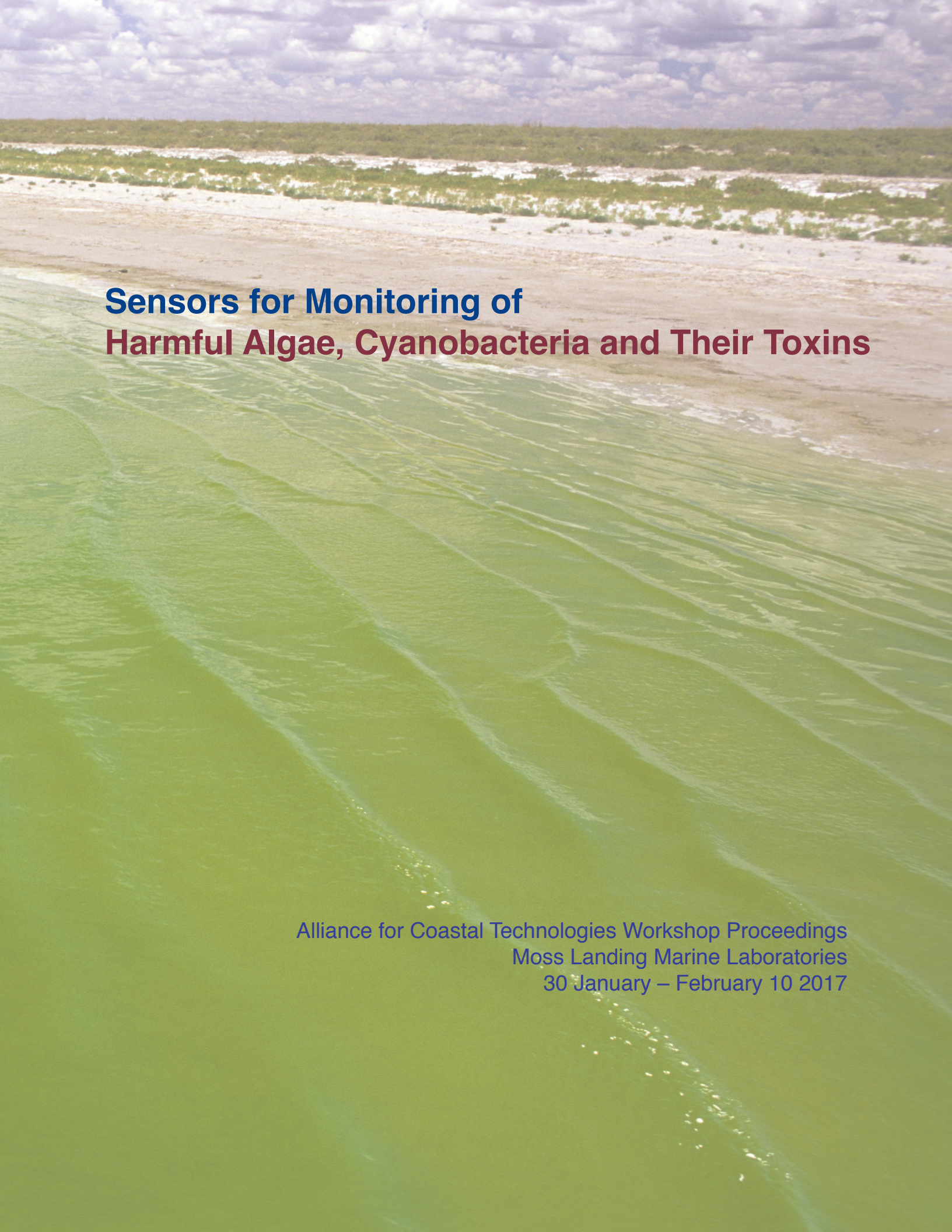

Sensors for Monitoring of Harmful Algae, Cyanobacteria and Their Toxins

Alliance for Coastal Technologies Workshop Proceedings
Moss Landing Marine Laboratories
30 January – February 10 2017

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Workshop Proceedings

Alliance for Coastal Technologies

Sensors for Monitoring of Harmful Algae, Cyanobacteria and Their Toxins

Moss Landing Marine Laboratories, Moss Landing CA

30 January – February 1 2017

ACT is a component of U.S. Integrated Ocean Observing System (IOOS) and

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EXECUTIVE SUMMARY

The Alliance for Coastal Technologies (ACT) and the U.S. Integrated Ocean Observing System (NOAA-IOOS) co-sponsored a workshop titled "Sensors for Monitoring of Harmful Algae, Cyanobacteria and Their Toxins" which convened 30 January – 1 February 2017 at the Moss Landing Marine Laboratories. Invited participants included a cross section of technology developers, vendors, agency representatives, regional managers and academic researchers engaged in harmful algae (HA) and toxin detection and monitoring from both freshwater and marine habitats. The workshop posed several questions that spanned use cases, needs, readiness levels, and advantages and limitations of current technology. By bringing together folks from different disciplines, these topics were discussed from a variety of angles. Specific goals were to:

1. Document the current use of technologies demonstrated to detect harmful algal blooms (HABs) and their associated toxins both in impacted U.S. coastal regions and internationally.
2. Discuss the suitability of currently available technologies to meet monitoring and forecasting needs.
3. Discuss the current state of performance verification testing of the available technologies.
4. Determine shared challenges that can be leveraged across the marine-freshwater continuum.
5. Define the role ACT (and others) could play to further the testing of currently available instruments and/or foster a competitive environment for the development of new sensors/technologies as identified by our regional stakeholders (a HAB sensor challenge akin to the Nutrient Sensor challenge) in light of concurrent efforts.

The need for sensitive and robust HAB and toxin detection capabilities are ongoing, as toxic events continue to threaten and affect economies, human health, and natural resources (i.e. drinking water, seafood) on a global scale. The desire for tools that predict and mitigate events is shared across the continuum of stakeholders, including managers, public health officials, researchers, and the public. The goals of this workshop directly align with the mandate of the Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA; authorized by Congress in 1998; reaffirmed and expanded in 2004 and 2014; submitted for re-authorization to Congress, August 2017), which requires NOAA to advance our understanding and abilities for HAB event detection, monitoring, assessment and prediction. Further, for the past several years NOAA has directed significant resources to developing an Ecological Forecasting Roadmap (EFR), a coordinated and systematic approach to ecological forecasts needed by the nation. HABs are a priority focus for this activity based on needs expressed by stakeholders, NOAA's maturity and capacity to develop HAB forecasts, and the national significance of the issue.

This workshop was a timely follow-up from prior HAB detection workshops (2002 and 2008) and resulted in updated recommendations including: 1) continued refinement of current methods to expand detection ranges and address cell physiology; 2) advancement of new strategies to further engage stakeholders to better define sensor technology for development or modification and identify realistic use cases; 3) tap into alternate sources of funding and partnerships to develop sustainable networks for long term data sets from regional HAB observing systems and mobile platforms; 4) retain a high performance computing network for efficient data storage and sharing; and 5) conduct a near-future ACT-based performance verification of commercially available HAB toxin kits to support the growing needs of the stakeholder community.

INTRODUCTION

Harmful algal blooms (HABs) are a continued threat to economies and marine/freshwater and human health throughout the US, including coastal regions encompassing the Pacific, Gulf of Mexico, Southeast Atlantic, Northeast Atlantic, and the Great Lakes. While blooms of varying intensities are common, highly toxic events have great impact and thrust the issue into the public spotlight. A few recent notable events include: 1) in the summer of 2014, more than half a million Toledo, OH residents were restricted from using tap water for three days because microcystin levels were measured as high as 2.5 ug/L, more than twice the 1.0 ug/L threshold for human consumption recommended by the World Health Organization (T. Henry, August 3, 2014); 2) in 2015, a sustained domoic acid event along the entire US West coast cost California more than $48 million in lost income for the Dungeness crab industry (Brown 2016); and 3) in 2016, the first domoic acid event was recorded in Maine, with toxin levels five times higher than what is considered safe for human consumption and resulting in the recall of approximately five tons of shellfish (P. McGuire, posted October 6, 2016). Monitoring efforts and long-term data sets are invaluable for developing strategies for prevention and mitigation of events such as these (Kudela et al. 2015). These data serve to inform now- and forecasting networks towards NOAA's mandate of developing an Ecological Forecasting Roadmap (EFR). In addition, the Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA) calls for predictive capabilities through advancing our understanding of and abilities for HAB event detection, monitoring, and assessment.

Anderson (1989) and Hallegraeff (1993) first outlined the rise in frequency, magnitude and geographical extent of HABs and their impacts during prior decades. Since then, continued long-term monitoring efforts on many levels (e.g. citizen scientists, tribal, NGOs, state and federally funded research) have confirmed this HAB expansion. While work is still needed towards the ultimate goal of predicting HAB events as a means of mitigating their impacts, seasonal periods of increased risk have been defined in some cases, which can serve to inform more efficient allocation of resources toward monitoring and research on triggers of bloom events. We are only beginning to understand how HAB events fit into the larger context of regional and global changes in climate and nutrient loadings (Sellner et al. 2003, Anderson et al. 2008, Hallegraeff 2010, Wells et al. 2015).

Decades of research have identified many of the harmful species responsible for toxic blooms, however the physiological drivers of toxicity have not been fully elucidated. Moreover, the genetic controls on toxin production and their variability within and among species and strains are still largely unknown. This is compounded by underlying cryptic diversity within genera/species that can affect detection capabilities for monitoring purposes. On a broader scale, factors controlling bloom initiation, persistence, and decline are not fully known. The variables involved can be very complex and work synergistically, including availability of micro- and macronutrients, physical parameters (e.g. irradiance, salinity, temperature, pH), biological influences (e.g. bacteria, grazers), and diversity (e.g. species and strains) within blooms (see Lelong et al. 2012 for review).

Several technologies exist or are in development for the detection of HAB species and their toxins (for review see Doucette and Kudela 2017). Instruments can be moored, mobile or handheld and scheduled for discreet sampling times or triggered remotely. Sampling can be passive or in response to fluorescence signatures that are broad (e.g. chlorophyll) or more group-specific (e.g. phycocyanin, phycoerythrin), while some sensors are coupled with imaging or genetic/toxin detection capabilities for more detailed species identification. The appropriateness of specific

analytical platforms is based on the scientific and/or management goals being addressed, the spatial/temporal sampling scales desired, but must be balanced with availability, capabilities, budget and expertise. Ultimately, tools that effectively integrate shore-station, offshore buoy, and autonomous vehicle monitoring with models of bloom dynamics, probability, and impact tracking are needed. The words of Jewett et al. (2008) are a concise reminder of the vision shared by all stakeholders: "To be useful to HAB management, observing systems must be located in areas where HABs frequently occur and must have sensors capable of detecting HAB cells and toxins and monitoring the environmental conditions that foster blooms. They must deliver integrated data sets that can be used in operational mode for forecasting HAB events."

In re-visiting recommendations from the 2008 workshop: "Technologies and Methodologies for the Detection of Harmful Algae and their Toxins", there are areas of both great improvement and ongoing need.

1. *One hurdle that remains constant is the small size and diversity of the marketplace interested in sensor technology.* This impacts product design and production costs, as these should be balanced with robustness and capabilities. The recommendation from the prior HAB workshop suggested development of sensors with broader applications while taking advantage of existing product designs (e.g. drinking water, human health, biomedical) to leverage costs associated with development and implementation. The second part of the recommendation was to garner more interest by local governments and regulators.
    - Large HAB events, particularly those that reach national coverage, continue to raise awareness among regional and state agencies for the importance of monitoring conditions both within and outside bloom events. For example, in response to the Toledo, OH drinking water ban, funding from the Great Lakes Restoration Initiative was awarded to deploy ESP's to measure daily levels of microcystin at source water intakes. These data are invaluable for alerting water resource managers to a potential toxic threat and making important decisions on water safety before they develop into an event.
    - Significant efforts have been made to include a broader range of stakeholders and incorporate relevant management sessions into national HAB meetings. The latest meeting held in Long Beach, CA (November 2015) included several relevant sessions: 'Bloom Prediction, Forecasting and Modeling'; 'Monitoring and Management'; 'Emerging Technologies, Instrumentation and Methodologies'. However, HAB researchers still make up the vast majority of attendees and ongoing efforts should be made to engage a variety of stakeholders (e.g. low-cost registration).
    - Water utilities (such as the City of Toledo) are recognizing the importance of sensors and sensor research and are beginning to design structural modifications to new and existing facilities to better accommodate researchers and sensor platforms.

2. *Development of real-time HAB sensors (deployable or handheld) for managers was a high priority*. The HAB community has been progressing in this area, with several examples of successful local agency partnerships.
    - The IFCB is currently being utilized off Catalina Island, CA to monitor for HAB species within a new offshore aquaculture facility and alert managers to potential ecosystem hazards.

- The IFCB has been used successfully over the past decade to alert managers with the Texas Parks & Wildlife Department and the Department of State Health Services to potential HAB events.
- The ESP is being deployed as part of monitoring efforts in the Great Lakes, as an early warning system for managers protecting public health. This initiative is a result of the ongoing microcystin impacts in that region.
- The Breve Buster has been deployed in Florida waters to monitor brevetoxin-producing *Karenia brevis* populations in order to alert officials and researchers to make decisions on closures and increased sampling, respectively.

3. *A disconnect was recognized between end-users and manufacturers.* This still remains an issue, as costs for detection platforms/kits can remain out-of-reach for many municipalities. Further, it can be difficult for managers to justify expense for limited technologies (e.g. detection restricted to one species/toxin), especially when events and their impacts are sporadic. In turn, manufacturers must weigh the investment of development versus potential client base. The integrated nature of ACT Workshops, and to some degree ACT Validation Testing, has worked to bridge this gap. Also, researchers play a vital role in advising non-technical end-users on the capabilities of the products and managing their expectations as to what the product can deliver. Technical Advisory Committees (TACs) have become more commonplace as an avenue for including more end-users. ACT incorporates a TAC, as do some granting agencies (e.g. PCMHAB under NOAA).

4. *The HAB community recognized the need for reliable support in developing community-derived standards (toxins and organisms) that are rigorous enough to accommodate a large portion of the end user group needs.* This was recognized as being highly challenging, as the question of 'what is good enough' is always one of complexity. The availability of toxin standards to the global HAB community has recently been expanded via a partnership between the Cawthron Institute (New Zealand) and Sigma-Aldrich. This builds on certified toxin reference materials available from the National Research Council (Canada). However, from the aspect of antibody production, detection systems can be challenged by numerous known and unknown toxin congeners. In addition, antibodies across the biological sciences are under validation scrutiny, as related to transparency in origin and reproducibility (see Blow 2017). Physiology and genetic differences in strains isolated from similar or disparate geographical locales can challenge detection systems and hinder advancements towards 'gold standards'.

5. *Given the diverse targets in geographic scale and limited funding availability, workshop participants proposed that ACT narrow its focus and play a more grass roots angled role by supporting existing programs that are working toward integration of HAB observing interregional ocean observing activities*. Over the past decade, ACT has solidified relationships with the regional associations of IOOS to prioritize testing technologies conducive to network platforms. This relationship ensures that both groups keep moving in the direction of meeting stakeholder and researcher needs.

The current workshop took place at a time when much of environmental science was being questioned and funding support for important monitoring and research efforts was limited. Meanwhile, the occurrence of toxic HAB events did not slow during this transition period. The 2017 season kicked off with several notable events, including 1) the closure of several Alaskan oyster farms due to high levels of saxitoxin (K. Lindsey, posted July 3, 2017); 2) postponement of

the scallop season in St. Joseph Bay (Florida) due to elevated domoic acid concentrations (K. Landeck, posted July 25, 2017); 3) blooms of toxic *Microcystis* in Lake Erie in summer 2017, the latest in a near-annual occurrence (T.J. Pignataro, posted July 25, 2017); and 4) bird mortality and sea lion illness events attributed to domoic acid in Southern California (C. Carlson, posted April 20, 2017).

The HAB community faces further challenge, as the single most important law focused on HAB research (Harmful Algal Bloom and Hypoxia Research and Control Act; HABHRCA 1998, P.L. 105-383; HABHRCA 2004, P.L. 108–456; HABHRCA 2014, Public Law 113–124) is currently up for re-authorization. This Act mandates that NOAA work toward advancing our understanding of and abilities for HAB event detection, monitoring, assessment and prediction. Additionally, NOAA has been developing an Ecological Forecasting Roadmap (EFR) for coordinated and systematic approaches to ecological forecasts, with HABs as a priority focus. Charge questions presented at the workshop were designed to address the goals of both mandates, as outlined in more detail below. This report serves to outline the consensus findings and lay groundwork for moving forward towards the next steps in increased platform integration, meeting the next levels of stakeholder needs and increasing partnerships and access to large data sets.

WORKSHOP OVERVIEW

The workshop brought together a broad range of experts from regional, national and international agencies. Participants (see *Steering Committee* and *Participants* sections below) were able to provide a breadth of knowledge from the fields of instrumentation, moored systems, molecular biology and public health. Many of these contributors are involved with state and regional partnerships focused on problematic HAB events in their respective geographic locations. Representatives of the U.S. Integrated Ocean Observing Program (IOOS) and Australia's Integrated Marine Observing System (IMOS) were also in attendance. The variety of professional backgrounds and experience provided dimension to discussion items, allowing for different aspects of the charge questions to be addressed in greater depth from across a range of perspectives.

The majority of the workshop focused on a series of breakout sessions and open discussions guided by a set of charge questions. However, the agenda included several short plenary presentations to provide examples of technology in use for management applications, updates on IOOS and the National Centers for Coastal Ocean Science (NCCOS) investments in HAB sensor technology and highlights from initiatives in Australia. R. Kudela (UCSC) presented examples of how we are advancing our ability for early detection and mapping of species and their toxins by 'wiring the ocean' with various sensors and platforms. T. Davis (NOAA-GLERL) provided an update on the multi-platform framework being utilized in Lake Erie to guide monitoring, prediction, forecasting and research of *Microcystis*. A. Lara-Lopez (IMOS) outlined Australia's multidisciplinary approach to systematic and sustained observing of the 80-100 HAB species along that country's coastline. J. Rhoades (NOAA-IOOS) and M. Suddleson (NOAA-NCCOS) gave an overview of their respective research programs and provided examples of NOAA supported HAB technology development, demonstration, and commercialization. The plenary sessions also enabled workshop participants to consider pathways to sustain existing and planned sensor networks currently funded via research grants; how sensors could support ecological forecasting initiatives; and how sensors might better meet needs of public water utilities, aquaculture operations and other HAB impacted industries. The group also recognized a need for improved data products to meet a variety of research, management, and public needs.

CHARGE QUESTIONS

A series of charge questions was posed to stimulate and guide discussion in the workshop, towards defining recommendations.

Breakout Session A - What are the challenges associated with current HAB and toxin technology/detection?

- Q1: <u>What are the cost, usability and readiness levels for current methods?</u>

Table 1 outlines various usability metrics associated with currently available technologies.

Platform/ Technology	**Purchase Cost, $[1]**	**Operational Costs / yr**	**Operational Space**	**Research/ Monitoring**	**Data Products *B/G/S/T*[2]**	**Non-Technical Usability[3]**	**Technical Readiness Level[4]**
Multispectral Remote Sensing	$	$$	Satellites, aircraft	M	B (G)	Med	9
Hyperspectral RS	$$$	$$	Aircraft, satellites	M	B, G	Low/Med	8-9
ESP-2G	$$$$	$$$$	Moored	R, M	B, G, S, T	Low	8
ESP-3G - LRAUV	$$$$	$$$$	Mobile	R	B,G, S, T	Low	5-6
Imaging Flow CytoBot (IFCB)	$$$$	$$$	Moored	R, M	B,G,S	Low	8
Breve-Buster / Optical Plankton Discriminator	$$$	$	Mobile	R	B,G,S	Med	9
FlowCAM	$$$$	$$	benchtop	R	B, G, S	Low/Med	8
HABscope	$	$	Field, benchtop	R	B, G, S	Med	8
Multichannel Fluorometers	$$-$$$	$	Field	R, M	B (G)	High	8
Isothermal Amplification AMG, NASBA	$$	$	Benchtop, handheld, moored	R, M	B, G	Med	6-7
Multiplex Molecular Assays	$-$$	$	Benchtop	R, M	B, G	Low/Med	7
LC-MS	$$$$	$$$	Benchtop	R, M	T	Low/Med	9
HPLC Pigments, toxins	$$$$	$$	Benchtop	R, M	B, G	Low/Med	9

ELISA's	$$	$ per kit	Benchtop	R, M	T	Med	9
SPATT	$	$	Field	R, M	T	Med	8
Dipsticks, Lateral Flow ELISA	$	$	Field	M	T	High	9

[1]Includes costs of hardware required to run or interpret sample composition
[2]***B***: biomass, ***G***: genus, ***S***: species, ***T***: toxin
[3]Includes consideration of whether individual operator or team operation is required
[4]On a scale of 1-9 following NOAA-NOS readiness level definitions

There are several variables to consider when assessing readiness levels of instrumentation, for example processing and reproducibility of data (can affect the ability to compare data sets over multiple years); the ease of procuring materials for chemical and molecular assays (some are in limited production); defining standard methods and QC equivalents for analysis (e.g beads for imaging, algal pigment spectra for fluorometers); spatial and temporal sampling capabilities and how they address the research/monitoring question(s); and the level of expertise needed (including the number of 'hands' required for operation, maintenance and data analyses).

- <u>What are improvement suggestions?</u>

1) Improve onboard data processing capabilities to reduce the burden of data transmission.

2) Tie in with IOOS platforms – utilize "platforms of opportunity" and consider the "scalable design of a system". This would involve current and future IOOS infrastructure initiatives, and ensuring new platforms are built to scale and can support additional co-deployed sensors. Improve on our ability to plug in additional analytics to more complex platforms such as the ESP.

3) Increase training and knowledge transfer in the form of classes, manuals, and a living online document.

4) Apply a suite of approaches. For example, generate cheaper, sentinel data sets to complement data from instrumentation. The community should think about complementary modalities for sampling, specifically for toxin detection. To this end, break away from the existing constraints from single projects / programs.

5) Implement portable systems for broader/cheaper use by more groups. An ELISA-based system (or passive sampler) for management level use is desirable.

6) Leverage platforms that enable improved spatial coverage and data transmission.

- <u>Q2: Identify region / HAB / water type-specific gaps and issues for HAB sensor technologies and their implementation.</u>

The working groups identified issues that fell within three major categories related to a) ***sensors***, b) ***biology of target organisms***, and c) ***transitioning results to public health relevancy***. Forecasting toxicity is a gap that cross-cuts all of these major categories, with

current limitations in detection capabilities, unknown factors that promote toxin production, and how values translate to public health threats.

a. Sensors

Myriad challenges were raised concerning sensor technology, many of which translate across platforms. One key aspect, which has been developed into a workshop recommendation, is **the storage and transmission of large data files**. While eleven agencies within the IOOS framework serve to fill a data hosting need, researchers still must rely on in-house hosting of certain complex data sets such as Flow Cytobot images. This ties into the question of how much data can/should be generated? When is generating species/toxin data more desirable than measurements of biomass (capturing both harmful and non-harmful species)? What type of data are needed for an early warning of an impending event? The desired data type(s) may not match available funding levels. Does the application require Tesla-level technology, or will a 'Honda Civic' (i.e. at a more moderate cost) do the job? As several participants pointed out, effective solutions to these overarching questions can best be addressed by engaging end-users of the data products early in the development cycle.

Additional gaps for implementation were identified:

- Regarding hand-held devices, researchers/managers desire more sensitivity/specificity.

- Development of specific molecular assays are needed for each system, for example the Sandwich Hybridization Assay (SHA) can be run on the ESP or a benchtop system, however both involve variations to the protocol and reagents used.

- For many sensors, the processes of QCing and intercalibration are intricate, and standardized protocols may or may not be available. Related to this is the long-term challenge of developing 'gold standard' reference materials (species and toxin) and performing rigorous testing using local isolates.

- Reagents can be proprietary, and often available from only one vendor or research group, which keeps operation costs high and the technology unattainable for many laboratories and/or resource managers.

- Balancing the current/future need for a platform conducive to routine monitoring versus HAB event sampling. This affects the type of sensor needed as well as the associated costs and assays available. Related to this is the desire in some cases to validate observations with multiple analytical techniques.

- Platforms / deployments have challenges associated with availability of supportive infrastructure, power supply, and readiness level for modularity.

- Given costs and complexity, redundancy in deployed instrumentation within a sentinel monitoring structure is often not feasible. For example, if there is one IFCB deployed and it malfunctions, there will be gaps in an otherwise continuous data set unless another instrument is available.

b. Biology

Coastal and freshwater systems often harbor multiple harmful algal species and toxins, which would benefit from assays and/or platforms that are multiplex in nature, as it is highly desirable to have the ability to detect the full suite of organism(s) and toxin(s) present. The importance of detection across multiple matrices (e.g. including benthic and water column communities) should not be overlooked. Another angle of HAB biology that is ripe for detection methodologies is trophic transfer (e.g. shellfish) and exposures to multiple species within the food web. This aspect of HAB research would open up much needed information on bloom initiation, persistence, and decline. Successful integration of sensor technologies depends on being able to couple biology and physics, towards fruitful measurements of the *in situ* ecology of HAB species and their toxins.

c. Public Health

One of the ultimate goals for management entities is the monitoring and prediction of HAB species that can affect human health (e.g. respiratory issues from brevetoxin [*Karenia brevis*], gastrointestinal issues from shellfish contaminated with okadaic acid [*Dinophysis spp.*]). In many instances, we currently have little knowledge of the ecosystem and health impacts of acute or chronic sub-regulatory (no-alert state) toxin exposures. Given our lack of knowledge/understanding about some HABs, some toxin exposure risks are considered to be 'theoretical' in terms of what effects they 'could' potentially have.

There is a need to increase awareness about HABs, perhaps through improved efforts for inclusion of epidemiologists and other health care practitioners within the context of multidisciplinary research and monitoring. This group can greatly enhance data sets with regards to reports of human health clusters that may be associated with exposure to HAB toxins. Some areas have developed these relationships outside of the HAB community (e.g. Florida). Increased knowledge among doctors (and other health care practitioners, clinics, etc.) about symptomology could serve to fill a gap in awareness between environmental exposure and illness. Health advisories are a useful strategy for focusing on the connection to and the need for environmental monitoring. This in turn garners support from the public for the need for funding these initiatives (e.g. Gulf of Mexico and Lake Erie HAB forecasts). *The synergy of these efforts would advance us towards long-term sustained funding to protect human health from toxic HAB events.*

There was discussion about how sensor users should/can alert the public regarding detection of HABs, as they can serve as an early warning by providing continuous detection results (cells and/or toxin). These needs are being met regionally, with some states having a tight collaboration between technology users, researchers, and state officials. For example, in Texas researchers are working with state health officials for early warning of HABs. IFCB data downloading, processing, and classification have been automated for the Texas sites, so when HAB species abundance exceeds a threshold (currently set at 2 cells/ml to avoid too many false positives), an automated email is sent to the Texas Parks & Wildlife Department (TPWD) and the Department of State Health Services (DHSH). Messages include information on cell abundance and a link to the IFCB dashboard (toast.tamu.edu) so that state officials from TPWD and DHSH can confirm identifications (Campbell et al. 2013). This approach allows state officials to be prepared for response. Since 2007, the IFCB has provided early warning of 8 HAB events to TPWD and DSHS, and there have been no reported human illnesses.

The US has experienced major threats to drinking water, and sensors can play a crucial role in assessing potential impacts in real-time. The 2014 microcystin event in the Great Lakes cut off tap water to more than half a million Toledo residents (T. Henry, August 3, 2014). This event served to thrust the issue of freshwater HAB toxins threatening public health to nation-wide attention, and resulted in prioritization of broad support for the subsequent deployment of ESPs to monitor microcystin levels in adjacent waters. Meanwhile, long periods of drought in California have ignited efforts to construct desalination plants in order to augment drinking water supplies. To be a viable source, plants must be able to detect potential contaminants, such as HAB toxins, entering the system in order to mitigate their impacts to the water supply (Caron et al. 2010). In both GL and CA coast water situations, sensors need to be economically feasible for municipalities while capable of detecting and rapidly alerting to toxins that could be making their way through treatment regimes.

- Q3: What are paths forward for transitioning to operational use for current and near-future technologies?

  There are recognized hurdles for adoption of sensor technology by regulatory agencies, including:

  a) How best to link environmental monitoring and observation to standards of exposure? In many cases, we still do not fully understand the full range of exposure levels that serve as a threat to human health.
  b) How to overcome obstacles to deployment? As with any instrumentation, a certain level of expertise is needed for initial deployment as well as troubleshooting. Although a variety of personnel can be trained on the technical aspects of instrumentation, researchers with an understanding of the biological angles (and associated caveats) are needed in order to ensure the generation of relevant data sets.
  c) How to address the need for stable long-term funding sources? Instrumentation (acquisition, maintenance, data storage) and supporting reagents/ assays/ personnel/ administrative support can present large costs that need to be covered by already funding-strapped regulatory agencies. Could this cost share be moved to commercial for-profit companies and/or public entities with investments in the data products?

  What are the paths forward?

  a) Harness the power of a HAB event/crisis: Punctuated HAB events thrust these issues into the public spotlight, as impacts can be devastating to local economies and well-being of communities. The three-day closure of drinking water for residents around Toledo, Ohio in 2014 is a prime example of a HAB event that became well-known nationally/internationally. These high profile events can serve as an opportunity to bring awareness to and educate not only impacted communities, but others that could potentially be affected by a similar event. The conversation can then be turned towards the importance of long-term monitoring frameworks. To further inform stakeholders and the public, the HAB community should be involved with providing solid communication

about what is known/unknown with regards to the target species and/or toxin(s) involved in the event.

b) Respond to crisis situations by using the opportunity to introduce/roll-out pertinent sensor technology. The idea is to be prepared with instrumentation, protocols and experience that can support an informed response to a local/ regional event. We also need to be prepared to assist with providing information/vetting for available instruments, thereby assisting in the 'fit-for-purpose' strategy needed in management.

c) Continue to work to increase stakeholder engagement: Over the years, the HAB community (which is relatively small) has been successful in developing partnerships with local, state and federal management officials across the US, but we still have work to do engaging and educating a broader stakeholder audience. Some of the suggested groups to include for increasing the partnership networks include the Interstate Shellfish Sanitation Conference, local aquaculture companies, and businesses that rely on access to safe water for production (e.g. the food and beverage industry).
    a. Engage stakeholders in the process of ongoing instrument development. The majority of instruments, if not all, undergo improvement iterations in hardware, software, capabilities, etc. Researchers and manufacturers need to strengthen collaborative efforts with managers and other operational stakeholders at the conception stage in order to ensure needs will be met in subsequent versions.

d) Securing long-term funding for monitoring networks: Expanding our reach to stakeholders within commerce can potentially open avenues for sustained funding of sensor platforms that benefit particular industries.

e) Be poised to justify the cost of monitoring efforts in local/ regional settings. This would include economics experts to measure the cost of impacts from past and potential events, in a manner that is informative but not alarmist. By homing in on a key management issue affecting the local community (economically, recreationally), we will have a better chance at successfully engaging public support for ongoing HAB monitoring initiatives.

f) To help offset costs associated with deployments, look to utilize existing platforms and consider this approach in expandability with new infrastructure build outs.

g) Support development of community resources and repositories for standardized reagents, probes, and detection algorithms. Such a repository could be modeled after existing non-profits such as www.addgene.org.

Breakout Session B – How do current technologies relate to stakeholder's needs?

- <u>Q1: Do currently available detection technologies meet stakeholder needs?</u>

  Some current technologies meet management needs, but that depends upon what the management goal is: take action/follow an event before it becomes a concern or wait until a metric (cell concentrations, toxin, etc.) reaches a level of concern. The former always has a lower limit of detection than the latter – thus, the answer to this charge question depends on the specific management need. Among many stakeholders, scientists, water utility, farmers, and government agencies, there are needs that are not being met towards assessing effects on natural resources.

a) How do we assess this?
The mechanisms by which we can garner feedback from stakeholders were discussed extensively. Topics discussed ranged, but included generating surveys that tried to identify specific end-user needs. This involves consideration of social science because how surveys are conducted (wording of questions and answers, audience, etc.) can dramatically impact the responses and thus how survey data are interpreted. There was a general consensus about utilizing social science in numerous capacities, while other recommendations included how to better communicate results and findings to managers and the general public.

b) What are the known challenges?

1. The expense of technologies can be a hindrance to adoption by stakeholders, which can directly affect the ability to spatially monitor a given area (e.g. throughout a water treatment plant system, beyond just intake/outtake sources). Despite this, there have been instances where timely data provided early warning of a potential threat. In 2008, an IFCB detected *Dinophysis* in Texas waters, which prompted officials to cancel an oyster festival due to the threat to human health (Campbell et al. 2010, Deeds et al. 2010).
2. Current surveillance projects and solutions have recognized holes (e.g. performance of satellites on cloudy days; seasonal deployments of moored instrumentation) and resolution issues.
3. The HAB community needs to arrive at a consensus regarding limits of detection and concentration triggers (cell abundance, toxin concentrations) to avoid confusion during an ensuing threat.
4. Natural biological constraints present unique challenges related to complex matrices and the presence of potential inhibitory substances.
5. The desire to detect the causative organism(s), associated toxin(s) and link those data to trophic transfer is not currently being met. Related to this aspect is the lack of measuring contributions from the benthic communities.

- <u>Q2: What constraints may limit widespread adoption of currently available technologies?</u>

Several constraints were identified:

1) A better integration of the valuation of technologies is badly needed. For example, is a $500K instrument worth the investment? Given massive events (example of the $800 million loss from recent Chilean fish kill was discussed; A. Esposito, posted March 9, 2016) that cost millions, this is a minor investment. Unfortunately, it is easier to show the economic benefits of expensive technologies after major catastrophes than when anticipating one. More coordination of how all of the current technologies can work together would be useful.

2) We need to better address 'Fit For Purpose': Technology exists but is it suitable for the job? Is timing of data generation/results and cost adequate for the need? For example, are FTC cards adequate enough to allow for rapid processing and interpretation of

potential blooms, over another more expensive technology like the ESP. We need to address the costs in relation to speed and applicability.

3) Research questions need to be secondary/complementary to practical management questions. Simple guidelines and interpretation are needed, in concert with technology and results that answer the main questions and decision-making needs.

4) Each instrument poses its own challenges, some overlapping and some unique to that platform.

    a. Initial costs – not only are costs associated with procurement of an instrument, but there are costs associated with supporting reagents, consumables, software, hardware, etc. that can significantly add to start-up costs that are out-of-reach for stakeholders.
    b. Operational costs – there are costs that extend beyond start-up and into the life of the instruments. Consumables, reagents, standards, software updates, maintenance etc. all contribute to these ongoing costs. Some of these costs are required to keep instruments in working order, regardless of if they are in use or not (e.g. to keep them from being moth-balled).
    c. Expertise – instruments can vary in terms of ease-of-use and expertise needed. Not all agencies will have access to a technician-type employee to dedicate to new instrumentation, in particular complicated platforms that require large time commitments.
        i. This feeds into reliability of the technology. A platform will be more readily adopted if it is stable and does not require frequent maintenance.
    d. Calibration performance – calibration of chemistries can be challenging, requiring funding and expertise to generate usable standard curves, etc.
        i. For example, qPCR and SHA are of great benefit because of their low limits of detection and dynamic working range, however calibration can be affected if cells are outside of log phase growth or experiencing nutrient limitation (Haywood et al. 2007, Main et al. 2014).
    e. Pathway to use – technology can have a long and arduous path towards approval/acceptance of SOPs within an agency; the process can be inefficient and slow. Furthermore, moving research into application is tough/challenging – when is research or data enough to justify putting into use?
    f. Limited species/toxin detection – adoption of technologies can be inhibited by limitations in the species/toxins targeted. Probe variability is well recognized and work continues to improve these. Some platforms are broad in their detection of pigments (chlorophyll, phycocyanin, phycoerithrin), while others are species specific. Stakeholders must weigh data generated with costs, expertise, etc. Also, limited capabilities can constrict knowledge of HAB genera in a given location.
        i. For example, currently, there is no method of detection and/or SOP for toxins in clinical specimens

5) Biology – the inherent biology from the organismal to systems level can have a profound effect on detection chemistries and platforms. If not well understood, this can lead to data inconsistencies. Examples include: pigment concentrations do not

necessarily correlate with toxin concentrations; different mussel species can exhibit different accumulation/depuration rates; large accumulations of cells can be harmful even if toxins are absent; cell/toxin thresholds may be different in marine versus freshwater systems, and this latter issue can be compounded by places like the San Francisco estuary where both marine and freshwater species can mix.

6) Management needs can be difficult to address with currently available technologies/platforms, since approval and adoption can be disjointed across the states. There is often a need/benefit for multiple parameter measurements (species abundance, toxin, supporting environmental data – chlorophyll, temperature, salinity, dissolved oxygen) that can dovetail with early warning at the species level. Continuous observations are important to integrate over large time scales and go hand-in-hand to inform the treatment/mitigation process. Networked platforms are highly favorable, leveraging broader spatial and temporal data acquisition. For example, toxin-only detection systems lack the ability of an agency to track cells in the water column as they lead up to a HAB event. To achieve the goal of networking current technologies, we need better inter-calibration between devices and applications. Furthermore, increased mobility is a high priority in some cases, as moored platforms can limit instrumentation use, particularly when they need to be manually moved to another location (including to depth). This can severely limit sampling coverage.

7) Lack of shared databases, regulatory frameworks and requirements can hinder smooth transition into adoption of technologies. Rules and laws can address needs and lead to financing for development and adoption.

8) Stakeholders (e.g., managers) often do not have the time or desire to sift through raw data produced by HA monitoring technologies. They want researchers to do this for them and then provide distilled and usable data for management. In some cases, researchers are able to provide usable information (e.g. binned estimates of cell/toxin concentrations, colored images for interpretation), but there is room for improvement. Further, interpretation and QC need to be easy to understand and comparable. Time to actionable results is not often ideal for making a management decision.

9) While regulatory limits are in place for tissue burdens of many HA toxins, there is no direct translation of these into alert levels for environmental toxin loads (particulate, dissolved, cell abundance) that can provide stakeholders early warning of HA impacts on ecosystems and public health. Related to this, how long should detection capabilities remain in place throughout a bloom event?

- <u>Q3: What surveillance needs are not being met?</u>

    1) The need to integrate physiological information to better inform models was discussed. It would help stakeholders if they had a wider picture of the ecological drivers that preceded blooms in addition to simple ‘alert levels’. We need to understand what causes an organism to escalate to alert concentrations or toxin production.

2) The complexity of 'what to regulate' was discussed. Specifically, what defines a 'bloom' varies widely between species and what defines a toxic event varies widely among compounds, so it's very difficult for regulators to make decisions about what levels and parameters constitute regulatory concerns. While this remains a challenge for several HAB genera/species, in other instances there are guidelines in place (e.g. the microcystin 1.0 ug/L threshold for human consumption recommended by the World Health Organization; though this may not be a direct correlation to abundance of cells in the environment).

3) Discussion focused on how data types often do not translate to ecological processes. When managers make observations they want to know processes that drive events (e.g., specific nutrients, grazers, food web disruption, presence/absence of important conspecific species). The idea behind this is if a causative agent can be managed (e.g. N or P, salinity intrusion, etc.), then we can more effectively prevent blooms in the first place. Management should be pro-active more than reactive, saving both time and money in the process.

4) Sample frequency is often seen as inadequate. Loss of info and data results in less certainty in decision process: increased sampling frequency may allow for better answers.

5) Interpretation of data and technology needs to be simple and clear to understand.

6) Technology platforms are not addressing trophic transfer. This is especially key in dynamic systems where currents and mixing from upwelling can result in the rapid movement of cells and toxin (e.g. Monterey Bay).

7) Large scale mapping capabilities are often inadequate, thereby providing a limited scope of an actual event or conditions leading up to that event.

8) Mobility can hinder the use of technologies, especially for mapping purposes, however platform development is currently underway to address this (e.g. 3G ESP). This is an important feature to work towards for both 'Tesla-level' and 'Honda Civic-level' platforms.

9) Surveillance and forecasts need to include both cells and toxins.

10) There would be great benefit to having more generic platforms that have the ability to house a variety of HAB sensors.

11) More assessment of current research is needed, with guidelines and algorithms to help stakeholders.

- <u>Q4: Are there viable solutions in the R & D pipeline?</u>
    1) New platforms are in development that allow for mobile adaptive sampling (e.g. mobile ESP, mobile IFCB)
    2) Better photo plankton class discrimination is being pursued on imaging platforms for better classification abilities (e.g. IFCB)

3) Improved microfluidics are under development
4) Hyperspectral capabilities are being used to assess spatial distributions of cells.
5) Methodology development for detecting both intra- and extracellular toxins, with an understanding of what exactly is being measured and how those constituents contribute to the total toxin profile.

Breakout Session C – What are the shared challenges across marine and freshwater ecosystems?

Are there agency/organization barriers to collaboration across the marine-freshwater continuum? What are some approaches for integration of data streams? What are the regional complexities for bridging knowledge gaps?

1) Standardized data presentations are needed for all end-users – stakeholders need streamlined, uncomplicated data outputs in order to make management decisions. Successful examples of this include bulletins (e.g. Lake Erie Harmful Algal Bloom Bulletin).
    a. Data products should be fit for purpose and not designed as 'pie-in-the-sky'. This further emphasizes the need to engage end-users of proposed technology data products early in the development cycle, especially for data product presentation.

2) Web application designs need to be developed in a manner such that end users find them both useful and easy to navigate (e.g. IFCB website at TAMU; toast.tamu.edu).

3) The role of IOOS is important for working in both ecosystems, especially with regards to supporting operational deployments. Those efforts can then be transitioned to NCCOS.

4) Increased development of a consortium of agencies dedicated to regional issues (e.g. Cyano Assessment Network) is favorable to ensuring communication and resource management across state lines. For example, the Chesapeake Bay watershed spans parts of six states and encompasses numerous freshwater systems that mix with oceanic water to form the bay estuary. As for the regions efforts at mitigating nutrient loading, a broad network of agencies is needed to understand the impacts of HAB events from both freshwater and marine species in order to ensure the safety of drinking water as well as seafood.

5) Both areas would benefit from more promotion of community engagement (e.g. Aqua-Hack Challenges). Involving the general public in local/regional HAB issues through educational activities can garner support and the realization of needed funding to protect health, and recreational use of coastal and freshwaters.

6) There is a need to bridge gaps in understanding and technologies for the marine/freshwater interface. While similar technologies can be used in both environments, the freshwater links directly to human health mean that protocols often come online faster. Further, the complexity of blooms in marine environments complicates the application of technology and SOPs.

Breakout Session D – How can detection methods be integrated into existing systems?

- Q1: What performance assessments (QA/QC) methods are in use for current technologies?

  The group discussed these methods in terms of three available platforms.

  ESP – analytical accuracy and precision are addressed in the lab, but in the field the same level is not expected given that an integrated time sample is collected. In the lab, arrays are "calibrated" with standards that are run pre- and post-deployment, and there is no in-field calibration performed. Standard curves are based on cell counts and array intensities. Getting a "same true sample" in the field is challenging due to the instrument pumping for hours to get the desired volume. As a result, cell and DA detection is a sequential process and not from the "same" water mass.

  IFCB – obtains results from a 5 ml sample approximately every 20 mins and data are typically reported as a mean of observation for 1 hour. This time series provides a much more realistic view than a traditional single bottle sample (daily or weekly) which offers a 'snapshot in time'. Comparisons with manual microscopy counts are performed for validation, and no standard QA/QC protocols have been established.

  ELISA – extraction efficiency can be measured through spiked samples and blanks. Standard curves are produced to determine dynamic working range, and internal controls should fall within the expected limit.

  Overall, QA/QC considerations can be a barrier to developing new methodology – the goal is to identify limitations in technology in prototypes, and to use that information to develop the next prototype. Some of the current metrics used in QA/QC may not be applicable to new technology. For example, CFU/mL is replaced by cycle time (CT) in qPCR, and CT is unique to this assay type.

  On the other hand, in practice QA/QC may not be the important driver. It could be pressures in the market place. Instrument makers should establish the metrics and provide performance specifications. In addition, there may be extra steps in the QA/QC process depending on application (e.g. FDA regulations versus ELISA field screening for toxins in shellfish) and agencies trying to develop standard protocols for calibration and operation.

- Q2: What ground-truth methods are in use for current technologies?

  Combining data from one or more sensors can be very helpful for ground-truthing each of those platforms. This can be explored on different spatial scales (or temporal scales, as in over a season), for example coupling an IFCB to a fluorometric profiler or using a coordinated network of in situ observations to support remote sensing models/forecasts. One challenge to these more complex networks is obtaining representative field samples. Boat-based CTD casts are often utilized for field sample comparisons matched to time of sampling by a deployed instrument.

  As for detection assays, there is a need for more standard protocols for new molecular qPCR approaches. These would include defining specificity of primers and quantitative

relationships. With these types of assays, you can "always get an easier answer, you just don't know how good it is" without implementing the proper QC/QA metrics. Comparison to "gold standards" may not always be the best approach in ground-truthing. For example, there can be user error and discrepancies among microscopists performing cell counts. Mouse bioassays, once used as the "gold standard" in toxin assays, are somewhat arbitrary and the community has been moving away from this technique in recent years due to ethical concerns.

- Q3: What's needed/feasible to expand on these approaches?

  Complexities in assay chemistries, engineering intricacies of platforms, and data output provide equally complex challenges for ground-truthing and QA/QC methodologies. Nevertheless, empirical approaches and identification of caveats have allowed the HAB community to continue to work towards providing valuable protocols for HAB detection.

  In choosing an assay/platform, it is important to focus on the application – for example, if all that is needed is accuracy and reliability at a given regulatory cutoff (e.g., X ug/100 g tissue) the accuracy and precision at concentrations well above or below this do not matter. In other words, ***the technology has to be "fit for purpose".*** Further, research applications (or even process monitoring at a water treatment plant) are different than regulatory applications. New technologies relating to food safety, clinical test procedures, or drinking water analysis require much more rigorous QA/QC, validations and even multi-laboratory validation or comparison of "approved or official methods". Following from this, we also need to define "operational technology". This concept spans the ultimate of "24/7, always on, never breaks, redundancy" (typical of the weather service) to a more simplistic "demonstration of principle" or "proof of concept" in the early stages of technology development. Defining the need will in turn define the required financial support.

  Technology developers should clearly distinguish developmental study data and performance or QA data for the finished prototype. The group discussed the goal of the ACT effort, e.g., helping new technology along for which there may not exist a sizable market. For many manufacturers, there is not enough return on investment to recoup development costs. The group discussed the high probability of failure for tech startup companies and the concept that the goal of ACT should not be to promote technology (for a long time) that "should" die in the market place. Further, there is a need for increased communication between researchers and companies, especially for young techniques in development.

  There can be issues with hardware performance versus biological/environmental (regional variation), as well as taxon specific needs. Optical platforms, such as the IFCB, were discussed as an example. There are issues related to hardware (e.g. beads for IFCB) versus data standardization, a need for defining windows for different growth phases, and considerations for packaging effects for fluorescence. Again, the issue of lack of standards for many congeners (e.g. > 200 for microcystin) was raised. Relying on blooms for ground-truthing is inherently difficult because results can be dependent on sampling time. Yet, there are differences in real world spatial/environmental data compared to lab calibration and a question of the appropriate number of samples to collect for ground-truthing – is >30 replicate samples over a season an adequate comparison for new versus existing methods? Furthermore, data interpretation can be problematic when decoupled from data collection

(i.e. different people), highlighting the need for comprehensive metadata. Modeling output errors need to be factored in as well. ***Overall, the community needs to agree on a consensus for standardization procedures for all platforms and assays***. There was a recommendation to approach an organization like IOOS for funding assistance to produce standards where none exist and/or provide them as an open resource.

Universal QA/QC is not likely feasible – there will never be a 'one-size fits all' solution, but there can be common guidelines for the approach: 1) data prioritization beyond IOOS core variables and resultant data products need to be regional in scope, and 2) introduction of new analytical tools need to be supported by in-field comparisons to existing methods. Further, the research community needs to communicate variability (uncertainty in analytical technique versus inherent biology based variation) in assays / platforms without confusing or discouraging stakeholders as to their efficacy, and work on building trust between all groups.

- Q4: What contextual data is required for interpretation of HAB detection patterns and alerts?

  Complementary 'contexual sensors' can be used alongside other technologies to devise measurements for temperature, pH, dissolved oxygen, salinity, nutrients, etc. in order to provide a more holistic picture of the environment in which species and toxins are detected. This approach is routinely being used to trigger ESP sampling (e.g. chlorophyll concentrations; increased salinity + decrease in temperature) since the platform has a limited number of possible sampling events per deployment.

  At the larger systems level, there is a need for multiple observing nodes coupled to circulation models so we better understand water masses (especially estuarine waters). When developing a model there is a desire to garner as much contextual information as possible but this needs to balance with monitoring efforts. Multi-faceted networks generate large amounts of metadata, and this requires consistency so that data can be pooled. The community needs to continue to work towards generating comparable data across these large scales.

- Q5: What end products are needed/desired?

  The community needs to be able to deliver data products that are usable, particularly for management and modeling applications. This includes being able to equate cell counts with ug toxin/kg mussel tissue. There was a suggestion to work through IOOS Regional Associations as a pilot to develop this process. Also, encourage IOOS to ingest/disseminate core in situ observations for remote sensing.

  To that end - Can we make enough measurements? How many are desired versus how many are feasible? At some point, the number of measurements and amount of ground-truthing can support a shift in methodologies used. For example, there have been ample places to establish confidence in in situ toxin testing to not need direct assessment of shellfish, an example of regulation based on more low-cost in situ measurements. To arrive at the balance between monitoring needs and cost feasibility, we need to determine which stakeholders care about a given technology. The community can facilitate this by being poised to respond to events in the news such as the HAB bloom in Ohio or the melamine

found in food, or similar. Further, the community should emphasize the importance of "connecting the dots" for businesses by demonstrating how this new technology will make them more competitive and profitable by averting/overcoming impacts related to HAB events.

Breakout Session E – What are the approaches to testing of HAB technologies?

- Q1: What level of verification testing is needed? (*i.e. what is "good enough" in the context of price versus performance for different data uses*)

  It is important that any verification testing be designed to match a well-articulated purpose – 'Fit for Purpose' and 'Intended Use'. For example, verification testing of technologies used for HAB forecasting support will be different from verification testing to support compliance with drinking water standards. In another example, PCR approaches may be best suited for "early warning" intended use, but once a bloom develops and the organism is known, the analytical task shifts to toxin detection rather than species identification. This can further be complicated when blooms contain more than one harmful species, particularly in marine systems. Also, verification testing may need to be system-specific. For example, HAB strains may vary lake to lake, so an assay validated (or verified) in one lake system may not be applicable in another lake system. This led to a discussion about the concept that "bloom" is a term that describes an intrinsically heterogeneous system that complicates the idea of verification testing. The testing framework can be challenged by a number of key biological factors, including independent variability in toxicity with respect to cell densities, unknown triggers of toxicity, timing of sample collection, and inherent influences from biodiversity, to name a few.

  There can also be platform functionality concerns for verification testing. One group discussed this using the ESP and IFCB technologies as examples. Questions arose such as: Are we testing cell counts versus speciation? Are the images themselves accurate? How well is the phytoplankton community being represented – chained organisms and those with higher mobility can be challenging for imaging systems; sampling intakes are working within the confines of inherently patchy surroundings; volumes sampled are often best guess estimates to not over/underwhelm detection capabilities. Are the processing algorithms (human and automated) accurate? Technologies provide surrogate measurements that require building a model to the parameter of interest. In the ideal scenario, these models could be built between remotely sensed or in situ fluorescence measurements with toxin concentration. In reality, that's not yet feasible in large part due to the uncertainty and variability that exists in all data inputs assimilated by a given model (e.g. remotely sensed ocean color, temperature, in situ fluorescence data vs cell counts or pigment concentrations vs. toxin concentrations). Therefore, the realistic best-case scenario is probably to develop models to estimate the abundance of known toxin producers.

  Verification testing was discussed as data for building and ground-truthing a model. For remote sensing, verification data should be collected across a range of water body types and environmental conditions. Target parameters are typically cell counts, abundance, or extracted pigment concentrations, and samples should ideally be collected weekly within 1 km and 8 hrs of the satellite data collection. For in situ sensors, weekly measurements for extracted pigment concentrations or cell counts are probably ideal given the variability

of algal composition and cell health over time. Although relationships can be built, they are very likely to be site specific. These statistical models should incorporate uncertainty given the other benefits of the measurements (e.g. large spatial coverage and frequent return for satellite; high frequency, real-time and low cost for in situ fluorometers).

- Q2: Are there 'gold standards' of reference?

The discussion around 'gold standards' in HAB research has been ongoing. While utilized for validation of system performance, in the biological realm they often need their own ground-truthing. One accepted method is microscopy, however there can be variability across microscopists' scoring of a split sample (Godhe et al. 2007). While having one person perform microscopy within a project/monitoring program is feasible, this consistency framework breaks down when comparing results across broad temporal and spatial scales. This is further complicated when genetic diversity within morphologically identical species is included. Accuracy can vary across taxa at the species or genus level (e.g. *Alexandrium* versus *Pseudo-nitzschia*), thereby limiting a universal approach for developing 'gold standards'. This accuracy ties into sampling practices, such as how to overcome the difficulties in species that are difficult to culture (e.g. *Dinophysis*), or exhibit different growth in culture (e.g. *Microcystis* exists as flocculated clumps in environmental samples, but remains dominantly uni-cellular in culture).

One group focused on 'gold standards' related to qPCR and ELISA, as these two methods are very common for species and toxin detection, respectively. Currently, there is no 'gold standard' consensus for extraction of material (genetic and toxin) and this is a critical yet unmet need. ***Extraction methods should be defined as operations requiring verification testing***. Microcystin was given as an example – the accepted protocol for toxin extraction is a triplicate freeze-thaw cycle, however it is unknown whether this method results in 100% cell lysis. Marine toxin extraction methods were not discussed in detail, other than to state that multiple extraction methods are required depending on the organism, sample matrix, and the complexity and importance of toxin profiles

Probe sets are not universally defined, and there was discussion about whether we are hitting the right genetic targets. Researchers and kit vendors have validated probe sets, but currently there is no agreement on universal loci, at least for the microcystins (MCY-D, MCY-A, MCY-E). Furthermore, our knowledge of toxin genes is limited for most toxic HAB species, with the only other target known (outside those for microcystin), is for saxitoxin A (produced by *Alexandrium* spp.). For other species, there may be a suite of genes involved, further complicating the development of standards. Once validated, these toxin gene standards should be provided as part of vendor kits.

For qPCR and SHA, cell counts are used to develop and validate standard curves, however we need to think about the reliability of using live cells. There was a suggestion to move towards the use of synthetic DNA molecules. Cell counts and extracted pigment concentrations are probably the realistic "gold standard" (although toxin concentration would be ideal if suitable models could be built). For future ACT testing of toxin kits, there will ideally be a set extraction method that can be used for verification/comparison. Even if the method is not perfect or the best, a consistently applied method is needed. Verification testing methods for immunoassays such as ELISA are generally well understood, covering topics such as recovery, precision, shelf-life/stability, and interfering

substances. While there are many known congeners for microcystin (> 200), there are only a few certified reference materials for verifying that an assay detects specific congeners.

- Q3: Are there shared metrics to assess performance across systems (eg. marine versus fresh water) and uses (e.g. research versus management)?

  Assessing performance across marine and freshwater systems and uses can often be gleaned through peer-reviewed publications. For example, results from platforms that can be deployed in either type of environment to address broad needs (research and management) are supported by metrics such as 1) error bars for cell counts (e.g. ESP) that are used for visualizing variability within complex systems; and 2) image comparisons within and across databases for in situ automated microscopy platforms (e.g. IFCB). For any freshwater or marine phytoplankton metric, QA/QC development goes hand in hand. The example of phytoplankton identification within both systems was discussed. While manuals are important resources for microscopy, there is a need for cross-method validation. Further, identification can be confounded based on sampling methods used: some phytoplankton cells are very delicate (e.g. athecate dinoflagellates), while others are quite robust (e.g. *Pseudo-nitzschia*). Both types of cells can be found in each system.

  Efforts for hindcasting and forecasting are common to both freshwater and marine systems. Although relationships are typically system dependent, most models are likely not. In both cases, there is uncertainty in evaluation of developing models and this can affect our ability to move forward with management decision capabilities. There is a need for a starting point for co-validation and for utility of methods for management decisions within both system types, e.g. is presence/absence of organism/toxin or quantitative analysis required? Technical components need to be addressed, but a proposal like that may or may not review well. Is this a priority? What is the first step? A successful pipeline would be: essential research – validation – an approved/functioning method. The group drew on parallels in other communities: developing advisory committees – publishing papers – establishing expert references. In the end, the community should "own" the validation process. QARTOD guidelines/manuals can be an important outlet for standardized information in a living document format. Their latest manual, Real-Time Quality Control of Passive Acoustics Data, is "written for the experienced operator but also provides examples for those who are just entering the field". The group also discussed the need for better methods communication, and suggested adding a chapter on extraction methods to the IOC Manual on Harmful Algae, reviewing the data and method-sharing approach used by the Interstate Shellfish Sanitation Conference (ISSC), and developing a living document.

- Q4: How would performance testing of these technologies ideally be conducted in the field?

  Lab testing would be performed in one location, however many HAB species from all regions could be tested.

  1) Choose cultures of interest
  2) Choose concentrations
  3) Change environmental parameters for exposure (e.g. salinity, temperature, turbidity)
  4) Alter automatic versus manual classification for ground-truthing

Field testing would ideally be performed in multiple locations that were strategically selected to test extremes (e.g. hot/icy/biofouling).

1) Perform verification using a matrix of paired samples – for microscopy, preserved versus live sampling is constrained by logistics, but a solution might be to create a few "mosaics" and send to a few microscopists around the country and average the results.

2) Adjust sampling interval on the fly to catch HAB events or anomalous conditions.

- Q5: What are end-user QA/QC needs for HAB data?
  Unfortunately, because of time constraints, this question received limited consideration from the workshop participants and no conclusions or consensus was reached. However, it is clear that QA/QC requirements remains an important issue to address.

  There is a need for stable designated funding to cover reagents and consumables for ongoing QA/QC needs during and between deployments, as these will directly influence data outputs.

RECOMMENDATIONS

The workshop presentations, charge questions and general discussions led to a range of recommendations addressing expanded observing platform capabilities and data storage, paths for transitioning to operational use among stakeholders and associated challenges, and methods communication. Consensus recommendation statements derived from these efforts include:

1. Need for a **national network** of regional HAB observing systems. Consider expandability with new infrastructure builds that support multiple sensors.

2. Identify suitable **reference ('Gold') standards**. The HAB community needs to address limited deployment opportunities, instrument availability, and system complexities as formidable barriers to assessing performance of 'gold' standards following the traditional ACT Performance Verification model.

3. As a community, prioritize **development of a cloud computing pipeline** that allows for large amounts of data storage, sharing and analyses. Support investment in approaches to alleviate access bottlenecks associated with high frequency image based monitoring systems and thereby facilitate inter-calibration of species assignment algorithms and deployment opportunities.

4. Continue efforts towards **inclusion and education of stakeholders** (including aquaculturists given the rise in that industry) regarding the latest in Harmful Algal Bloom detection technologies. Reach out to this community by attending shellfish meetings (devise a list of regional meetings and request attendance of a geographically close HAB community representative), requesting a HAB section at meetings, inviting representatives of the

industry to HAB-related meetings (national and regional), and offering to accompany stakeholders in the field with various technologies (e.g. hand held qPCR machine). New technology development or application customization pipelines should engage stakeholder input at an early stage to ensure products of technology investments are fit for purpose. Given both the biological and regional diversity of HA species and toxin profiles it should be acknowledged that detection assays will likely require regional tuning rather than a one size fits all strategy.

5. Further **refine microcystin detection levels** in order to improve early warning. Additional research should include extracellular toxins and how those may contribute to an event.

6. Seek **sustainable funding for continuous measurements** of environmental parameters and species/toxin detection via mobile and moored platforms for continued expansion of long-term data sets. A pathway for this support should include researchers, manufacturers and stakeholders. As a community, begin creating a space for these relationships to form and build in order to demonstrate the need to funding agencies. The NOAA-OAR funded Lake Erie HAB Bulletin is a successful model between partnerships, long-term funding sources and platform operations.

7. Support investment in both autonomous in situ HA detection technology along with end-user accessible, highly portable formats to **spatially expand HA detection** at autonomous sentinel monitoring sites.

8. Strategically identify and tap into **alternate sources of support/partnerships** to advance sensor technologies including obvious HAB-impacted industries (e.g. aquaculture), larger market capitalization industries (e.g. flour/milling) which rely on clean/toxin-free water, and industries (e.g. cosmetics, medical) which may have more lucrative applications for technologies currently being developed for HABs.

9. Continue to **support the mandates** of the Harmful Algal Bloom and Hypoxia Research and Control Act (HABHRCA) and the Ecological Forecasting Roadmap (EFR).

10. Focus on HAB toxin detection assay kits for near-future ACT Performance Verification.

11. For future ACT verification tests, consider requesting funded groups to participate in order to lessen the burden on small vendors that are unable to loan out multiple instruments for an extended period of time.

REFERENCES

Anderson, D.M. 1989. Toxic algal blooms and red tides: a global perspective. In *Red Tides: Biology, Environmental Science and Toxicology*, eds. T. Okaichi, D.M. Anderson, T. Nemoto, pp. 11–16. New York: Elsevier.

Anderson, D.M. Burkholder, J.M., Cochlan, W.P., Glibert, P.M., Gobler, C.J., et al. 2008. Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. *Harmful Algae* 8:39–53.

Blow, N. 2017. Fighting for the future of antibodies. *Biotechniques* 63: 51-56.

Brown, E.G. 2016. Office of the Governor to the US Department of Commerce. https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=116284.

Campbell L., Olson R.J., Sosik H.M., Abraham A., Henrichs D.W., Hyatt C.J. and Buskey, E.J. 2010. First harmful *Dinophysis* (*Dinophyceae, Dinophysiales*) bloom in the U.S. is revealed by automated imaging flow cytometry. *J. Phycol.* 46:66-75.

Campbell, L., Henrichs, D.W., Olson, R.J., and Sosik, H.M. 2013. Continuous automated imaging-in-flow cytometry for detection and early warning of *Karenia brevis* blooms in the Gulf of Mexico. *Environmental Science and Pollution Research* 20:6896-6902.

Carlson, C. "Toxin likely to blame for sea lion, bird deaths". Ventura County Star. Posted April 20, 2017.

Caron, D.A., Garneau, M.E., Seubert, E., Howard, M.D.A., Darjany, L., Schnetzer, A., Cetiníc, I., Filteau, G., Lauri, P., Jones, B., and Trussell, S. 2010. Harmful algae and their potential impacts on desalination operations off southern California. *Water Research* 44:385-416.

Deeds J.R., Wiles K., Heideman G.B. VI, White K.D, and Abraham A. 2010. First U.S. report of shellfish harvesting closures due to confirmed okadaic acid in Texas Gulf coast oysters. *Toxicon*. 55:1138-1146.

Doucette G.J. and Kudela R.M. 2017. In situ and real-time identification of toxins and toxin-producing microorganisms in the environment. In *Comprehensive Analytical Chemistry: recent advances in the analysis of marine toxins*, eds. J. Diogène and M. Campàs, pp. 411-443. Cambridge: Elsevier.

Esposito, A. "Chile's salmon farms losing up to $800 million from algal bloom". Reuters. Posted March 9, 2016.

Godhe, A., Cusack, C., Pedersen, J., Andersen P., Anderson, D.M. et al.. 2007. Intercalibration of classical and molecular techniques for identification of *Alexandrium fundyense* (Dinophyceae) and estimation of cell densities. *Harmful Algae* 6:56-72.

Hallegraeff, G.M. 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32:79–99.

Hallegraeff, G.M. 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *J. Phycol*. 46:220–35.

Haywood, A. J., Scholin, C.A., Marin III, R., Steidinger, K.A., Heil, C., and Ray, J.. 2007. Molecular detection of the brevetoxin-producing dinoflagellate *Karenia brevis* and closely related species using rRNA-targeted probes and a semi-automated sandwich hybridization assay. *J. Phycol*. 43:1271-1286.

Henry, T. "Water crisis grips hundreds of thousands in Toledo area, state of emergency declared". Posted August 3, 2014.

Jewett, E.B., Lopez, C.B., Dortch, Q., Etheridge, S.M., and Backer, L.C. 2008. *Harmful Algal Bloom Management and Response: Assessment and Plan.* Interagency Working Group on Harmful Algal Blooms, Hypoxia and Human Health of the Joint Subcommittee on Ocean Science and Technology. Washington DC, 76 pp.

Kudela, R.M. 2015. Harmful Algal Blooms: A Scientific Summary for Policy Makers. IOC/UNESCO, Paris (IOC/INF-1320).

Landeck, K. "FWC postpones scallop season". Panama City News Herald. Posted July 24, 2017.

Lelong, A., Hégaret, H., Soudant, P. and Bates S.S. 2012. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. *Phycologia* 51: 168–216.

Lindsey, K. "Deadly toxin shuts down some Southeast Alaska oyster farms". Alaska Dispatch News. Posted July 3, 2017.

Main, C.R., Doll, C., Bianco, C., Greenfield, D.I., and Coyne, K.J. 2014. Effects of growth phase, diel cycle and macronutrient stress on the quantification of *Heterosigma akashiwo* using qPCR and SHA. *Harmful Algae* 37:92-99.

McGuire, P. "Brain-damaging toxin closes Down East shellfish industry". Portland Press Herald. Posted October 6, 2016.

Pignataro, T.J. "Toxic algae starts to bloom in open Lake Erie waters". The Buffalo News. Posted July 25, 2017.

Sellner, K.G., Doucette, G.J., and Kirkpatrick, G.J. 2003. Harmful algal blooms: causes, impacts and detection. 2003. *J Ind Microbiol Biotechnol*. 30:383-406.

Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S.O., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D.M., and Cochlan, W.P. 2015. Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae*, 49:68-93.

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WORKSHOP AGENDA

SENSORS FOR MONITORING OF HARMFUL ALGAE, CYANOBACTERIA AND THEIR TOXINS – Current Status and Integration Into Observing Systems

- An Alliance For Coastal Technologies Technical Workshop -

Day 1 – Monday, 30 January 2017

8:15 – 9:00 Arrival at MLML; coffee and treats in seminar room

9:00 – 9:30 Welcome & Introductions

- MLML logistics
- quick intros (name, affiliation, interest in workshop)
- quick review of recommendations from 2008 workshop
- outline overall goals for this workshop

9:30 – 10:30 **SESSION 1**: OVERVIEW: Current State of HAB Detection Technologies and Integration with Regional Observing Systems

- 20 min: R. Kudela – overview of technology in use (in situ, remote, modeling), toward management applications
- 20 min: T. Davis – the Great Lakes example
- 20 min: Ana Lara-Lopez – highlights from the Nov 2016 HAB workshop in Australia

10:30 – 10:45 BREAK

10:45 – 12:30 **SESSION 2**: HAB and TOXIN TECHNOLOGY/DETECTION CHALLENGES

10:45-11:30 Breakout into four groups (groups will be mix of industry/ research/stakeholders) to discuss charge questions

- Q1: What are the cost, usability and readiness levels for current methods? What are improvement suggestions?
- Q2: Identify region/HAB/water type-specific gaps and issues for HAB sensor technologies and their implementation.
- Q3: What are paths forward for transitioning to operational use for current and near-future technologies?

11:30-11:45 Rep from each group will give 3-5 minute group summary
11:45-12:30 Open discussion

12:30 – 1:30 LUNCH ON SITE

1:30 - 3:15 **SESSION 3**: STAKEHOLDER NEEDS

1:30-2:15 Breakout into four groups (groups will be mix of industry/ research/stakeholders) to discuss charge questions

- Q1: Do currently available detection technologies meet stakeholder needs?
- Q2: What constraints may limit widespread adoption of currently available technologies?
- Q3: What surveillance needs are not being met?
- Q4: Are there viable solutions in the R & D pipeline?

2:15-2:30 Rep from each group will give 3-5 minute group summary
2:30-3:15 Open discussion

3:15 – 3:30 BREAK Group photo on deck

3:30 – 4:30 **SESSION 4**: SHARED CHALLENGES ACROSS MARINE AND FRESHWATER ECOSYSTEMS

- Open discussion - What are the shared challenges across ecosystems? Are there agency/organization barriers to collaboration across the marine-freshwater continuum? What are some approaches for integration of data streams? What are the regional complexities for bridging knowledge gaps?

4:30 – 5:00 Daily Wrap up and return to Monterey

Day 2 – Tuesday, 31 January 2017

8:15 – 9:00 Arrival at MLML; coffee and treats in seminar room

9:00 – 9:15 Recap of Day 1 Outcomes, quick intro to sections for this day

9:15 – 9:30 Jen Rhoades – update on IOOS investments in HAB sensor technology
9:30 – 9:45 Marc Suddleson – update on NCCOS involvement/investments in sensors for observing systems

9:45 – 12:00 **SESSION 5**: INTEGRATING DETECTION WITH EXISTING SYSTEMS

9:45-10:30 Breakout into four groups (groups will be mix of industry/ research/stakeholders) to discuss charge questions

- Q1: What performance assessments (QA/QC) methods are in use for current technologies?

- Q2: What ground-truth methods are in use for current technologies?
- Q3: What's needed/feasible to expand on these approaches?
- Q4: What contextual data is required for interpretation of HAB detection patterns and alerts?
- Q5: What end products are needed/desired?

10:30-10:45 Rep from each group will give 3-5 minute group summary

10:45 – 11:00 BREAK

11:00 – 12:00 Continue Session 5 with Open Discussion

12:00 – 1:00 LUNCH ON SITE

1:00 – 3:00 **SESSION 6**: HAB TECHNOLOGY TESTING / 'CERTIFICATION'

1:00 – 1:15 T. Johengen – Overview of ACT Performance Verification Process

1:15 - 2:00 Breakout into four groups (groups will be mix of industry/ research/stakeholders) to discuss charge questions

- Q1: What level of verification testing is needed? (i.*e. what is "good enough" in the context of price versus performance for different data uses*)
- Q2: Are there 'gold standards' of reference?
- Q3: Are there shared metrics to assess performance across systems (eg. marine versus fresh water) and uses (e.g. research versus management)?
- Q4: How would performance testing of these technologies ideally be conducted in the field?
- Q5: What are end-user QA/QC needs for HAB data?

2:00-2:15 Rep from each group will give 3-5 minute group summary
2:15-3:00 Open discussion

3:00 – 3:15 BREAK

3:15 – 5:00 Continue SESSION 6 – Performance Testing

3:15 – 4:00 Breakout into four groups (groups will be mix of industry/ research/stakeholders) to discuss charge questions

- Q1: What opportunities can we utilize to demonstrate newly verified sensors?
- Q2: In what ways can we involve other organizations/programs in the verification/demonstration process?

4:00 – 4:15 Rep from each group will give 3-5 minute group summary
4:15 – 5:00 Open discussion

5:00 – Return to Monterey

Day 3 – Wednesday, 1 February 2017

8:30 – 9:00 Arrive at MLML, Coffee, continental breakfast

9:00 – 12:00 **SESSION 7**: Steps Forward – Group Discussion

9:00 – 9:20 T. Johengen/T. Davis – development of an operational HABs forecasting system in Lake Erie

- recommendations for challenges
  - related to stakeholder needs
  - related to system integration
- recommendations for moving forward with coordination of marine and freshwater approaches
- recommendations for verification and knowledge-sharing

ALLIANCE FOR COASTAL TECHNOLOGIES

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