How to Track a HAB: New Technologies and Methods for Identifying and Monitoring Harmful Algal Blooms

Part 3: Summer Webinar Series to Build Awareness About Harmful Algal Blooms and Nutrient Pollution

Tuesday, August 20, 2013
1:00pm – 2:30pm ET

Speakers:
Steve Morton, PhD Research Oceanographer, Marine Biotoxin Program, National Oceanic and Atmospheric Administration
Project Leader, and Phytoplankton Monitoring Network
Don Anderson, PhD Senior Scientist, Biology Department, Woods Hole Oceanographic Institution
Director, Cooperative Institute for North Atlantic Research (CINAR)
Director, U.S. National Office for Harmful Algal Blooms

Moderated by: Christina Badaracco, ORISE Intern, US EPA

Today’s Schedule

• Introduction and GoToWebinar Logistics
• Steve Morton
  – Algae and HABs
  – Phytoplankton Monitoring Network
  – Case Studies
• Don Anderson
  – Molecular techniques
  – New technology: Environmental Sample Processor and Imaging FlowCytobot
• Polling Questions
• Q&As
• Final Announcements
Webinar Logistics

• To ask a question — Type your question in the “Questions” tool box on the right side of your screen and click “Send.” Our panelists and moderator will respond to the entire audience.

• To report any technical issues (such as audio problems) — Type your issue in the “Questions” tool box on the right side of your screen and click “Send” and we will respond by posting an answer in the “Questions” box.

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Today’s Topic and Speakers

Phytoplankton Monitoring Network and Case Studies

Steve Morton, PhD
• Research Oceanographer and PMN Program Leader
  • National Oceanic and Atmospheric Administration

HAB Illnesses and Public Communication

Don Anderson, PhD
• Senior Scientist
  • Woods Hole Oceanographic Institution
• Director
  • U.S. National Office for Harmful Algal Blooms
Citizen Scientist Monitoring HABs and Changes in Environmental Conditions

Promoting a better understanding of Harmful Algal Blooms by way of volunteer monitoring.

Steve L. Morton, Ph.D.

Road Map

- What are Harmful Algae
- Identification Techniques
- Citizen Scientist
- Case Studies
- How you can get involved

NOAA Marine Biotoxins Program

http://www.chbr.noaa.gov/pmn/
ALGAE BASICS

General Characteristics

– Phyto: Plant
– Plankton: Drifting/Wandering
– Single-celled organisms
– Smaller than the width of a human hair (< 100µm (microns))
– Photosynthesis (autotrophic)
– Found in marine and freshwaters
– Three major toxin producers
  • Diatoms
  • Dinoflagellates
  • Cyanobacteria

PHYTOPLANKTON IMPORTANCE

– Start of the marine food web
– Use and store carbon as part of the carbon cycle
– Source for much of the world’s oxygen
– Source of many natural products
– Good indicator of environmental change
TOXIC HARMFUL ALGAL BLOOMS (HABs)

Many different algal species are responsible for HABs. Some produce toxins that can endanger the lives of marine animals and human health.

HUMAN HEALTH SYNDROMES – Associated with Phytoplankton

<table>
<thead>
<tr>
<th>SYNDROME</th>
<th>SPECIES AND TOXIN</th>
<th>SYMPTOMS</th>
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<tbody>
<tr>
<td>Amnesic Shellfish Poisoning (ASP)</td>
<td><em>Pseudo-nitzschia</em></td>
<td>Permanent short term memory loss</td>
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<td></td>
<td><em>Domoic acid</em></td>
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<tr>
<td>Ciguatera Fish Poisoning (CFP)</td>
<td><em>Gambierdiscus toxicus</em></td>
<td>Temperature Sensation Reversal</td>
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<td><em>Ciguatoxin &amp; Maitotoxin</em></td>
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<tr>
<td>Diarrhetic Shellfish Poisoning (DSP)</td>
<td><em>Dinophysis</em></td>
<td>Diarrhea</td>
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<td></td>
<td><em>Okadaic acid</em></td>
<td>Nausea</td>
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<td></td>
<td><em>Prorocentrum lima</em></td>
<td>Vomiting</td>
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<tr>
<td>Neurotoxic Shellfish Poisoning (NSP)</td>
<td><em>Karenia brevis</em></td>
<td>Gastrointestinal and Neurological Problems</td>
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<tr>
<td></td>
<td><em>Brevetoxin</em></td>
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<tr>
<td>Paralytic Shellfish Poisoning (PSP)</td>
<td><em>Alexandrium</em></td>
<td>Loss of motor control</td>
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<tr>
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<td><em>Saxitoxin</em></td>
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Reports of harmful algal blooms have drastically increased in the past three decades. Researchers attribute the increase partly to excessive nutrient pollution of the water and partly to better detection of HABs by coastal monitoring programs.

Identification and Detection Tools

- Light Microscopy
- Electron Microscopy
- Genetic Analysis
- Antibody Recognition
- Imaging cytometry
MARINE BIOTOXINS PROGRAM SERVICES

**Phytoplankton Monitoring Network**
Network that monitors distribution of harmful algae and species composition throughout the coastal US. Observations and samples by PMN monitors assist the research that is being done by the Marine Biotoxins program.

**Analytical Response Team**
Provides rapid and accurate identification and quantification of marine algal toxins in suspected harmful algal blooms, marine animal mortality events and human poisonings.

Identification and analytical capability provides support for management agencies that can then make timely and informed decisions impacting stakeholders involved in coastal wildlife, human health and commerce.
Phytoplankton Monitoring Network

PMN Mission ~
“To educate the public on harmful algal blooms (HABs) while expanding the knowledge of phytoplankton that exist in coastal waters.”

Program Goals

• Monitor and maintain an extended survey area along coastal waters throughout the year
• Create a comprehensive list of harmful algal species inhabiting coastal marine waters
• Promote an increased awareness and education to the public on HABs
• Identify general trends where HABs are more likely to occur
• Isolate areas prone to harmful algal blooms (HABs) for further study by researchers in effort to assist state managers in mitigating the effects of HABs
• Create a working relationship between volunteers and researchers
• Increase the public’s awareness of research conducted by federal workers on HABs
**HAB Sampling Plan**

**Step 1:** Conduct 3-min net tow, record water temperature and salinity

**Step 2:** Collect 1L & 30mL live whole water grab samples

**Step 3:** Analyze net tow sample for **Target Species**

**Step 4:** If **Target Species** are identified, prepare necessary samples for UPS shipment

**Volunteer Equipment**

Volunteers are loaned all sampling equipment

- Refractometer
- 20 um mesh plankton net
- Thermometer
- 5 gridded slides
- Cover slips
- 250 mL bottles
- 1L bottles
- 15mL of Lugol's solution for preservation

*Region specific volunteer manual

*The PMN Manual has data sheets, phytoplankton ID sheets, and HAB information specific to your local coastal waters.

Photo credit: Elizabeth Zerai
Use of Technology

WebEx communication

Digital microscopes/photography

Interactive web site and GIS mapping

“Phyto” Smart Phone App

Developed by PMN volunteer Shawn Gano, to assist with and to improve volunteer’s identification skills of marine algae in the Gulf of Mexico region.
When a Bloom is reported

Phytoplankton Monitoring Network
Promoting a better understanding of Harmful Algal Blooms by way of volunteer monitoring.

Examples of Data Usage and Case Studies
Bloom Events (2001-2012)
NOAA Phytoplankton Monitoring Network

Total Blooms: 210  Non-toxic: 173  Toxic: 37

Phytoplankton Monitoring Network Identifies First Recorded Bloom of a Toxic Pseudo-nitzschia species in North Carolina Waters

A bloom of Pseudo-nitzschia was observed by students of First Flight High School and preserved samples sent to the Marine Biotoxins Program were positively identified using scanning electron microscopy as *Pseudo-nitzschia multiseries*, and shown by LC-mass spectrometry to produce domoic acid. The identification of *P. multiseries* in North Carolina’s waters is another example where a volunteer monitoring program is useful in developing a species list and record of distribution patterns, as well as alerting NOAA scientists to the presence of potentially harmful species.

Windows to Research & Response

PMN Findings Help Lead to the...

First time Identification of Domoic Acid in Marine Mammals in Southeastern U.S Waters

Domoic acid was detected exclusively in *Kogia* spp. stranding in the absence of observed HAB activity. The frequency of occurrence and concentrations of domoic acid suggests potential chronic animal exposure in a region with virtually no history of HABs.
*Pseudo-nitzschia* in the Southeast

- November 1, 2006: First Flight High School in Kill Devil Hills observed a bloom of *Pseudo-nitzschia* and sent both a preserved and live sample to NOAA.
- November 6, 2006: College of the Albermale reported a bloom of *Pseudo-nitzschia* from the Bonner Bridge and sent preserved, live sample and oysters to NOAA.

Multi-species *Pseudo-nitzschia* bloom
North Carolina Governor's Award for Environmental Stewardship

Kogia story

41 Samples from 1997-2008 being tested for DA, 87% of the samples contained DA
Pilot Program Collaborators

Community members that have expressed concerns about lake quality and whether the presence of HABs and HAB toxins in the local waters has any adverse health impacts on people and animals (e.g. respiratory complaints and headaches).
Pilot Project Impacts:

*Develop partnerships with communities impacted by HABs, public health practitioners, and scientists and engineers dedicated to addressing HAB-related public health issues.*

- Enhance environmental awareness about HABs
- Increase community knowledge of the occurrence and extent of freshwater HABs

Target Species

- *Microcystis*
- *Anabaena*
- *Aphanizomenon*
- *Cylindrospermopsis*
- *Oscillatoria*
Wisconsin: 9 Lakes with 34 Volunteers
Minnesota: 2 Lakes with 3 Volunteers
Over 300 data points during Summer 2010
5 Microcystis blooms and 3 Oscillatoria blooms
Forecasting of HABs

**Observations**
(satellite imagery, buoys, field samples)

**Model output**
(physical, ecological, health impacts)

**Analysis of data and models**

**HAB Bulletin (managers)**

**Conditions Report (public)**

- Harmful algae has been identified in northern Brazoria and Southern Galveston Counties. Very low impacts are possible in northern Brazoria and Southern Galveston Counties today through Thursday. No impacts are expected in any other Texas Counties through today.

- Monday, April 20, 2020

**Conditions Report**

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- Monday, April 20, 2020
Conclusion

• Volunteer Phytoplankton Monitoring Networks are a proven key asset in the detection of harmful algal blooms.
• Data generated by volunteers are very useful in many aspects of HAB research and management decisions.

Funding

NOS/NCCOS CCEHBR Marine Biotoxins Base funding
NOS/NCCOS CCMA HAB forecast funds
NESDIS/NOCD/NCDDC Database/IMS backing
NOAA/MERHAB funding

HABISS funding for HAB Surveillance in SC
Novel Sources of Surveillance Data for Climate Change
New technologies for HAB cell identification and monitoring

Donald M. Anderson
Woods Hole Oceanographic Institution
Common needs:
- Rapid and accurate cell identification and enumeration in the laboratory
- In situ, real-time estimates of cell abundance

Challenges:
- Dozens of HAB species
- Genetic differences between strains from different regions
- Toxic and non-toxic strains of the same species co-occur
- Sometimes cells are in low in abundance
- Huge geographic areas, dynamic hydrographic environments
Example of cruise sampling plan in the Gulf of Maine

Molecular approaches to cell identification and counting

- Antibody probes
  - target -> cell surface proteins
- Oligonucleotide probes
  - target -> ribosomal RNA or other molecules
    - Multiple formats and platforms being tested
Antibodies

Indirect immunofluorescent detection

Ag= cell surface protein specific for target organism
One problem with antibody probes is that they may not work over broad geographic areas due to differences in cell surface proteins between strains of a given species.

Oligonucleotide probes (oligos)

Assay formats:

1. Whole cell (FISH)
2. Quantitative PCR
DNA Base Pairing

http://upload.wikimedia.org/wikipedia/en/f/f0/DNA_Overview.png

Ribosomes

FISH method development and cross-reactivity testing using rRNA-targeted probes for identifying whole cells

Ribosomes

Cell

Probe-ribosome Complex

Labeled Cell

Fluorescent Probe, no PCR required

http://upload.wikimedia.org/wikipedia/en/f/f0/DNA_Overview.png
Dual-labeled sample:
Texas red - *Alexandrium fundyense*
FITC - *Alexandrium ostenfeldii*

*Cylindrospermopsis raciborskii* LB 2897

(a) No probe, Cy3 filter      (b) No probe, FITC Bandpass filter
(c) Probe 2, Cy3 filter      (d) Probe 2 (6-FAM), FITC Bandpass filter

FITC bandpass filter negates the autofluorescence visible with the Cy3 filter, allowing for easy probe detection when labeled with the fluorochrome 6-FAM.
Quantitative Real-Time PCR (qPCR)

qPCR measures the accumulation of PCR product as an increase in fluorescence. Fluorescence is proportional to the number of copies of target DNA. Cells are lysed (broken apart) for this procedure.

- High efficiency
- High sensitivity
- Large dynamic range of detection

Live, WC, and qPCR contours - EN437-07
Comparison of cell counting methods

<table>
<thead>
<tr>
<th></th>
<th>Traditional light microscopy</th>
<th>FISH (WC)</th>
<th>SHA</th>
<th>qPCR</th>
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</thead>
<tbody>
<tr>
<td>Sample throughput</td>
<td>8-12 samples / d</td>
<td>20 samples / d</td>
<td>32 samples / d</td>
<td>96 samples / d</td>
</tr>
<tr>
<td>Cost / sample</td>
<td>&lt; $1</td>
<td>$6</td>
<td>$7</td>
<td>$6</td>
</tr>
<tr>
<td>(not including labor)</td>
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Fiber-optic based HAB species detection using sandwich hybridization

Utilizes an optical fiber sensor array to detect and enumerate multiple target species simultaneously with high specificity.
Fiber Optic Bundles

Each fiber carries its own light signal and consequently, arrays can be used to build up images using a pixel-by-pixel image reconstruction (similar to a complex insect eye).


Microspheres in Etched Wells

Fiber cores can be selectively etched relative to the clad using HCl

Silica beads can be loaded into the wells by applying a small aliquot of bead solution directly to the fiber tip. Upon drying, beads are held firmly within the wells.

Different DNA probes are first attached to different bead types.

Probes are combined into a library.

Probe bead library is distributed randomly into wells on fiber array surface.

1) Encoding image: used to construct a “bead map table” listing all beads and the type of receptor on each.

2) Background Image taken.

3) Specific Hybridization of target rRNA to probe; rRNA is directly labeled with fluorophore.

4) Increased fluorescent signal for hybridized and labeled sensors.
The image cannot be displayed. Your computer may not have enough memory to open the image, or the image may have been corrupted. Restart your computer, and then open the file again. If the red x still appears, you may have to delete the image and then insert it again.
Multiplexed array with 3 sensors

- Multiplexed array was tested with cultured cells of 3 organisms (A. fundyense, A. ostenfeldii, and Pseudo-nitzschia australis)
- Each sensor showed positive signal only with its target organism - no significant cross reactivity

Common needs:

Rapid and accurate cell identification and enumeration in the laboratory

In situ, real-time estimates of cell abundance

Historically there have been in situ sensors for chemical or physical parameters, but not for species
The Environmental Sample Processor (ESP) (DNA-based detection)

The Imaging FlowCytobot (IFCB) (Optical detection)
2012 deployment:
- Successful detection of *Alexandrium fundyense* at 100 – 200 cells/L
- Counts consistent with ground-truth surveys in the area of the ESP mooring
- Concurrent, successful detection of toxic *Pseudo-nitzschia*

ESP issues

- Communications
  - Freewave versus cell phone versus Iridium
- Mooring deployment and recovery operations are expensive
  - Dock or pier-based deployment is simpler than in situ moorings
  - Need to lengthen deployment cycles (e.g., adaptive sampling; instrument redesign)
- Size, cost (>200K) and complexity
- Limited sample number at present – typically 44 HAB analyses per deployment (multiple HAB species per array)
- Expand capability for HAB toxin detection (only DA and STX thus far)
We are here

Wilbur Wright, 1902

The future

• 3rd generation instruments on AUVs are under development
• horizontal & vertical coverage in situ

The Imaging Flow Cytobot (IFCB)

Lisa Campbell and dock-mounted IFCB, Port Aransas, Texas
The Imaging FlowCytobot (IFCB) is an automated, underwater microscope

Analysis

• The IFCB generates huge numbers of images. Automated image analysis and classification are essential

• Computer software is manually “trained” to identify individual species
IFCB Development

• First generation instruments required power and data cables from shore
• Second generation instruments have lower power consumption and are smaller and lighter; power needs still an issue
• Automated biofouling control measures have enabled continuous deployments of up to 6 months
• McLane Research Laboratories, Inc. now manufacturing second generation instruments for commercial sale

Time series of Dinophysis and Karenia from dock-mounted IFCB at Port Aransas Texas

Campbell et al. 2010

Campbell et al. 2013
• Two objectives at WHOI
  – Develop a cable-free mooring platform for the IFCB
  – Test the IFCB on *Alexandrium fundyense*
Time series from the 2012 Salt Pond bloom

Hourly mean *A. fundyense* abundance

- **A. fundyense**
- **Other**

<table>
<thead>
<tr>
<th>Date</th>
<th>Hourly mean A. fundyense abundance</th>
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<tbody>
<tr>
<td>3/27</td>
<td>0 cells mL⁻¹</td>
</tr>
<tr>
<td>4/1</td>
<td>0 cells mL⁻¹</td>
</tr>
<tr>
<td>4/6</td>
<td>0 cells mL⁻¹</td>
</tr>
<tr>
<td>4/11</td>
<td>0 cells mL⁻¹</td>
</tr>
<tr>
<td>4/16</td>
<td>0 cells mL⁻¹</td>
</tr>
<tr>
<td>4/21</td>
<td>0 cells mL⁻¹</td>
</tr>
<tr>
<td>4/26</td>
<td>0 cells mL⁻¹</td>
</tr>
<tr>
<td>5/1</td>
<td>0 cells mL⁻¹</td>
</tr>
</tbody>
</table>

[Graph showing hourly mean A. fundyense abundance with dates 3/27 to 5/1 and abundance values from 0 to 3000 cells mL⁻¹]
IFCB issues

- Power needs still high
- Discrimination of low abundance, non-descript HAB species uncertain
- Volume of water sampled is small (~5 ml);
- Larger colonial or filamentous organisms (e.g., cyanoHABs) are more difficult to sample
- Training sets and classifiers needed for new species and areas
- Cost (~$125K) and complexity
A vision for the future - proposed locations for HAB sensors moored in the Gulf of Maine, with data assimilated into forecast models.

Summary

- There are many challenges facing HAB scientists and managers, and among these is the need to rapidly and accurately identify and count HAB species in the laboratory.
- Another need is for real-time, situ measurements of HAB cell abundance at remote locations.
- Historically, biosensor development has been slow compared to sensors for physical and chemical parameters. Rapid developments in robotics, optics and molecular biology are now changing this imbalance.
- A range of laboratory-based methods using molecular probes of various types are now routinely used. New assay platforms are being developed as well.
Summary

- Robotic instruments such as the ESP and IFCB show considerable promise for use with a range of HAB species. Both are commercially available as 2nd generation instruments, with 3rd generation designs underway.
- However, both need strong testing and support from the HAB research and management community if they are to continue to develop further as products and as HAB research and monitoring tools.

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- Woods Hole Center for Oceans and Human Health - NSF Grants OCE-0430724, OCE-0850421, OCE-0911031, OCE-1314642 and NIEHS 1P50-ES01274201, 1P01-ES02192301
Questions?

Contact Information

Steve Morton
Marine Biotoxin Program, Phytoplankton Monitoring Network
National Oceanic and Atmospheric Administration
Phone: 843-762-8857
Email: steve.morton@noaa.gov
jeff.paternoster@noaa.gov (PMN)

Phytoplankton Monitoring Network: http://coastalscience.noaa.gov/research/habs/pmn

Don Anderson
Biology Department and Cooperative Institute for the North Atlantic Region
Woods Hole Oceanographic Institution
Phone: 508-289-2351
Email: danderson@whoi.edu

Lab: http://www.whoi.edu/groups/andersonlab/
CINAR: http://www.cinar.org
Harmful Algae: http://www.whoi.edu/redtide
Watershed Academy Certificate

- If you would like to obtain a participation certificate, type the link below into your web browser:

- You can type each attendee’s name into the PDF and print the certificate.

Additional Resources

EPA HABs website: http://www2.epa.gov/nutrientpollution/harmful-algal-blooms

Facebook: https://www.facebook.com/EPAWaterIsWorthIt

Twitter: @EPAWater

Flickr: http://www.flickr.com/photos/usepagov/sets/72157634706332559/

State of the Environment blog: http://blog.epa.gov/epplocations/

Watershed Academy Webcasts: www.epa.gov/watershedwebcasts