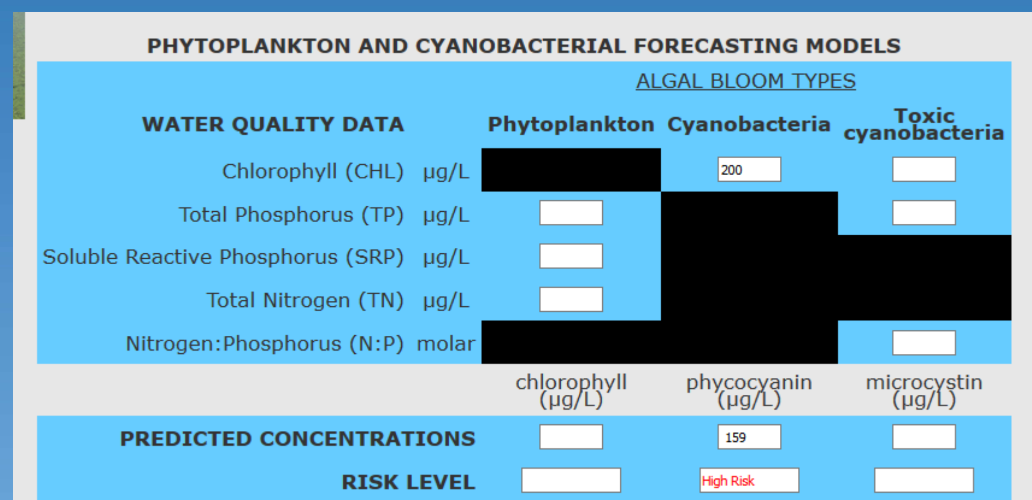


Computed probability of microcystin based on total chlorophyll

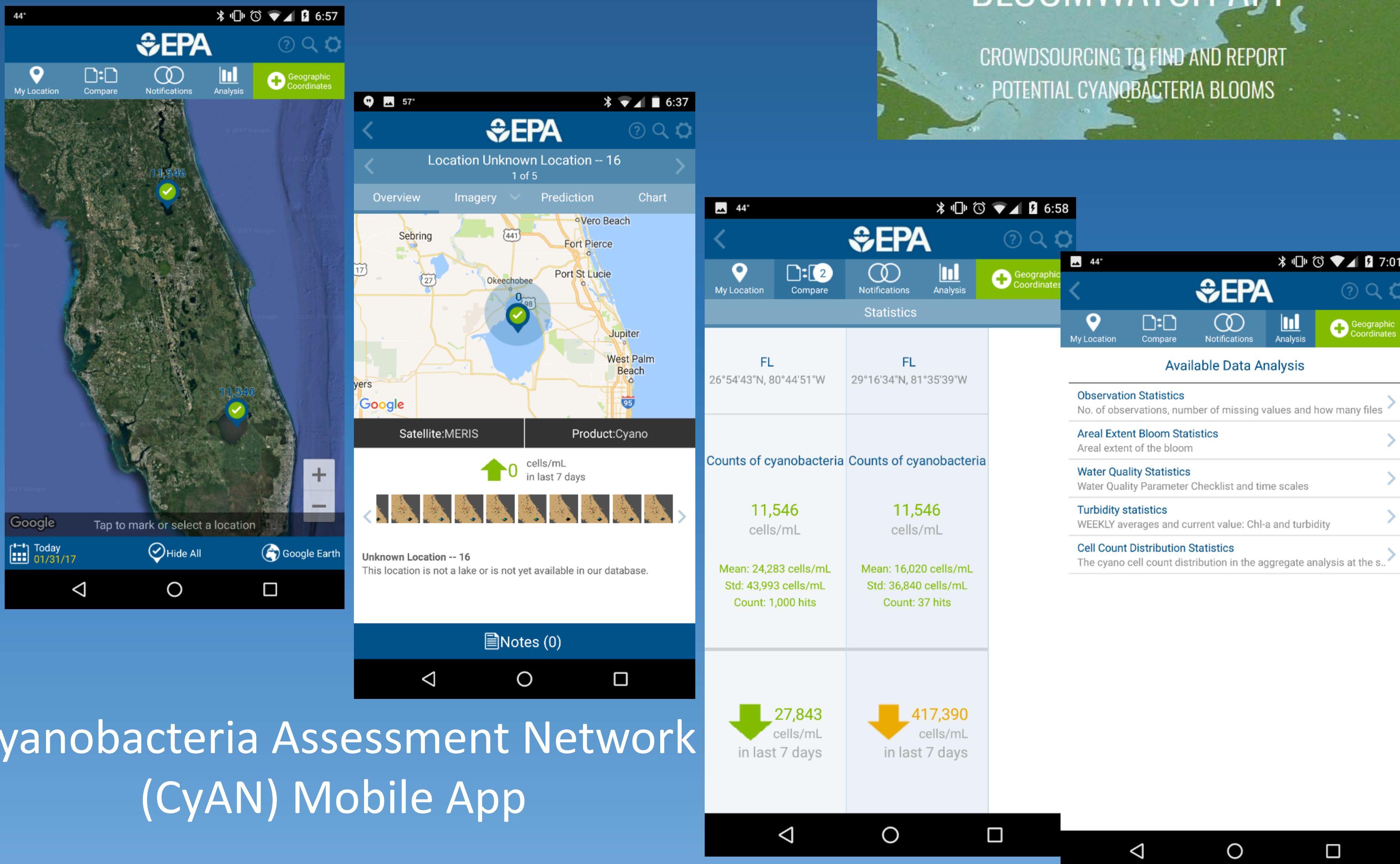
Stone, M.L., Graham, J.L., and Gatotho, J.W., 2013. http://pubs.usgs.gov/of/2013/1123/.

Model Developed By Alan Wilson
Auburn University, Alabama
http://wilsonlab.com/forecasting.html





Smartphone Bloom Reporting Apps



Cyanobacteria Assessment Network (CyAN) Mobile App


Questions?
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