Molecular methods for detecting and quantifying cyanobacteria

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Harmful Algal Blooms

- HABs are caused by cyanobacteria (*Microcystis*, *Planktothrix*, and *Anabaena)* that produce toxins such as microcystin

- Many factors can trigger toxin production

- Methods are needed to understand the potential for toxin production, what triggers toxin production, and provide an early warning
  - Molecular methods
  - Optical sensor measurements

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Molecular method

Quantitative polymerase chain reaction (qPCR)

- Looks for a specific genetic sequence in a specific type of microorganism
- Makes copies of the sequence so that it can be detected and quantified

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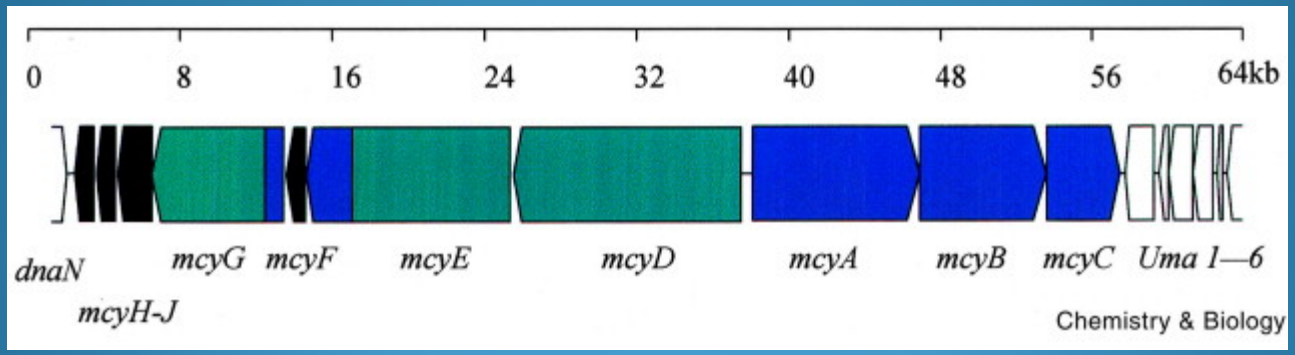
Microcystin Toxin Production

- Toxic strains contain microcystin synthetase (*mcy*) gene clusters in their genome.
- General and genus-specific qPCR assays have been created using the *mcy*E gene at the USGS Ohio Lab



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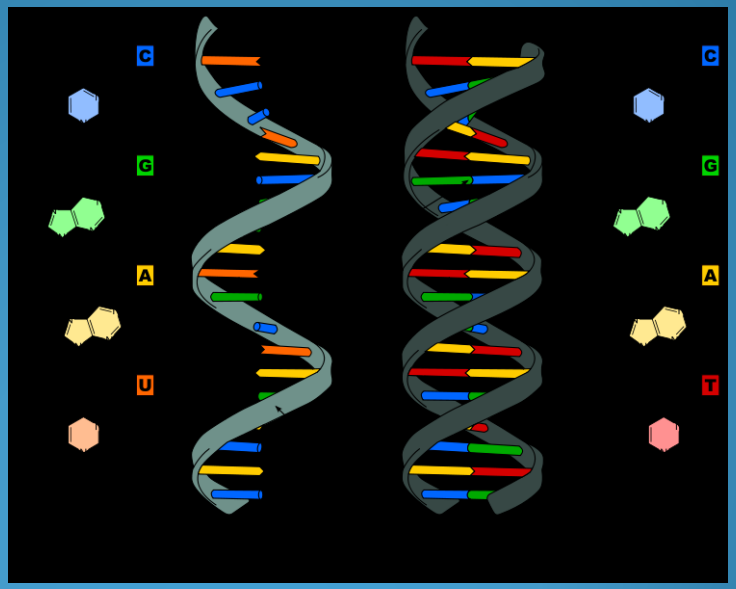
DNA vs. RNA

- DNA-based methods reveal the presence of the toxin gene.
- RNA-based methods can detect the cyanobacteria that are taking the first step in actively expressing the gene to produce the toxin.



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qPCR cyanobacterial assays

- Applied on four levels
  1. Total Cyanobacteria
  2. Total *Microcystis*
  3. Genus-specific DNA *mcy*E assays for *Microcystis*, *Planktothrix*, and *Anabaena*
  4. Genus-specific RNA *mcy*E assays for above genera

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Optical sensor measurements

- Measure algal pigments based on fluorescence
- Chlorophyll—total phytoplankton abundance
- Phycocyanin—cyanobacterial abundance

Pictures from YSI

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Two current USGS studies in Ohio

- DNA and RNA methods comparison in samples from Maumee Bay State Park Lakeside Beach—2012
  - Quantified variability of the assays
  - Investigated holding times for RNA analyses
  - Determined relations between DNA and RNA results
- Using tools to better understand and predict harmful cyanobacterial algal blooms in Lake Erie and Ohio inland lakes—2013–2015

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Maumee Bay State Park Lakeside Beach 2012

- Eleven samples from mid June to late August
  - All samples collected from one location at Lakeside Beach
- Analyzed for:
  - Microcystin by ELISA (Kansas Organic Geochemistry Research Lab)
  - DNA-based qPCR assays
  - RNA-based qRT-PCR assays

Funded by Ohio Lake Erie Protection Fund and USGS

NASA satellite photo from October 5th, 2011

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Hold time for RNA analyses

Sample date	Microcystis toxin mcyE at time 2 hours	Microcystis toxin mcyE at time 24 hours	Log difference
6/26/2012	<166	<166	ND
7/10/2012	1,200	<166	>0.86
7/24/2012	870	450	0.29
7/31/2012	2,400	1,400	0.23
8/9/2012	310	760	-0.39
8/14/2012	780	180 E	0.64
8/22/2012	1,200	<166	>0.86
8/29/2012	7,600	6,300	0.08
		Average log difference	>0.37

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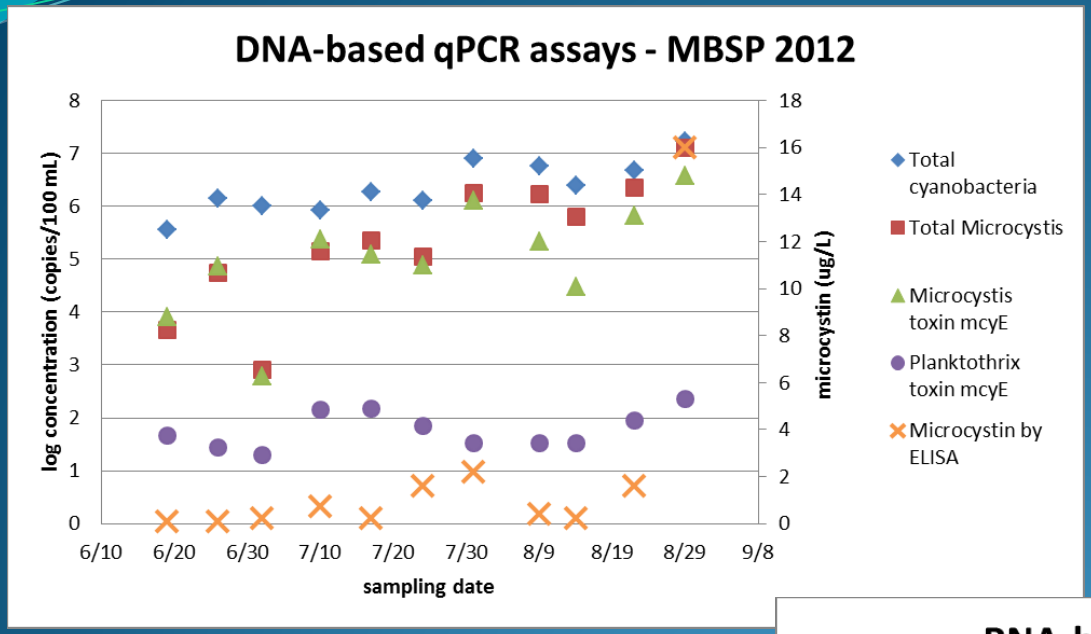
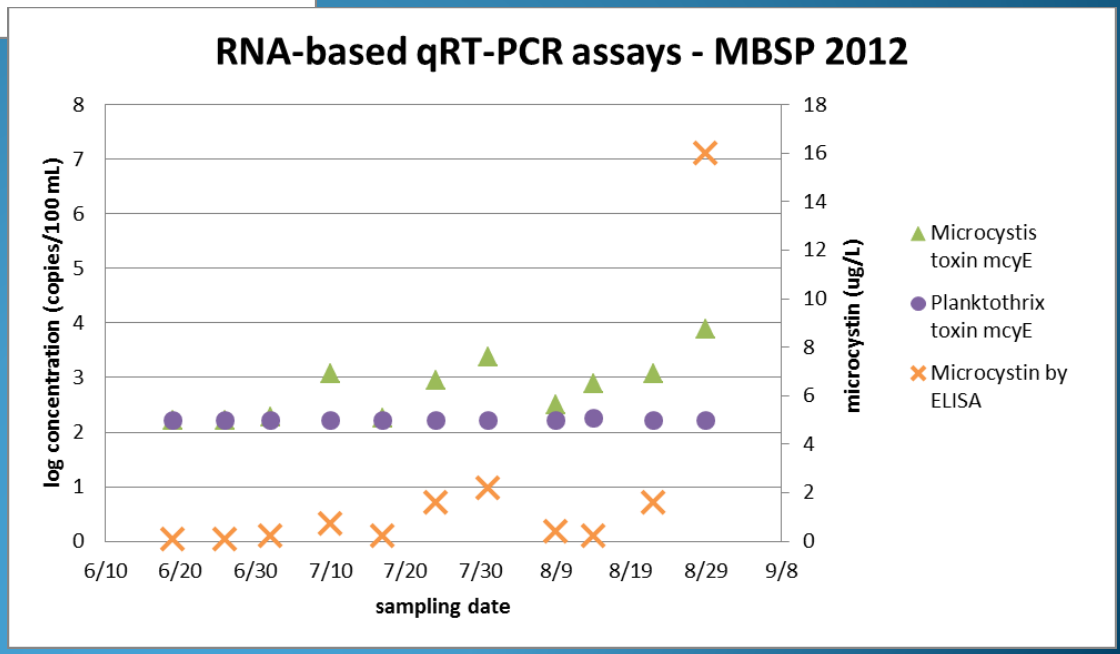
DNA and RNA methods comparison



DNA measures presence of the toxin gene.

RNA measures first step towards expression of the toxin gene.



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Tools to understand and predict HABs 2013–2015

- At Ohio beaches, understand how the community of the bloom progresses
  - From nontoxic to toxic strains
  - From unexpressed to expressed genes
- Determine relations between toxin concentrations and environmental and water-quality variables to help support predictive capabilities for HABs
- Determine if molecular assays and optical sensors can be used as early warning indicators

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Project team and funding

- USGS Kansas Water Science Center
- University of Toledo and Erie County Health Dept—sampling at Maumee Bay and Sandusky Bay sites
- USEPA—data sharing at Harsha Lake
- USACE—algal classification by FlowCam imaging particle analyzer
- Clark County Combined Health District—Buck Creek SP
- Ohio Dept of Natural Resources and Ohio EPA—technical advisors

Funded by Ohio Water Development Authority and USGS

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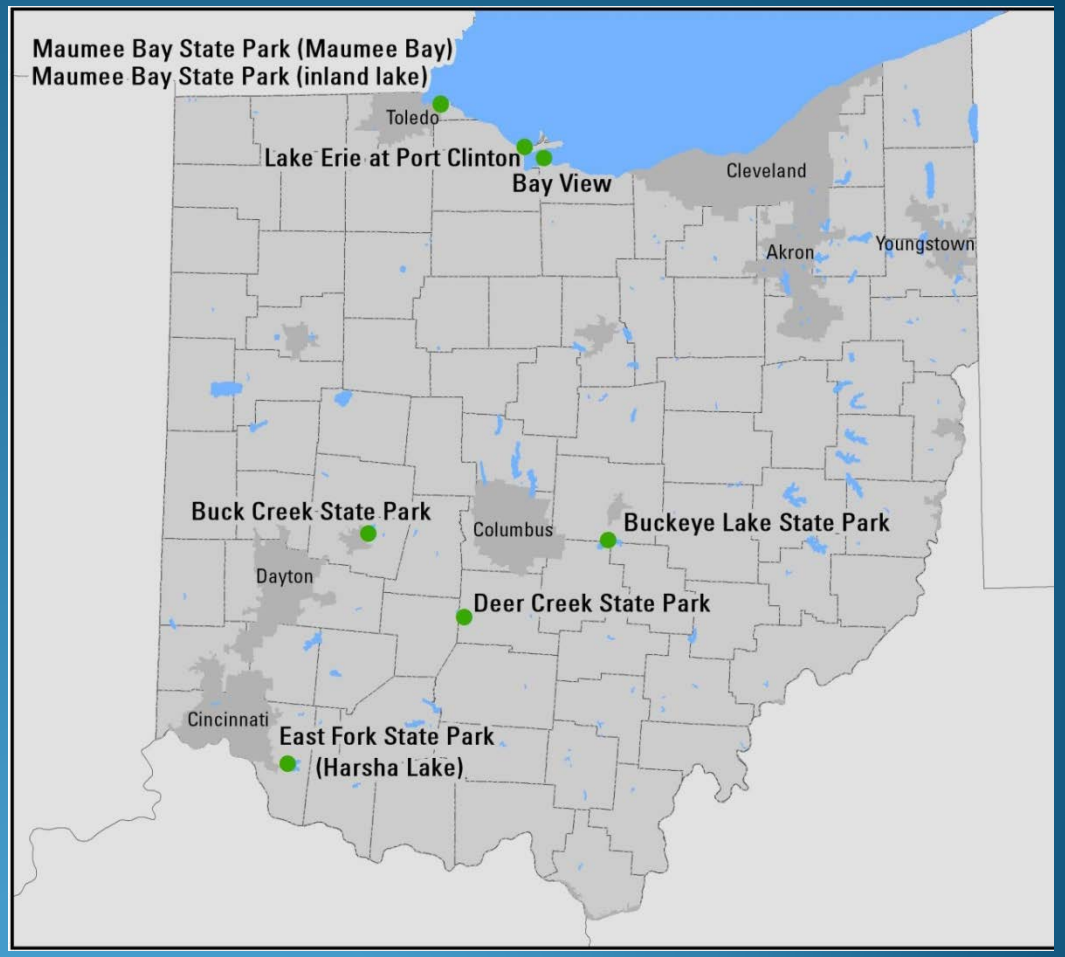
Phase 1—2013

- General survey of 8 beaches
- Sampling from weekly to monthly from April–Oct
- Nutrients, toxins, physical and algal pigment measurements, phytoplankton, cyanobacteria by qPCR, environmental data



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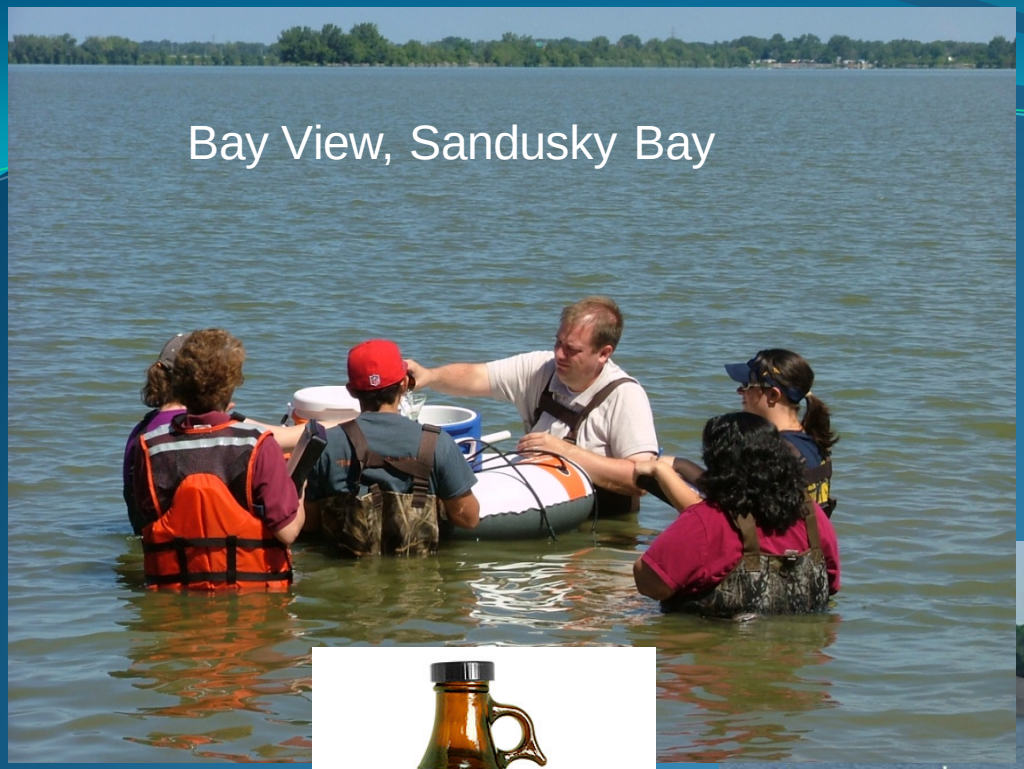

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Phase 2—2014

- More frequent sampling at 3 sites where early warning indicators are promising
  - Significant relations between qPCR results, optical sensor readings, phytoplankton, and (or) toxin concentrations
  - Patterns in these and water quality and environmental factors
- Understand temporal changes in community structure and toxins

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Questions?

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