Workshop Proceedings

GENETIC SENSORS FOR ENVIRONMENTAL WATER QUALITY

St. Petersburg, Florida
January 5-7, 2005

Funded by NOAA's Coastal Services Center through the Alliance for Coastal Technologies (ACT)
An ACT 2005 Workshop Report

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

Genetic Sensors for Environmental Water Quality

St. Petersburg, Florida
January 5-7, 2005

Sponsored by the Alliance for Coastal Technologies (ACT) and NOAA's Center for Coastal Ocean Research in the National Ocean Service.

Hosted by ACT Partner organization the 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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ACT WORKSHOP: GENETIC SENSORS FOR ENVIRONMENTAL WATER QUALITY

EXECUTIVE SUMMARY

The Alliance for Coastal Technologies (ACT) Workshop "Genetic Sensors for Environmental Water Quality" convened in St. Petersburg, Florida, January 5th - 7th, 2005, sponsored by the University of South Florida (USF) College of Marine Science, an ACT partner institution. Participants from various sectors including research/academia, resource managers, and industry, collaborated to foster the exchange of information and ideas on present and future automated genetic sensor technologies for use in coastal monitoring.

Deterioration of water quality is a global issue. Understanding the processes that compromise the quality of surface water, groundwater, and the coastal ocean are important for public health, ecological, economic, and since September 11th, national security reasons. Coastal and estuarine water quality can rapidly deteriorate in response to episodic events or global climatic oscillations that are best monitored in real time. Previous ACT workshops on Biosensors for Harmful Algal Blooms (CBL, March 2002) and on Rapid Identification of Coastal Pathogens (MLML/MBARI, May 2003) recognized that automated genetic sensor technologies held the greatest promise for the detection of harmful algal blooms, water-borne pathogens, and bio-warfare agents in coastal waters.

Development of autonomous genetic sensors for monitoring environmental water quality is advancing on many fronts. Field-deployable sensors that employ a variety of techniques to detect viral and bacterial human pathogens and harmful algal blooms are being tested by several research groups. This workshop explored the present state of these technologies, identified the major impediments to their advancement, and recommended necessary steps to make genetic sensors part of an operational coastal ocean observing system.

Workshop participants established that genetic sensor technologies are effective in monitoring water quality in coastal environments. Advancement in genetic sensor technology depends on the sensor development community to clearly understand the needs of the end-user. The impediments to understanding the users' needs can be resolved if the particular application(s) and problem(s) in question can be identified. Additionally, participants agreed that the genetic sensor design must be flexible and robust, and that the molecular technology used to detect the target must be integrated within the instrument packaging. Participants thoroughly discussed issues with sensor design including: defining potential field targets, defining target choices, deployment issues, biogeochemical factors, software needs, field transfer basics, technology transfer, building a workforce, funding, costs, education/outreach, collaborations/partnerships, and government mandates. Potential solutions addressing several of these issues include: defining needed applications and field targets by funding a market analysis, customizing engineering to
applications by conducting sampling tests/studies, creating a climate for additional funding, establishing partnerships, and encouraging collaborative efforts for overarching science in an educational outreach capacity. Participants also agreed that it is necessary for users to agree on datum and methodological standards that to satisfy requirements as defined by the Integrated Ocean Observing System (IOOS) as well as a Global Ocean Observing System (GOOS). To meet these goals, customization options will need to be integrated for different geographic regions.

**ALLIANCE FOR COASTAL TECHNOLOGIES**

There is widespread agreement that an Integrated Ocean Observing System (IOOS) is required to meet a wide range of the Nation's marine product and information service needs. There also is consensus that the successful implementation of the IOOS will require parallel efforts in instrument development and validation and improvements to technology so that promising new technology will be available to make the transition from research/development to operational status when needed. Thus, the Alliance for Coastal Technologies (ACT) was established as a NOAA-funded partnership of research institutions, state and regional resource managers, and private sector companies interested in developing and applying sensor and sensor platform technologies for monitoring and studying coastal systems. ACT has been designed to serve as:

- An unbiased, third-party testbed for evaluating new and developing coastal sensor and sensor platform technologies,
- A comprehensive data and information clearinghouse on coastal technologies, and
- A forum for capacity building through a series of annual workshops and seminars on specific technologies or topics.

ACT Headquarters is located at the UMCES Chesapeake Biological Laboratory and is staffed by a Director, Chief Scientist, and several support personnel. There are currently seven ACT Partner Institutions around the country with sensor technology expertise, and 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. Finally, a larger body of Alliance Members has been created to provide advice to ACT and will be kept abreast of ACT activities.
ACT Workshop on Genetic Sensors

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 goals are to both help build consensus on the steps needed to develop and adopt useful tools while also facilitating the critical communications between the various groups of technology developers, manufacturers, and users.

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 http://www.act-us.info/.

### GOALS FOR THE GENETIC SENSORS WORKSHOP

The underlying goal of the ACT workshop on Genetic Sensors for Environmental Water Quality was to explore present and future genetic sensor technologies for environmental water quality measurement and identify the steps necessary to incorporate them into an operational coastal ocean observing system. Specifically, the participants were charged with the following tasks:

1) Summarize the current state of genetic sensors for water quality observation.

2) Discuss actions needed to incorporate genetic sensor technologies as part of an operational observing system.

3) Define major obstacles to furthering genetic sensor technologies and suggest achievable actions to overcome these impediments.

### ORGANIZATION OF THE GENETIC SENSORS WORKSHOP

The workshop's organizing committee included Drs. Steve Weisberg (Southern California Coastal Water Resources Program [SCCWRP]) and Mark Luther (USF), and Mr. Roger Schaller (Cepheid). Participants included researchers, federal/state/regional environmental managers, and industrial representatives interested in the development and implementation of genetic sensors for
water quality applications. A list of participants is included at the end of these workshop proceedings.

The two and a half day workshop commenced on the evening of January 5th, 2005, with Dr. Mark Luther summarizing ACT's missions and goals to the invited participants. The following morning opened with a formal presentation by the workshop coordinator, Dr. John Paul, summarizing the status of genetic sensors technologies. Participants were divided into three groups for breakout sessions. The first breakout session was based on each participant's background: research/academia, resource managers, and industrial representatives. The purpose of this was to facilitate focused discussions on the common issues in each sector regarding the charges of the workshop. After lunch, the groups reconvened and a chair from each group provided a summary of the group's findings. Participants then separated into three groups, with members of each sector evenly distributed between the groups. Each was charged to revisit the same workshop goals. Afterwards, a chair from each group provided a summary of the group's findings. Specifically, these working groups discussed:

1) Identifying key obstacles for implementing genetic sensor technologies into operational observing systems.

2) Recommend specific, achievable actions needed to overcome these impediments and make genetic sensor technologies a component of operational observing systems.

On January 7th, the workshop concluded with a prioritized ranking of specific action items. After the workshop, Mr. David Fries and Dr. Matthew Smith, from Center for Ocean Technology (COT), provided interested participants a thorough overview of the Autonomous Microbial Genosensor (AMG) and other genetic and non-genetic sensor technologies in development at COT. The workshop-related notes and Dr. Paul's formal presentation are available via the web (http://act.marine.usf.edu/ACT_Workshops.html).

Unattended genetic sensors are needed to monitor microbial populations in situ. These sensors should be able to detect their targets at near ambient concentrations and be able to telemeter data to shore-based receiving stations in near real time. Such sensors should also be able to be deployed for long periods of time with minimal service. At this time, these goals for genetic sensors have yet to be realized.

One approach to the development of autonomous sensors is to begin by developing hand-held sensors, which still require human involvement to operate (a microbial ecologist's "Tricorder"). Several groups have developed such sensors based upon nucleic acid amplification, electrochemical nucleic acid detection technology, and other approaches. These sensors have
sample processing steps performed by operator intervention, but the detection modules can be identical or similar for an autonomous mooring. There are four key components to genetic sensors. Genetic sensors must 1) efficiently sample for their target organism 2) extract nucleic acids efficiently 3) detect and, ideally, quantify the target genes and 4) store or telemeter data. Each one of these issues will be discussed below.

To efficiently sample a target organism, it is imperative to know the abundance of the organism in the environment to be sampled. For example, sampling for human enteroviruses in certain highly impacted marine environments requires only minor sample concentration, whereas in most estuaries in North America, it is commonly necessary to filter hundreds to thousands of liters. The need for environmental abundance data for a target organism is also determined in part by the sensitivity of the assay. For detection of the Florida Red Tide organism, *Karenia brevis*, it may only require sampling tens of milliliters. If filtration is to be used, the efficiency of the filtration process also needs to be known. Chris Scholin of MBARI has put forth the concept of hierarchical sampling; *i.e.*, all samples are put through a general genetic test to see if a particular group of organisms is present. If that particular group of organisms is found, then a more exhaustive or specific test is employed to detect the organism of interest.

For nucleic acid extraction from the collected sample, it must be determined if simple cell lysis or permeabilization will suffice, or if nucleic acid purification is necessary. The former are often sufficient for nucleic acid hybridization while the latter is usually required for amplification.

The detection technology may require direct hybridization to probes and detection by fluorescence, chemiluminescence, or electrochemical detection. Although these methods are usually not as sensitive as amplification, there are advantages because of less specificity/selectivity. As with amplification techniques, the sensitivity/specificity of hybridization is dependent on probe design and the stringency of the hybridization conditions. Macroarrays and microarray technology show promise for incorporation into sensors that use hybridization. However, there has been some concern about the lack of sensitivity that traditional array approaches possess. Advantages of amplification are sensitivity, and if required, specificity (differentiating closely related species). Polymerase chain reaction (PCR) and Nucleic Acid Sequence-Based Amplification (NASBA) are the major amplification strategies employed. Usually the target product is detected by an internal fluorescent probe. Disadvantages of amplification are the requirement for enzymes in the sensor, the need for fairly rigorously purified nucleic acids, and the limited number of targets that can be assayed simultaneously. Other potential detection strategies include Luminex technology and the US Genomics Gene Engine platform, although these have not been examined in respect to autonomous sensors.

Current genetic sensors developed to date include the Environmental Sample Processor (ESP) of the MBARI group under the direction of Chris Scholin and its successor, the ESP II, as well as the Autonomous Microbial Genosensor (AMG) under development by the USF College of Marine Science and Center for Ocean Technology (Figures 1 and 2). The ESP collects samples onto filters that have specific probes immobilized on them. After a microbial community is filtered, detection of target organisms is performed by chemiluminescent probing and imaged by
a CCD camera. This format enables detection of cell lysates or intact cells. When intact cells are used, the filters can be archived for retrieval and examination by microscopy.

A second generation ESP (ESPII) is currently under development by the MBARI group. For more information on the ESP see http://www.mbari.org/microbial/ESP/. The AMG uses filtration and RNA extraction prior to amplification by NASBA and detection by hybridization with fluorescent Molecular Beacons. NASBA is an amplification process that starts with RNA and produces an RNA product. Thus, this approach has the potential to detect transcriptionally active target genes, implicit that the organism detected was alive and metabolically active, or recently so. The NASBA reaction is isothermal, and does not require thermal cycling as does PCR. The detection module need only be a thermostable fluorometer. For more information on the AMG see http://www.marine.usf.edu/microbiology/sensor-research-main.shtml and www.marine.usf.edu/systems.
I. Current Status

The molecular methods employed by genetic sensor technologies are robust. These methods can be adapted to a variety of targets, such as bacteria, dinoflagellates, viruses, toxin genes, and source tracking markers. However, to best advance the next steps in genetic sensor technology, the particular application(s) and problem(s) in question should be identified, so that the sensor development community can develop a clear understanding of the users' needs. This will also help with the identification of potential markets for commercial interest. Identification of commercial interest is necessary in order to leverage funding for sensor production. The use and controls for the application need to be defined to ensure accuracy and performance of the developed technology. The process of identifying applications of greatest need will, in turn, help identify what is required for sampling protocols to address issues including, but not limited to, knowing the target organisms and concentration ranges, patchiness, temporal variability, and the physical dynamics of the surf zone (e.g., currents, waves, and turbidity). Understanding such parameters is required to define deployment protocols (in both time and space) and establish instrument design. Data concerns also should be defined and adapted to the user's needs (e.g., rate and frequency required). In addition, optimizing the molecular methods themselves differs between targets with regard to issues such as required sample volume, sampling efficiency, sensitivity, rates of false positives/negatives, and rapidity. Overall, defining the problem in question helps define the operational needs of the sensors, and input from the overarching scientific community is needed. However, some applications still have basic science questions that need to be answered in order to best guide the sensor development community. For example, the public health sector is in need of epidemiological studies to address non-point and non-human sources of pollution and also to address the use of indicator organisms versus pathogen detection, particularly for areas that show re-growth of indicator organisms. Sensor development should be done in concert with efforts for guidance and verification of design.

II. Sensor Design

The molecular technology used to detect the target must be integrated into an instrument package. Overall the system is comprised of several components or layers that must be integrated:

1) Sample filtration/concentration
2) Nucleic acid processing
3) Nucleic acid detection

Overall, the design must be flexible and robust, specifically regarding fluidics, data channels, adaptability and configurability.
The first layer of the system, upstream sample processing, is a critical component. Concentrating rare targets was identified as the largest technical impediment to pathogen detection. For example, virus detection with present methods can require filtration of several hundred liters of water depending on the source of water. Moreover, during this process numerous other organisms and organic/inorganic molecules are included in that sample, some block filters and tubes, and others cause unintended reactions with several of the reagents. Additionally, many non-target organisms interfere with the detection of the pathogens. Pre-concentration needs pose challenges with regard to engineering, power requirements, and concentration of molecular biological inhibitors. Cross-contamination and over-loading also need to be avoided. In addition, dynamic sampling capability is needed so that a range of volumes can be sampled in order to detect targets present at varying concentrations (e.g., feedback mechanism to sample more or less volume in order to stay within the dynamic range of the molecular assay). Other sampling concerns need to address patchiness, temporal variability, and depth profiles. Engineering designs also should include the ability for high frequency detection.

The second layer, nucleic acid processing, should be designed to maximize sensitivity and minimize sample preparatory steps, again to avoid cross-contamination.

The third layer is the genetic sensor. A mobile, stand-alone sensor that can be plugged into a sensor package would be convenient. Many present sensor designs include disposable parts. A regenerable sensor, would have advantages and is currently being considered. At present, regeneration is often achieved chemically, and the practicality of this needs to be investigated.

The genetic sensor design is dependent upon the market and user needs modulated by the state of technology development. For example, "rapid" sampling depends on the end-users audience and application. Rapid for water quality managers has been defined as "less than four hours" and this is a realistic goal for genetic sensors. However, real-time in the sense of data per minute is not a realistic goal. Once the application has been defined, the target organism and the sensitivity required for detection can be determined. For example, greater sensitivity is needed for non-amplification methods. Sensitivity issues tie into sample preparations such as target separation (e.g. size fractionation), pre-concentration processes, and nucleic acid extraction and purification. Pre-concentration processes are a concern, for they consume large quantities of time and power. Attention needs to be focused in this area.

III. Needs and Potential Impediments for Operational Autonomous Genetic Sensing

This workshop resulted in a collaborative brainstorming among the genetic sensor developers and engineers, end-users, stakeholders, and environmental researchers resulting in a list of needs or concerns to be incorporated into future designs of genetic sensors. One aim is to provide standards among users, satisfying requirements as defined by the IOOS as well as a global OOS. To meet these goals, customization options will need to be integrated for geographic regions.
Define Potential Field Targets
Defining which target(s) is best to use is a matter of debate within the overarching science of water quality monitoring. For most applications, sensors must deliver quantitative detection to be useful to users. In addition, the ability to detect multiple targets simultaneously is highly desirable in the sensor design. The total maximum daily loads (TMDL) definition is unfolding as a model and is a motivating need, particularly for source tracking. Genetic sensors are a potential solution for water quality monitoring to protect human health. For example, sensors could eliminate the burden of needing to transport samples back to the lab before the hold time is exceeded. However, issues of quantification, viability, and sensitivity need to be addressed. The workshop identified several potential field targets that are presently used or predicted for use for different applications:

1) Enterococcus
2) Bacteroides
3) Fecal coliform
4) E. coli
5) Viruses -- e.g., norovirus, Norwalk virus, any human virus? (Norwalk-like virus are noroviruses that have sickened many passengers on cruise ships and hotels (visit www.cdc.gov website for more information)
6) Salmonella
7) Campylobacter

Questions Surrounding Target Choice
Because sensor development is guided by target choice, there was general discussion regarding targets. Topics discussed included the following:

1. Risk assessment partners are needed. Sensor developers and end-users need additional input (e.g., EPA, epidemiological data) to make informed choices about the best targets to choose for monitoring. For example, should we measure pathogens vs. indicators? Does the presence of Enterococcus mean sewer failure? What are the issues and concerns regarding the use of indicators in tropical waters?

2. A major impediment to being able to choose appropriate targets is the state of public health science. Where will the funding come from to do the work that needs to be done?

3. What is the best approach to take with regard to choosing targets to protect public health - restrict to present regulations or expand to what the future may look like? We now look at measurements with respect to present regulations, but is this the best approach? Perhaps we need to expand the approach to what is useful or will be useful in the field. For example, end-users need to be able to source track spatially while in the field, so that they can find the physical origin of a contamination source (e.g., boat, tree, dock). The requirements to achieve that define how the genetic sensor needs to operate.

4. Shell fish safety could benefit from genetic sensors, however, the standards seem complex - e.g., what if detect pathogens (zero tolerance) closure requirements are more stringent than if detect by the indicators?
5. Fisheries science could also benefit from sensors designed to monitor fish larvae. Fisheries partners are needed. Again, where will funding come from for the science to be done to guide the sensor development?

**Deployment Issues**
The physical dynamics within the surf zone, such as waves, currents, and turbid conditions, could negatively impact the sensor both its housing as well as sampling capacity. Additionally, due to the instrument's placement, it could be vulnerable to bird excretions and corrosion which can contaminate the instrument or damage its housing. In addition to environmental factors and sampling design needs, there is concern that sensors would need to be placed in areas in which they would be prone to theft or vandalism.

**Biogeochemical Factors**
Biogeochemical factors including humics and oils could contaminate the sampling process. Specifically, variable ion exchange between ocean waters and sediments and suspended particles can influence microbial growth and heterogeneous media, *i.e.* ocean waters and solids, such as suspended particulates, sediments, rocks, man-made structures, create a complex environment in which the microbial community grows and thus, could alter levels of interference with reagents/assays. Harmful algal blooms (HAB) are usually followed by the retention of toxins within living shellfish, for example, with little free toxin in the ocean waters. This time-varying, highly non-uniform distribution of pathogenic biological materials presents major challenges to reliable sampling and analysis. Bio-fouling issues must be addressed and preferably resolved by developing a method to retard or prevent growth. A resolution for this problem would be invaluable to the instrument design and eliminate most issues associated with biological contamination.

**Software Needs**
Data collected by a genetic sensor must comply with the user's needs and must be regulated, standardized, and quality controlled and quality assured. Adaptive and evolvable software system designs must include flexibility in its techniques, tools, and applications. Additionally, software engineering support must be available. A workforce to support the above mentioned criteria should be established.

**Field Transfer Basics**
The impetus for designing an automated genetic sensor is to integrate numerous manually performed laboratory functions into a workable package that can survive field conditions. Several basic requirements that need consideration include the instrument's power source, size and weight. Available options for powering the instrument include using batteries, fuel cells, or solar technologies. Quality controlled regulations will need to be established regarding storing and metering of reagents. Consequently, addressing this issue will require more support and research.

**Technology Transfer**
There is a large need to formally deal with the many issues surrounding technology transfer. Issues include intellectual property (IP) issues, mass production of the designed instrument, and cultural differences (e.g., researchers, engineers, end-users, industry). There needs to be industry
support for cost-effective and efficient design and testing. In turn, the industry needs to understand what problem the scientist is trying to solve. Specifically, industry needs to understand the role of the target. Targets tailored to the market have a better chance of acquiring support. Funding agencies need to be engaged to help solve cultural issues. For example, academic standards associated with the grant award process stress prolific publications, and a parallel exists between the number of publications and the number of grants awarded. This standard is not compatible with the realistic time frame of engineering an instrument, and it sets up a culture clash. Instead, grant agencies could extend the time-frame of defining success and shift focus to production of an operational product rather than the number of publications per year. Additionally, evaluation of grants should be linked with current public issues which would support and promote the drive for advancing technology.

Establish the Workforce
This endeavor requires development of a workforce at all levels. In order to complete design, there must be a meeting of molecular biologists and engineers. Once the genetic sensor design is developed and mass produced, an additional workforce must be established on all levels - local, federal, governmental, and global. Staff with various levels of experience will need to be placed together and trained on this technology. Incorporating this technology into a usable application for IOOS will also require trained technicians and an educated workforce. Lab scientists and/or field technicians would assist in deployments. Educated staff would be needed to archive, process, and ensure QA/QC of the data. This workforce must have the capacity to troubleshoot and promote connectivity among user and developer.

Funding Needed
Overcoming the various impediments to allow genetic sensors to become operational will require further research on various fronts and equally challenging, gaining financial and political support behind the endeavor. Groups interested in data collected or products obtained from the genetic sensor analyses need to be targeted. Public involvement can supply a push for funding and implementation of the sensors. For example, non-governmental organizations (NGO) and citizens can promote the positive aspects of this technology, such as increased rapid monitoring to protect human health. This support would allow for more rapid response to be available for biological emergencies such as sewer overflows, containment facility breaks, and acts of bio-terrorism.

Evaluate Costs
What is low in cost and deemed acceptable depends on the market definition. Currently, $10K is considered acceptable or standard for oceanographic instrumentation, and this appears to be a realistic goal for an off-the-shelf autonomous genetic sensor. However, this does not include the cost of sustaining the sensor. Assessment of funding needs should include the costs of developmental science, employment, consumables, maintenance, and workforce.

Education and Outreach
The scientific community involved with genetic sensors needs to collaborate and develop methods aimed at assisting and educating its applications to all groups (stakeholders, congress, lobbyists, community, etc.). Content should focus on the purpose and benefits coming from the data such as economic benefits and quality of life. Science and data products need to be clearly
explained. Education outreach will result in promoting awareness by gaining support, interest, and involvement from the targeted audiences.

Need for Collaborations/Partnerships
In order to move this technology further, stakeholders must be involved (e.g. National Bureau of Standards (NBS), National Institute of Standards and Technology (NIST), and the International Organization for Standardization (ISO)). Congressional support and lobbyists need to come together and promote this utility. Collaborations and partnerships should be built between industry-academia, biology-engineering, agencies-scientists/technicians/resource managers.

Need for Government Mandates
New legislation should push forward to support genetic sensor technology by promoting networking with regards to promoting research, development, implementation, and deployment. Responsibilities need to be defined locally, regionally, and globally. NGOs should be involved, holding both beach water and general recreational water to higher standards and preventing governmental agencies from becoming complacent on these important issues.

IV. Possible Solutions

The following items were identified as possible solutions to address some of the needs and impediments outlined above.

Define Needed Applications and Field Targets
- Fund a market analysis with the possible following players: IOOS, ACT, NOAA, American Water Works Association (AWWA), American Water Works Association Research Foundation (AWWARF), Environmental Protection Agency (EPA), and National Science Foundation (NSF)

Customize Engineering to Applications
- Acquire funding to conduct sampling tests/studies related to adaptive sampling, sensitivity, environmental variability, etc.

Create a Climate for Additional Funding
- Define the positives of autonomous genetic sensors and the data products. Communicate this information to various groups in order to draw in support, involvement, awareness, and funding.

Support Efforts for Overarching Science
- Establish partnerships and aid/encourage funding of projects that address questions regarding appropriate field targets, epidemiological studies (e.g., tropical climates), source tracking, fish larval needs.

Improve Technology Transfer and Workforce Establishment
- Establish incubators to develop technology transfer.
At the close of the workshop, the participants constructed an itemized list of recommendations that would facilitate advances of genetic sensors for environmental water quality for coastal ocean observing systems. These recommendations were prioritized in order of importance by vote of all participants, with each participant casting five votes.

**General Recommendations:**

1. Establish a working group on sample collection and/or preparation. This working group would be responsible for addressing issues and concerns regarding adaptive sampling, especially for genetic sensors.

2. Facilitate collaborative research to demonstrate relations between measurements (by new technology) and health effects (or other relevant end-points). Research efforts could be in the form of epidemiological studies.

3. Develop multiplex sensors.

4. Fund customer needs survey and/or market analysis for the genetic sensor technology; include cost benefit analysis and ecological evaluations.

5. Develop incubator and/or technology transfer facilities through collaborations with federal / academic / industry as well as the Small Business Innovation Research (SBIR) Program and IP. Groundtruthing is incorporated into this action item.

6. Work with NIST & EPA to develop standards appropriate for new technology.

7. Fund additional research to find new indicator organisms.

8. Develop and implement outreach programs utilizing presentations and education modules for citizens groups, NGO's, etc.

9. Build work group to establish guidelines for regulatory approval.

**Recommendations Specific to ACT:**

- Align ACT with AWWA and outreach to fisheries

- Facilitate collaborations on pilot applications and prototyping for developers and/or end users via the ACT forum. Broad scale indicators and actual pathogens issues and concerns need to be addressed in this utility.
• ACT should fund a survey of resource managers, scientists, and end users modeled after its existing customer needs assessment survey.

ACKNOWLEDGMENTS

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