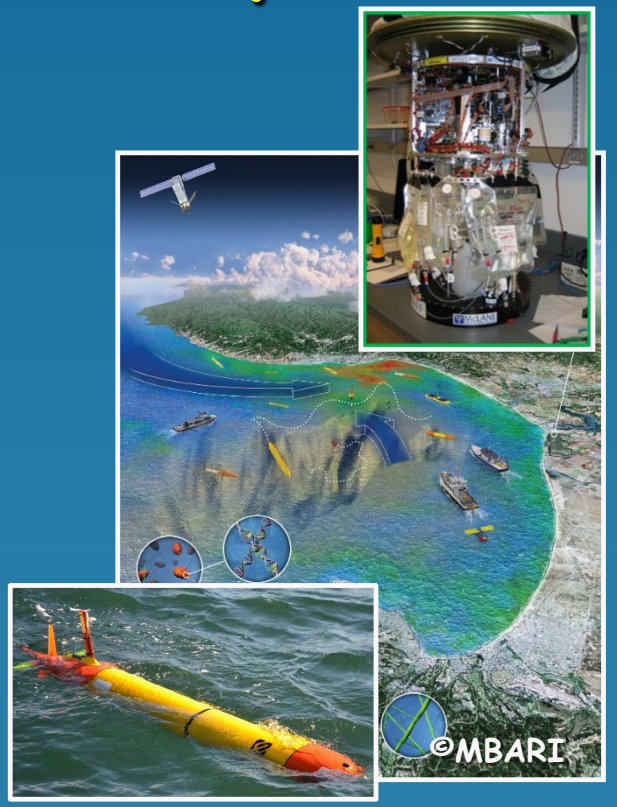
Being there - autonomous, in-situ detection of biotoxins associated with harmful algal blooms in marine and freshwater systems

Gregory J. Doucette

Christina M. Mikulski



NOAA/National Ocean Service

National Centers for Coastal Ocean Science

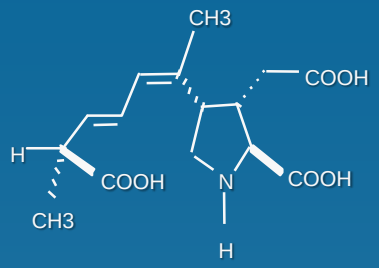
Harmful Algal Blooms (HABs)

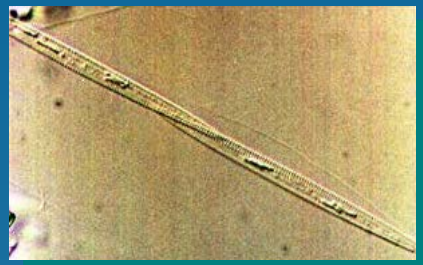
A harmful algal bloom is an aggregation or accumulation of either toxic or non-toxic, micro- or macroalgae that causes harm due to:

- production & trophic transfer of highly potent toxins
- accumulation of high biomass levels
- physical effects of cells on susceptible organisms

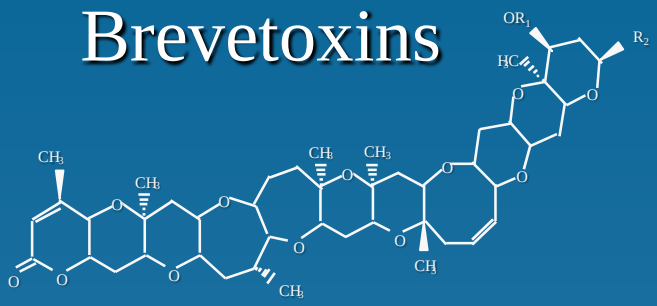
Diversity of HAB Species & Toxins

Domoic Acid

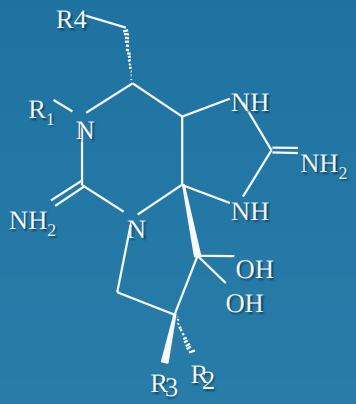


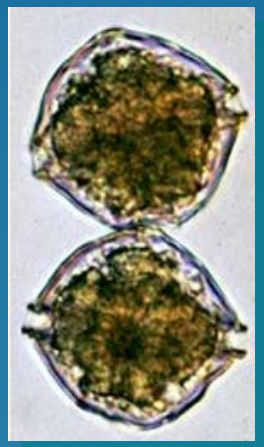


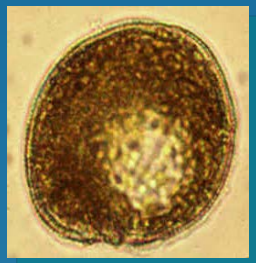
Brevetoxins



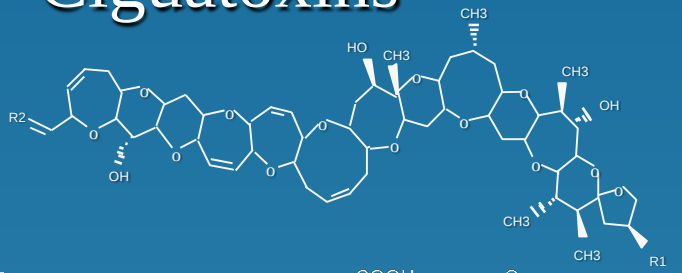
Saxitoxins

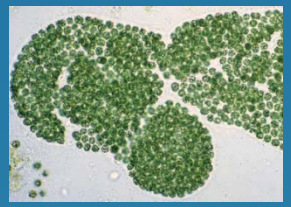


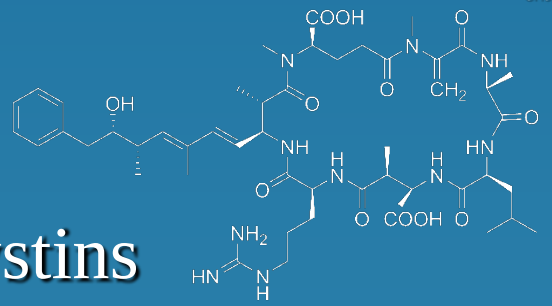




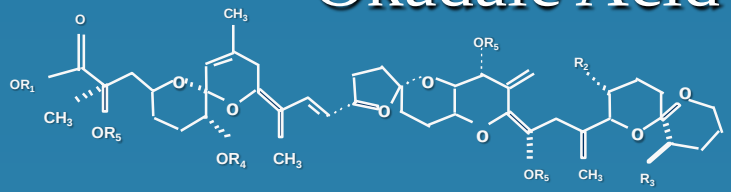
Ciguatoxins







Okadaic Acid



Microcystins

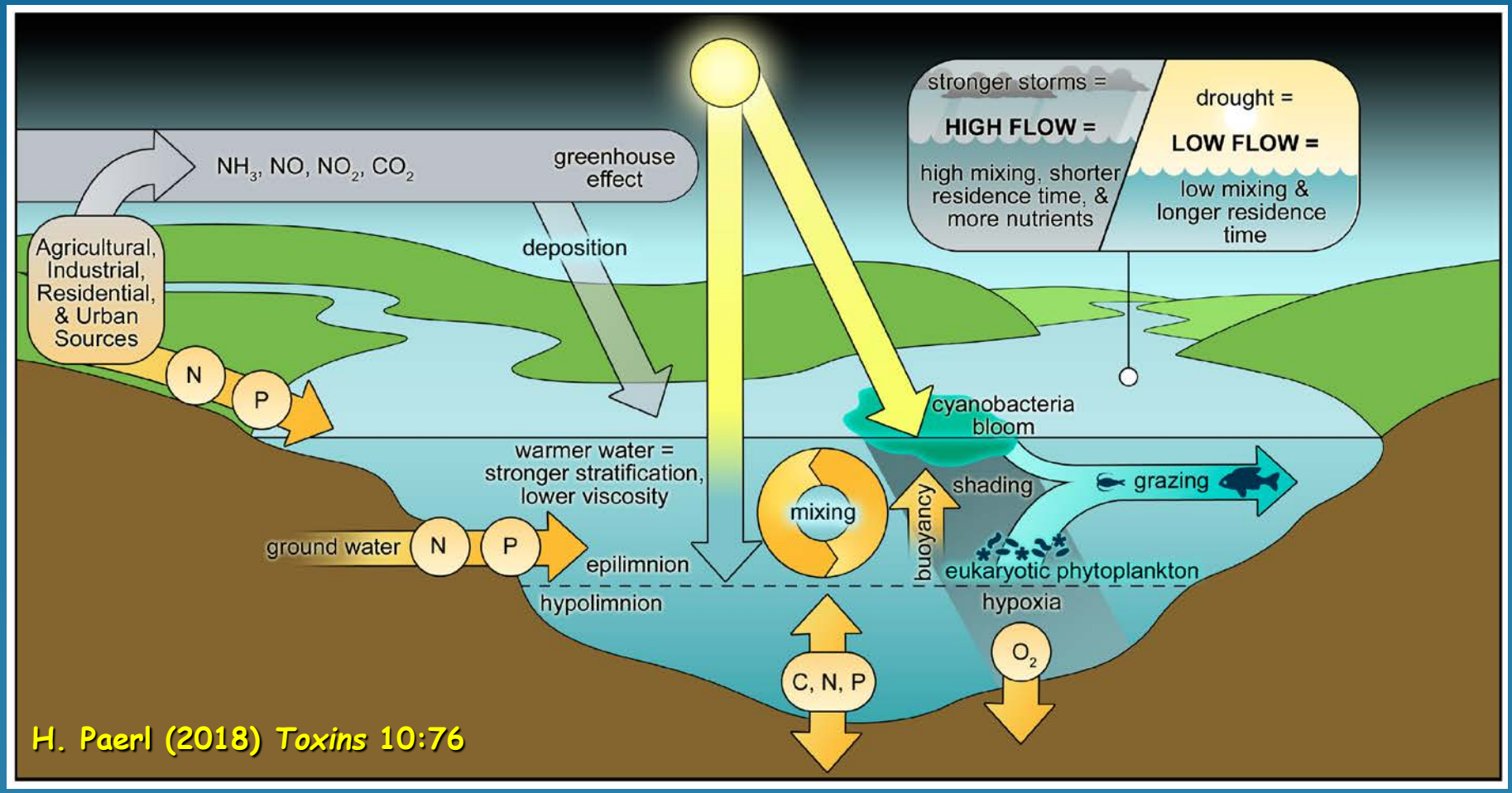
Cylindrospermopsin

Azaspiracids

Spirolides

Unknown Toxins?

Complex interactions between external & internal environmental/ecological factors control growth, accumulation, toxicity & fate of freshwater (marine) HABs



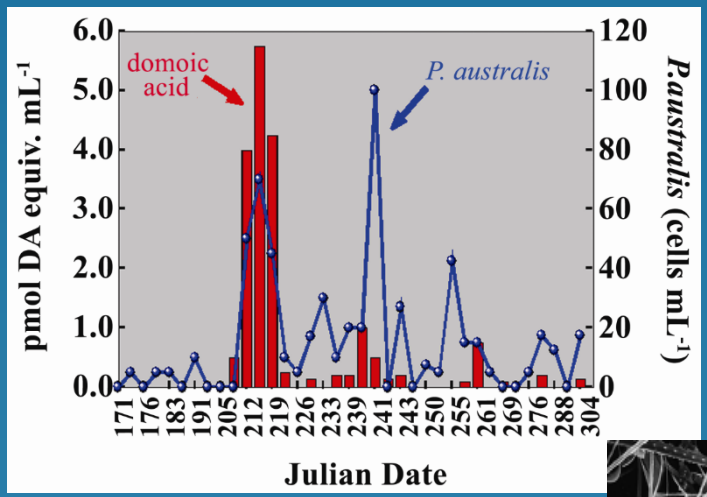
HAB monitoring, management & research in marine and freshwater systems requires near-real time, in-situ observations

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

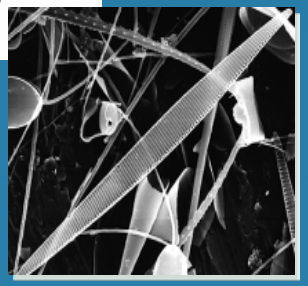
Why is it important to design sensors to detect both HAB species and toxins?

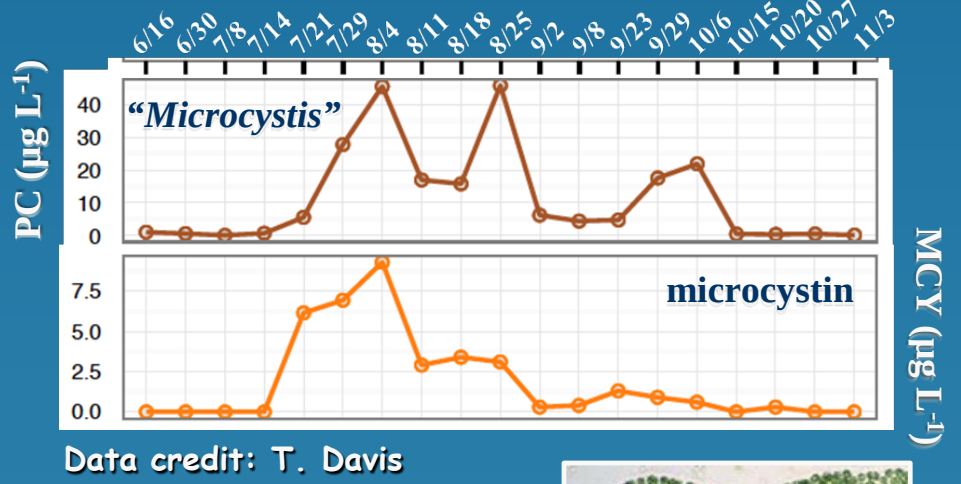
For HAB management & mitigation, it is critical to detect organisms *and* toxins due to fluctuations in toxicity caused by a changing (marine/freshwater) environment -

high cell numbers don't always mean high toxin levels



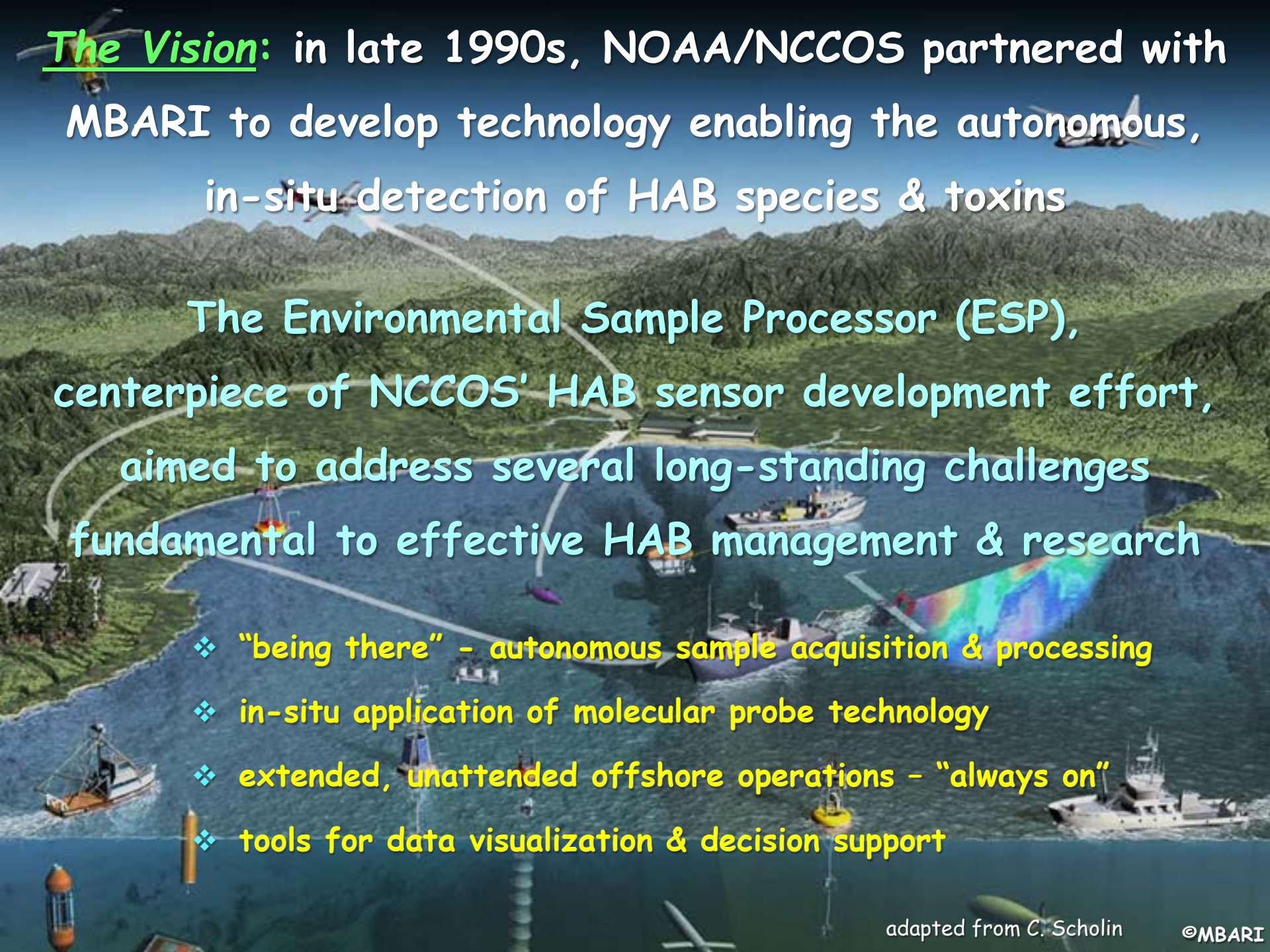
Pseudo-nitzschia/
domoic acid





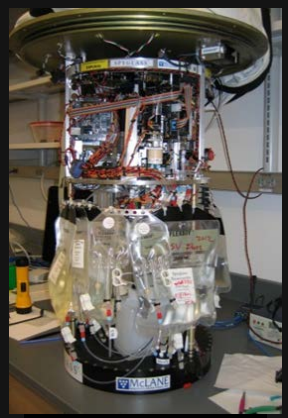
Data credit: T. Davis

Microcystis/
microcystin


The Vision: in late 1990s, NOAA/NCCOS partnered with MBARI to develop technology enabling the autonomous, in-situ detection of HAB species & toxins
The Environmental Sample Processor (ESP), centerpiece of NCCOS' HAB sensor development effort, aimed to address several long-standing challenges fundamental to effective HAB management & research
❖ "being there" - autonomous sample acquisition & processing
❖ in-situ application of molecular probe technology
❖ extended, unattended offshore operations - "always on"
❖ tools for data visualization & decision support
adapted from C. Scholin
©MBARI

Autonomous, underwater detection of HABs with the ESP...
when you can't actually be there!

1G ESP



2G ESP

SMART-SRAUV

3G ESP-LRAUV

Sensor development - Stage 1: 1G & 2G Environmental Sample Processor – autonomous, in-situ HAB spp/toxin detection

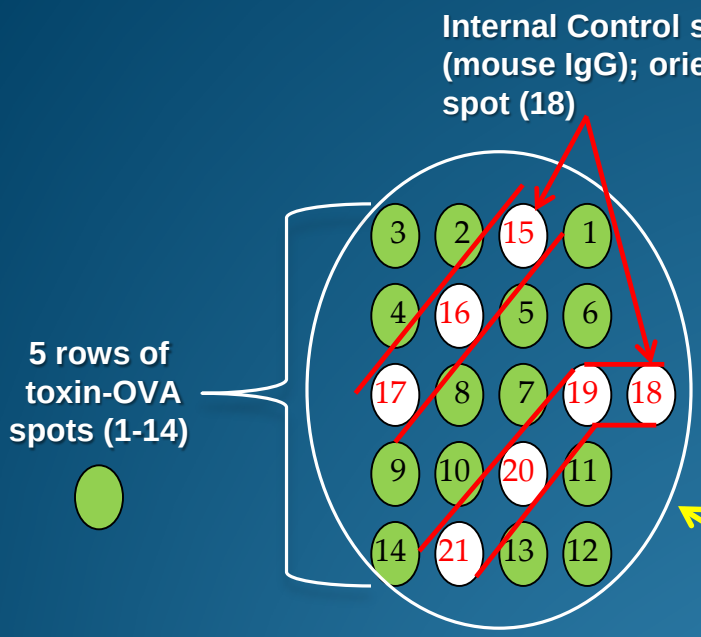
Current Capabilities of 2G ESP

2G ESP instrument located at NOAA/NCCOS Laboratory

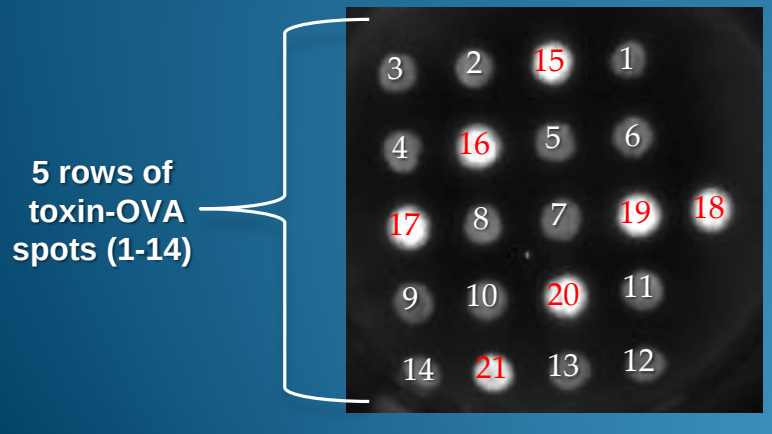
- Real-time application of DNA probe arrays (SHA); algae, bacteria, inverts
- Real-time application of protein/Ab arrays (cELISA); phycotoxins
- Sample archival
  - whole cell microscopy/FISH
  - nucleic acids (gene libraries)
  - phycotoxins
- Real-time application of quantitative PCR (qPCR); various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

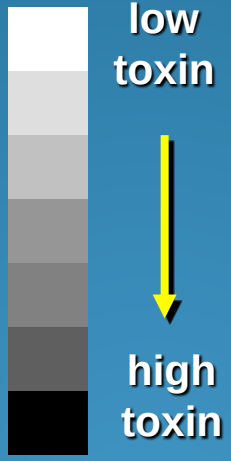
Developing an Array-based cELISA

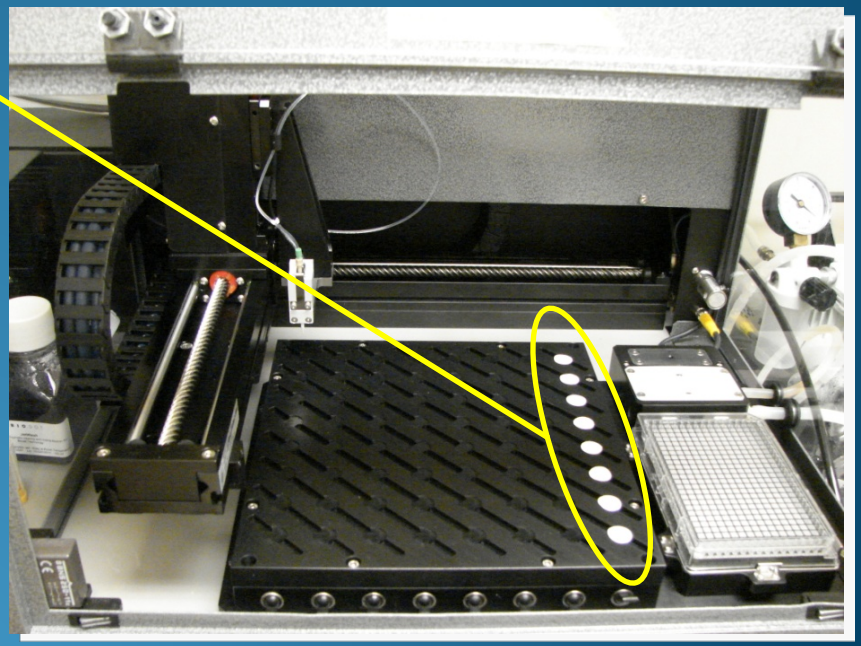
- competitive (c) ELISA membrane-based array employs toxin-protein (OVA) spots for detection
- α-toxin antibody (monoclonal or polyclonal) is selected based on target affinity and cross-reactivity among toxin variants or congeners
- 'in-assay' detection limit in low ng/mL range



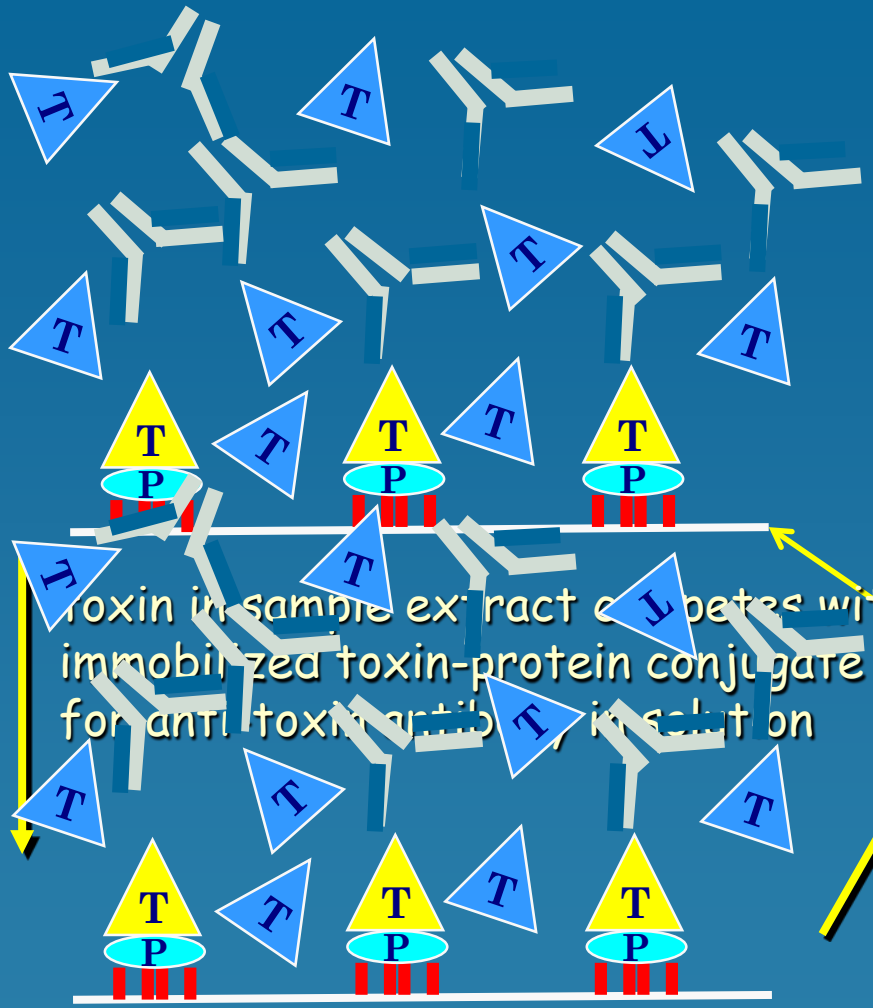
array map for toxin assay using α-toxin Mab



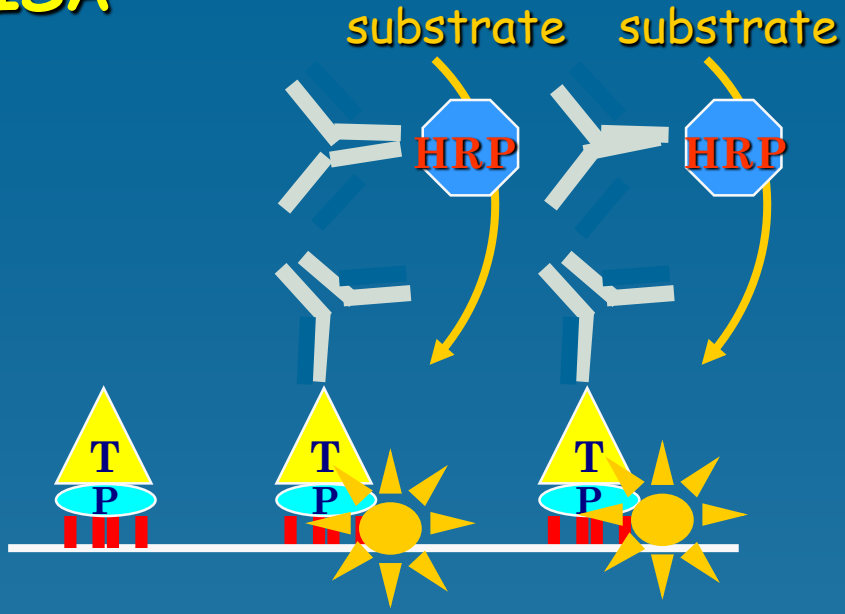


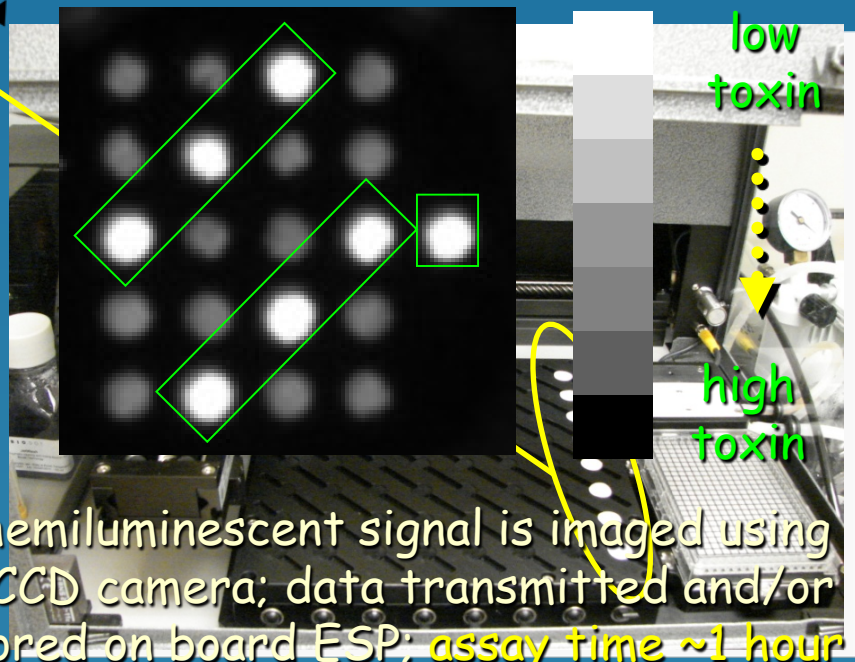


Toxin Detection Strategy: cELISA



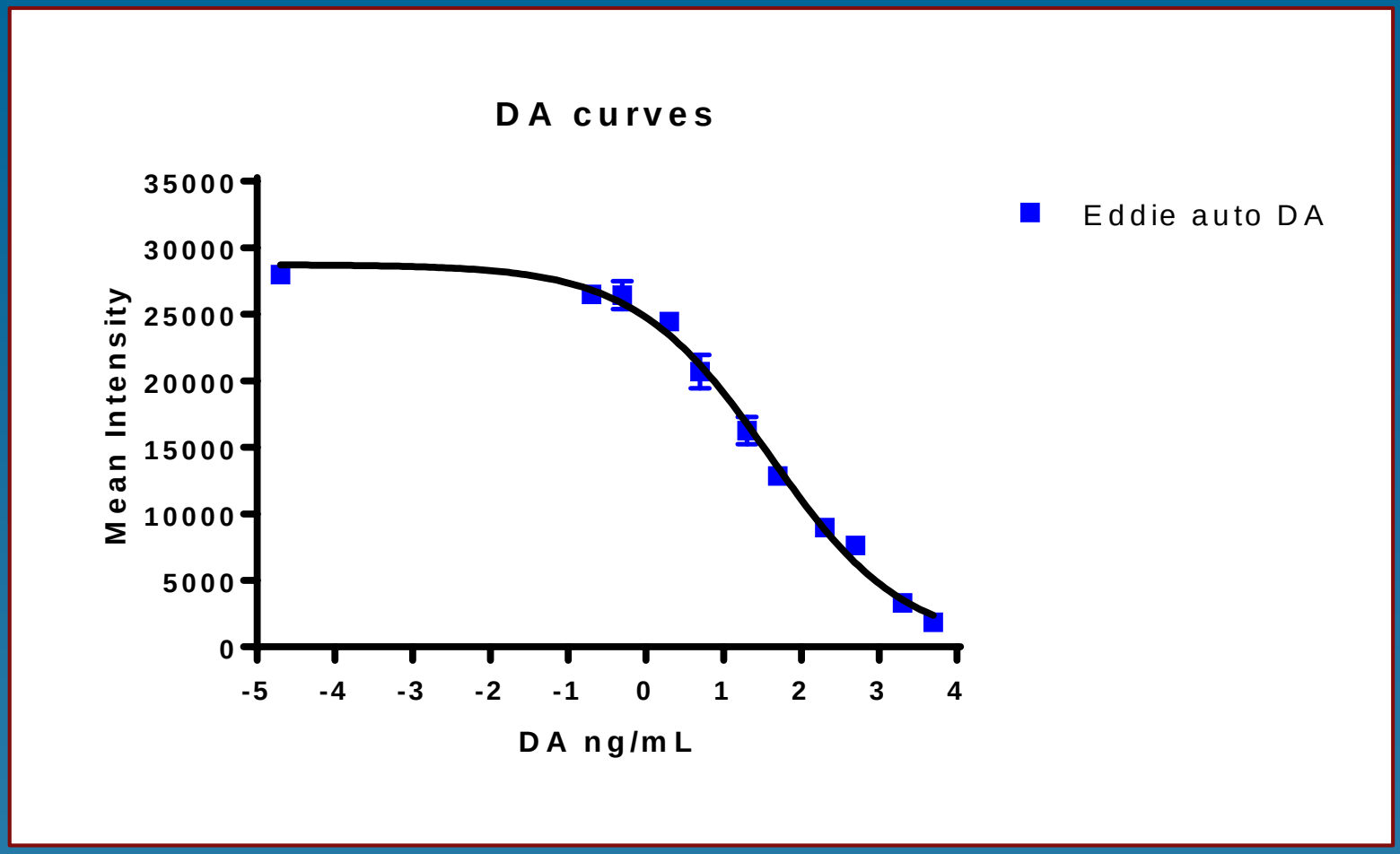
toxin in sample extract competes with immobilized toxin-protein conjugate for anti-toxin antibody in solution





free antibody and that bound to toxin in the sample extract are washed away, leaving only antibody bound to the immobilized toxin-protein conjugate

chemiluminescent signal is imaged using a CCD camera; data transmitted and/or stored on board ESP; assay time ~1 hour



Detection and quantification limits

(in water concentrations based on 1L sample; PNW pDA alert level ~200 ng/L)

EC50 = 78 ng/L

ULOQ = 1240 ng/L

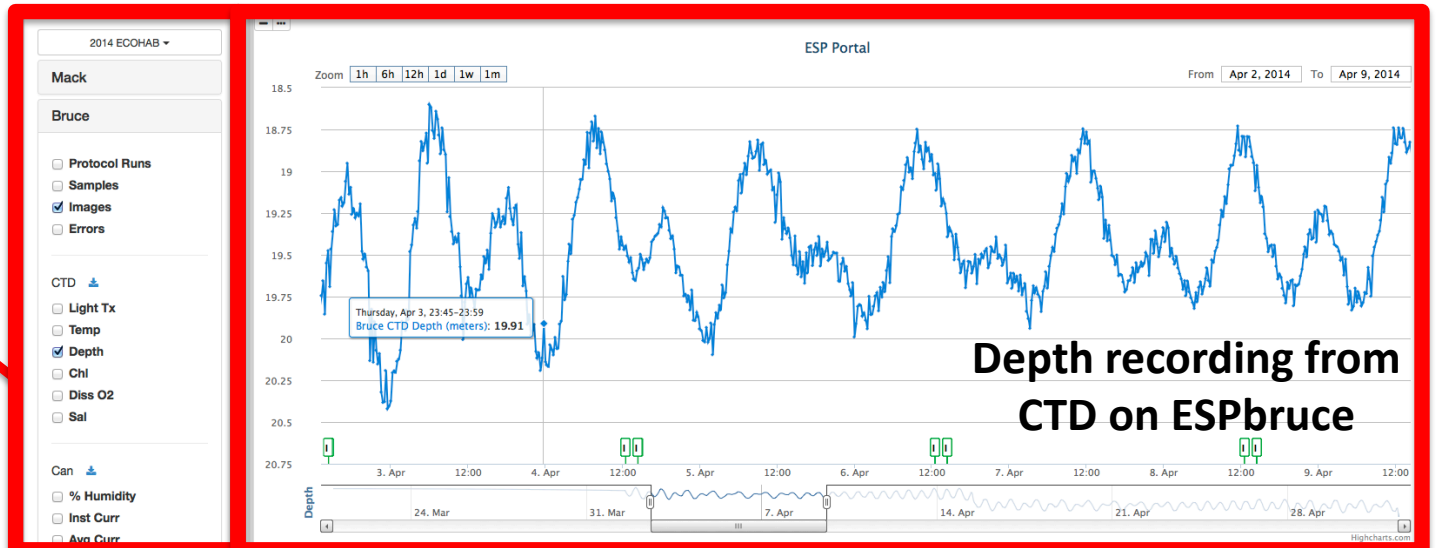
LLOQ = 4.9 ng/L

LLOD = 2.6 ng/L

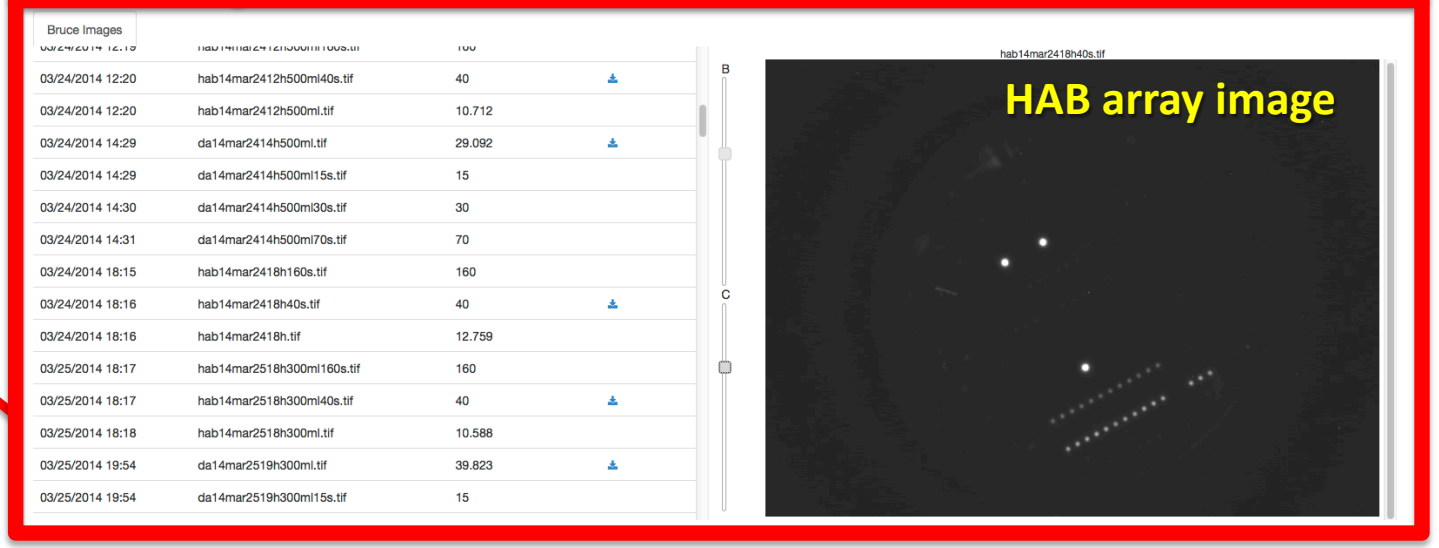
Web Portal for ESP data access

MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

Select source & type of data to display



Select HAB or toxin array image to display



Inbox (3,051) - greg.douce | esp-bruce | MBARI Slack

https://mbari.slack.com/messages/esp-bruce/

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gdoucette

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- esp-dennis
- esp-don
- esp-friday
- esp-jake
- esp-mack
- makaikoaspr

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- dpargett
- hollybowers
- jbirch
- kgomes
- kyamahara
- methane
- preston
- ryjo
- scholin

#esp-bruce

2015-06-05 12:31:28 -0700

Sample Completed

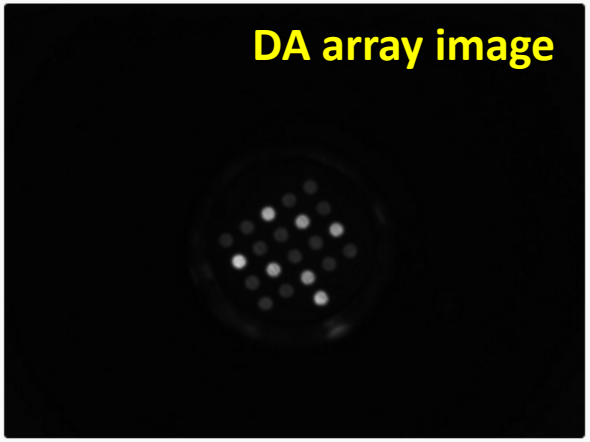
> Actor: MAIN
> Target Volume: 25.0
> Actual Volume: 25.0
> Volume Diff: 0

esps BOT 5:27 PM

2015-06-05 13:51:03 -0700

Image Taken: da15jun0513h25ml.tif (36.291s - 696px X 520px)

http://services.mbari.org/espweb/data/instances/Bruce/deployments/2015%20Spring%20CANON/data/processed/esp/da15jun0513h25ml.jpg (8KB)



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2015-06-05 13:51:53 -0700

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2015-06-05 13:53:10 -0700

Image Taken: da15jun0513h25ml70s.tif (70s - 1392px X 1040px)

June 7th, 2015

slack.com

- **desktop or smartphone app**
- **24/7 communications with team members before, during, and after ESP deployments**
- **real-time, remote access to review ESP operations & array images**

Many 2G ESP HAB-Related Applications in US Coastal Waters & Great Lakes

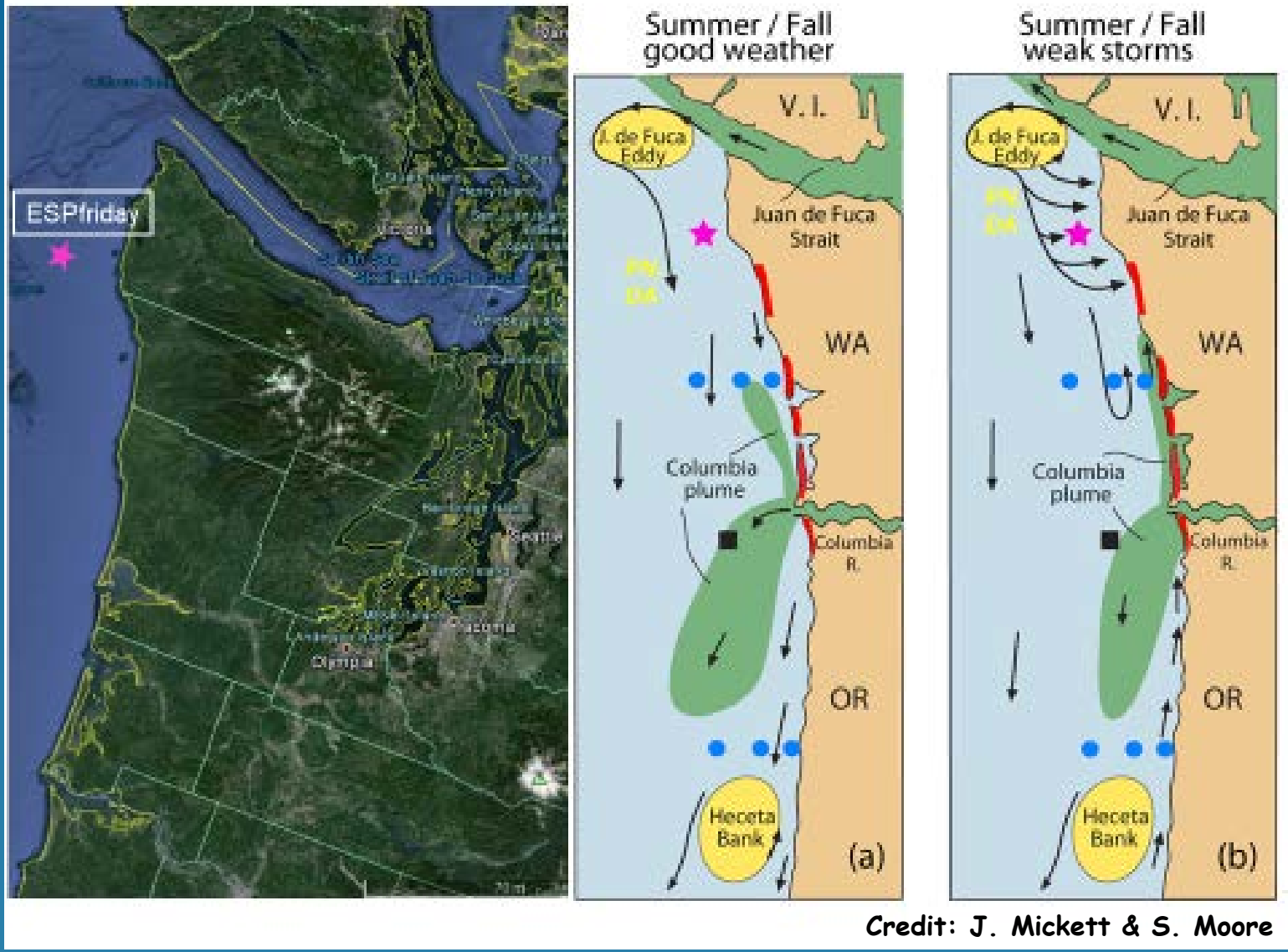
4 deployments - WA coast

3 deployments – near Toledo water intake (L. Erie)

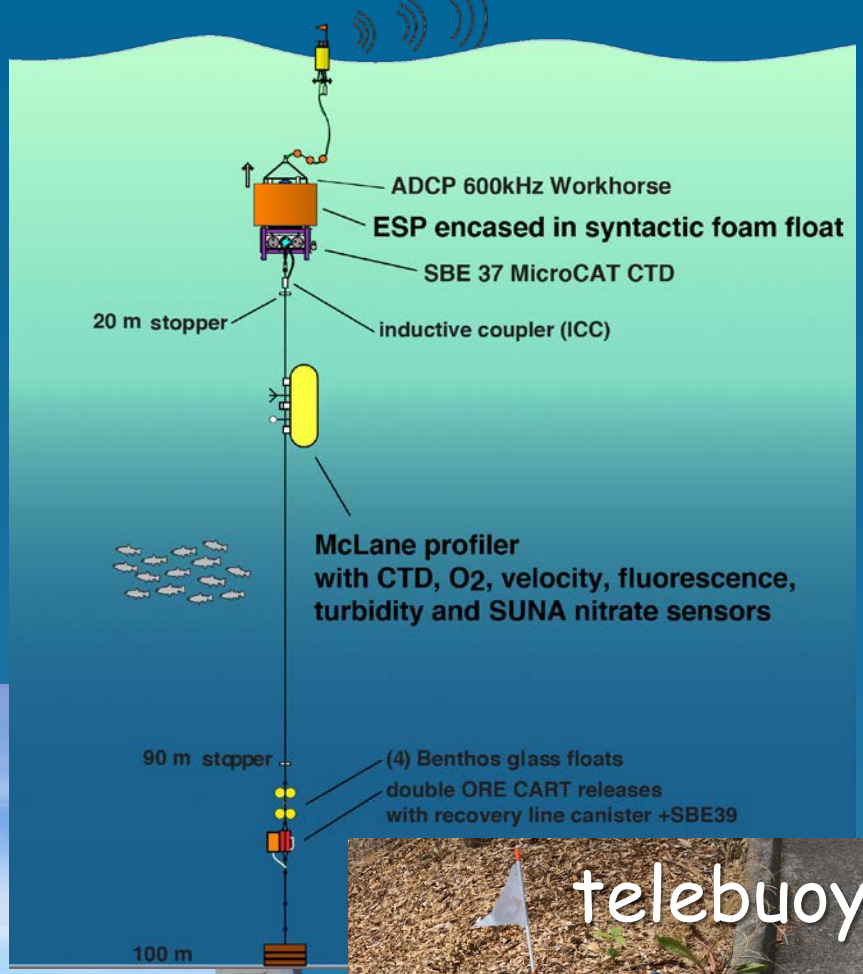
many deployments – MB, S. CA

many deployments - Gulf of ME

2G ESP deployments in the PNW: *P-n.* transport path from Juan de Fuca Eddy to coast



Credit: J. Mickett & S. Moore



Credit: S. Moore

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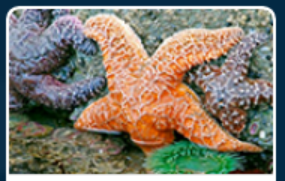
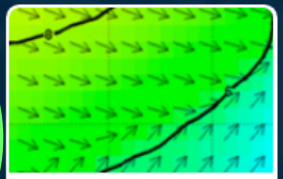
Oregon Tsunami Clearinghouse



Pacific Coast Habitat Server

ProbCast: Probability Weather Forecasting

Real-time HABs



Regional PNW Wave and Wind Forecasts

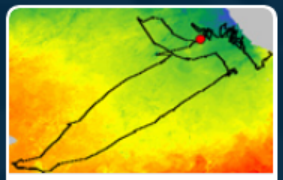
Rhythms Of Our Coastal Waters

Salish Cruises

NVS

Shellfish Growers

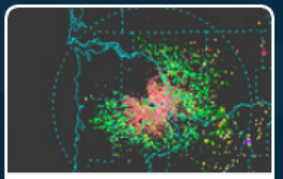
NVS



Tagging Pacific Pelagics

Tsunami Evacuation Zones

NVS



REALTIME HABs

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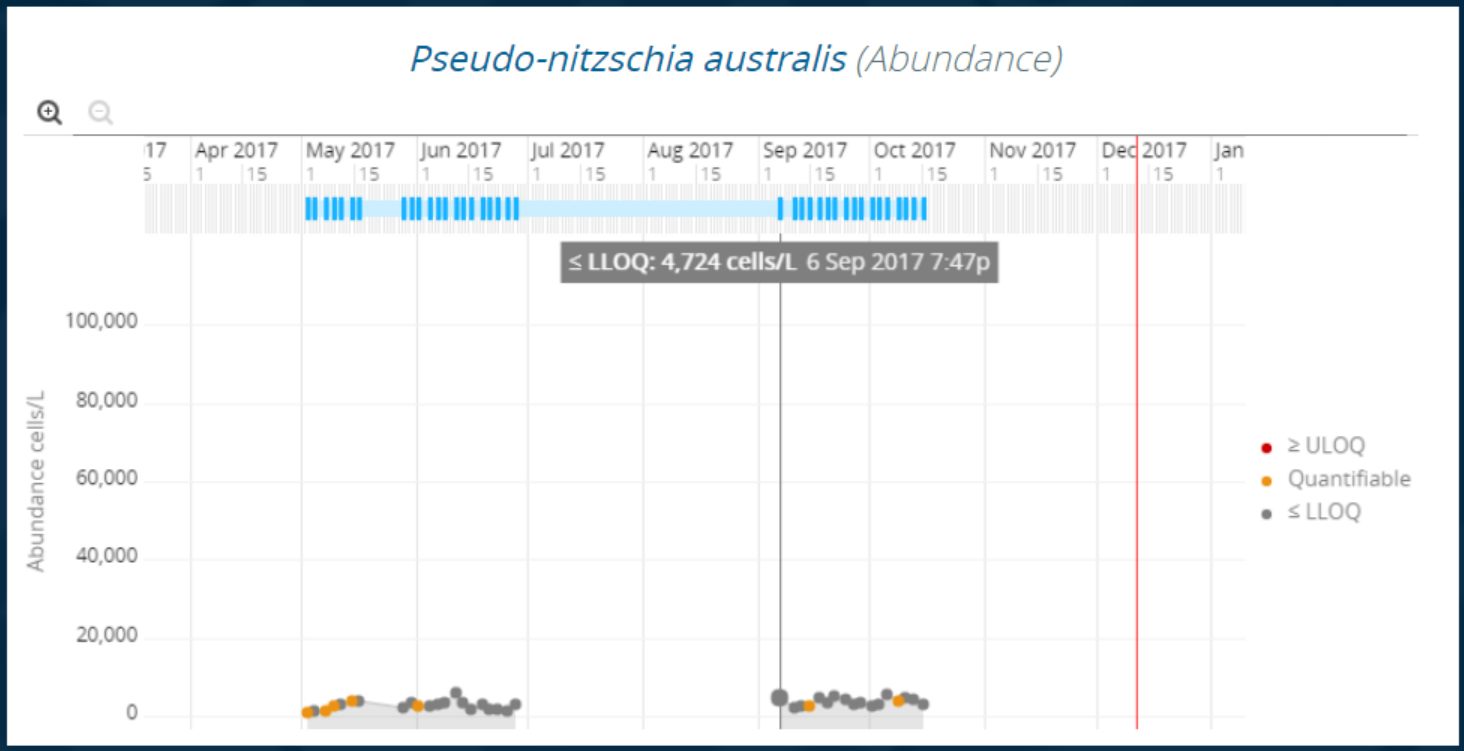
Species Abundance	Species Present / Not Detected	Toxins
Pseudo-nitzschia australis	*Alexandrium Species*	Domoic Acid Concentration
Pseudo-nitzschia multiseries	*Heterosigma akashiwo*	
Pseudo-nitzschia fraudulenta		
Pseudo-nitzschia pungens		

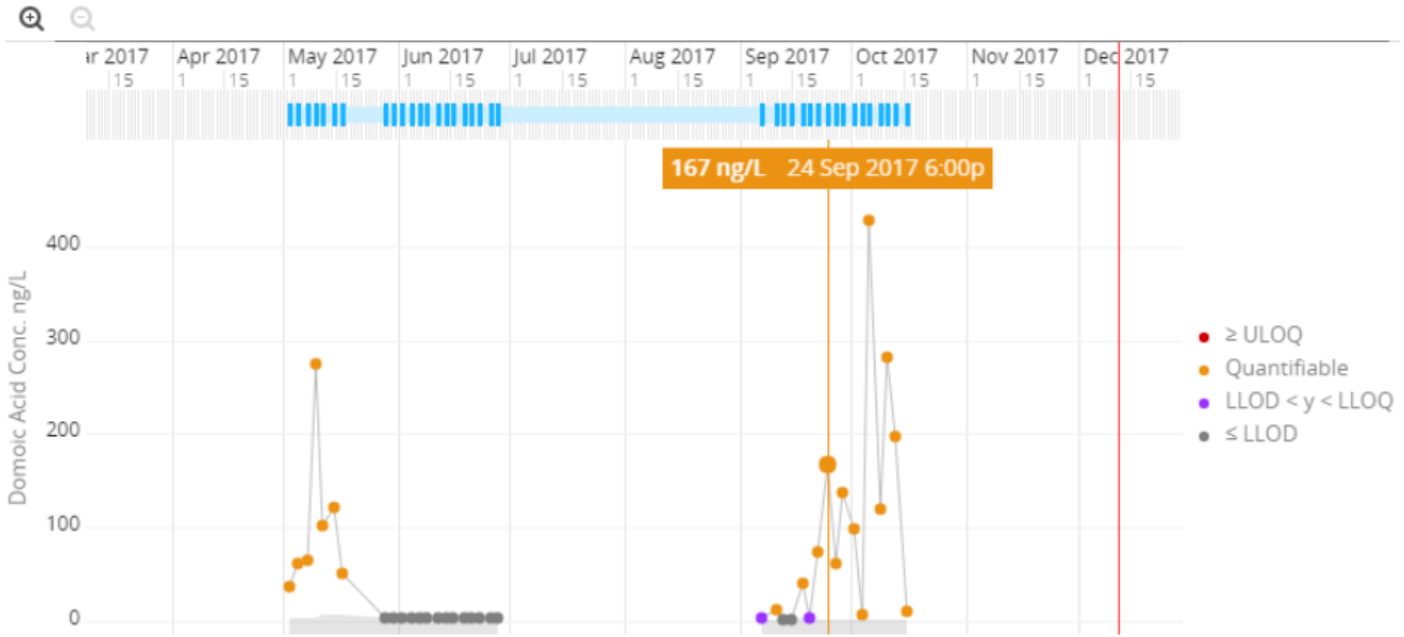


Quantitative cell abundances of *Pseudo-nitzschia australis*. This species can sometimes produce the toxin domoic acid which can cause amnesic shellfish poisoning in humans.

Pseudo-nitzschia fraudulenta

Pseudo-nitzschia pungens

Domoic Acid Concentration



Concentration of particulate domoic acid in seawater. Domoic acid is a toxin produced by some species of phytoplankton in the genus *Pseudo-nitzschia*. If domoic acid concentrations are detected above the Lower Limit Of Quantification (LLOQ, see description below), this means that one or more *Pseudo-nitzschia* species are producing the toxin. There is no regulatory threshold for domoic acid in seawater, rather the toxin is regulated based on its concentration in the tissues of shellfish where 20 ppm is a "no-harvest" limit (see the Washington State Department of Health Beach Closures site). However, a high seawater domoic acid concentration may provide an early warning of a HAB event.

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Pseudo-nitzschia australis
Pseudo-nitzschia multiseries
Pseudo-nitzschia fraudulenta
Pseudo-nitzschia pungens
Alexandrium Species
Heterosigma akashiwo

Domoic Acid Concentration

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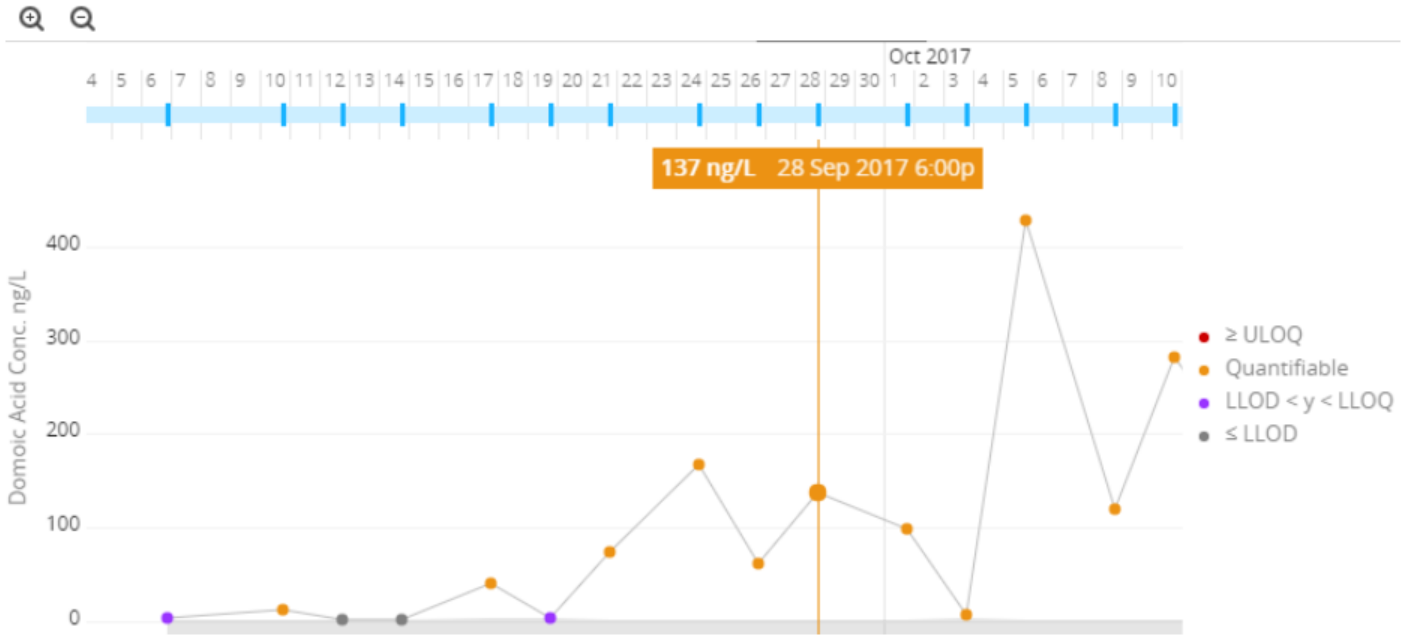
Domoic Acid Concentration



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Pacific Northwest Harmful Algal Blooms Bulletin

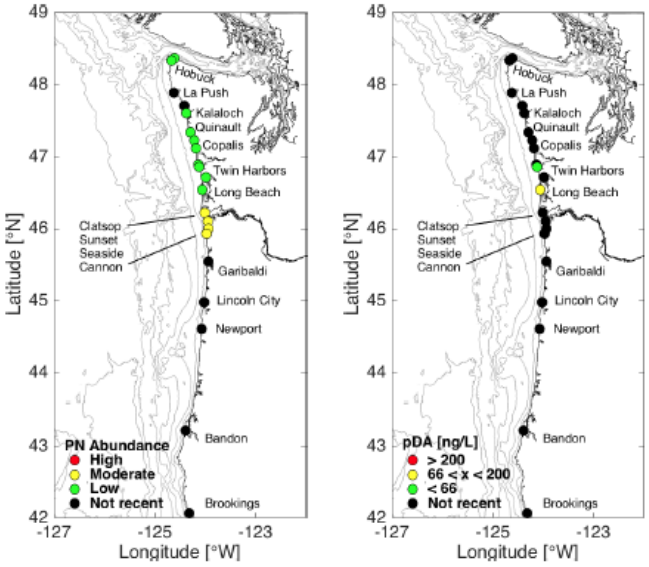
Oct 25, 2017 HAB risk =

HAB risk key:
- = low
- = medium
- = high

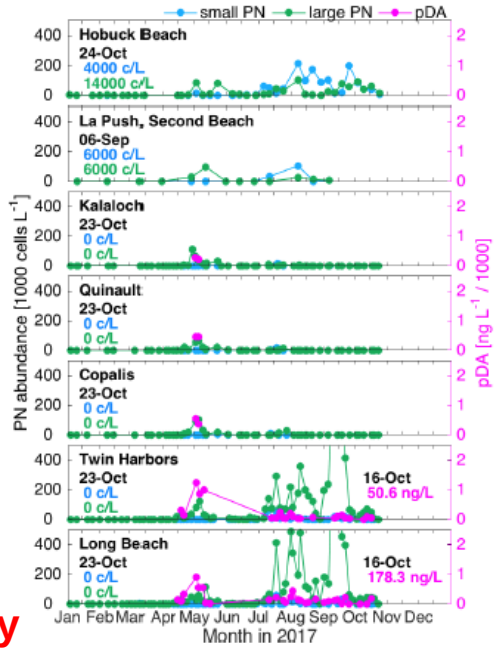
JISAO · School of Oceanography, University of Washington · ORHAB Partnership

Beach Sampling

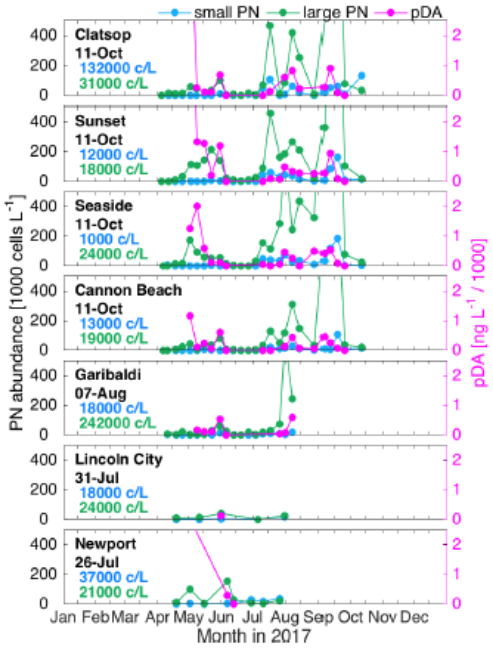
(*Pseudo-nitzschia*) (particulate domoic acid)



WA *Pseudo-nitzschia* & domoic acid



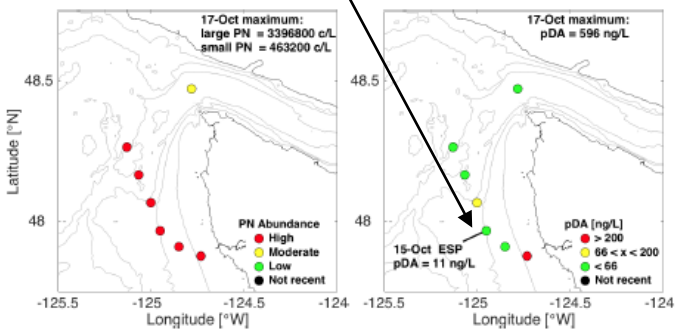
OR *Pseudo-nitzschia* & domoic acid



ESP offshore toxicity

Offshore Sampling

(*Pseudo-nitzschia*) (particulate domoic acid)



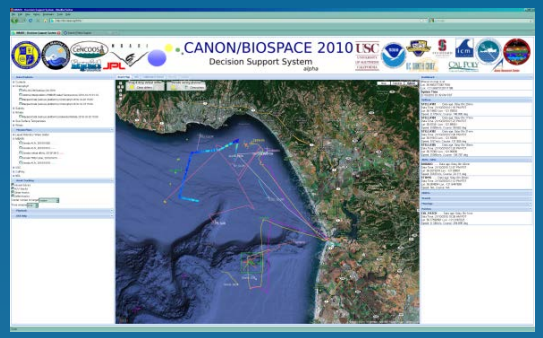
Pseudo-nitzschia (*PN*) abundances are quantified for large and small cell morphologies using light microscopy. Threshold values of 50,000 cells/L for large cells, and 1,000,000 cells/L for small cells trigger additional testing for water column particulate domoic acid (pDA). Water column pDA values >200 ng/L often lead to toxin accumulation in shellfish such as razor clams. Sampling sites, colored by relative *PN* abundance (*high:* > threshold value for either cell morphology; *moderate:* > 1/3 threshold value; *low:* < 1/3 theshold value) and pDA, are shown in the upper left two panels. "*Not recent*" indicates that there were no data within the previous 15 days. Time series of *PN* abundance (cells per liter = c/L) and pDA at select beaches are shown in the upper right main two panels. Offshore samples (lower left) are collected and analyzed at ~2 week intervals during late summer/early fall. Additional samples are collected by a remotely operated Environmental Sample Processor (ESP) that is moored off La Push, WA, in late spring and late summer.

Decisions regarding shellfish harvest closures at individual beaches are made by the Washington Department of Health and the Oregon Department of Agriculture after measuring toxin levels in shellfish collected from each beach (WA link; OR link), and not from the information presented here. However, the information presented here aids coastal managers in better understanding and predicting the onset, duration, and magnitude of toxin outbreaks as well as their impacts.

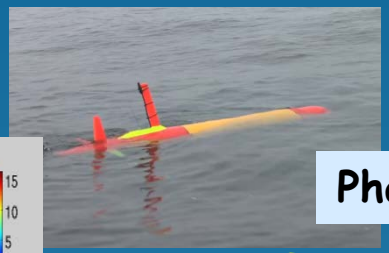
Sensor development - stage 2: 'Smart'- SRAUV intelligent HAB tracking/sampling

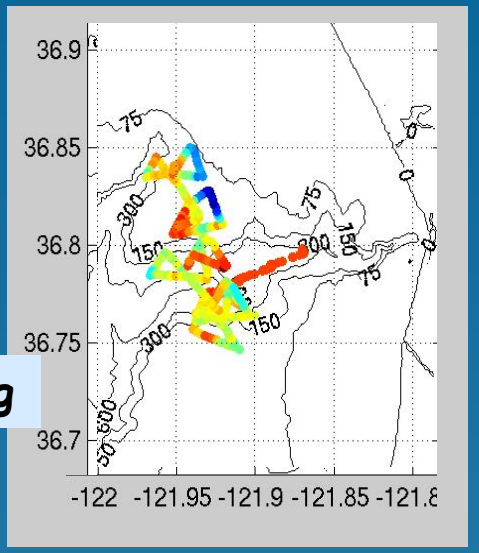
HAB growth and toxicity, and thus their potential impact, change continuously over time and space…so instead of a stationary instrument (e.g., 2G ESP), wouldn't it be cool if we could actually ***track a bloom*** and intelligently/adaptively sample the population & water mass

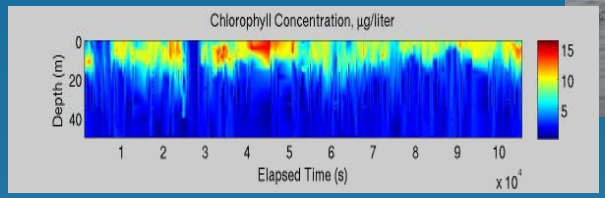
CANON: Controlled, Agile, Novel Observing Network: uses multiple platforms to track & sample HABs



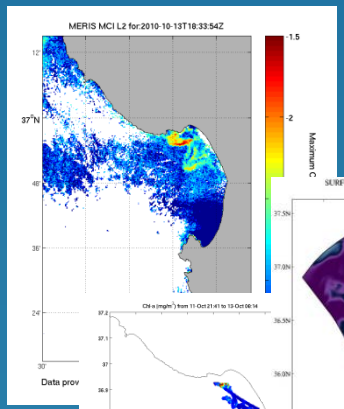
M B A R I

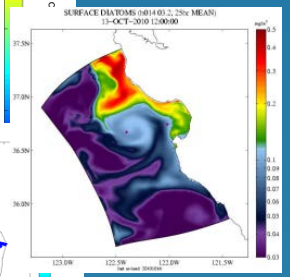






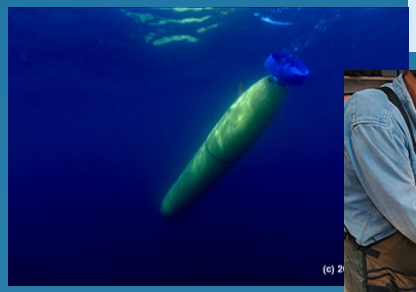
Phase 3: Patch Tracking

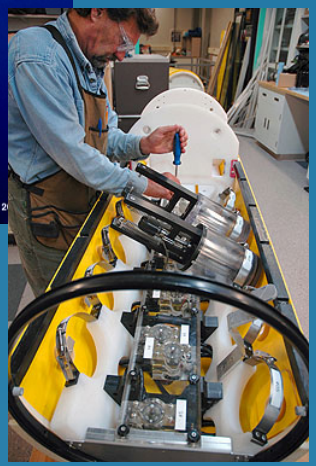




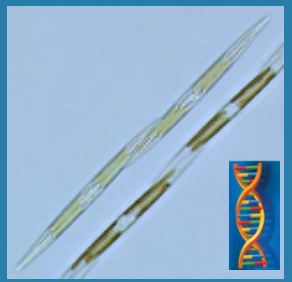
Phase 2: Patch selection

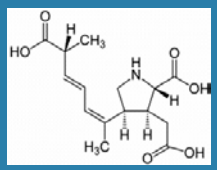
Phase 4: Targeted/intelligent sample collection (time series of patch)





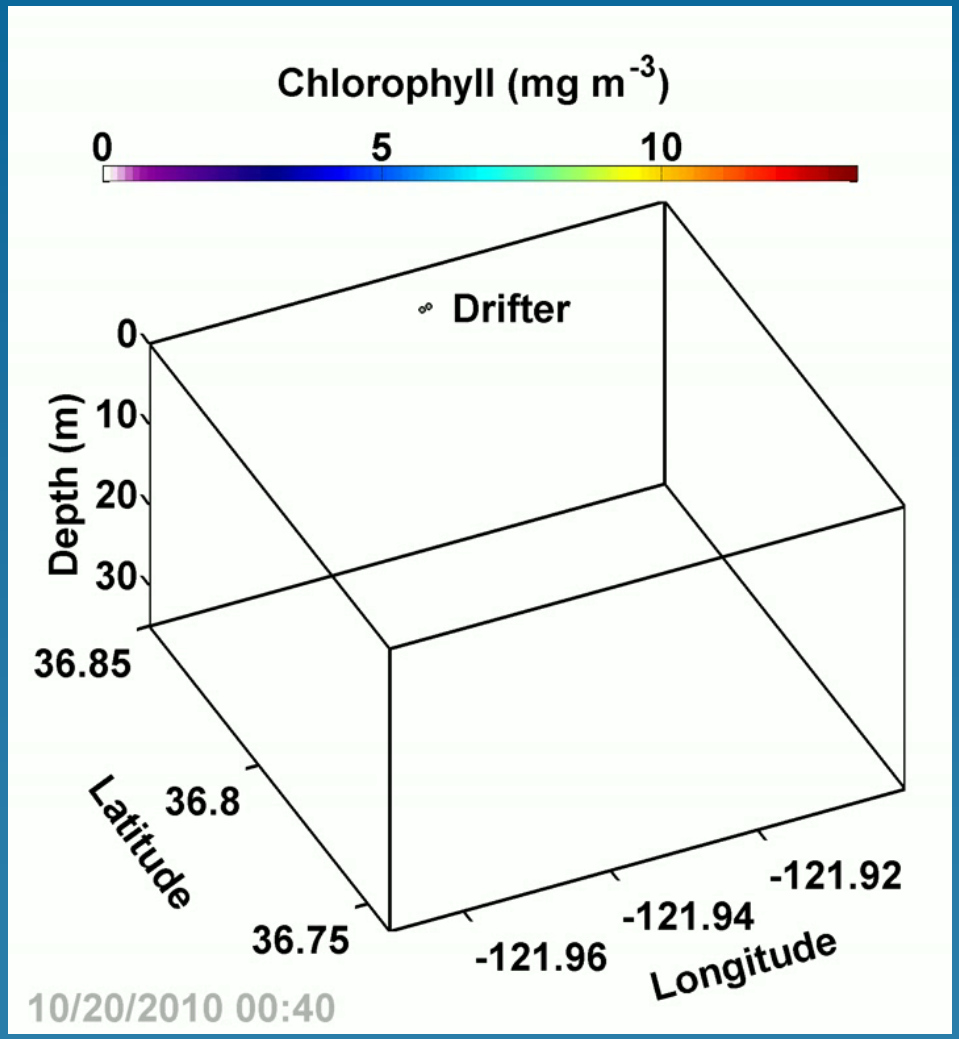
Phase 5: Analysis (laboratory-based; 'human-in-the-loop')

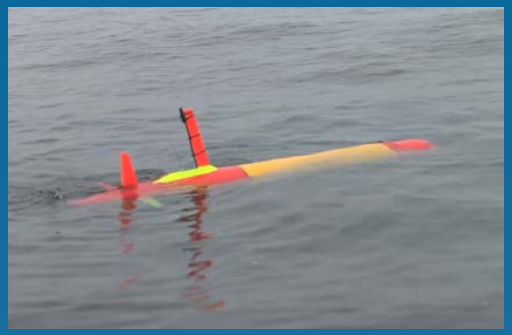




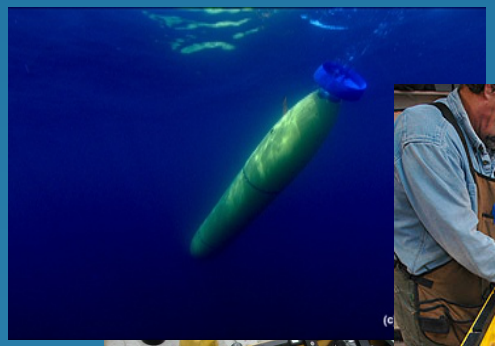
Phase 1: Bloom reconnaissance

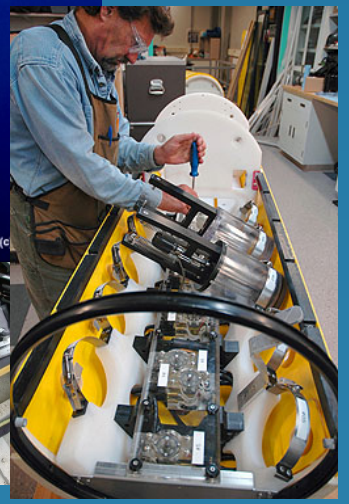
The 'Robot Ballet'…





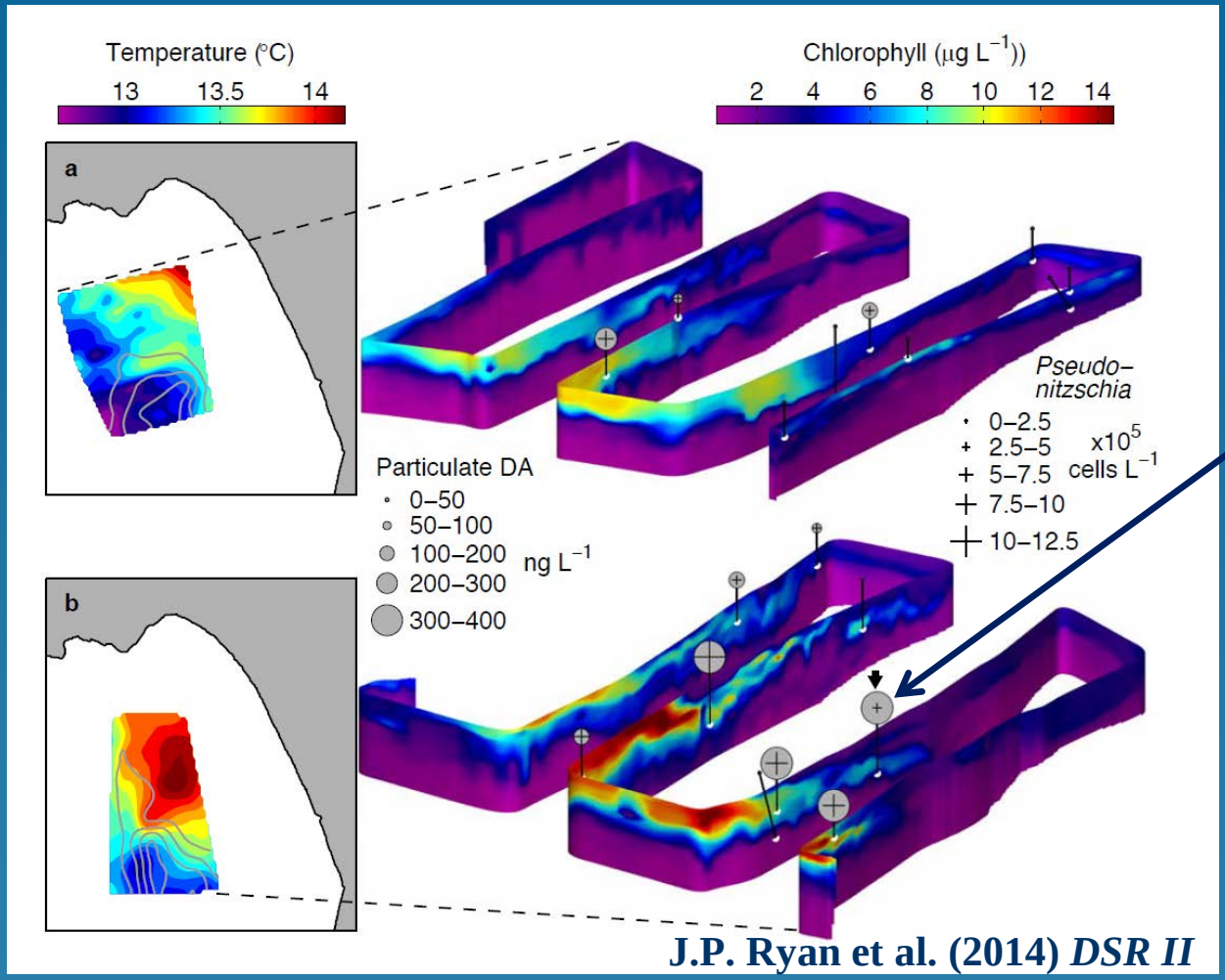
Tethys: long range (10 days) AUV for bloom patch mapping





Dorado: 'smart' short range (24 hrs) AUV with 'gulpers' for intelligent sampling of microorganisms

What can we learn from this new approach?

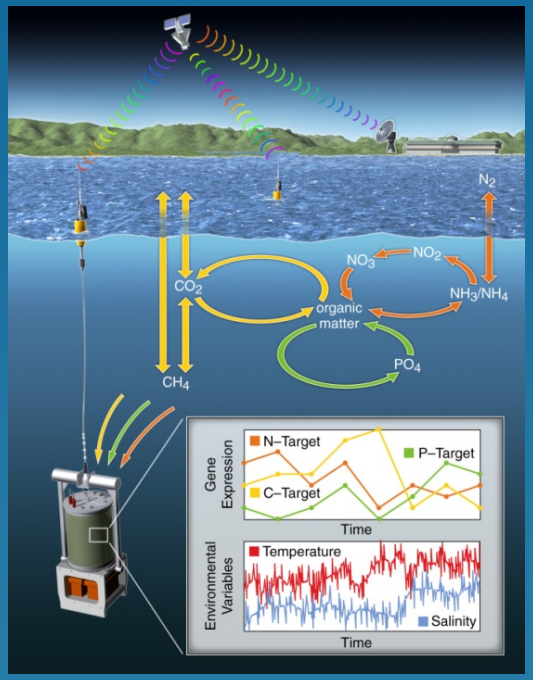


J.P. Ryan et al. (2014) *DSR II*

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

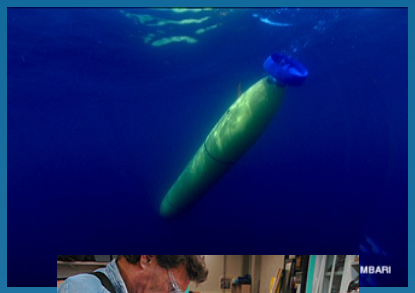
adaptive/intelligent AUV-based sampling provides a '3-D picture' of phytoplankton assemblage & water column structure over time; shows changes in *Pseudo-n.* and pDA conc. (bloom toxicity)

Sensor development – stage 3: 3G ESP-LRAUV autonomous detection + mobility/tracking



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

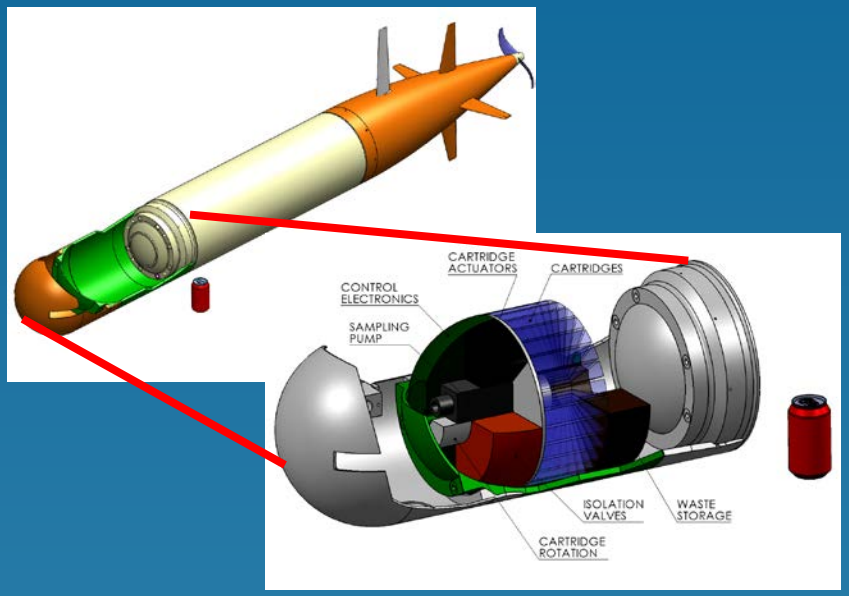
+



patch tracking & intelligent sampling for molecular analyses

=

3G ESP-LRAUV

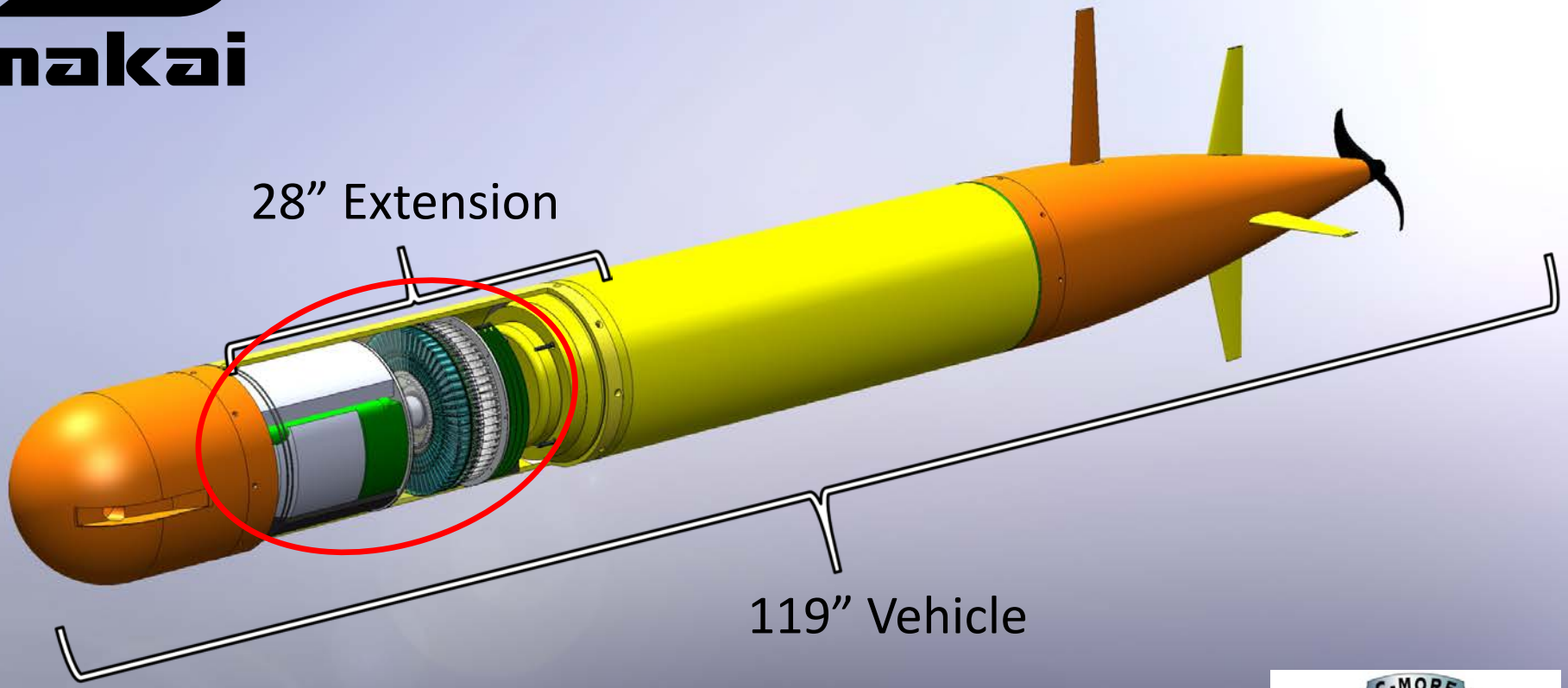


ESP integrated with Tethys-class long-range AUV (LRAUV)

- synergism of ESP sampling/ processing technology & LRAUV mobility and 'smarts'
- flexible repertoire of analytical modules (SPR, dPCR)

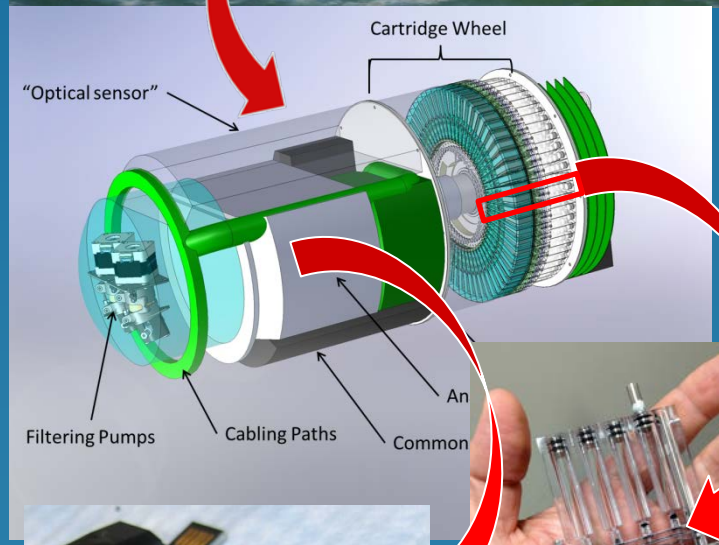
3G ESP/Long-Range AUV

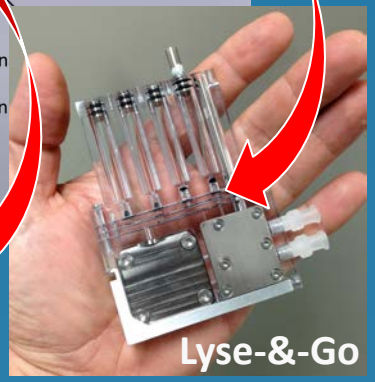
Mission Duration: 3 weeks/1800 km

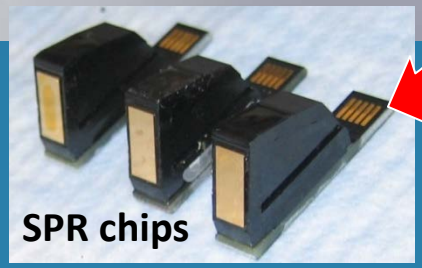


Creating positive outcomes for future generations.

Re-engineering the 2G ESP = 3G ESP-LRAUV







- first *MOBILE*, long-range AUV-based HAB spp. and toxin detection
- adaptive, intelligent tracking & sampling of target features
- novel, self-contained sample prep cartridge & miniature toxin sensor chips
- all results transmitted in near-real time
- deployed in marine and freshwater (in 2018) systems

3G ESP-LRAUV Prototype: The Deployments

makai

C-MORE
CENTER FOR MICROBIAL OCEANOGRAPHY: RESEARCH AND EDUCATION
Science
Technology
Education and Training
Communication and Outreach
EXPLORE-EXPERIMENT-EDUCATE

Credit: J. Birch

3G ESP: showing off its 'smarts'!

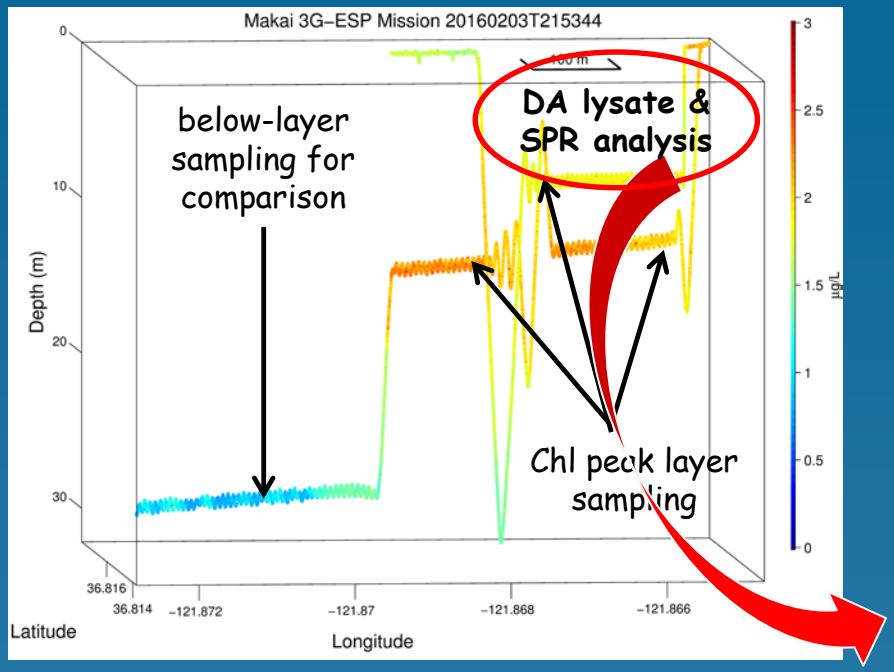


Image credit: Y. Zhang

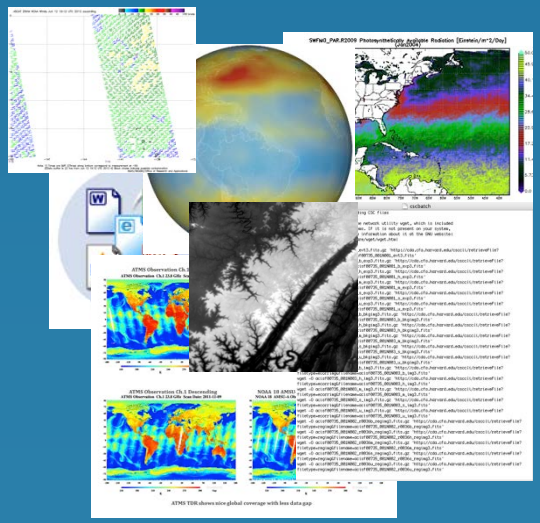
- multiple 3G prototype flights in Monterey Bay, CA since Nov. 2014
- 3 Feb mission demonstrates ability to locate/sample near Chl peak
- *MILESTONE: first autonomous, end-to-end SPR-based domoic acid measurement on 3G ESP-LRAUV*

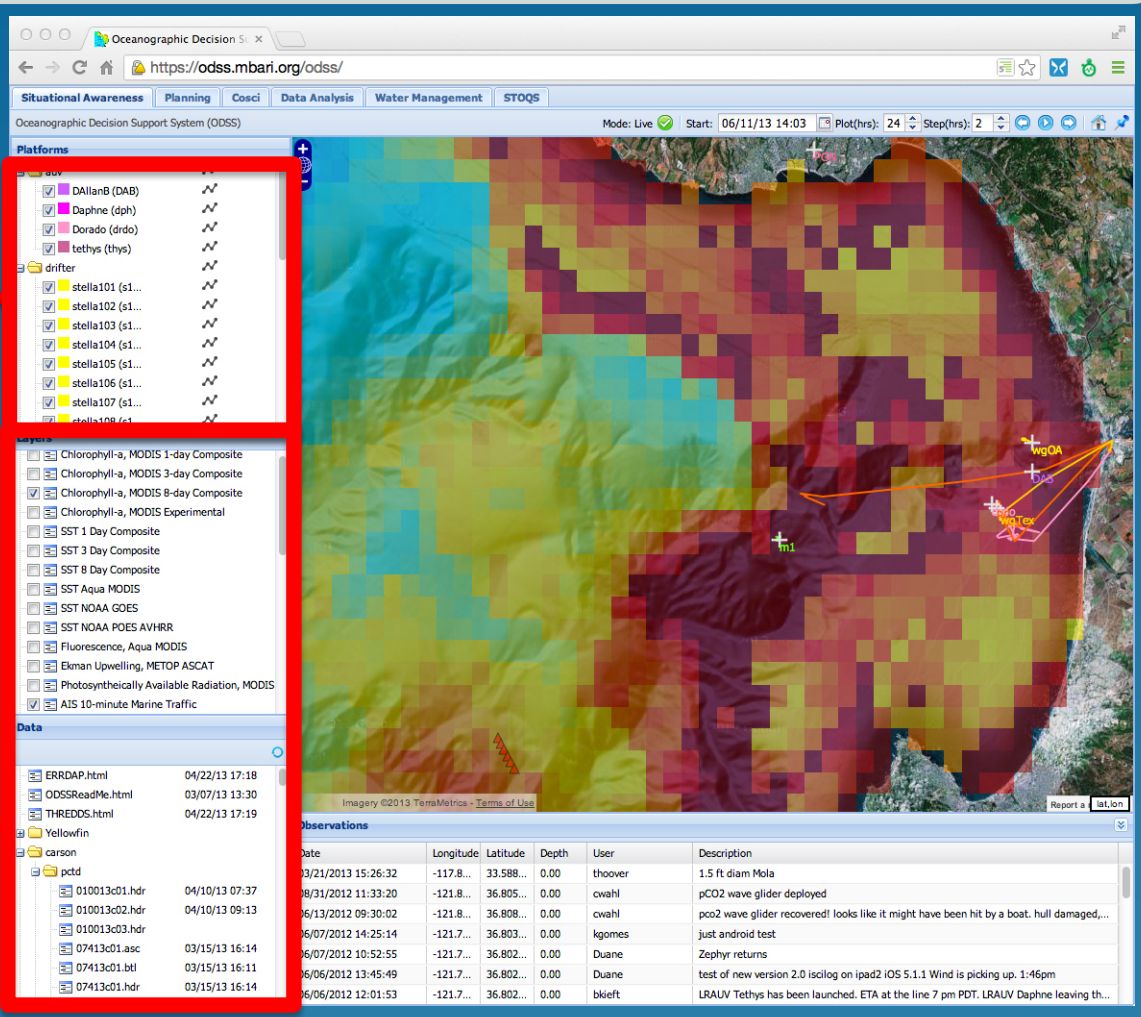
3G ESP SPR-based MCY – full 60-cartridge field test planned for late summer 2018 in Lake Erie

Emerging Role of Observing Systems in HAB Detection & Forecasting – Many Challenges!

Emerging Role of Observing Systems in HAB Detection & Forecasting – Many Challenges!

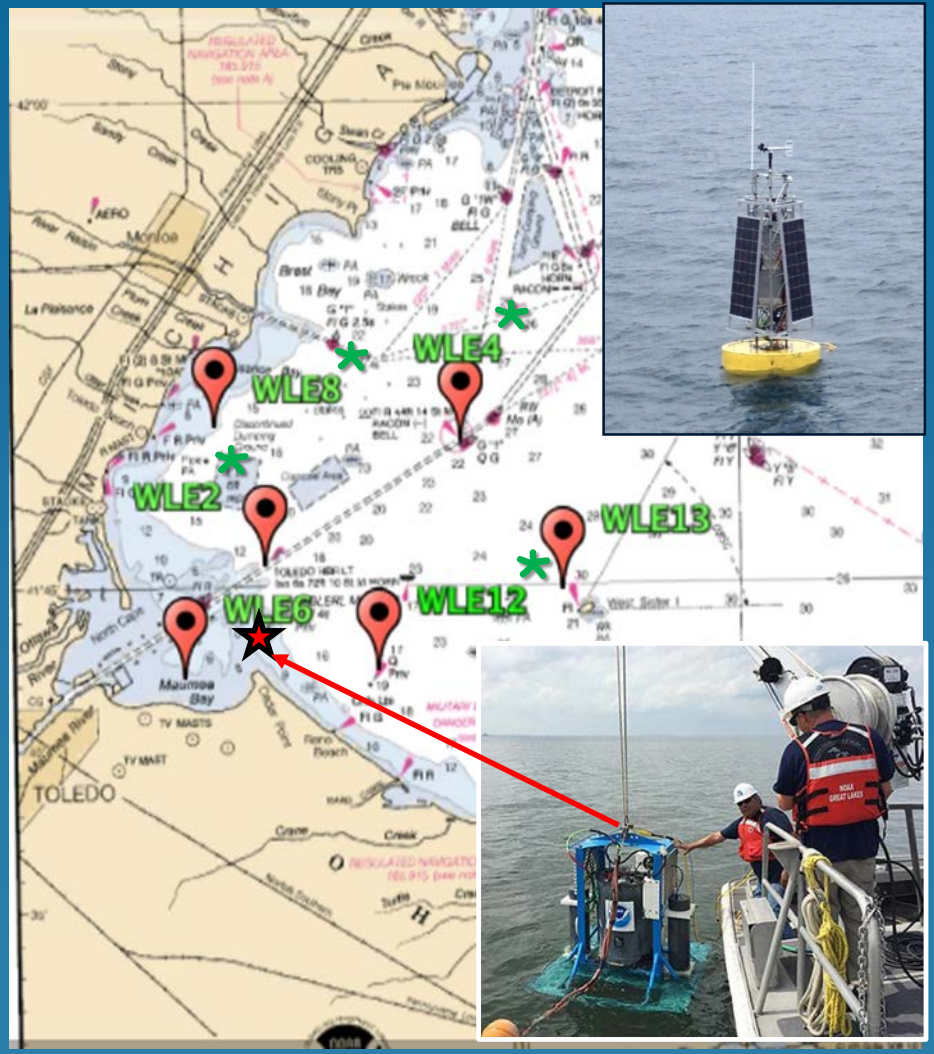
Asset planning, deployment, and coordination





Data acquisition, analysis, and assimilation into models

Emerging Role of Observing Systems in HAB 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*
- *early warning of CHAB events & understanding of environmental drivers of bloom growth/toxicity*
- *support for CHAB forecasts*

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

Emerging Role of Observing Systems in HAB Detection & Forecasting – NOAA HAB OFS

Lake Erie Harmful Algal Bloom Bulletin

18 September, 2017, Bulletin 20

The *Microcystis* cyanobacteria bloom continues in the western basin, along the Michigan and Ohio coasts, extending around the islands and east to the central basin. Observed winds since Thursday (9/14-18) caused an increase in surface concentrations, and allowed for the formation of scum. Scums were visible northwest of the Bass Islands. Measured toxin concentrations are below recreational thresholds throughout most of the bloom extent, but concentrations can exceed the threshold east of Maumee Bay State Park and northwest of the Bass Islands where the bloom is most dense (appearing green from a boat).

Forecast winds (5-11kn) today through Thursday (9/18-9/21) may reduce the potential for scum formation and minimize transport of remaining *Microcystis* concentrations. The water temperature is approaching or below 68˚F (20˚C) throughout the western basin, limiting the growth of *Microcystis* concentrations.

Please check Ohio EPA's site on harmful algal blooms for safety information: http://epa.ohio.gov/habalgae.aspx. Keep your pets and yourself out of the water in areas where scum is forming. NOAA's GLERL provides additional HAB data: https://www.glerl.noaa.gov/res/HABs_and_Hypoxia. The persistent cyanobacteria bloom in Sandusky Bay continues. Cyanobacteria is visible in the central basin extending offshore Headlands Beach State Park to Presque Isle State Park. --Ludema, Davis

The images below are "GeoPDF". To see the longitude and latitude under your cursor, select "Tools > Analyze > Geospatial Location Tool".

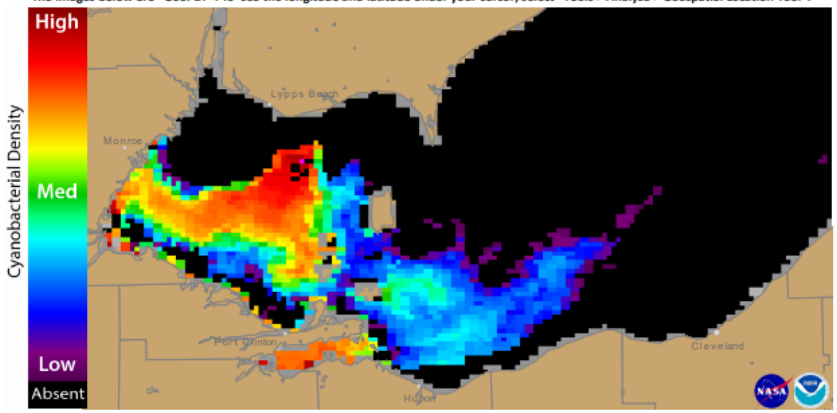


Figure 1. Cyanobacterial Index from NASA MODIS-Aqua data collected 17 September, 2017 at 13:31 EST. Grey indicates clouds or missing data. The estimated threshold for cyanobacteria detection is 20,000 cells/mL.

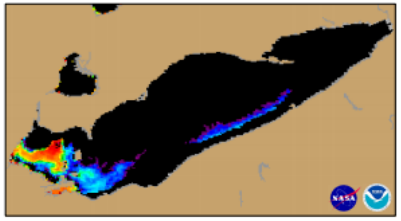
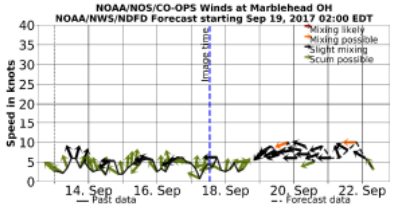


Figure 2. Cyanobacterial Index from NASA MODIS-Aqua data collected 17 September, 2017 at 13:31.

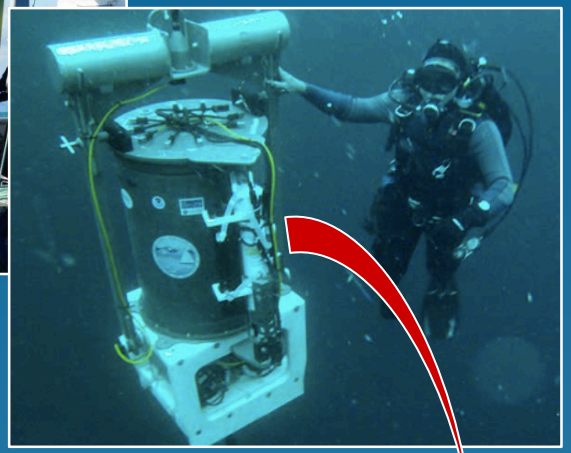


Wind speed and direction from Marblehead, OH. Blooms mix through the water column at wind speeds greater than 15 knots (or 7.7 m/s).

For more information and to subscribe to this bulletin, go to: https://tidesandcurrents.noaa.gov/hab/lakeerie.html

- provide user-friendly data tools & accurate forecasts to resource managers (fisheries, drinking/ recreational water)
- support <u>timely decision making</u> to protect public health, coastal resources, ecosystem services, and local economies
- assimilate data streams from autonomous, <u>*in-situ sensors*</u> into predictive models for forecasting bloom biomass, trajectory, toxicity

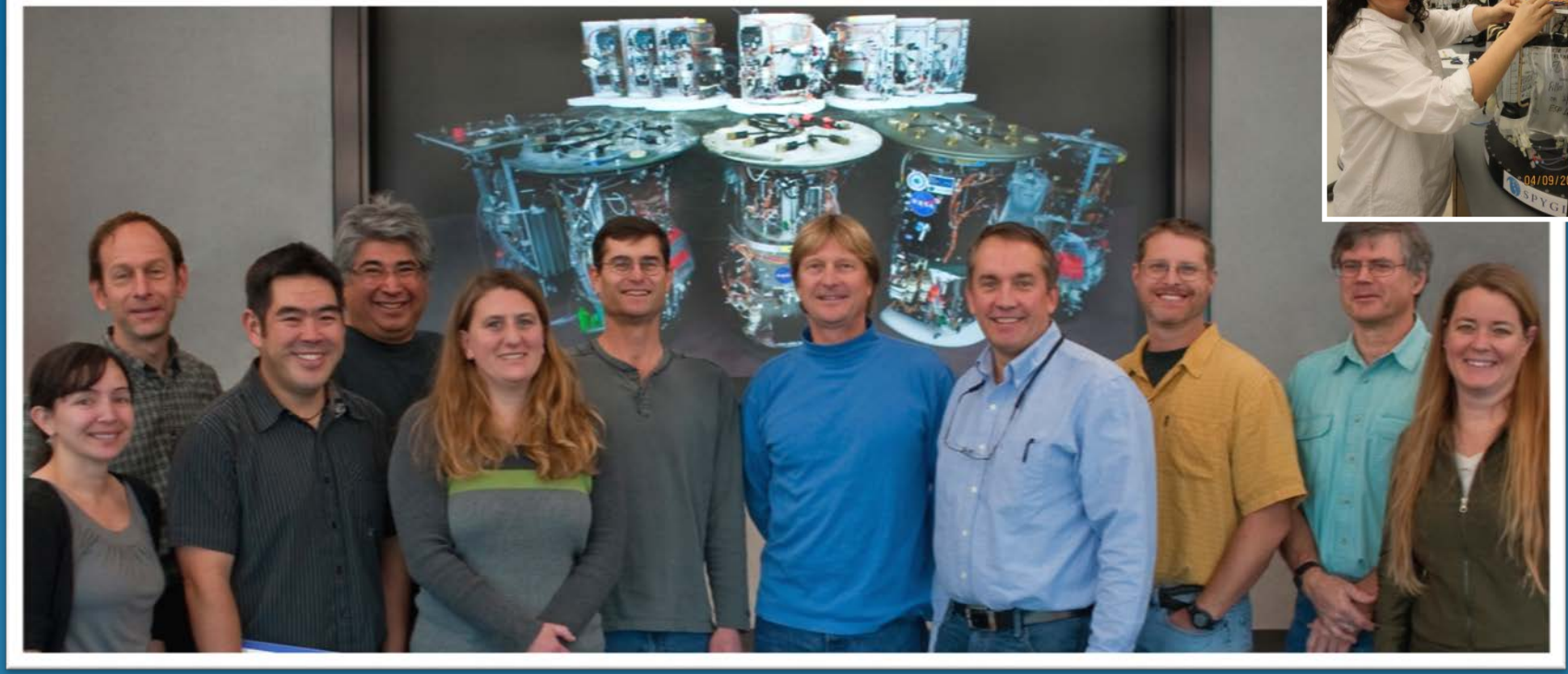
What will the future look like & what are some of our new challenges?



- **Imagining the next steps**
  - smaller, better, faster, cheaper, easier to manufacture
- **Adopting interface standards**
  - 'plug-and-play' modular sampling and analytical modules
- **Exporting the technology**
  - transitioning research to application & operations
  - enabling others to address diverse questions

Adapted from C. Scholin

MBARI & NCCOS ESP TEAM



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M B A R I SM

NOAA National Oceanic and Atmospheric Administration U.S. Department of Commerce

Gordon and Betty MOORE FOUNDATION

NSF

SCCWRP Established 1969

NASA

C-MORE Center for Microbial Oceanography: Research and Education Explore-Experiment-Educate

JHT INCORPORATED

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GLERL: T Davis (BGSU), E Davenport, S Ruberg

NWFSC: S Moore, N Adams; UW: J Mickett

Queen's University Belfast: C Elliott, K Campbell

The David and Lucile Packard Foundation

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