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Workshop Proceedings

TECHNOLOGIES AND METHODOLOGIES FOR THE DETECTION OF HARMFUL ALGAE AND THEIR TOXINS

St. Petersburg, Florida
October 22 - 24, 2008

Funded by NOAA's Coastal Services Center through the Alliance for Coastal Technologies (ACT) at the University of South Florida, the Cooperative Institute for Coastal and Estuarine Environmental Technology, and the Florida Fish and Wildlife Conservation Commission

An ACT Workshop Report

A Workshop of Developers, Deliverers, and Users of Technologies for Studying and Monitoring Coastal Environments:

Technologies and Methodologies for the Detection of Harmful Algae and Their Toxins

St. Petersburg, Florida
October 22 - 24, 2008

ALLIANCE FOR COASTAL TECHNOLOGIES

Sponsored by ACT; the Cooperative Institute for Coastal and Estuarine Environmental Technology; and the Florida Fish and Wildlife Conservation Commission.

Hosted by ACT Partner Organization, University of South Florida.

ACT is committed to develop an active partnership of technology developers, deliverers, and users within regional, state, and federal environmental management communities to establish a testbed for demonstrating, evaluating, and verifying innovative technologies in monitoring sensors, platforms, and software for use in coastal habitats.

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Executive Summary

The Alliance for Coastal Technologies (ACT) Workshop "Technologies and Methodologies for the Detection of Harmful Algae and their Toxins" convened in St. Petersburg, Florida, October 22-24, 2008 and was co-sponsored by ACT (http://act-us.info); the Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET, http://ciceet.unh.edu); and the Florida Fish and Wildlife Conservation Commission (FWC, http://www.myfwc.com). Participants from various sectors, including researchers, coastal decision makers, and technology vendors, collaborated to exchange information and build consensus. They focused on the status of currently available detection technologies and methodologies for harmful algae (HA) and their toxins, provided direction for developing operational use of existing technology, and addressed requirements for future technology developments in this area.

Harmful algal blooms (HABs) in marine and freshwater systems are increasingly common worldwide and are known to cause extensive ecological, economic, and human health problems. In US waters, HABs are encountered in a growing number of locations and are also increasing in duration and severity. This expansion in HABs has led to elevated incidences of poisonous seafood, toxin-contaminated drinking water, mortality of fish and other animals dependent upon aquatic resources (including protected species), public health and economic impacts in coastal and lakeside communities, losses to aquaculture enterprises, and long-term aquatic ecosystem changes.

This meeting represented the fourth ACT sponsored workshop that has addressed technology developments for improved monitoring of water-born pathogens and HA species in some form. A primary motivation was to assess the need and community support for an ACT-led Performance Demonstration of Harmful Algae Detection Technologies and Methodologies in order to facilitate their integration into regional ocean observing systems operations. The workshop focused on the identification of region-specific monitoring needs and available technologies and methodologies for detection/quantification of harmful algal species and their toxins along the US marine and freshwater coasts.

To address this critical environmental issue, several technologies and methodologies have been, or are being, developed to detect and quantify various harmful algae and their associated toxins in coastal marine and freshwater environments. There are many challenges to nationwide adoption of HAB detection as part of a core monitoring infrastructure: the geographic uniqueness of primary algal species of concern around the country, the variety of HAB impacts, and the need for a clear vision of the operational requirements for monitoring the various species. Nonetheless, it was a consensus of the workshop participants that ACT should support the development of HA detection technology performance demonstrations but that these would need to be tuned regionally to algal species and toxins of concern in order to promote the adoption of state of the art technologies into HAB monitoring networks.

Alliance for Coastal Technologies

The Alliance for Coastal Technologies is a NOAA-funded partnership of research institutions, resource managers, and private sector companies dedicated to fostering the development and adoption of effective and reliable sensors and platforms. ACT is committed to providing the information required to select the most appropriate tools for studying and monitoring coastal environments. Program priorities include transitioning emerging technologies to operational use rapidly and effectively; maintaining a dialogue among technology users, developers, and providers; identifying technology needs and novel technologies; documenting technology performance and potential; and providing the Integrated Ocean Observing System (IOOS) with information required for the deployment of reliable and cost-effective networks.

To accomplish these goals, ACT provides these services to the community:

- Third-party testbed for quantitatively evaluating the performance of new and existing coastal technologies in the laboratory and under diverse environmental conditions.
- Capacity building through technology-specific workshops that review the current state of instrumentation, build consensus on future directions, and enhance communications between users and developers.
- Information clearinghouse through a searchable online database of environmental technologies and community discussion boards.

The ACT workshops are designed to aid resource managers, coastal scientists, and private sector companies by identifying and discussing the current status, standardization, potential advancements, and obstacles in the development and use of new sensors and sensor platforms for monitoring, studying, and predicting the state of coastal waters. The workshop's goal is to help build consensus on the steps needed to develop and adopt useful tools, while facilitating critical communication among the various groups of technology developers, manufacturers, and users.

ACT is organized to ensure geographic and sector involvement:

- Headquarters is located at the UMCES Chesapeake Biological Laboratory, Solomons, MD.

- Board of Directors includes Partner Institutions, a Stakeholders Council, and NOAA/CSC representatives to establish ACT foci and program vision.

- There are currently eight ACT Partner Institutions around the country with coastal technology expertise that represent a broad range of environmental conditions for testing.

- The ACT Stakeholder Council is comprised of resource managers and industry representatives who ensure that ACT focuses on service-oriented activities.

ACT Workshop Reports are summaries of the discussions that take place between participants during the workshops. The reports also emphasize advantages and limitations of current technologies while making recommendations for both ACT and the broader community on the steps needed for technology advancement in the particular topic area. Workshop organizers draft the individual reports with input from workshop participants.

ACT is committed to exploring the application of new technologies for monitoring coastal ecosystem and studying environmental stressors that are increasingly prevalent worldwide. For more information, please visit www.act-us.info.

Workshop Goals

The underlying goal of the workshop on harmful algae was to focus discussion on the technologies and methodologies developed to detect/quantify harmful algal species and their toxins found along the US coastal waters of the Pacific, Gulf of Mexico, Northeast Atlantic, and the Great Lakes. Specifically, the participants were charged with the following tasks:

1. Review the current state of technologies/methodologies and their success at addressing user needs through brief invited presentations.

2. Develop strategies for the commercialization and transition to operations of new technologies/methodologies based on an assessment of potential markets and operational needs.

3. Build community consensus on approaches and foci of demonstration activities, from laboratory inter-calibrations to field testing of detection technologies.

Organization of the Workshop

The workshop's advisory committee included Quay Dortch, NOAA/ECOHAB; Cindy Heil, Florida FWC; Raphael Kudela, HABMAP/UCSC; Rich Langan, CICEET/UNH; G. Jason Smith, ACT/MLML; Mario Tamburri, ACT/CBL; Dwight Trueblood, NOAA/CICEET; and Steve Weisberg, SCCWRP. Workshop participants included researchers, coastal decision makers, and vendors interested in the development and implementation of harmful algae detection technologies. A list of participants is included at the end of these workshop proceedings.

The two-day workshop commenced on the evening of October 22, 2008 with Dr. Mark Luther summarizing ACT's missions and goals to the invited participants. Following dinner, our guest speaker, Larry Langebrake (SRI International), gave a presentation on strategies for "Bridging the Gap Between Innovation, applied Research, and Production". The following morning opened with a formal plenary presentation by Dr. Kevin Sellner (Chesapeake Research Consortium, Inc.) summarizing the current technologies and methodologies for the detection of harmful algae and their toxins. Participants were then divided into three groups for breakout discussion sessions. For the first breakout session, three break groups were formed with representation from each of the standard ACT technology focus sectors (research/academia, management end-users, and manufacturer representatives). The purpose of this session was to update and assess the current state of technology and identify user needs. The groups then reconvened, and a chair from each group provided a summary of the group's findings. Participants then separated into four groups, with members representing their geographic area of concentration (Gulf of Mexico, US East Coast, US West Coast, and Great Lakes). The purpose of this session was to discuss particular regional needs. Specifically, these working groups were charged with the following:

1. Identify the current state of technology, methodology, and user needs
2. Discuss specific regional needs
3. Prioritize species of harmful algae and summarize current applications
4. Discuss challenges to the development, commercialization, and operational use of harmful algae/toxin detection/quantification technologies and methodologies and how ACT could focus their technology/methodology demonstration priorities.

Afterwards, a chair from each group provided a summary of their findings. The workshop concluded after a morning session on October 24, following the development and prioritization of a list of specific recommendations guiding development of robust HAB technology demonstrations.

Summary of Existing Technologies and Methodologies

The Needs and Progress Towards Development of HA and Toxin Monitoring Technologies

HABs have become persistent, if not continually expanding, phenomena impacting marine and freshwater coastal zones and fishery activity at both national and global scales. While this can be tied in part to anthropogenic influences on the coastal zone (e.g., nutrient loading, land use patterns, ballast transport), HABs are by no means a new environmental phenomena. With expanding human utilization of coastal resources, the motivation for monitoring and ultimately predicting the occurrence and distribution of these natural events has also increased.

For most HAB events, mitigation of harmful effects will often entail operational integrated monitoring systems (networks) for robust early detection of HA species distributions and relative toxicity. Changes in HAB cell abundance and toxicity need to be monitored, so that the distribution and potential risk of HAB species and their toxicity can be predicted, resulting in proactive and effective management of impacted resources (fisheries, beaches, water intakes). Historically, human health and terrorism concerns have fueled some of the concern and early investigation of HABs and their toxins.

The critical importance of monitoring both species and toxins in the context of their regional environments has recently been highlighted in a variety of national and international reports. Two reports submitted to Congress in response to the 2004 reauthorization of the Harmful Algal Bloom and Hypoxia Research and Control Act make a strong case for the development, design, and integration of HAB cell and toxin sensors into observing systems (Jewett et al., 2008, Lopez et al., 2008).

“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 also deliver integrated data sets that can be used in operational mode for forecasting HAB events” (Jewitt et al., 2008).

The Interagency Oceans and Human Health Research Implementation Plan (Sandifer et al., 2007) also emphasizes the need for development of biosensors, especially those to be deployed as part of observing systems. From an international perspective, the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) Science Plan (GEOHAB, 2001) has also issued calls for new tools for detecting harmful and potentially harmful organisms.

A number of national and international programs have recognized and are addressing this problem. The NOAA-led interagency program on Ecology and Oceanography of Harmful Algal Blooms (ECOHAB), supports research leading to new and innovative HAB detection technology. The NOAA's Monitoring and Event Response for Harmful Algal Blooms (MERHAB) program promotes sustainable partnerships that are directly responsible for the development of new technologies to observe and mitigate HAB impacts. Small Business Innovation Research Initiative (SBIR) programs are used by the federal government to ensure that small and innovative businesses become part of the government's R&D efforts. Over time, they have funded some of the commercialization of HAB detection technologies. Through a series of regional science implementation plans, the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) program is addressing specific recommendations about needs for HAB sensors. In addition, HAB monitoring and prediction have been among the core criteria supporting the need for GOOS, IOOS, and related ocean health initiatives.

Past Workshops

The fundamental work linking specific phytoplankton species to specific intoxication of humans and aquatic vertebrates coincident with consumption of contaminated shellfish or aerosol exposures has traditionally relied on coordinated, but rarely co-located, laboratory-based microscopic analysis by expert taxonomists and rigorous chemical analysis of impacted foodstuffs and potential phytoplankton sources. Needless to say that while these approaches are invaluable and provide the ultimate 'gold standard' for HA description, it is recognized that they are retrospective analytical tools and cannot by themselves be considered as operational components of ocean observing systems. Hence, the development and application of technological innovations for higher throughput and ultimately in situ detection of HA species has been encouraged for some time. In March of 2002, ACT held its initial HAB workshop entitled "Biosensors for Harmful Algal Blooms" in Solomons, Maryland that identified some initial steps the community should focus on to accelerate access for coastal managers to HAB biosensors. Industry representatives clearly wanted to help bridge the gap between available technologies and user needs, but commercial considerations indicated a need for outside funding to cover initial R&D costs. This initial meeting was also very important because it gave resource managers an opportunity to identify their short-term technological needs (sampling frequency, spatial scales, operational costs), focusing on some of the newer portable technologies with a desire for inexpensive yet reliable products.

The ACT Workshop on Rapid Microbiological Indicator Methods (Moss Landing, CA 2003), while not focused on HABs, did highlight a variety of technological approaches being leveraged from other agencies' R&D efforts towards the detection and quantification of human pathogens in coastal waters. These discussions highlighted challenges also pertinent to design and performance assessment of HAB detection systems, such as sample pre-concentration to obtain sufficient target for detection and the role of established regulatory criteria (or decision limits) to motivate adoption of new technological approaches into monitoring schemes.

The main objective of the HABWATCH meeting in June of 2003 was to provide the user community with the data and knowledge to detect and forecast physical, chemical, and biological changes in coastal and open-ocean ecosystems through presentations, demonstrations, and practical tutorials. While presented as a whole ecosystem approach, much of the workshop's core content was devoted solely to the detection of HABs and their toxins (Babin, Roesler and Cullen, 2008). This workshop provided much needed cross-community exposure to existing HA detection technologies and requirements for their integration into observing system infrastructures available at the time.

The ACT Workshop, Genetic Sensors for Environmental Water Quality (St. Petersburg, FL 2005), provided an interim update on the rapid advances in gene-detection technologies and prototype in situ platforms for a range of biological targets providing proxies of ambient water quality. It was felt that, at this time, robust technologies were available to support assay development but that this should best be focused on clear definition of end-user needs and that the molecular proxies be well correlated with the managed impacts. Sample collection and preparation were highlighted as critical integral processes controlling the success and accuracy of these assays in field applications.

Combining these efforts and ongoing programmatic support for technology development and field validation studies (e.g. ECOHAB, MERHAB, CICEET, NSF, and Small Business Innovation Research) has lead to development of a variety of portable, yet laboratory-based assays for species and toxin detection, as well as significant development of field compatible assay platforms. However a "clear need for facilitating the final step of technology adoption and movement through the high capital-demanding commercialization phase ('Valley of Death') into operational use still remains" (Dortch et al. 2008). This workshop was convened to address this challenge, as well as the impact of region specific monitoring information needs on the design requirements for technology demonstration projects. Multi-tiered approaches and operational requirements for integration of HA detection systems into HA alert and forecasting systems were also highlighted. Towards this end, the following section provides an overview of currently available HA monitoring tools.

Survey of Current Technologies for HAB Monitoring

A variety of microscopy techniques (light, epifluorescence, SEM, and TEM) provide the gold standard for phytoplankton species description based on morphological criteria. The chemical diversity and structural complexity of phycotoxins have limited commercial efforts towards their de-novo synthesis and the majority of toxin reference standards are currently purified in bulk from natural sources. Availability of this reference information and materials have enabled the exploitation of the diversity of HA species and toxins to provide a wealth of alternate targets for development of both laboratory and field compatible HAB detection systems. Potential diagnostic targets for HA range from cell morphology to cell surface molecules, intracellular pigments to genes, gene products and the toxin molecules themselves. The capacity to incorporate these targets into monitoring applications has also been facilitated by advances in detection and reporting technologies. Several common core technological approaches have been leveraged for detection of HA-specific targets and include: optical characterization, immunological recognition, nucleic acid hybridization and nucleic acid amplification along with appropriate reporter strategies in each category (Tables 1 and 2).

While molecular assays are attractive back-ends for HAB detection systems due to their potentially high sensitivity, this feature also requires that they be extensively evaluated against heterogeneous field samples in order to assess potential for false negative or positive calls due to detritus or novel cross-reactivity. In order to benefit existing monitoring efforts and promote their expansion, several considerations need to be addressed in final detection system design including: assay cost efficiency, operational constraints (ease of use, maintenance, required support infrastructure), data types provided (raw vs. relative vs. quantitative) and compatibility with other water quality monitoring assets. The refinement of data types reported would be facilitated by clear definition of action levels being used or targeted for cell abundance, particulate or dissolved toxin loads in whole water samples. While these may vary regionally some consistency in required units could be agreed upon. Ultimately, the aim is to combine and "tune" these various approaches to provide region- and/or HA-specific detection capabilities useful to coastal managers. Moreover, the resulting data streams should be compatible with automated assimilation into remote sensing products (see below) to improve their accuracy.

Table 1. The existing methodological approaches that have been employed for detection (presence/absence) and quantification (concentration measurement) of HA species and their toxins. '*'includes all detection systems, as well as MS/MS. 'Qr' denotes relative quantification. 'Lab / Field' denotes primary application with (F) indicating engineering refinements needed to make field compatible or remotely deployable. See Paul et al. 2007 and Scholin et al. 2008 for detailed assessments of these technologies.

Base Technology	Method	Target	Reporter	Detect / Quantify	Standard	Lab / Field
Microscopy						
	Light	Cell morphology, pigmentation	tional waters)	D, Q	Voucher specimens, taxonomic keys	L
	SEM/TEM	ultrastructure	image	D	"	L
Analytical						
	HPLC methods*	Toxins, metabolites, pigments	Chromatogram, mass information	D, Q	Specific analytes	L
	Mouse Bioassay	toxin	toxicity	D , Qr	"	L
	UV-VIS spectroscopy	Toxins, pigments	absorption spectra	D, Q	", spectral libraries	L, F
	Fluorescence spectroscopy	pigments	fluorescence spectra	D, Qr	", "	L, F
Molecular						
	FISH	Nucleic acid (NA) sequence in cells – gDNA, RNA	Fluorescence with cell morphology	D, Q	Reagent controls, voucher samples	L
	Sandwich Hybridization	NA sequence – gDNA, RNA	Colorimetric, chemiluminescence, fluorescence	D, Qr	"	L, F
	Microarrays	Multiple NA targets (species) – gDNA, RNA	Spatially indexed fluorescence	D, Qr	"	L, F
	NASBA – nucleic acid sequence based amplification	NA sequence – RNA, ssDNA	Fluorescence kinetics - isothermal amplification	D, Qr	"	L, F
	PCR – polymerase chain reaction	NA sequence – gDNA, cDNA from RNA	Fluorescence kinetics - thermal cycling amplification	D, Qr	"	L, F
	ELISA	toxin	Colorimetric, fluorescence	D, Q	Toxin standards	L

Base Technology	Method	Target	Reporter	Detect / Quantify	Standard	Lab / Field
	Lateral Flow ELISAs 'dip sticks'	toxin	Colorimetric	D, Qr	Toxin standards	L, F
	RBA –Receptor Binding Assay	toxin	isotopic	D, Q	Toxin standards	L
	SPR – Surface Plasmon Resonance	Toxin Nucleic acid sequence	Refractive index dynamics associated with molecular binding	D, Q	Toxin standards, reference sequences	L, (F)
	Fiber Optic Bead Microarrays	Nucleic acid sequence (cells)	Spatially indexed fluorescence	D, Qr		L,(F)
Optical						
	In situ fluorescence	Chlorophyll or other taxonomically informative pigments	Relative fluorescence	D, Qr		F
	In situ absorbance	Inherent optical properties of water mass	Attenuation and scattering spectra	D, Qr		F
	Inverse modeling	IOPs	Characterization of phytoplankton community	D	Cell specific spectra	F
	4th derivative analysis	Cell specific spectra	Identification of characteristic absorbance or fluorescence peaks	D, Qr	Cell specific spectral features	L+ F
Remote Sensing						
	SeaWiFs	Ocean color	Chlorophyll distributions	Dr	Ground verification	F
	MODIS	Fluorescence line height	" and proxy for photosynthesis	Dr	"	F
	MODIS	Remote sensing reflectance	Spectral inputs for inverse modeling	Dr	" and IOPs	F
	Hyperspectral imagers, aircraft	Meter scale Rrs	"	Dr	"	F

Table 2. Field-ready derivative systems designed for specific detection of HA species and/or their associated toxins in water or tissue samples. All have undergone extensive field testing. See Paul et al. 2007 and Scholin et al. 2008 for detailed assessments of these technologies. '#' indicates commercially available.

Analytical Package / Vendor	Target	Reporter	Detect / Quantify	Primary Application Lab / Field
Abraxis Kits#	Cyanotoxins, Brevetoxin, DA, Okadaic Acid, STX	Colorimetric ELISA - microtiter plate	D, Q	Water quality and shellfish testing, lab based
BioSense Kits#	DA, Okadaic Acid, STS, YTX	Colorimetric ELISA - microtiter plate	D, Q	Shellfish testing, lab
Mercury Science kits#	DA	Colorimetric ELISA - microtiter well strips	D, Q	Water quality, shellfish testing, lab based, field format in beta
Jellet Rapid Tests#	DA, STX	Colorimetric lateral flow ELISA	D, Qr	Shellfish testing, lab and field
Environmental Sample Processor 1G & 2G - MBARI	Cells – multiple species, toxins – DA, Sample archiving	SH, ELISA, PCR	D, Qr	Remote or pier based deployments, data telemetry along with WQ context, sample archiving possible
Autonomous Microbial Genosensor USF-COTS	*Karenia brevis* - can be extended to other taxa	NASBA	D, Qr	Remote or pier based deployment, handheld version for spot surveys
Breve-buster Mote Marine Lab	*Karenia brevis*	Spectral matching to *K. brevis* Potential to extend to other taxa	D, Qr	Glider/AUV pier based deployments
Sequoia Scientific - *LISST*	Particles	Particle scattering distributions (cell size, shape, abundance)	D	Variety of deployment options and portable versions for lab or field use
Fluid Imaging Technologies - *FlowCam*#	cells	Images and abundance – automated particle classification	D, Q	Lab, dock, and pier deployments FW and marine
Flow CytoBot - WHOI	cells	Images and abundance – automated taxon classification	D, Q	Lab, dock, pier and moored deployments, FW and marine

Remote Sensing Tools

A limitation of shore and mooring based HAB monitoring is that they only provide data at limited spatial coverage and are dependent on blooms impinging on those points. In contrast, airborne and satellite remote-sensing platforms can generate broad spatial scale synoptic images of water quality parameters. This synoptic capability, although limited by weather restriction of water surface fields of view, will prove essential for development of phytoplankton bloom forecasts and identification of persistent bloom initiation sites. Although satellite technology developed in the 1970s was immediately applied to bloom detection, such image products provide only estimates of chlorophyll biomass distributions, not HA per se, and require the collection of in situ surface truth data for validating the image interpretation (see ACT WR06-02).

The NOAA HAB bulletin (Stumpf et al., 2003, Stumpf and Tomlinson, 2005), which utilizes an algorithm that tracks changes in Chl *a* concentrations over time in conjunction with wind data and validation of Chl *a* as *K. brevis*, has proven effective in monitoring the maintenance stages of blooms in the eastern Gulf of Mexico and predicting respiratory and shellfish impacts. It is, however, of limited usefulness for detection of early bloom stages or during large, prolonged blooms (eg. 2005 *K. brevis* bloom in Florida coastal waters) when high concentrations of bloom associated Chl *a* are present for extended periods. Its application to *K. brevis* blooms off the Texas shoreline has also been complicated by issues of Chl *a* detection in the presence of extensive resuspension of sediments and benthic microalgae (Wynne et al., 2005).

MODIS fluorescence line height has also been used to detect and trace the movements of Karenia blooms on the west Florida shelf (Hu et al., 2005). Documentation of a novel relationship between Chl a and particulate backscattering in Karenia blooms also holds promise for discriminating between blooms of Karenia, the N2 fixing cyanobacterium Trichodesmium and diatoms in the Gulf of Mexico (Cannizzaro, 2005) using satellite imagery, and is currently being assessed as a tool to guide HAB sampling by managers.

All satellite detection technologies for HAB tracking and forecasting require adequate groundtruthing. To date, this has involved the collection of water samples with subsequent microscopic enumeration of HA cells to the species level. It is important to keep in mind that remote sensing tools do not directly detect species of HA and provide no information on their toxicity, but are invaluable for informing adaptive sampling schemes which can be used to assess the hazard potential of remote or offshore blooms. For these multi-tier monitoring approaches to be robust, cross-validation of new detection technologies against microscopic counts is required prior to the routine use of these technologies in verifying satellite-based HAB forecasting.

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Monitoring Programs

To varying degrees states have developed water monitoring programs to ensure safe water supplies and monitor treatment efficacy by major dischargers and water quality changes around critical habitats. The intensity of these monitoring efforts can vary widely (i.e., continuous in situ to event driven) and, until recently, generally fell off seaward of estuaries or with distance from discharge or runoff sources. The continuity and accessibility of these water quality monitoring efforts is also problematic as they are often managed by multiple independent agencies and NGOs (e.g., regional water boards, natural resource agencies, public health, EPA, NERRS, and academic research groups) with different data requirements and management goals. The situation should improve with the establishment of the national and regional IOOS infrastructure with a goal of improving coordination, standardization, and accessibility of core water quality (temperature, salinity/conductivity, currents, chlorophyll, turbidity, dissolved oxygen, nutrient) time-series. These environmental characterizations are essential for HAB monitoring applications. A prime motivation for much of the research community's effort is ultimately the development of predictive capabilities for HAB initiation, persistence, and movement in order to effectively mitigate their impacts. Unfortunately, HA monitoring is currently infrequently co-located with WQ monitoring stations confounding efficacy of model development based on these disparate datasets. In order to ensure their overlap, enhance inter-comparisons, and guide development of management action levels across regions, cell and toxin monitoring programs and systems should incorporate at minimum sonde-type WQ instrumentation.

Standardization

The effectiveness and sensitivity of the new generation technologies for detection of harmful algae (and planktonic organisms in general) and toxins has been well demonstrated in the literature. However, the diversity of detection targets employed, ranging from microscopic images to DNA or RNA sequence motifs, presents challenges to the interpretation of the equivalency of their respective data outputs. These potential discrepancies can be further compounded by the known heterogeneity of plankton distributions, especially during pre-bloom conditions or for taxa like Alexandrium or Dinophysis, which can impart harmful effects at low abundance (< 1000 cells / L). The issue of defining data equivalency for these technologies is critical for useful integration of HAB detection systems into an observing system framework and can only be assessed by cross-standardization of detection systems and sample collection processes. While these technologies undergo in-house evaluations against known cell isolates or toxin standards with assay performance refined through successive deployment activities during R&D, many of these applications are at this time not widely available until they pass beta development or are adopted for commercialization, and the natural tendency is for retention of current sampling and analysis protocols at the local level.

At minimum, detection applications should include reagent and system detection controls to guard against false negative calls and alerts to declines in system performance. Provision of positive reference standards is more problematic. On one hand, they are needed to calibrate system response characteristics, but depending on system design and stability, may only be needed for pre- and post-deployment system tests as long as results are incorporated into the associated metadata. On the other hand, decisions on where in the sample processing stream to apply reference materials are not as straightforward and may constrain the interpretation of data equivalency. This consideration may have lesser impact on toxin detection or imaging flow cytometric applications, but in the case of cell detection and quantification by molecular methods, variations in lysis, permeability, and variability in target abundance with physiological state sample load relative to probe availability can all affect the accuracy of prior calibration and more critically, the interrelationship among methods. For example an intercomparison conducted with field samples from the Gulf of Maine revealed that microscopic, whole cell FISH and SHA derived counts of Alexandrium spp. were not consistently correlated across seasons or depths (Anderson et al., 2005) and suggested contributions of the aforementioned issues.

While the HAB monitoring community desires access to 'gold standards' to verify performance of field compatible detection systems, the brief discussion above indicates that this is not a straightforward problem, particularly in the realm of cell detection or quantification. While direct cell counts are a logical first choice, they are equally problematic, especially for morphologically similar taxa of dissimilar toxicity. Supply of fixed cells, while possible, may not be compatible across methods. It would seem that the best alternative is to encourage organization of intercomparison / intercalibration programs through which a particular method's performance can be assessed using natural samples along with reference material spiked and select processing (e.g., filter collected, bulk nucleic acids, cell lysates, etc.) as input samples. Finally, the utility of such exercises would be greatly enhanced by adoption of action criteria for each of the data types (i.e., cells/toxins present / absent vs. more than X / L) that could be used to inform testing activities by the concerned regulatory agencies.

Summary of Breakout Group Discussions

Breakout Session I

In contrast to the standard Workshop structure and based on the focused expertise of the current workshop participants, the initial breakout discussion groups were formed haphazardly from the participant pool. It was felt that this breakout design would facilitate identification of salient issues needed to guide the design of HAB technology demonstration and inter-comparison projects. For clarity, bulleted summaries follow of each group's major issues identified from their discussion around the issue of: ***Assessment of the current state of HAB detection technology, potential applications, operational requirements, and end-user needs for HAB monitoring data.***

Group 1:

- Research community, private sector developers, and funding sources would benefit from a clearer understanding of the managers' needs (i.e, what needs to be measured, to what degree of accuracy, and how it should be reported).
- To pursue new technologies, commercial developers need to know market size, what will sell, what type of test is required (presence/absence – large spatial distribution, inexpensive versus or very expensive species-specific instruments).

- Regulatory standards need to be established for certain toxin classes based on human health effects (e.g., Ciguatera). This will help guide the design of and need for monitoring assays. In general, motivation for commercialization of toxin or cell detection assays is dependent on the knowledge of how well the assay target relates to an accepted regulatory standard.
- Need predictive capabilities to identify hotspots for bloom initiation.
- Need better understanding of linkage between acute vs. chronic low level exposures to HAB-derived toxins with human and wildlife health (e.g., microcystins and liver damage, brevetoxins and respiratory distress, domoic acid and sea lion brain disorders).
- Technologies should be integrated and complementary.

Group 2:

- Design requirements: The R&D process needs to be based on need for quantitative vs. qualitative measurements (cell counts). It is important to clarify user needs that may not be dependent on stringent qualitative requirements. Qualitative products might be sufficient to inform downstream regulatory activities. User needs should be defined and considered as part of the design process.
- Sensor vs. Sensing: It is critical to have a holistic view during a possible technology demonstration.
- Implementation: Bringing all technologies together for a demonstration would be very useful but very challenging. However, this won't answer the questions related to instrument or methodology performance. The answer needs to come from field validations.
- Challenges:
  - Defining standards (e.g., a limited number of cultures is not a good representation of what is happening in the field)
  - Validating field in situ measurements. There is large variation in samples in the field, and this may require a statistician,
  - Units (for cells it is cells/liter, but for molecules it is different). How do you visualize the data in units?
  - Metadata is also very important to enable new users to assess performance variation (devise service history, reagent lot identification and certification metrics all need to be accessible)
  - Buffers and reagents in different labs are not always the same.
- Field validation: Data presently exist that may indicate where a technology or methodology demonstration should take place. It would be appropriate to apply multiple techniques at these locations to assess how these sampling matrices work in different areas with different people. The community should coordinate with other field programs to provide a context for the demonstrations.
- Logistics: Sample collection, analysis, dissemination.
- Need: A tiered approach, a statistician.

Group 3:

- The regional specificity of monitoring needs must be considered. This includes: HA species of concern, oceanographic forcing of phytoplankton distributions vs. description of regional triggers promoting HA growth *and* toxin production that can be highly variable even within regions. Observational needs would ideally be met by a combination of platforms equipped with regionally tuned HA sensing systems. Core platforms include: mobile platforms with horizontal and vertical sensing, fixed platforms, and remotely sensing both in-situ and satellite systems to generate early warning based on assimilation of standardized HAB sensor data types.
- Generic monitoring needs include: sensors detecting phytoplankton bloom formation (e.g., fluorometers) that would inform deployment or activation of hierarchical or tiered sampling assets; design of regional-minimal sensors; ability to take advantage of the cheap, reliable sensors to determine how much needs to be deployed, coupled with specific sensing to generate an early warning system; coupling these activities with regional needs for water quality monitoring and sampling programs for detection of pathogens for regulation.
- Make use of biological platforms, not only as platforms for minimal sensor packages, but also for isolation of toxin-specific biomarkers to determine an organism's integrated exposure in its foraging environment.
- Data needs and uses: monitoring groups, developers and users need to work together to translate available datasets into products that can be assessed retrospectively for utility as an early warning system; For example, with knowledge of *Alexandrium* spp. cell count distributions, New England managers are able to make more informed decisions with shellfish beds and preserving economic and human health.
- Models will generally need to be developed on a regional basis due to oceanographic forcing. Regional cross comparisons of models for specific HA species could facilitate identification of common bloom and toxin accumulation triggers. IOOS and RAs need to be engaged as data clearing houses.

Clearly the participants as a whole felt that a clear definition of the monitoring data types and level of resolution needed by managers and other users would aid adoption, refinement, and commercialization prospects for HAB detection technologies currently available. A possible barrier to this process is the recognition of the regional variability of major species of concern, the resources impacted and the environmental forcing of phytoplankton bloom dynamics. These considerations will ultimately require regional tuning of high resolution systems but do offer the prospect that a market is available for accurate but lower resolution, possibly low cost, binary type HA/toxin detectors (i.e., present vs. absent) to provide better spatial coverage for early warning of HAB development. Developmental feasibility for such systems again hinges on definition of user data requirements and action levels.

Breakout Session II

In recognition that a 'one size fits all' approach to HAB detection technology demonstration or evaluation is not feasible due to the heterogeneity in HAB dynamics and impacts, the second breakout session sought to stimulate regional centric discussions on HAB monitoring needs. Four breakout groups were formed based on broadly defined coastal regions, including: East Coast, Gulf of Mexico, Pacific Coast, and Great Lakes / freshwater resources. The discussion charge sought to develop regional priorities for: ***Harmful algal species of concern, continued development and operational use of HA detection technologies, knowledge gaps and guidance for ACT design and support of HA detection technology demonstration projects.***

West Coast Group

- The West Coast group identified the following species and toxins with known impacts:

  Alexandrium catenella / PSP - Paralytic Shellfish Poisoning is the intoxication syndrome caused by saxitoxin and its analogues.

  Pseudo-nitzschia australis, Pseudo-nitzschia multiseries, Pseudo-nitzschia delicatissima / ASP / DAP- Amnesic Shellfish Poisoning / Domoic Acid Poisioning is the illness caused by domoic acid accumulated in food injested by humans or wildlife respectively.

  Gambierdiscus toxicus - Ciguatera is the illness caused by the ingestion of the toxin, ciguatoxin.

  Heterosigma akashiwo, Chaetoceros concavicornis, Coclodinium polykrikoides and other red tide formers. These phytoplankton have been associated with fish kills in the aquaculture industry, largely through biomass and exudates affects rather than novel toxins.

  Microcystis aeruginosa / microcystins impacting limited freshwater resources.

- The existing needs for toxin detection were divided into three categories: regulatory, monitoring, and research applications.

  Regulatory applications include: Shellfish collection (natural intertidal, bagged for sentinel sites, aquaculture) with certified lab analysis using approved methods (e.g., Association of Analytical Communities (AOAC) and the American Public Health Association (APHA)). For PSP: AOAC mouse bioassay (only approved for regulatory), Jellett Rapid Testing, Ltd. kit (approved for screening shellfish), Lawrence HPLC (AOAC approved but not yet for regulatory applications); For ASP: Quilliam HPLC (AOAC approved, used for regulatory purposes), Biosense Laboratories AS (AOAC approved, but not for regulatory); For microcystin: liquid chromatography/mass spectrometry.

  Monitoring applications include: Mercury Science Inc. for domoic acid (DA)(not approved, used for monitoring), Jellett Rapid Testing, Ltd. for PSP and DA (new extraction protocol; threshold), microcystin can be detected through protein phosphatase inhibition (PPI) and the enzyme-linked immunosorbent assay (ELISA).

Research applications include: Receptor binding for PSP/DA, Mercury Science, Inc., competitive-ELISA for DA, surface plasmon resonance (SPR) for DA, LC/MS, Cigua-Check, and Abraxis, Inc. for PSP, yessotoxin (YTX), Jellett Rapid Testing, Ltd. for DSP, and Abraxis, Inc. and LC/MS for microcystins.

- The topic of current needs for species detection was divided into the same three categories:

  Regulatory applications include: Microcystis cell counts (drinking water and recreational waters).

  Monitoring applications include: net tows and whole water sampling (majority from shore-based stations); sample analysis by microscopy (both field scope, laboratory grade microscopes for optical and fluorescence observations); molecular analytical methods: fluorescence in situ hybridization (FISH), sandwich hybridization assay (SHA) PCR/qPCR.

  Research applications and technologies in use include: geographic range, autecology, physiology of toxin production, food web interactions, SPR, Flowcam, laser in situ scattering and transmissometer (LISST), environmental sample processor (ESP), remote sensing, predictive modeling, toxins and species from water samples. Integrating molecular methods with "operational oceanography" is critical.

- Challenges to implementation of these applications in routine operations include: sample collection methods, including processing time, integration with observational observation systems, data dissemination/visualization, maintenance, method standardization and intercalibration; reagent production QA/QC; non-radioactive, rapid, reliable, easy-to-use, and cost effective analyses; moving technologies from the lab to the field; commercialization if targeted at a small market; users need to define requirements, consensus building, approval for advisory/regulatory purposes; and leveraging other markets/applications (e.g., HAB species detection combined with existing mandated water quality monitoring). HABMAP (Harmful Algal Bloom Monitoring and Alert Program, SCCWRP 2008) represents a grass roots organization of academic and public sector monitoring groups in California, coordinating efforts to develop a HAB alert network within state and integrating with efforts along the Pacific coast.
- ACT can help focus by: clarifying what technologies /assays are 'shovel-ready' versus earlier stages of development; supporting a regional focus for technology demonstrations; identifying cross-cutting needs by asking "what should be national, regional, and local?"; facilitating more planning that is needed for a possible demonstration, QA/QC; statistics to support credible results; refining documents to include standards; producing protocols that are required ahead of demonstration; assessing what regions are "ready now."

East Coast Group

- The East Coast group identified the following taxa of concern: *Alexandrium*, *Pseudo-nitzschia*, *Karenia*, *Karlodinium*, *Pyrodinium*, and cyanobacteria.

- The following were identified as technologies or methodologies to measure or concentrate species or toxins of interest in this region: microscopy, FISH, SHA, PCR, mouse bioassay, ELISA, and SPATT (solid phase absorption toxin tracking, used for in situ toxin collection) resins.

Gulf of Mexico Group

- The Gulf of Mexico group identified the following taxa of concern: *Karenia*, *Pseudo-nitzschia*, *Dinophysis*, *Gambierdiscus*, *Alexandrium*, *Pyrodinium bahamense*, *Karlodinium*.
- The main challenge is that the toxicity is not known for the multiple species of *Karenia*. Most information is regarding *Karenia brevis* but others are unknown. There are potentially new species with unknown toxicities.
- General challenges related to methodological issues: cell counts, volume sampled, sensitivity (what level needed?) or precision of counts, access to reference culture strains, toxin analyses needed to detect 5x under regulatory level, reliable supply of calibrated standards, challenge of a 4D environment (time, nearshore, offshore, depth) to the interpretation of point-based monitoring data.
- Bias in sampling methods.
- Challenges of commercialization: identify user needs and market.
- Need a good market analysis. There may be a potential market in aquaculture.
- HAB-specific: consider developing an instrument with multiple applications/targets; the market could be larger/broader in the Gulf of Mexico.
- Operational challenges: money, practicality of operations (biofouling, collisions, vandalizing), telemetry, servicing, ship time, personnel, data management, product development (for public or scientists) --- packaging of data.
- Longevity of reagents is a consideration in servicing of the instruments. Need to have a small number of operational instruments because of the weekly need to service these instruments. The alternative is to keep a maintenance staff to devote time to regular cleaning and calibrating.

Freshwater Group

- The freshwater group identified *Microcystis aeruginosa* as the most commonly encountered HAB. This is a well studied species in the Great Lakes region forming massive blooms in summer.
- Many states that have a monitoring program use a tiered approach with cell and toxin monitoring but are personnel-intensive.
- No sensors are currently available to do a *reliable job* of measuring microcystins.

- The main challenge is in sampling; there can be lots of variability with depth.
- There is a need for chronic long-term effects research for human health concerns.
- Challenge of commercialization is the lack of regulation in drinking water plants.
- Future needs include research on a biomarker for human exposure.
- More information is needed on existing technologies; lots of technologies exist that are unknown.
- Given the widespread threat to drinking water supplies, a workshop on cyanobacteria and their toxins is justified

Workshop Recommendations

The following is a summary of the recommendations that came out of the closing plenary session where workshop participants were asked to make suggestions on how to develop strategies for the commercialization and transition to operations of new technologies/ methodologies and build consensus on approaches of the ACT Demonstration activities. The first discussion resulted in a list of community-wide recommendations that were relevant in all geographical regions. Priorities were then categorized by region.

One obstacle to commercial development of HAB sensors is the small size and diversity of the market. In order to create a better market for HAB sensing technologies, the community can begin by taking advantage of existing product designs in related environmental assessment technologies (drinking water & water quality standards, bioterrorism, hypoxia issues, human health, biomedical). This may distribute the cost for research and implementation, drive and foster development, and create a wider market for HAB sensors. Conversely, HAB sensing technologies that can be used for broader applications (drinking water and water quality issues, bioterrorism, hypoxia, human health, biomedical, environmental public relations, recreational users) can be emphasized to attract interest by local governments and regulators. If local governments can see an immediate benefit of a near real-time "beach condition" report gained from hand-held devices that measure indicators, perhaps they can stimulate fiscal support. By taking advantage of a HAB sensing technologies' broader market, perhaps other sources of funding for development and engineering may step up (SAIC, SRI, NASA).

A high priority was the development of real time HAB sensors, either for deployment in the water as part of an observing system or for use by managers in the field to test water or animal tissues. Currently, there are broader market applications for easy-to-use handheld devices that can detect a suite of pathogens, such as the dipstick devices to detect fecal indicators required for beach warnings and shellfish harvesting closure applications. However, shellfish closures and beach health depend on much more than just a HAB. If manufacturers want to develop a detection technology for a rapid assessment, the technology should encompass the broader suite of potential pathogens. Some possibilities that have already shown promise are the waveguide, field portable surface plasmon resonance sensors (SPR), fiber optics, and handheld nucleic acid analyzer based on NASBA or electrochemical detection. Sample preparation may be the largest hurdle when it comes to real-time measurements of HA and their toxins in the field. Although there are some technologies that perform sample preparation in the field (e.g., Autonomous Microbial Genosensor, Environmental Sample Processor), technology itself cannot substitute for good resources and the required infrastructure to effectively disseminate both alert and normal condition information to the public.

The disconnect between the end users and the manufacturers was a common thread throughout all regions. Industry representatives need specific functional requirements and specifications from the end users to move forward. The question was asked "Does the technology have to be cheap if it does exactly what you want it to do?" in response to the request, frequently heard at ACT workshops for design of 'accurate, portable, faster and cheaper' technologies. Everyone agreed that there is not an easy answer to this question since costs will ultimately limit extent of coverage and would be further confounded in the case of HA since each organism and toxin may have to be sampled differently for detection or identification. It was suggested that a subcommittee be formed to develop these specifications which although likely to vary regionally, could serve to identify cross-cutting specifications.

As in most discussions of sensor technologies, the workshop participants as a whole agreed that there needs to be reliable support for development of community-derived standards that are rigorous enough to accommodate a large portion of the end-user group needs. It was recognized that this represents an ambitious recommendation as standardization for each methodology or HA target will be different. For example, cell counts currently are not standardized across laboratories and can be highly variable depending on the counter.

When participants were asked for region-specific recommendations, the focus morphed into summarizing the ongoing projects in the various regions and discussing how existing efforts in these areas can participate in any potential technology or methodology demonstration. Given the diverse targets and geographic scale and limited available funding, workshop participants proposed that ACT narrow its focus and play a more "grass roots" angled role by supporting existing programs working toward integration of HAB observing into regional ocean observing activities and one focusing on the issue methods intercomparison as a critical step towards development of HAB alert networks.

Region-Specific Recommendations

West Coast

- Involve HABMAP along with monitoring groups from OR, WA, and AK. Focus on *Pseudo-nitzschia* spp and *Alexandrium* spp as cosmopolitan species of concern.
- Define and quantify differences in standard methods, and propose a 'Gold Standard" so sharing and comparing data can become easier. The outcome of this effort will be helpful to the regulatory community. Community should make use of the intercomparison exercise conducted in Sweden (Godhe et al., 2007) as the initial design model and utilize traditional optical cell counts as the best reference method (putative Gold Standard) available at this time. This effort can also give ACT and others a sense for how labor intensive an exercise like this can be. Standardization is difficult since the number of cells is dependent on the counter. The test can utilize multiple counters in order to see variation between counts by microscopy and use this variance to set reference levels for methods under comparison.

Flow cytometry could also be used to verify cell counts data from unialgal or low diversity field samples.

- Invest time in identifying robust 'gold standards' useful for referencing field samples for cell counts and toxin levels.
- Standardized nomenclature, as basic as, "What is a HAB?"
- Create a 'standards team' committee, and the standards that are agreed upon may be region-specific.
- Standardize to Lugols Fixative, for reference counts (Godhe et al., 2007).

Gulf Coast

- Involve GOMA 2 year project.
- Only *Karenia* cell detection; best management uses for *Karenia* detection (cells only).
- Association of Official Analytical Chemists (AOAC) International – they certify reliability of toxin methods for a cost.
- Need to distinguish between where toxin is present in water, shellfish, etc.

East Coast

- Need to detect and quantify *Alexandrium* cells; toxicity and cell counts; need to test shellfish.
- Using Utermohl-based cell counts for a standard needs a lot of volume for field sampling.
- Right now (for cyanobacteria), the East coast has a tiered approach where they look at chlorophyll levels, then species, then toxicity.
- For *Alexandrium* detection methods, there needs to be calibration of whole cell counting, FISH, and PCR-based assays.

Great Lakes

- The highest priority for the freshwater community is an *in situ* sensor for microcystin toxins.
- Individual cell counts vs. colonies.
- Toxin easier to tackle.
- Detection of the genes that are responsible for toxin production is also very important and is available as a research tool.

Recommendations for ACT

As the discussion began to shift towards the potential for an ACT Sensor Demonstration, participants stressed the difficulty in performing a comprehensive verification. Would ACT focus their efforts on hand-held sensors, in situ technologies, or laboratory-based methodologies? These questions should necessarily be answered with the goals of resource managers and the IOOS community in mind. Notwithstanding the small community cross section sampled by Workshop events, it was clear from the rigorous discussions during this workshop that the promise of current HA detection technology developments is partly held in check by poor definition of detection criteria requirements. It was therefore felt that intercomparison studies across technology platforms would enable assessment of the degree of equivalency of monitoring data provided by diverse detection systems.

- ACT could address challenges facing conduct of HAB detection technology demonstration and intercomparison studies (including involvement of a statistician and economist) in order to produce useful outcomes.
- ACT could support development of community-defined standards, at least for cell detection applications and required inter-comparison studies. Analogous issues hold for toxin detection following guidance from underway efforts by FDA and AOAC.
- ACT could prioritize sample concentration methods as a workshop topic in the near future.
- At present, ACT can provide more of a support role for West coast and Florida studies. Based on ACT's technology verification experience, it can take a lead in guiding development of QA/QC procedures associated with these efforts. Great Lakes region that has a well defined HA target should also be considered for an ACT support demonstration as funds become available.

It became clear that in the short-term ACT would best serve the HAB community by taking a more supportive role for projects that are already in place to study and compare different technologies.

- The Florida Fish and Wildlife Conservation Commission has received funding to evaluate various optical, molecular, and hybrid detection methods for Karenia against the accepted "cell count" method. The goal of this project is to evaluate how these technologies or methodologies can be integrated into existing sensor platforms.
- As part of a community driven development of a HAB monitoring alert network along coastal California (SCCWRP, 2008) and in support of the development of a west coast regional HAB forecasting system, an inter-comparison of both field and laboratory monitoring practices has been called for to provide a foundation for these efforts. This project is in its planning stage, with the goal of enhancing and ensuring comparability among HA monitoring data from disparate monitoring efforts along the Pacific Coast.
- Already in place in the Great Lakes is the development of an integrated HAB alert system using remote and molecular methods. With emerging technologies and methodologies for detection of the cyanobacterial toxin, microcystin, this region should also be prioritized for cyanobacteria-specific technology evaluations.

To help lay useful foundations for meaningful technology demonstrations, it was recommended that ACT should facilitate formation of regional subcommittees (including statistical and economic expertise) to identify region-specific user requirements (i.e., frequency, reporting metrics, sample matrix) for HAB cell and toxin detection. Understanding regional needs would be invaluable to the manufacturers who need more information on the market value of such sensors. This subcommittee would also discuss the standard methods that are used by different agencies in their region and come to a consensus on community-defined standard(s) to ensure meaningful inter-comparison efforts. ACT could subsequently make use of such reference materials in the development of a formal HA Detection Technology Demonstration as these methods become more accessible.

It was also recognized that disparities at the 'front-end' of HAB and other in situ monitoring technologies, including sample collection and concentration, can have a significant impact on interpretation of field inter-comparison efforts. As a cross cutting issue for interpretation of both biological and chemical monitoring activities, it was recommended that ACT should prioritize sample collection and concentration technologies for a future technology workshop.

Concluding Remarks

The vigorous discussions during this workshop have provided ACT with invaluable guidance on how the program can best focus its efforts to support the implementation of new analytical approaches for detecting cells and / or toxins as useful components of regional HAB monitoring programs. It was highlighted by the workshop participants that, whether from in situ platforms or grab samples, it must be ascertained that these detection systems must provide comparable data of quality needed by the end users. Federal (ECOHAB, CICEET, MERHAB, OHHI) and private (e.g., Packard Foundation) funding of R&D efforts have supported the development of diverse yet effective analytical approaches for cell and/or toxin detection. Rather than force selection of 'winners or losers' and risk stifling future innovations, it was felt that the next steps require broad support for technology intercomparison efforts to guide appropriate use and promote the routine adoption of these HAB detection applications into ocean observing and regional management operations.

As stated in the recent HAB RDDTT report (Dortch et al 2008):

"*An effective HAB observing system will include the transfer or incorporation of novel HAB-specific sensing technologies into operational use. Laboratory-based protocols for species and toxin detection are now routine and efforts to shift these technologies to in-water platforms are in progress. …… Specific mechanisms need to be identified to facilitate the inclusion of emerging sensors into HAB observing systems. In particular, partnerships with organizations such as the Alliance for Coastal Technologies to foster the further development, user training, and third-party evaluation of HAB-specific sensors"*.

The workshop discussions also highlight the substantial challenges for the design and conduct of useful evaluations / intercomparison / demonstrations of HAB detection technologies. In recognition of these challenges, ACT reissued its HAB Detection RFT directing applicants to consider participation in two distinct regional demonstration efforts: The Gulf of Mexico Alliance (GOMA) and the Gulf of Mexico Coastal Ocean Observing System (GCOOS) have combined efforts to create a Gulf of Mexico-wide plan for HAB observing. This effort, HABIOS, is focusing on detection of the dinoflagellate *Karenia brevis* and brevetoxin, and details are being drafted in the HABIOS Plan. On the west US coast, HABMAP (Harmful Algal Bloom Monitoring and Alert Program) is an outcome of collaborations among the California Ocean Science Trust (CA OST), the Southern Coastal Water Research Project (SCCWRP), NOAA-CSCOR, and regional research groups to provide end users with the information necessary to respond to HAB events in a timely manner with correct data and data products. As a key step in developing a robust HA alert network, HABMAP seeks to conduct intercomparison exercises for cell and toxin detection methodologies targeting *Pseudo-nitzschia* spp. and *Alexandrium* spp. as their respective toxins domoic acid and saxitoxins. ACT will be providing personnel and logistical support to both of these critical efforts, and it is anticipated that through cross agency partnering, other regional activities will be supported in future years.

It is widely recognized that many harmful algal taxa are now persistent if not native components of regional phytoplankton communities. As economic pressures mount to further exploit coastal ocean resources for aquaculture, desalination based water supply, energy development, and recreational purposes, the risk of chronic exposure to low levels of phycotoxins also exists in addition to quantifiable impacts of observable HAB events. These considerations alone demonstrate a need for HAB monitoring to become an integral service of the larger ocean observing effort. This can be accomplished by taking advantage of coastal water quality monitoring activities, local resource management, and leveraging the substantial R&D investments that have yielded an array of effective HAB detection technologies. However, some level of interpretation or packaging may need to be in place to assist end users in applying the data correctly. This multi-tiered observational approach, while challenging for data integration, is necessary to obtain practical understanding of HAB event triggers, knowledge required as an underpinning for development of robust HAB forecasting capabilities.

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Appendix A. List of Attendees

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