Detecting Cyanobacterial Blooms & Toxins: Current and Emerging Technologies… and Future Priorities

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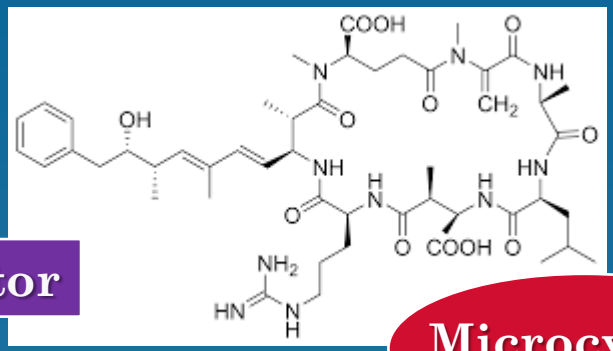
Topics to Consider

- **Cyanobacterial bloom/organism detection**
  - microscopy/visual; optical/spectral; molecular/immuno.
- **Cyanotoxin detection**
  - functional; structural; analytical; tiered strategies
- **Emerging sensor and sampling technologies**
  - in-water; ship-/shore-based; passive sampling
- **Observing systems/networks**
- **Future priorities**

WHY do we want to detect CHAB organisms and toxins?

- early warning of CHAB development and toxin production in support of management decisions
- regulatory applications mandated to ensure water is safe for drinking and recreational activities
- generate data for assimilation into predictive models for CHAB forecasting & long-term trends
- assess CHAB growth/toxicity for ecophysiological studies to ID environmental drivers/predictors

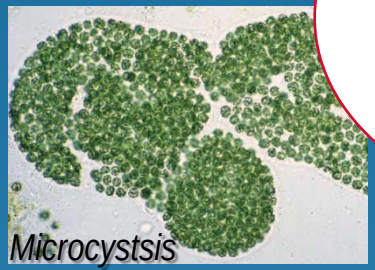
The Targets: CyanoHAB Taxa & Cyanotoxins (peptides)

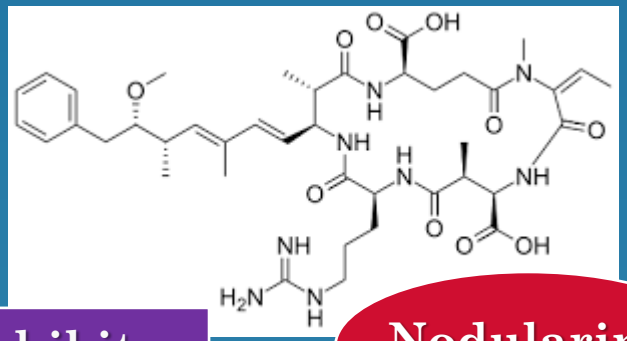


PP inhibitor

Microcystins

Anabaena
Anabaenopsis
Microcystis
Nostoc
Planktothrix





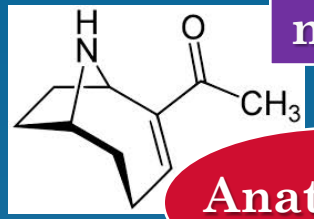
PP inhibitor

Nodularins

Nodularia

The Targets: CyanoHAB Taxa & Cyanotoxins (alkaloids)

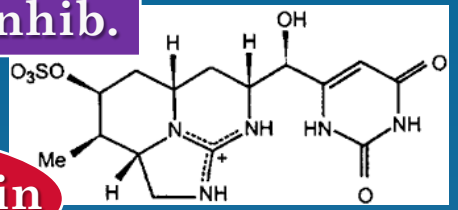
nAchR agonist



Anatoxin-a

Anabaena
Aphanizomenon
Microcystis
Planktothrix

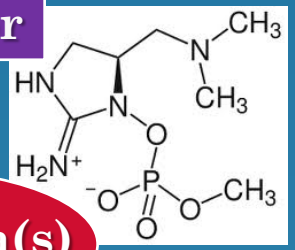
protein synthesis inhib.



Cylindrospermopsin

Aphanizomenon
Cylindrospermopsis
Raphidiopsis
Umezakia

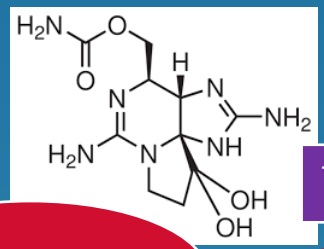
AChE inhibitor



Anatoxin-a(s)

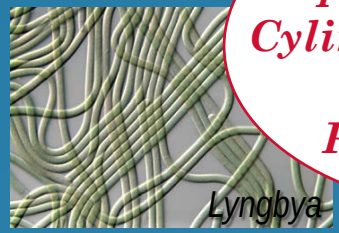
Anabaena
Aphanizomenon

VGSC blocker

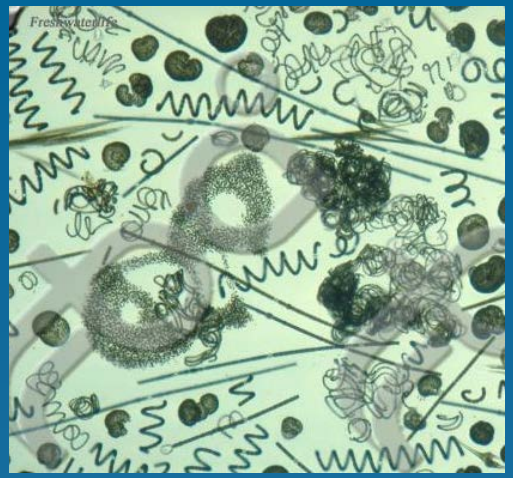


Saxitoxins

Anabaena
Aphanizomenon
Cylindrospermopsis
Lyngbya
Planktothrix



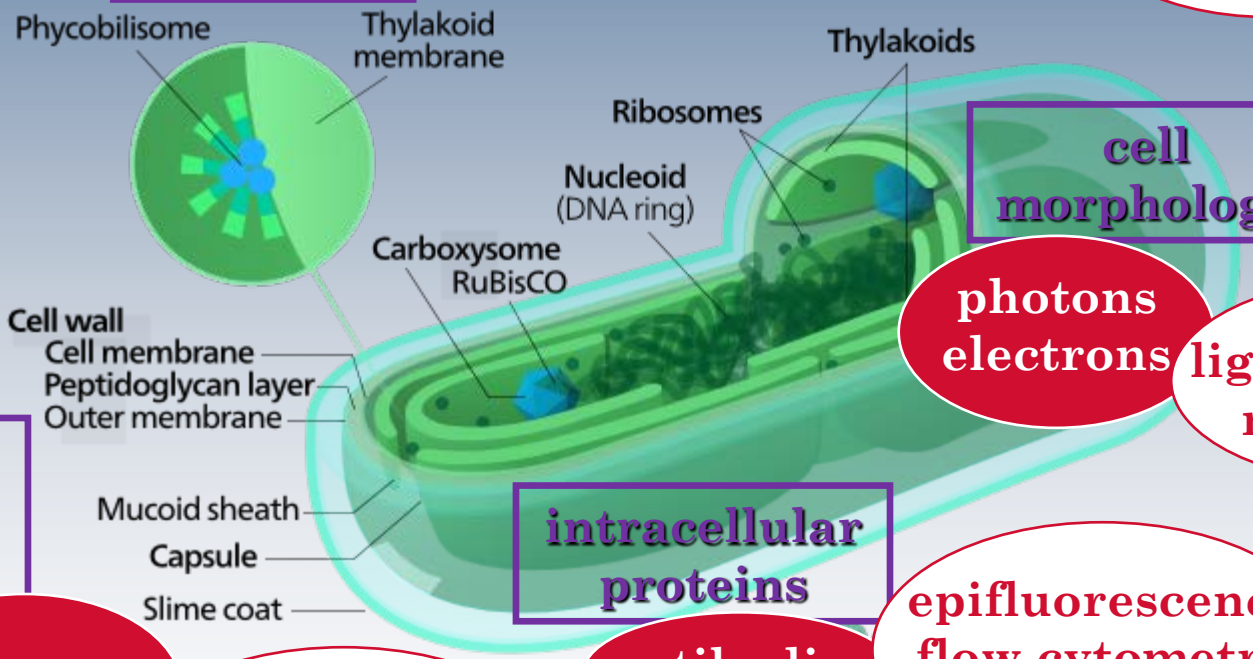
Detection of CHAB Organisms



What are some of the challenges?

- genetic variation w/in morphospecies
- molecular target levels variable (e.g., 16S rRNA)
- target taxa at low *relative* abundance (sample matrix interference)
- target taxa at low *absolute* abundance (requires adequate biomass/sample volume)
- suspended material; CDOM (remote sensing apps)

Targets & Tools for CHAB Organism Detection



remote sensing
in vivo fluor.
flow cytometry

radiometry
fluorometry
laser excit.

optical properties

nucleic acids

probes
primers

FISH/SHA
flow cytometry
qPCR

cell morphology

photons
electrons

light & electron microscopy

cell surface moieties

antibodies

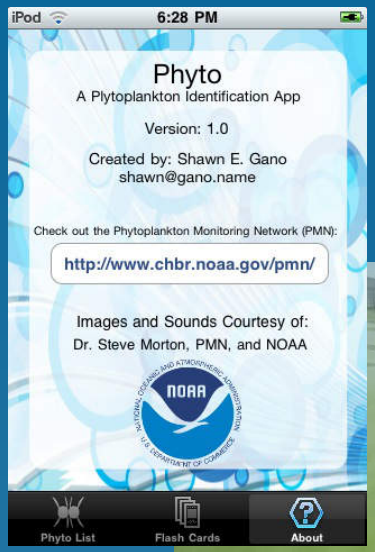
epifluorescence
flow cytometry
immunoassay

intracellular proteins

antibodies

epifluorescence
flow cytometry
immunoassay

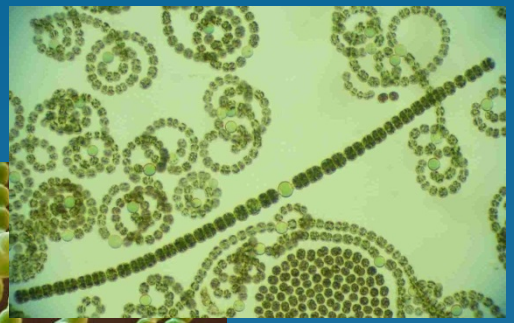
Microscopic/Visual Techniques

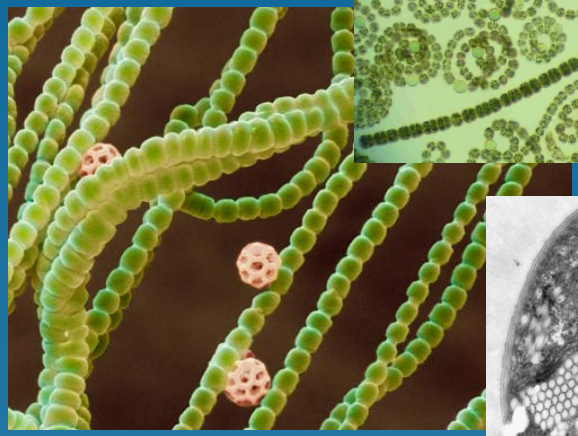


NOAA Cell Phone App
v2.0 incl. cyanos

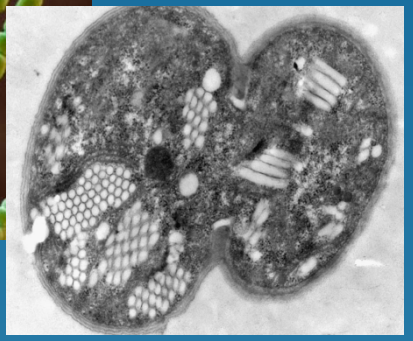
EYE

LM





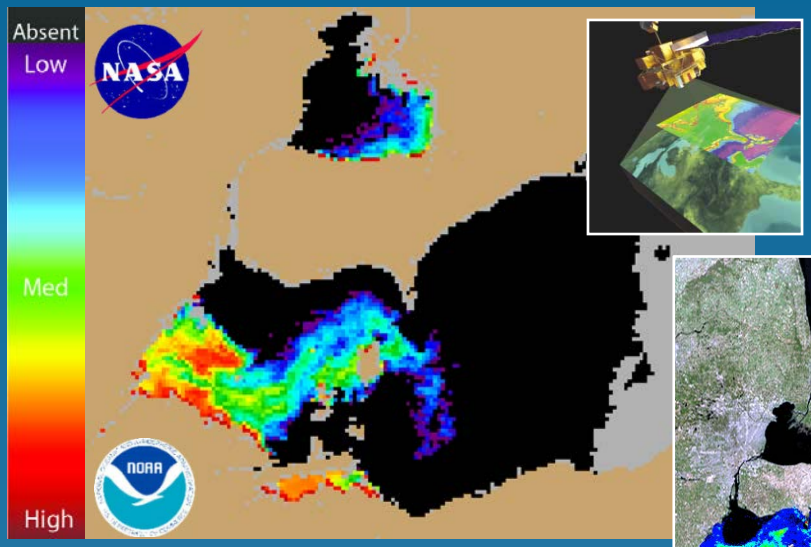
SEM



TEM

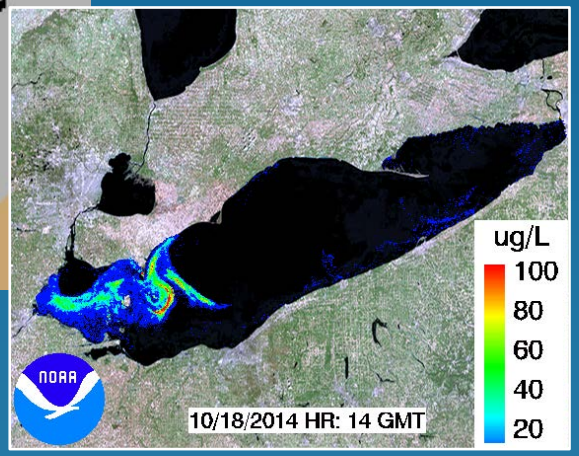
- **Light microscopy:** lab & field-portable units; genus-species level ID; low-cost; variable turnaround time; cell phone apps & LM camera attachments…internet connectivity for data distribution
- **Electron microscopy:** lab only; high cost & technical proficiency; ID confirm., extra- & intracellular ultrastructural details

Optical/Spectral Techniques



Cyanobact. Index; NASA MODIS-Aqua; 3 Aug 2014

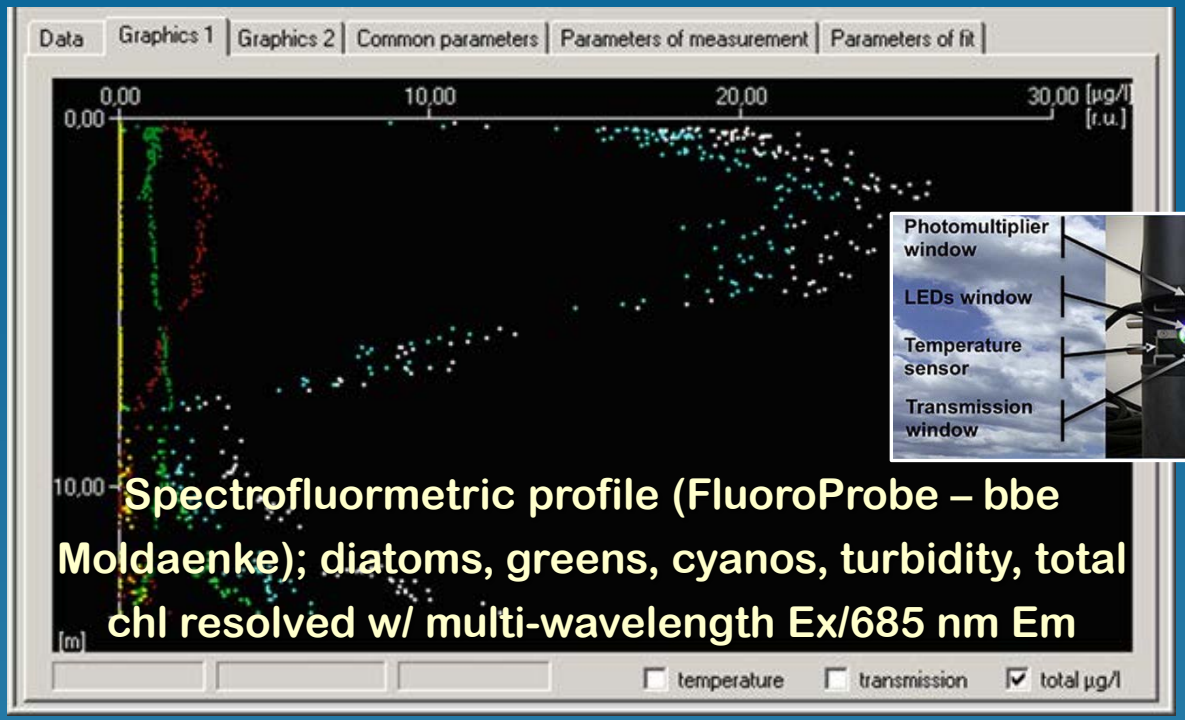
GLERL/CILER HABs Tracker



- **Space- or airborne RS:** spectroradiometers detect algal pigment signatures over large area; focus on surface waters, *but* can be limited by cloud cover/suspended particles/CDOM; RS data currently support *NOAA Experimental Lake Erie HAB Bulletin* & GLERL/CILER experimental Lake Erie *'HABs Tracker'* forecast

Optical/Spectral Techniques

fluorometers for benchtop & field apps; chl a, PC

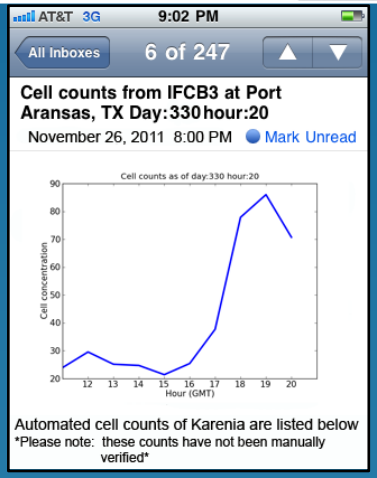


Spectrofluormetric profile (FluoroProbe – bbe Moldaenke); diatoms, greens, cyanos, turbidity, total chl resolved w/ multi-wavelength Ex/685 nm Em

- *in vivo* fluorometry (chl a, PC): *used extensively* as lab & field-portable units (hand-held; *in-situ*; profiles); estimate pigment conc. as biovolume proxy; *however,* can be issues with calibration, interference from turbidity; data influenced by species composition & cell physiology (NSW Tech Rpt #11433 (2012))

Optical/Spectral Techniques

Imaging Flow Cytobot & FlowCAM; imaging flow cytometers



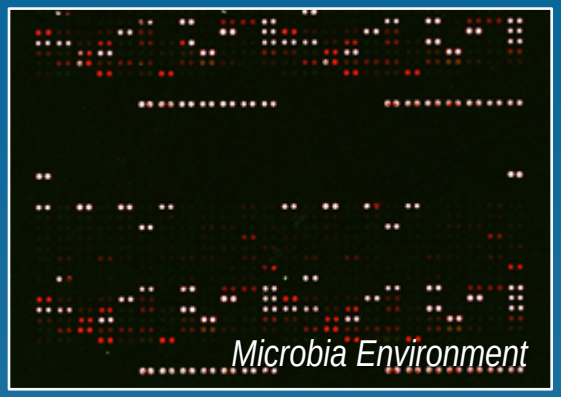
IFCB data reported over cell phone app showing increase in *Karenia* cell conc. at Pt. Aransas, TX (credit: L. Campbell, TAMU)

- **laser-induced fluorescence/light scatter**: flow cytometer (particle characterization); coupled with LM imaging (IFCB, FlowCAM; visual imaging, analysis, and ID of target cells); benchtop & *in-situ* units
  - IFCB provided early warning of 7 HAB events on TX coast (*Dinophysis* & *Karenia*; Campbell et al.); *issues with cyanobacteria colonies/filaments?*

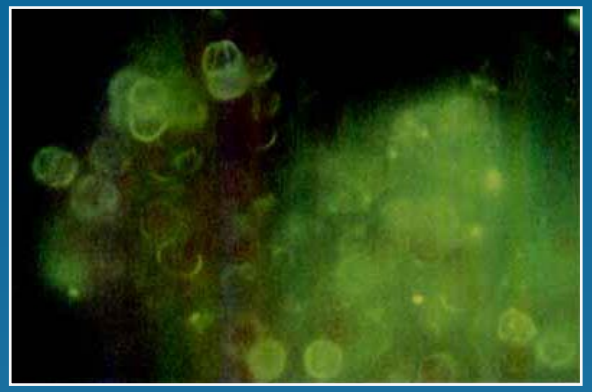
Molecular Techniques

- **qPCR/dPCR:** lab and field-portable units; *powerful & increasingly adopted detection technol.;* target taxon- and/or toxin-specific genes (*mcyE, mcyE/ndaF, cyrC, sxtI*; *multiplexed detection* – AusDiagnostics; Baker et al. 2013); sensitive, specific; *however,* variable sample extraction/reaction efficiency (e.g. PCR inhib.); not always 'cell specific' (amplify extracell. target)

Molecular/Immuno Techniques



microarray for detection of toxic algae; EU-MIDTAL project



antibody labeling of *Microcystis* colony (Lopez-Rodas & Costas 1997)

- **microarray:** DNA/oligo probes (rRNA target) for organism detection; slide-, microelectrode-, liquid-(Luminex) based formats; *multiplexed* detection of numerous targets; can 'tune' for taxonomic specificity; *EU-MicroAqua project:* univ. microarray for FW quality (50 targets)
- **immunoassay:** antibodies for cell-surface & intracellular antigens; generally use fluorescent reporters & microscopy; can automate detection (flow cytometry); *however,* susceptible to variation in target antigen expression & interspecific cross-reaction

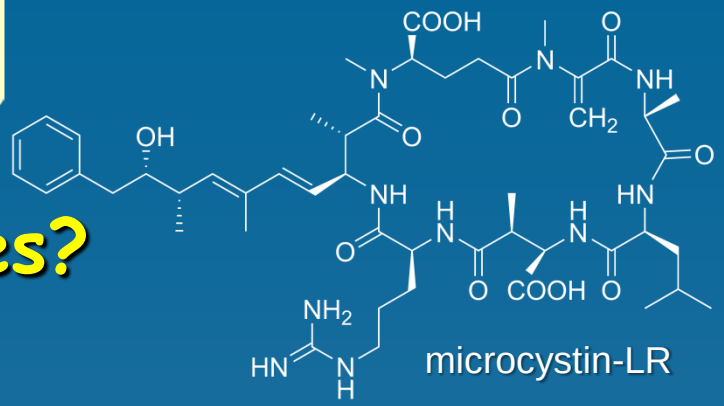
CHAB Detection – bottom line…

All CHAB detection methods & technologies have strengths & weaknesses:

choice of a given approach dictated by the needs of a specific application…

e.g., spatio-temporal coverage, specificity/selectivity, cost, speed, etc.

Detection of Cyanotoxins



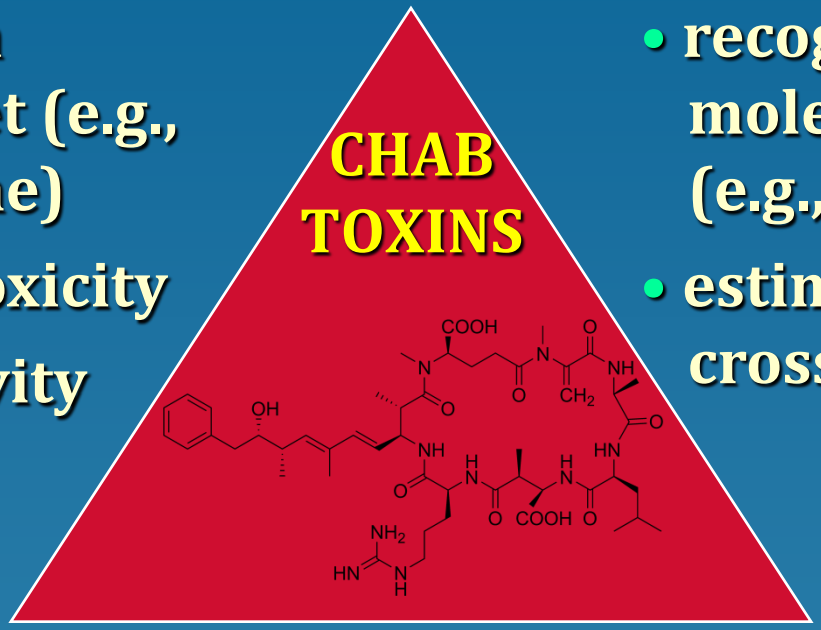
What are some of the challenges?

- multiple congeners/variants for single toxin class
- variable intrinsic toxicity across congeners
- particulate (incl. 'bound') & dissolved (extracell.); depends on species, growth stage, physiological status
- abiotic & biologically-mediated transformations (Schmidt et al. (2014) *Toxins* 6:3354)
- sample matrix interference
- low toxin conc. (requires adequate biomass/sample size)

Approaches to CHAB Toxin Detection

Functional Assays

- interaction with molecular target (e.g., receptor, enzyme)
- estimate total toxicity
- variable selectivity



Structural Assays

- recognition of molecular structure (e.g., Ab, aptamer)
- estimate conc. of cross-reactive variants

Physico-chemical (Analytical) Methods

- based on chemical properties/structure
- quantitative measurement
- confirmatory identification

Functional Assays

microplate-based radio-receptor binding assay for saxitoxins (AOAC OMA 2011.27)

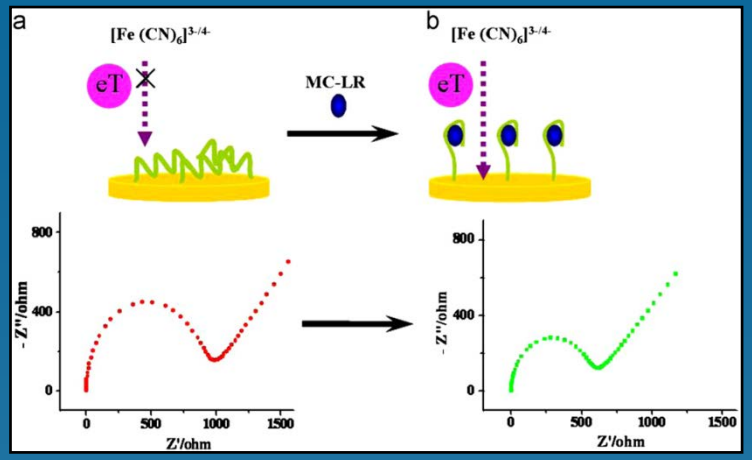
PPIA (MicroCystest; ZeuLab SL) for MC; performance validated - USEPA Environ. Technol. Verification Progr.

- **receptor binding assays:** e.g., VGSC for saxitoxins; nAChR for anatoxin-a (Abraxis); rapid, high throughput, nM sensitivity, variable end-points (isotope, color), *integrated toxic potency* for congeners present; *but, no congener ID*
- **enzyme inhibition assays:** e.g., protein phosphatase for MC, NOD (ZeuLab SL (Abraxis); microplate; color; 0.25-0.5 µg/L LOD); AChE for anatoxin-a(s) (cross-rxn w/ organo-P pesticides)

Structural Assays

microplate-based ELISA kit

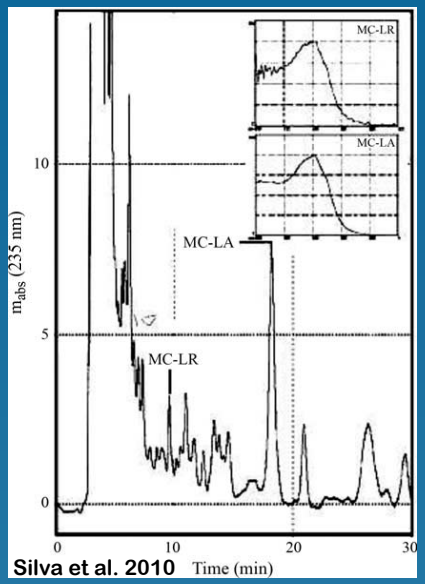
SPR optical biosensor & chips



aptamer-based electrochem. biosensor for MC (Ng et al. 2012)

- **immunoassays:** *most commonly used screening method;* MC/NOD (congener-spec. or –indep.), CYN, STX; formats: ELISA (plate/strip/tube; multiple vendors), SPR (optical biosensor), electrochemical biosensor; LOD ~ 0.04-0.4 µg/L
- **aptamer (ssDNA/ssRNA oligimer)-based assays:** non-commercial, emerging technol.; electrochemical biosensor for MC (Ng et al. 2012 *EST*); SPR biosensor for STX (Handy et al. 2012 *Toxicon*)

Analytical Methods



Silva et al. 2010

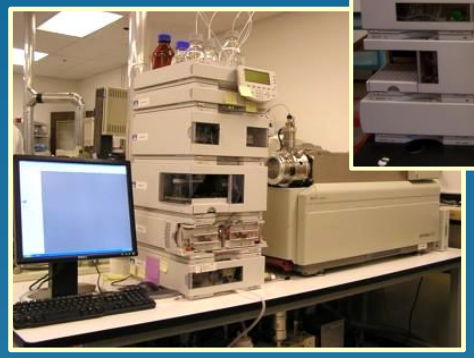
HPLC-PDA

HPLC-FLD

- **HPLC-UV/PDA:** liquid chromatogr.(RP C18) – UV/photodiode array; *commonly used*; toxin ID based on retention time + UV absorb. max. or full UV spectrum; low-mod. sensitivity; SPE clean-up/conc. lowers MDL; ISO 20179:2005 HPLC/PDA (valid. for MC-LR, -YR, -RR)
  - **HPLC-FLD (saxitoxins):** HILIC column; fluorescence (pre- or post-column deriv.)

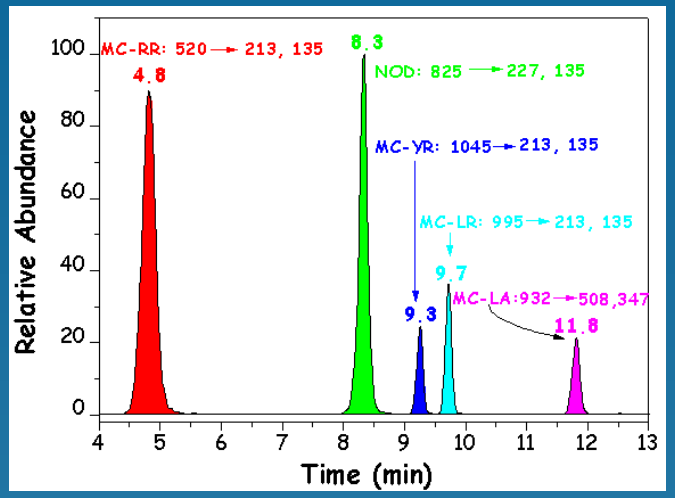
Analytical Methods

LC-triple quad. MS



LC-IT MS

LC-QTOF MS

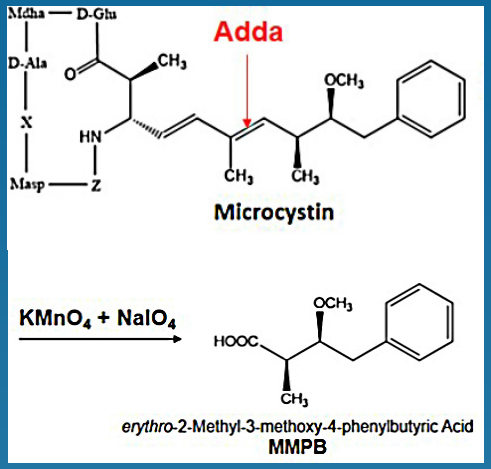


- **LC-MS/MS (triple quadrupole; ion trap; QTOF)**: liquid chromatogr.–tandem MS – *considered 'gold standard'*; high selectivity, sensitivity & confirm ID; capable of *multiplexed detection*; **triple quadrupole** – most commonly used for cyanotoxin quantification & ID; **QTOF** – *accurate mass* and high sensitivity across rapid full-scan of mass range for product ion spectrum (effective for ID *toxin metabolites*)

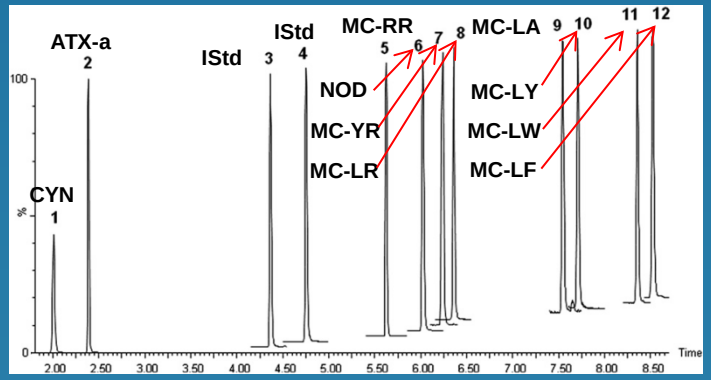
Analytical Methods - Multiplexing

Detecting multiple cyanotoxins in a single analysis

- Roy-Lachapelle et al. (2014, *Anal Chem Acta*): total MC (bound & unbound) analyzed in water using MMPB oxidation product; simple oxid. step; 15 s analysis time with laser diode thermal desorption-APCI-MS/MS; LOD = 0.2 µg/L



- Oehrle et al. (2010, *Toxicon*): 7 MCs, NOD, CYN, ATX; UPLC-MS/MS (MRM); LOD ~ 0.5 µg/L *w/o clean-up & enrich.*

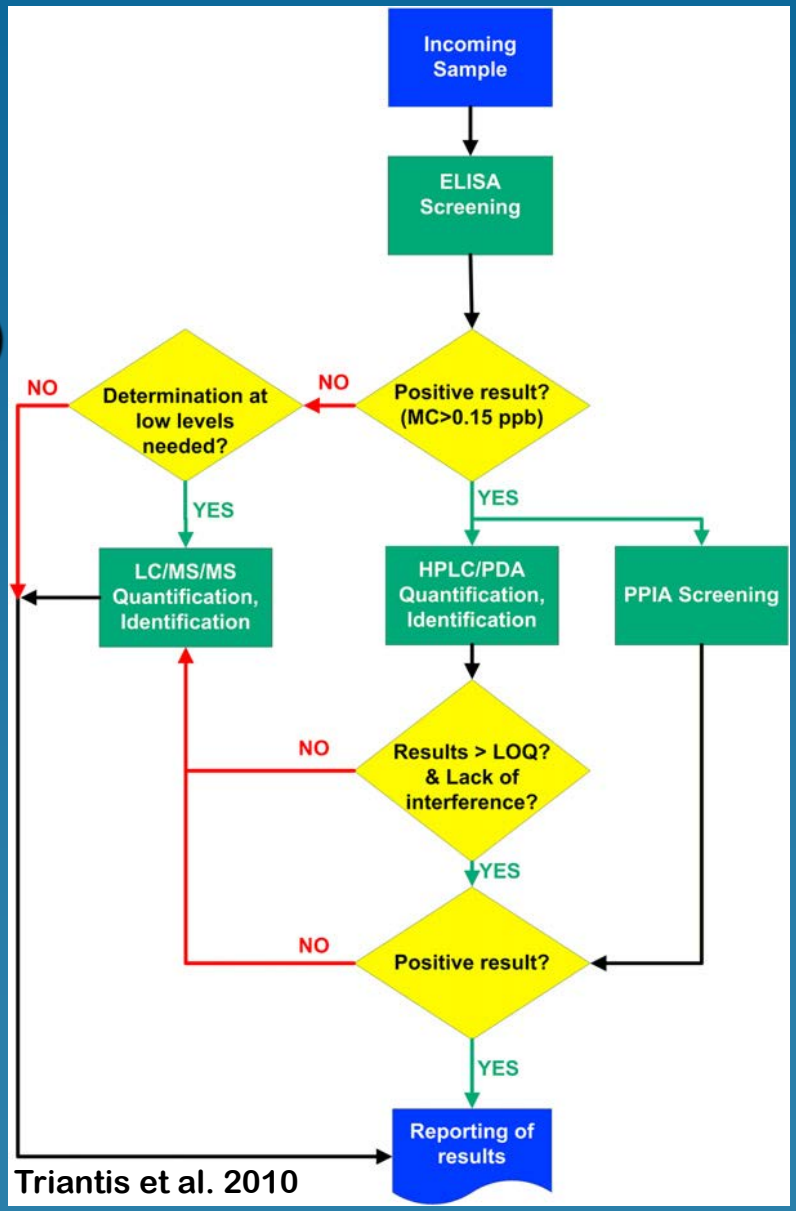


- Yen et al. (2011, *Toxicon*): 6 MCs, NOD, CYL, ATX; *dual-cartr. SPE* (C18 (MC, NOD, ATX) + carbon (CYN, ATX)); LC-ESI-MS; LOD: 2-100 ng/L, depends on toxin; LOD ↑ 5-10x w/ environ. smpls.

Tiered Cyanotoxin Detection Strategies

Depending on 'use case', rapid *in-vitro* screening assays (e.g. ELISA) ID putative positive samples; confirm. by anal. meth. (e.g. HPLC-PDA; LC-MS/MS)

- rapid screening can be used 'on-site' to provide real- or near real-time data
- elimination of negative samples for further analysis maximizes efficient use of laboratory resources
- overall cost of analysis can be reduced even if all screened positive samples are run by an analytical method

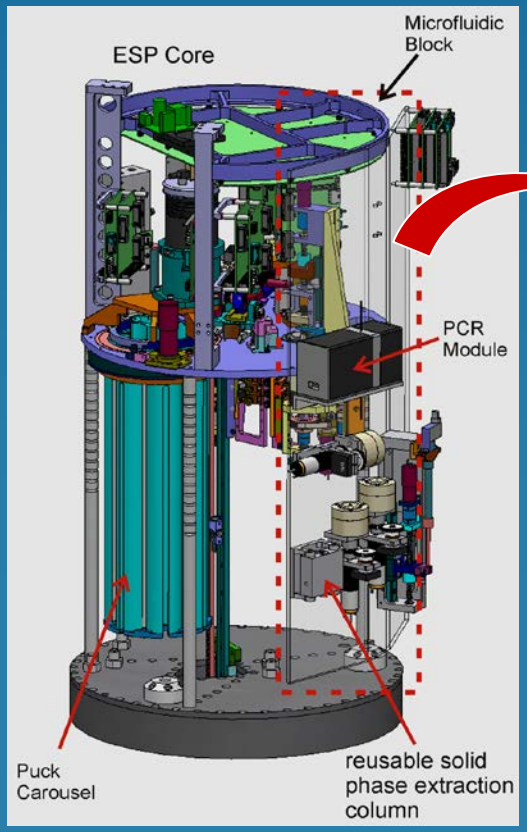


Triantis et al. 2010

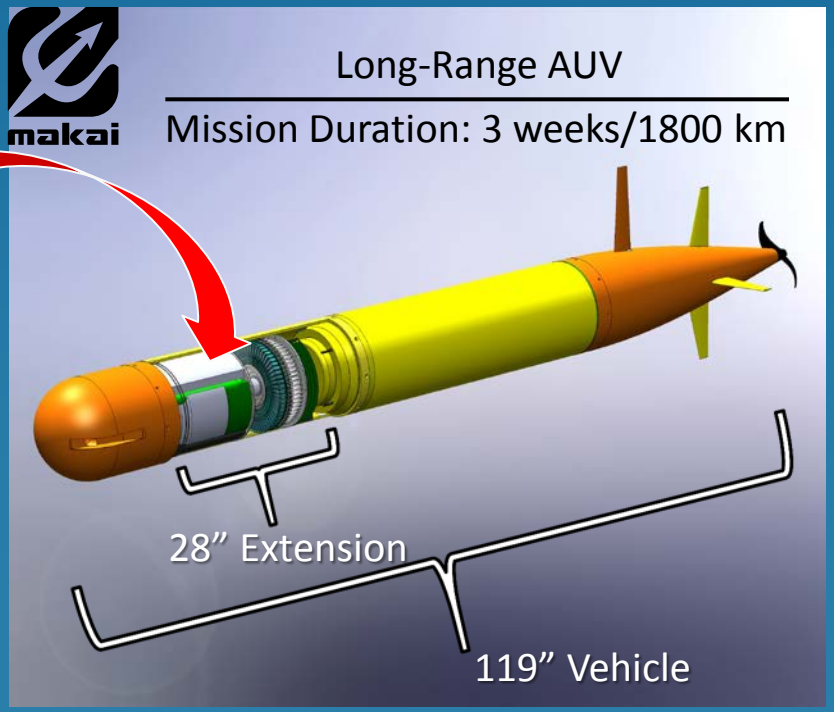
Emerging Sensor Technologies

In-water & ship/shore-based sensor technologies capable of multiplexed detection of cyanobacteria AND cyanotoxins

2G ESP



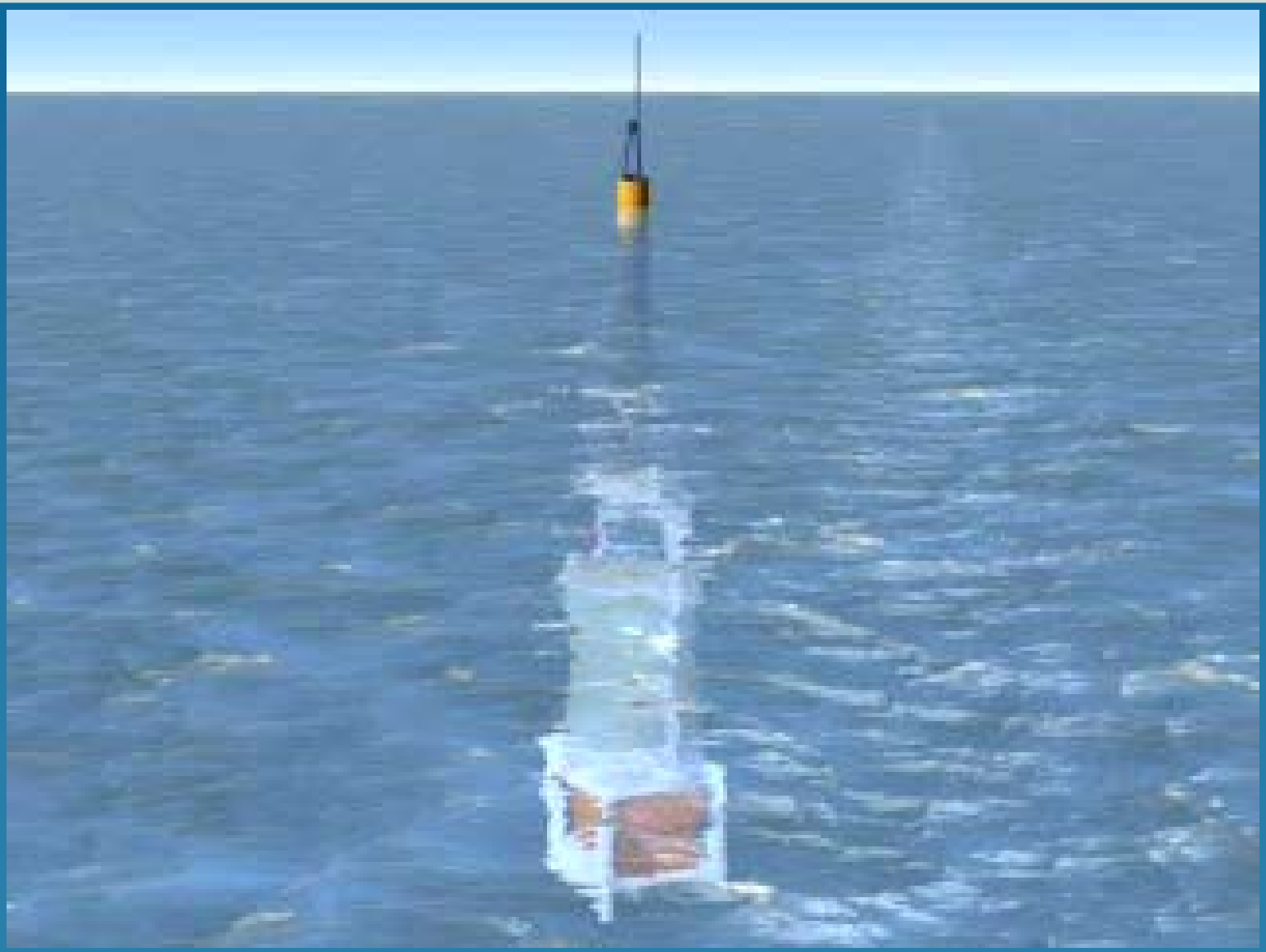
3G ESP



MBio Waveguide

Planar Waveguide Assay Cartridges and Reader

The Environmental Sample Processor – autonomous, in-water HAB/toxin detection



MBARI

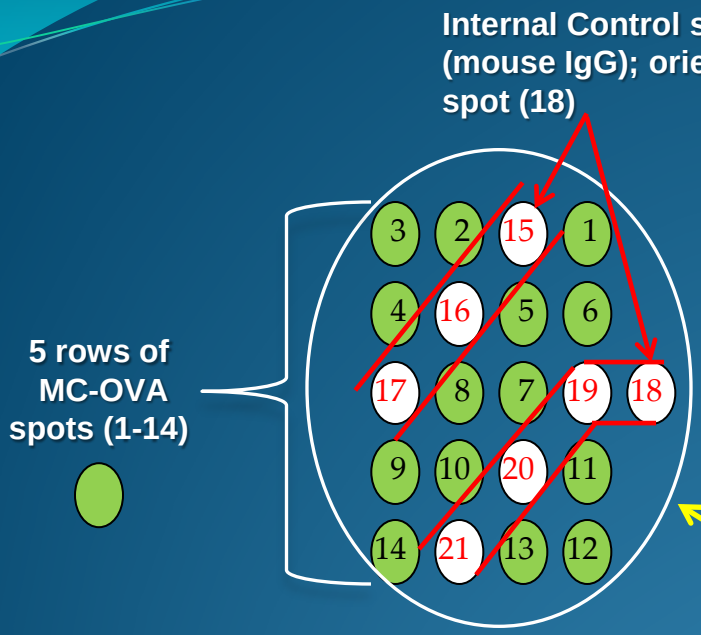
Current Capabilities of G2 ESP

2G ESP instrument located at NOAA/CCEHBR Laboratory

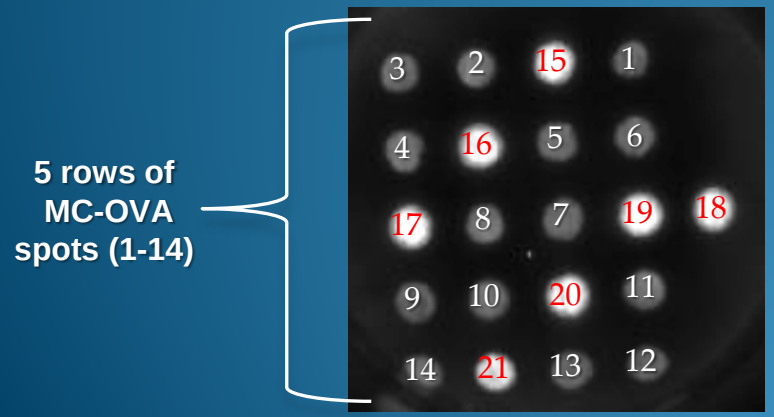
- Real-time application of DNA probe arrays (SHA); algae, bacteria, inverts
- Real-time application of protein/Ab arrays (cELISA); phycotoxins
- Sample archival
  - whole cell microscopy/FISH
  - nucleic acids (gene libraries)
  - phycotoxins
- Real-time application of quantitative PCR (qPCR); various target genes
- In-water & pier/land-based deployment
- Mfg by McLane Research Laboratories

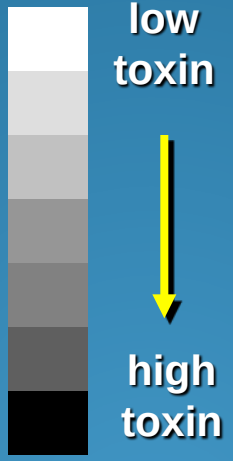
Developing a Microcystin/Nodularin Array-based cELISA

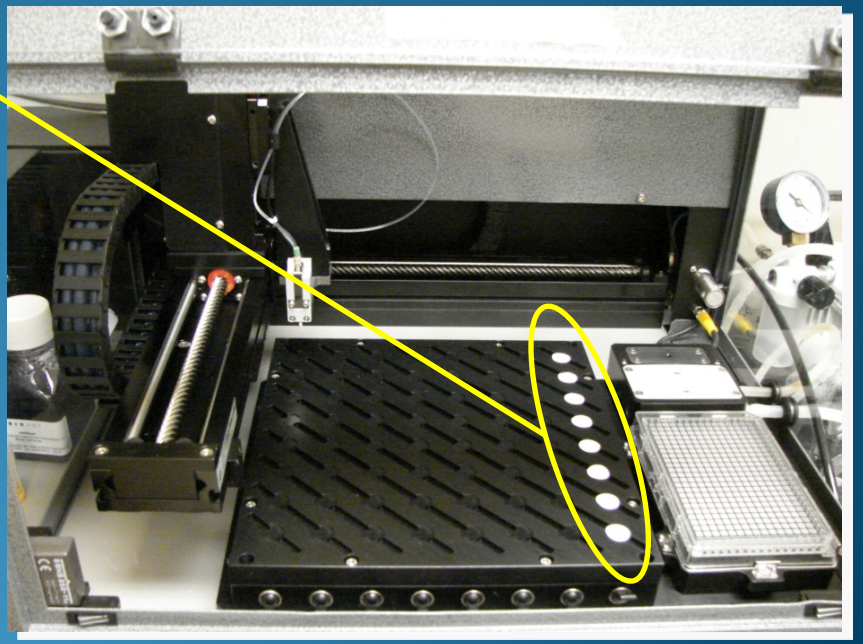
- competitive (c) ELISA membrane-based array employs MC LR-OVA spots for toxin detection
- application of α-MC monoclonal Ab (5C4): MC-LR, 100%, MC-RR, 108%; MC-YR, 68%; MC-LA, 69%; MC-LW, 71%; MC-LF, 68%; NOD, 94%
- 'in-assay' detection limit in low ng/mL range
- ***anticipate deployment in WLE 2016 w/ GLERL***



array map for MC/NOD assay using α-MC Mab; MC-LR-BSA employed as immunogen



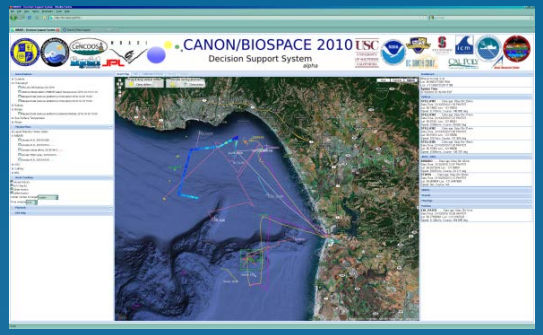




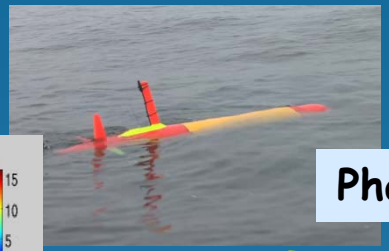
Intelligent AUV-based HAB detection

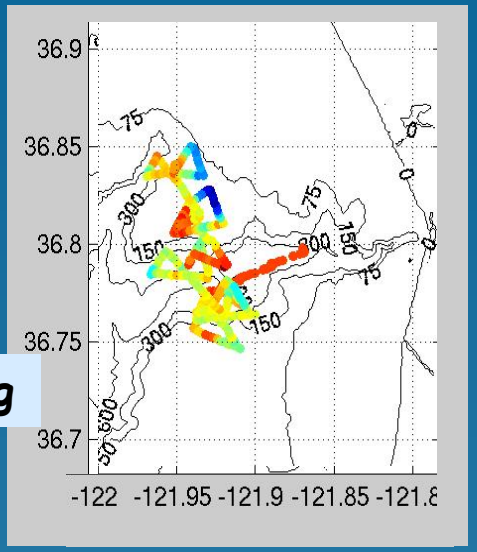
HAB growth and toxicity, and thus their potential impact, change continuously over time and space…so instead of a stationary instrument (e.g., 2G ESP), need the ability to actually ***track a bloom*** and adaptively/intelligently sample the population & water mass

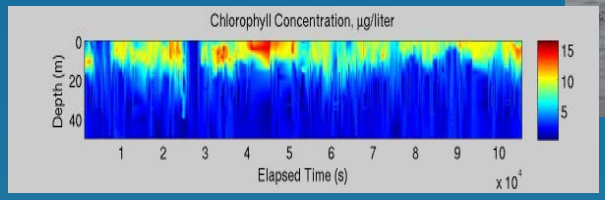
CANON: Controlled, Agile, Novel Observing Network: uses multiple platforms to track & sample HABs



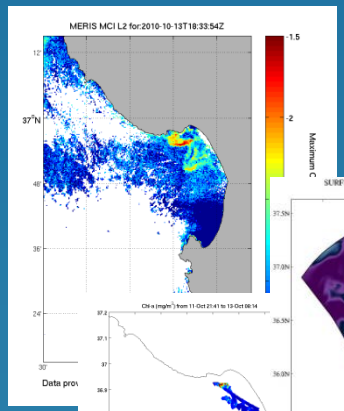
M B A R I

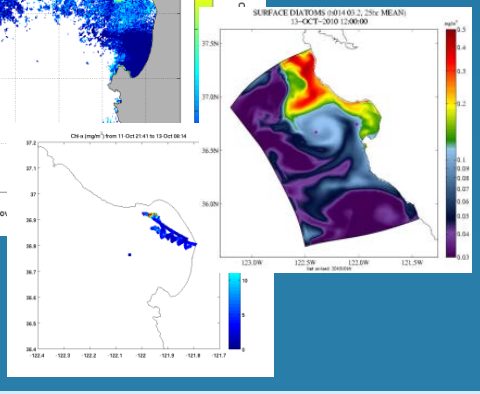




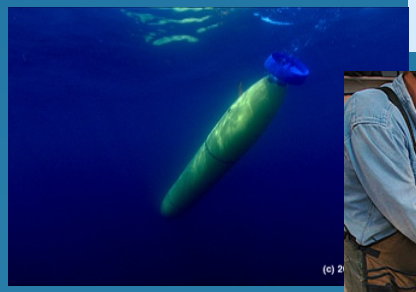


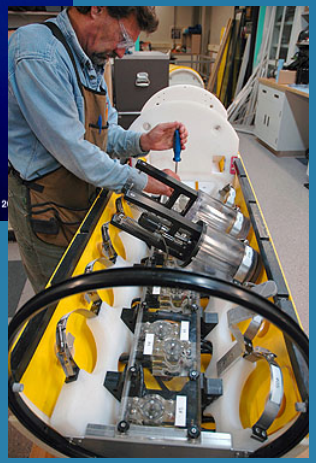
Phase 3: Patch Tracking





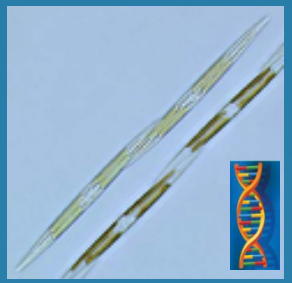
Phase 2: Patch selection

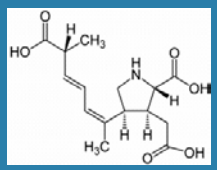




Phase 4: Targeted/intelligent sample collection (time series of patch)

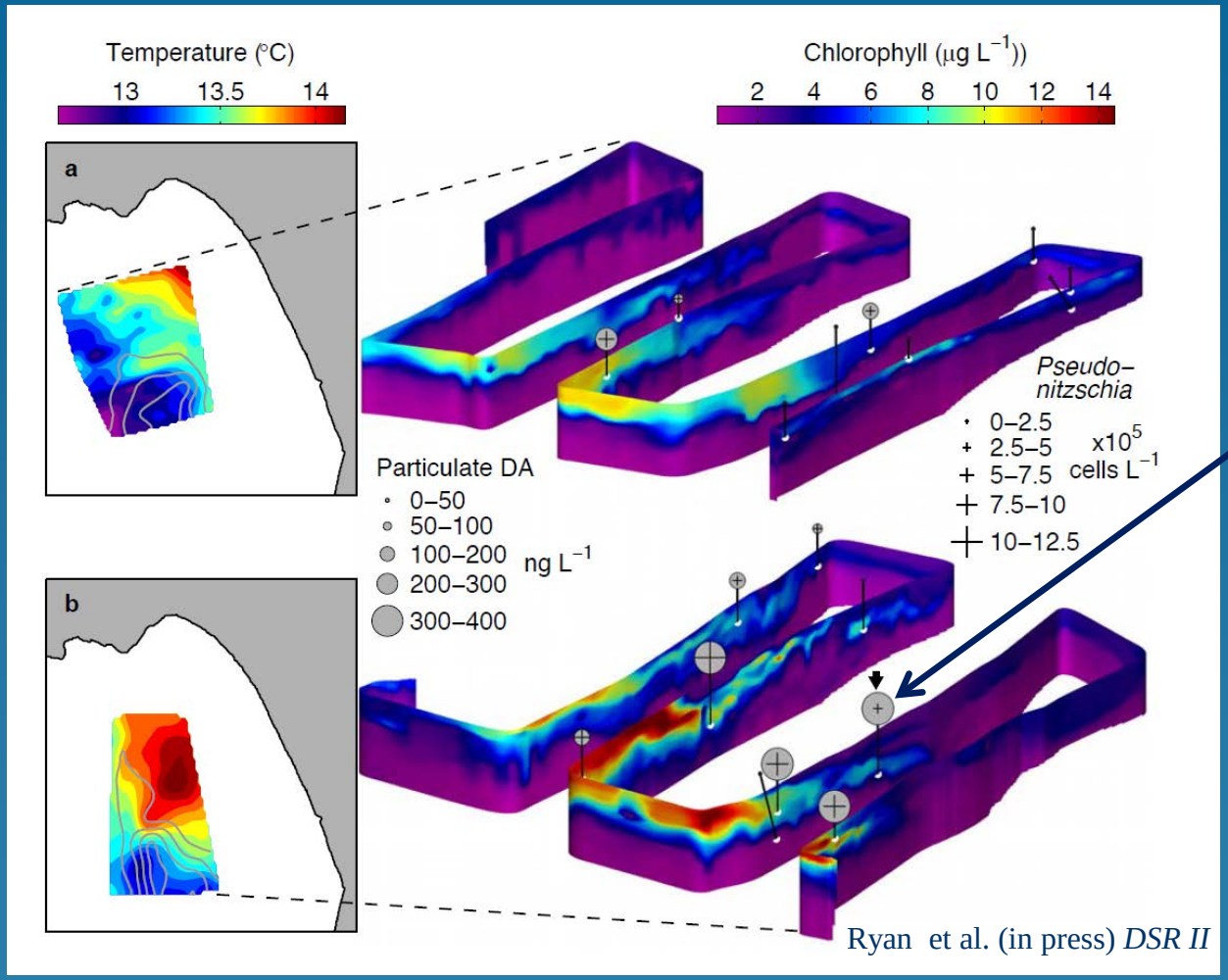
Phase 5: Analysis (laboratory-based; 'human-in-the-loop')





Phase 1: Bloom reconnaissance

What can we learn from this innovative approach?

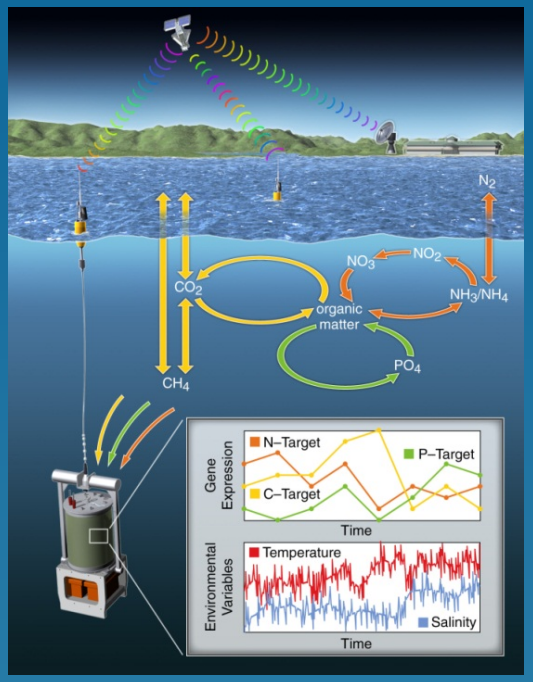


high cellular toxicity (hi DA/lo *P-n*)

- adaptive/intelligent AUV-based sampling provides a *'3-D picture'* of bloom population & water column structure over time; shows changes in HAB species & particulate toxin conc. (i.e., *bloom toxicity*)

Autonomous detection + AUV-based coverage

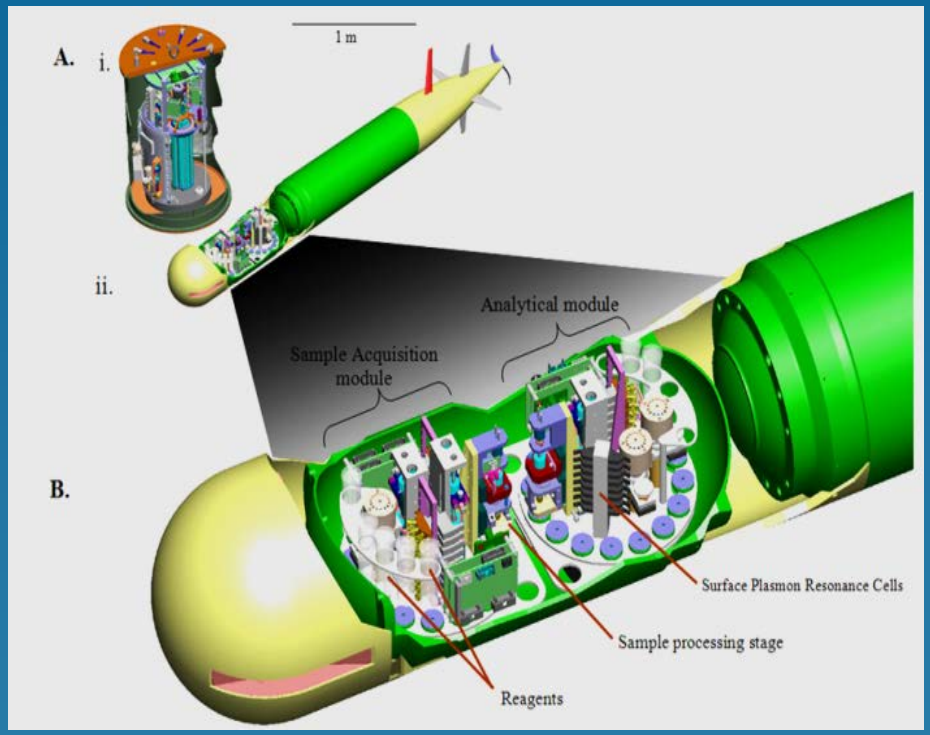
Current



2G ESP on moorings, drifters, benthic installations for autonomous detect.

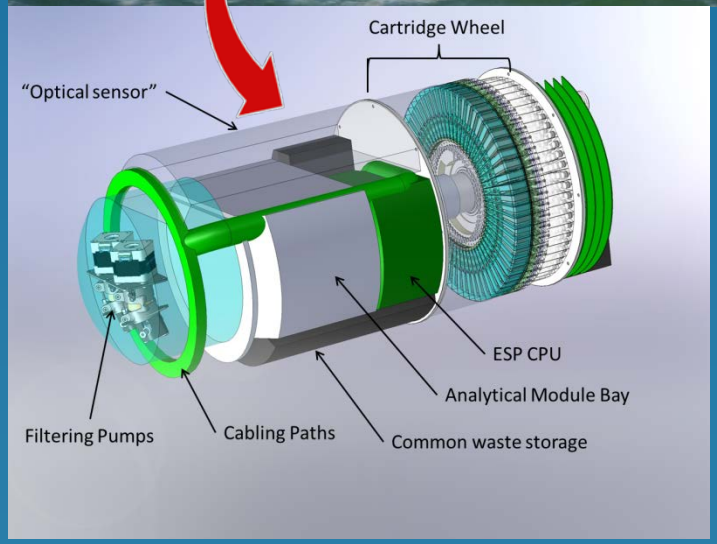
patch tracking & intelligent sampling for molecular analyses

Now under development (NSF-OTIC)



3G ESP deployed on long range AUV with patch tracking & intelligent sampling capabilities coupled with autonomous in situ *molecular analyses*

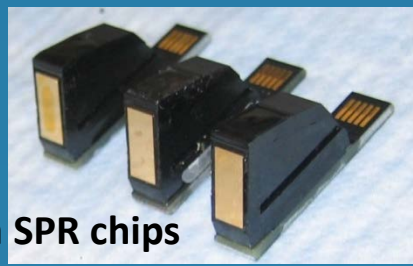
3rd Generation Environmental Sample Processor



3G ESP engineering design & assay implementation

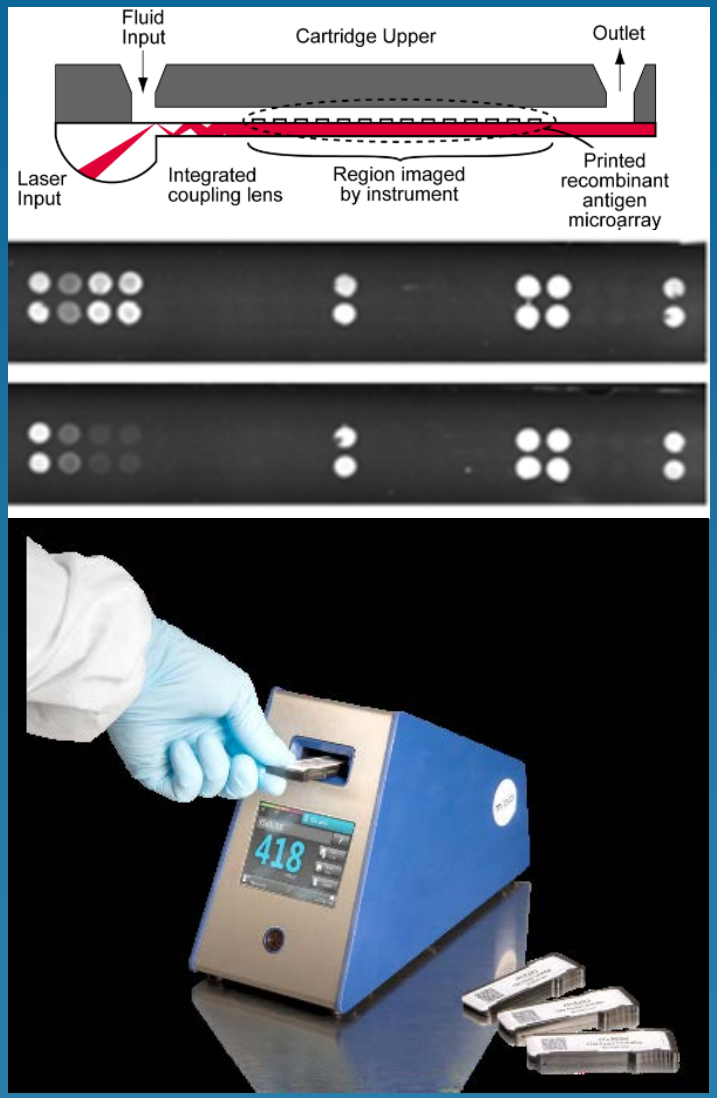
Summary of 3G ESP development

- 2G ESP form factor re-engineered for deployment in payload of MBARI long-range AUV (LRAUV)
- re-designed, self-contained sample processing cartridge; arranged as 'fins' on carousel for efficient use of reagents/space and > sample capacity
- toxin/organism assays transitioned from single-use membrane arrays (2G) to multi-use, miniature SPR sensor chips regen. between assays (3G)



Spreeta SPR chips

MBio Diagnostics LightDeck® Waveguide System

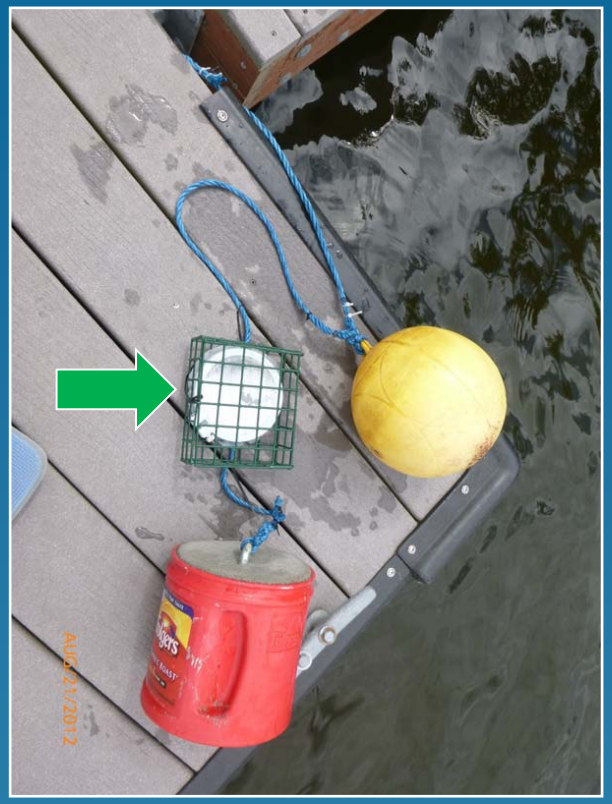


MBio Diagnostics, Inc. LightDeck® optical system: Snap-Esi ® planar waveguide assay cartridges & reader

TIRF waveguide-based sensor for multiplexed toxin/organism detection

- small, robust, field-portable format
- single-use, disposable cartridge; target-specific array, on-board waste storage
- assay is rapid (~ 15 min); ~ $20/test
- published protocol (Devlin et al. 2013) for MC/NOD immunoassay; commercial kit under development
- *potential to integrate cyanotoxin & cyanobacterial species detection on a single cartridge*

SPATT Autonomous Passive Sampling for Cyanotoxins



Dock-based deployment of SPATT passive sampler (photo credit: S. Davis & C. Hansen, 2013)

Solid Phase Adsorption Toxin Tracking (SPATT) Passive Sampling

- *autonomously sample for multiple cyanotoxins* (e.g., MC, STX, ATX) depending on sorbent
- very cost-effective; integrates over time; *but,* targets dissolved phase so may underestimate total toxin conc.
- deploy 1-4 wk; elute toxins in 50% MeOH; analyze by multiple methods
- *excellent potential as low-cost monitoring tool*

Emerging Role of Observing Systems in CHAB Detection & Forecasting: GLERL-ReCON Network

Aim to leverage existing and emerging observing network infrastructure for locating CHAB-specific platforms

- *integrate real-time physico-chemical data & CHAB-specific (cells/toxins) measurements*
- *contribute to early warning of CHAB events and understanding of environmental drivers of bloom growth & toxicity in support of ecoforecasting*

Cont. Monitoring: SRP, T, C, CHL, PC, PE, Turbidity, CDOM, pH, DO

NOAA's Phytoplankton Monitoring Network: Citizen Scientist Observing Network

Freshwater Target Taxa:

- *Microcystis, Anabaena, Aphanizomenon, Cylindrospermopsis, Oscillatoria/Planktothrix, Euglena*

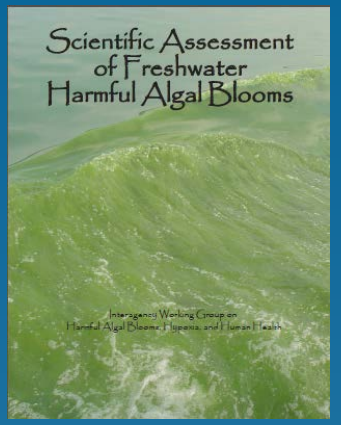
- *> 200 volunteers sample 140+ sites in 17 states*
- *data avail. on GIS database served by NOAA-NCDDC*
- *NOAA-EPA Partnership:*
  - *expands PMN into freshwater sites around Great Lakes region*
  - *funds 'Phyto v2.0' smartphone app add FW spp.; improve ID accuracy*
- *valuable infrastructure for deploying low-cost CHAB/ toxin sensors over wide area*

The Take-Home Message…

The detection methods & technologies summarized here are key to providing timely & accurate observations that are the foundation of efforts to understand, manage, and forecast CHABs…*but there is still considerable work to be done!*

Future Priorities for CHAB/Toxin Detection

Harmful Algal Bloom & Hypoxia Research & Control Act (HABHRCA): Scientific Assessment of Freshwater HABs; 2008)



- **NEED: Certified Reference Materials** are available only from NRC-CRMP for MC-LR, -RR, [Dha7] MC-LR, NOD-R, CYN, ATX-a; 9 STX congeners
  - non-certified, 'analytical' (≥ 99% purity) & 'reference' (≥ 95% purity) standards available (7-15 MC variants, NOD, ATX-a, CYN)

CRMs & reliable commercial stds are critical to support management, regulatory, & research (e.g., toxicology) apps, including standardized/validated analytical methods

Future Priorities for CHAB/Toxin Detection

USEPA published Standardized Analytical Method for: Determination of MC & NOD in drinking water by SPE and LC-MS/MS (Method 544; v1.0; Feb 2015)

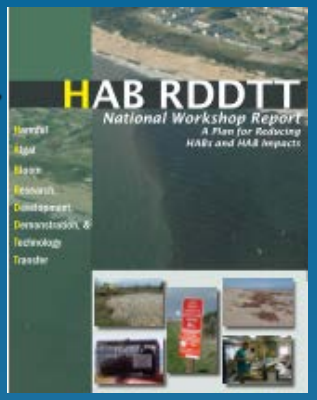
- **NEED: Standardized Analytical Methods** for ATX-a, and CYN, which are identified on the USEPA Draft Contaminant Candidate List (CCL) 4, along with MC-LR

CRMs & reliable commercial stds are critical to support management, regulatory, & research (e.g., toxicology) apps, including standardized/validated analytical methods

Future Priorities for CHAB/Toxin Detection

"applied development, demonstration, and technology transfer [are] needed to translate promising ideas into a wide range of operational tools…"

(HABHRCA: HAB Research, Development, Demonstration, & Technol. Transfer Rpt. 2008)



- **NEED:** Portable/manual, low-cost, rapid, easy-to-use assays and/or sensors (incl. sample prep.) for *multiplexed* toxin/organism detection

- **NEED:** Observing networks of moored & mobile *autonomous*, *in-situ* sensors for (near) real-time, *multiplexed* toxin/organism detection

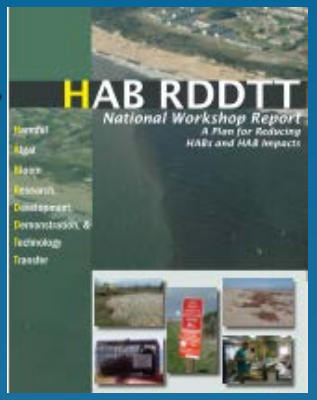
- **NEED:** *In-line, cyanotoxin sensor* deployable in water treatment facilities to continuously monitor finished drinking water

diverse data streams are critical for timely decision making & assimilation into predictive models supporting CHAB forecasts

Future Priorities for CHAB/Toxin Detection

"applied development, demonstration, and technology transfer [are] needed to translate promising ideas into a wide range of operational tools…"

(HABHRCA: HAB Research, Development, Demonstration, & Technol. Transfer Rpt. 2008)



- **NEED:** Local, state, regional, national *infrastructure* to standardize/ harmonize detection methods, coordinate development of data management/visualization tools, and disseminate QC'd monitoring data to end-users (e.g., managers, health officials, stakeholders, and researchers)

access to reliable, 'actionable' CHAB/cyanotoxin data is critical to support risk assessment, management/public health decisions, and research applications

Acknowledgments

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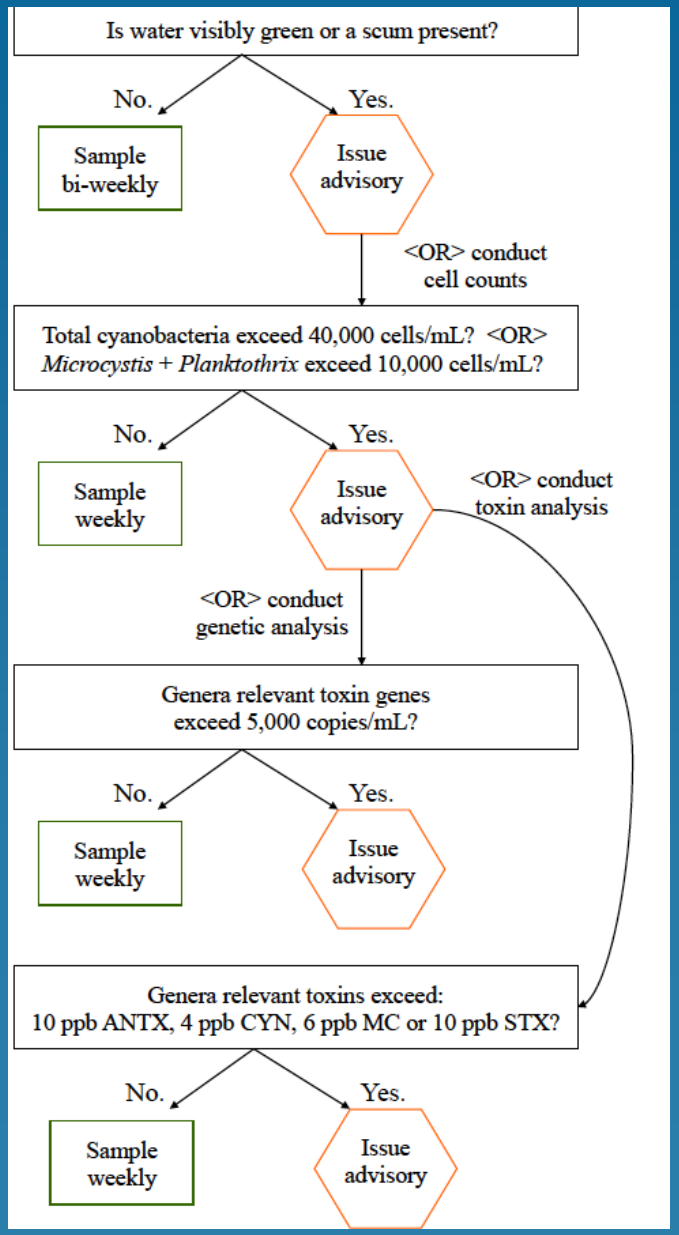
Breakout group discussion topics

1. **Do you currently use a decision framework to support your sampling?**
   a. If so, what are the threshold numbers you use for decision making (e.g. cells/mL, gene copies/L, toxin concentrations.)? Why did you choose these values?

   a. If you do not have a framework, why not?

2. **What detection methods are you currently using during your routine sampling?**
   a. Would the development of a SOP for genetic detection of cyanobacteria encourage you to incorporate these techniques?

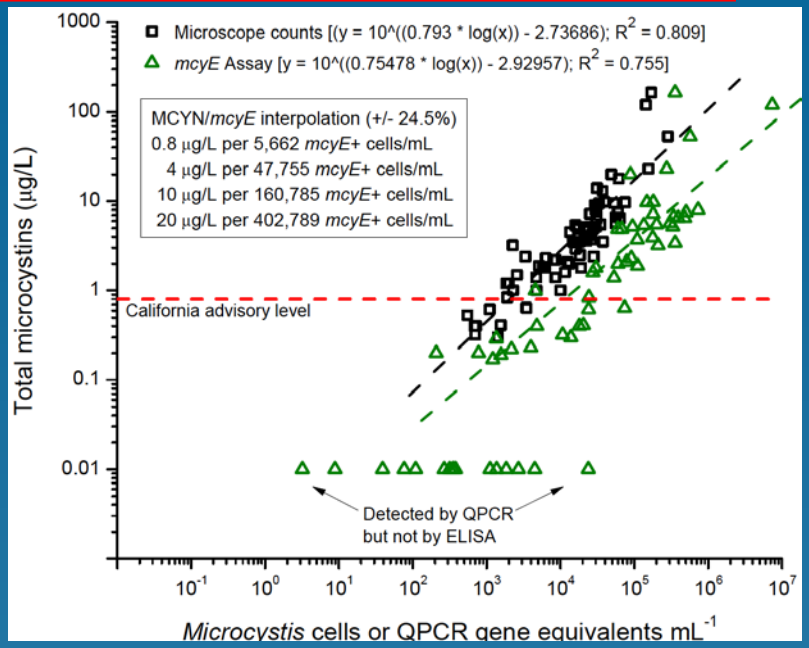
   b. Would you be willing to participate in a working group to develop SOPs for detection of cyanobacteria, if so which ones?

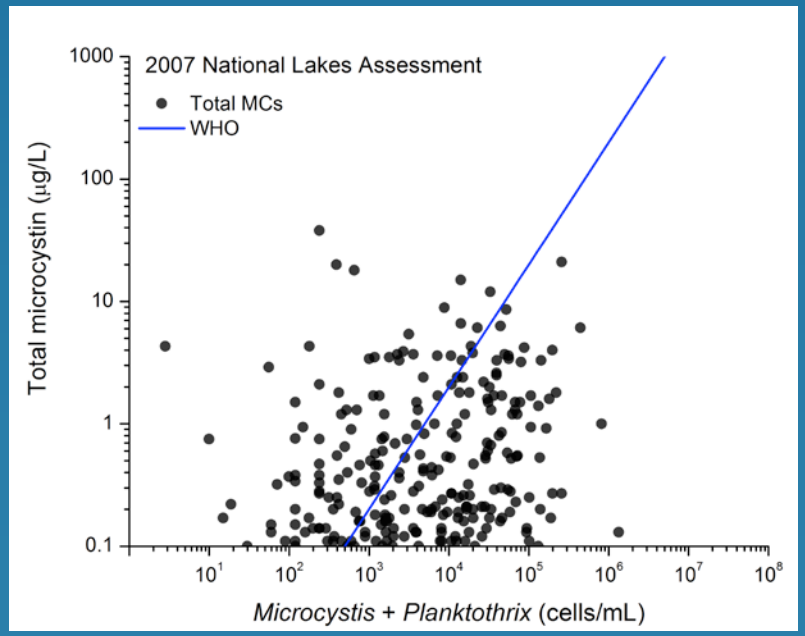


National molecular detection framework study

1. Currently there are no standard protocols for molecular detection of cyanobacteria making comparisons between studies, and sometimes even graduate students within the same laboratory, very difficult.

2. Development of meaningful large molecular databases will be hindered because observed differences may be artifacts of sample collection, extraction, gene used or a combination of all.



1. A study similar to the NLA but focused on generating a national framework for genetic detection of cyanobacteria using standard protocols to minimize variance

2. Water managers can use these validated protocols for management decisions