APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

☐ Pre-application ☑ Application
☐ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION *** Organizational DUNS:** 1783417720000

* Legal Name: Monterey Bay Aquarium Research Institute

Department: | Division:

* Street1: 7700 Sandholdt Road | Street2:

* City: Moss Landing | County: | * State: CA: Californ

Province: | * Country: JNITED ST | * ZIP / Postal Code: 95039

Person to be contacted on matters involving this application

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	Chris		Scholin	

* Phone Number: 831 775 1700 | Fax Number: | Email:

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):*

77 0150580

7. * TYPE OF APPLICANT:

M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):

8. * TYPE OF APPLICATION: ☑ New

☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Small Business Organization Type

☐ Women Owned ☐ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration

☐ D. Decrease Duration ☐ E. Other *(specify)*:

9. * NAME OF FEDERAL AGENCY:

Office of Naval Research

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

12.300

TITLE: Basic and Applied Scientific Research

* Is this application being submitted to other agencies? Yes ☐ No ☑

What other Agencies?

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployment, Technoloyg Transfer, Commercial Eva

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*

ME, NH, MA, RI, CT and CA

13. PROPOSED PROJECT:

* Start Date	* Ending Date
09/01/2008	08/31/2011

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant	b. * Project
CA-17	CA-17

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

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OMB Number: 4040-0001

Expiration Date: 04/30/2008

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding	1,527,404.00
b. * Total Federal & Non-Federal Funds	1,527,404.00
c. * Estimated Program Income	0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☐ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☑ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☑ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

19. Authorized Representative

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
	Patrice	A	Carroll	

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* Email:	grants@mbari.org		

*** Signature of Authorized Representative**
Completed on submission to Grants.gov

*** Date Signed**
Completed on submission to Grants.gov

20. Pre-application Add Attachment | Delete Attachment | View Attachment

21. Attach an additional list of Project Congressional Districts if needed.

SCHOLIN NOPP Congressional Districts.c | Add Attachment | Delete Attachment | View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Monterey Bay Aquarium Research Institute
CFDA: 12.300 NOPP
BAA: ONR-BAA-07-040
PI: Scholin

AREAS AFFECTED BY PROJECT

Monterey Bay, California and New England Coastal States

State	Congressional District
Maine	**1, 2**
New Hampshire	**1**
Massachusetts	**3,4,6,7,8,9,10**
Rhode Island	**1,2**
Connecticut	**2,3,4**
California	17

Mbari cover page

Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployments, Technology Transfer, and Commercial Evaluation

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Volume 1: Technical Proposal

A. Project Summary

This proposal is in response to ONR-BAA-07-040 Topic 3A and 3B.2. Specifically we aim to:

- Construct two copies of the Environmental Sample Processor (ESP) and transfer those instruments to federal and academic partners.
- Demonstrate *in situ* detection of harmful algal bloom (HAB) species and toxins in estuarine and open ocean regimes of the northwest Atlantic.
- Integrate data products derived from the ESP into the Gulf of Maine regional HAB monitoring network to enhance time series measurements and predictive capabilities for forecasting HAB events.
- Explore options for commercializing the ESP technology in collaboration with McLane Research Laboratories Inc. (McLane; http://www.mclanelabs.com/) and Applied Biosytems Inc. (ABI; http://www.appliedbiosystems.com/).

The ESP (www.mbari.org/esp/) is a novel device for conducting molecular biological analyses of organisms and their metabolites in real-time below the ocean surface. We seek support to transition this new instrument from its current stage of development toward a commercially viable status. We propose to build copies of the instrument and dedicate them to the NOAA/NOS Marine Biotoxins Program (Doucette et al.) and to the Woods Hole Oceanographic Institution (WHOI; Anderson et al.) for the duration of this study. The ESP transferred to NOAA/NOS will be dedicated to laboratory-based refinement and calibration assessments of the existing assay for domoic acid (DA; algal toxin), as well as development of new assays for other phycotoxins (saxitoxin, brevetoxin, etc.). The instrument provided to WHOI will be evaluated in both laboratory and field studies with the data assimilated into nowcast/forecast models (Anderson and McGillicuddy et al.). The primary target will be detection of the dinoflagellate, *Alexandrium fundyense* and associated toxins in the Gulf of Maine (GOM).

Alexandrium fundyense is a well-known harmful algal bloom (HAB) species that produces the potent neurotoxin saxitoxin and derivatives, the causative agents responsible for a potentially fatal human illness known as paralytic shellfish poisoning (PSP). PSP is the most widespread of all of the HAB poisoning syndromes, and affects dozens of countries worldwide (Hallegraeff 1993). In the GOM, it is a recurrent and serious problem that annually closes shellfish beds for harvesting along vast expanses of coastline, on occasion causing damages in excess of $50M from a single outbreak (Anderson et al. 2005c). Extensive laboratory, field, and modeling work to date (e.g. Anderson et al. 2005a, b) provides an excellent model system in which to deploy ESPs in key locations in ways that can augment ongoing and planned PSP monitoring and forecasting capabilities.

This project provides a unique opportunity to evaluate the utility of the ESP and affiliated molecular assays in both field and laboratory settings by groups who are not part of the instrument development team. So doing we will transfer ESP technology and its underlying detection chemistries from MBARI to a number of potential user groups, thereby providing new opportunities for validating the utility of the instrument in a variety of operational settings. **Such demonstrations are essential if commercialization of this new technology is to be successful**.

B. Statement of Work

B.1a *Project overview and rationale.* Here we propose a project and partnership that will extend in-water application of the Environmental Sample Processor (ESP) by facilitating deployment validation studies in two different coastal environments, both of which have

relevance to harmful algal bloom (HAB) management and to water quality testing in general. In addition to open-ocean applications, the instrument will be configured for shore-side deployments where there are potential applications for HAB monitoring, water quality assessments, and other programs where frequent, automated measurements of organisms and chemical compounds are needed for regulatory purposes. The instrument's capabilities to detect HAB toxins will be expanded as well, and field deployments will be made to fully evaluate the reliability, sensitivity, specificity, and overall capability of the ESP to contribute to HAB monitoring and forecasting programs. Throughout this process, commercial partners will evaluate the instrument and associated detection chemistries and move toward commercialization of this technology. We note that this specific project is focused on HAB cell and toxin detection, but emphasize that the approaches and techniques being developed are applicable to many other pathogens, organisms, and chemicals of interest to regulators, managers, and scientists. The Gulf of Maine (GOM) is an excellent proving ground for this instrument given the long history of an important HAB poisoning syndrome in the region (see Mercer and Mickelson support letters), with annually recurrent, well-understood outbreaks that allow us to deploy instruments in key locations/times to ensure toxic cells will be encountered. Furthermore, the GOM will be the site of a NOS-sponsored operational HAB forecasting system slated to begin in 2009 – a system that would be greatly augmented with real-time observations of *Alexandrium* cell abundance and toxicity (see Stumpf support letter). The on-shore, shallow water location was selected because it also is a site with annually recurrent, toxic *Alexandrium* blooms, and is part of an estuarine system that has significant water quality monitoring needs as well (see Hickey support letter).

B.1b *Development and current status of the ESP system.* The application of molecular analytical techniques is pervasive in the ocean sciences, yet the vast majority of work typically occurs in a shore-based laboratory after return of a discrete set of samples collected at sea. The delayed analysis may be too late for critical resource management decisions that rely on those measurements to protect the public health. For example, timely detection of HABs would require an immediate action such as increased shellfish monitoring and immediate closure of the resource for harvesting. Likewise, the forecasting systems that are under development will require real-time data that can be assimilated into model runs to improve accuracy and predictive skill. The ESP was designed with these issues in mind by allowing on-site (*in situ*) collection and analysis of water samples and wireless transmission of data from natural environments.

Figure 1. Second generation ESP instrument (16"d x 30"h). bottom left: puck magnified ~2x relative to those loaded in the carousel. See also: http://www.mbari.org/ESP/esp2G_compare.htm

The instrument is an electromechanical fluidic system designed to collect discrete water samples, concentrate microorganisms or particles, and automate application of molecular probe technologies. It can be configured to identify microorganisms and their gene products (e.g., metabolites). Results from both laboratory- and field-deployed ESP experiments demonstrate that this approach can be used to detect species or groups of species, ranging from bacterioplankton, harmful algae and their toxins, and invertebrate larvae using a common sample collection and processing protocol (e.g., Paul et al. 2007, Goffredi et al. 2006, Greenfield et al., 2006, Jones et al. in press).

Early work sponsored by MBARI centered on the "first

generation" (1G) ESP. Based on lessons learned from that work, a "second generation" (2G) ESP (Fig. 1) was built under the auspices of a NOPP/NSF grant (NSF OCE-314222) to advance the system towards use by the oceanographic community. We now seek funds to transition this instrument towards operational use and commercial development.

The 2G ESP consists of three major components: the core sample processor ("core ESP"), analytical modules and sampling modules (Scholin et al. 2006). The core ESP is designed to handle small- to moderate-sized samples (ml's to several l's) at depths to 50m. "Analytical modules" are conceived of as stand-alone molecular detection systems that can be added to or removed from the core ESP to impart different suites of analytical functions downstream of common sample processing operations (e.g., PCR, capillary electrophoresis, microbead arrays, etc.). "Sampling modules" are devices external to the core ESP that can be added to meet specialized needs such as operating in the deep-sea. This proposal focuses on the core ESP for use in the laboratory and near surface waters only.

The ESP utilizes DNA probe and protein arrays to detect target molecules indicative of species and the substances they produce. Within the core ESP, DNA probe arrays specific for target species capture ribosomal RNA (rRNA) sequences from a crude sample homogenate using a sandwich hybridization assay (SHA; e.g., Fig. 2). The protein arrays utilize a competitive ELISA (cELISA) technique for detecting target substances such as DA (e.g., Fig. 3). The ESP can also archive samples for laboratory analyses after the instrument is recovered, including fluorescent *in situ* hybridization (FISH), various nucleic acid analyses (cloning, sequencing) and algal toxin measurement (e.g., Greenfield et al. 2006).

The instrument executes user-defined scripts that specify a sequence of steps to accomplish high-level tasks such as collecting a sample and generating a lysate, developing a probe array, collecting and archiving a sample, or flushing the system. Sample manipulations are carried out in reaction chambers, called "pucks" that are loaded into and removed from various stations by robotic mechanisms. The automated process from collection of a live sample to broadcast of an imaged DNA or protein probe array takes ~2 hours and can occur subsurface. The system operates on 12V (nominal). A complete sample processing cycle, from collecting a sample for developing a probe array, archiving a sample and broadcasting results of the assay, consumes ~30Whr. The instrument can perform ~30-40 of those operations before servicing is required[1].

The instrument is bundled with contextual sensors such as a CTD/fluorometer/transmissometer and ISUS (http://www.mbari.org/chemsensor/ISUShome.htm). Data from the external sensors along with results of the probe assays are uploaded periodically from the deployed instrument to a shore station for analysis and interpretation. Two-way communication allows for rescheduling of mission sampling profiles if desired. We currently operate the ESP using either lead acid rechargeable batteries, or stacks of primary alkaline D cells. We have successfully tested the ESP under laboratory conditions with ambient temperatures ranging from 4-25°C. Further details on the instrument's design and operation are described elsewhere (Greenfield et al. 2006, Scholin et al. 2006, Roman et al. 2007, Paul et al 2007; see also http://www.mbari.org/esp).

[1] The current limitation is the number of pucks stored in the carousel. There are many options for increasing the number of sampling/analytical events and decreasing power consumption that are beyond the scope of this proposal.

Field testing of the 2G ESP began in 2006. Since that time we have deployed the device seven times in surface waters for periods of several weeks to a month. In addition, the core ESP used in surface waters has undergone two field campaigns to 1000m, with each campaign composed of three dives aboard an ROV[2]. All of these deployments were in Monterey Bay, CA. While moored in shallow water, the core ESP has successfully automated application of three different classes of DNA probe arrays (HABs, bacteria/archaea, invertebrate larvae) and the DA assay in single deployments lasting up to 30 days (e.g., Figs. 2, 3). The instrument was programmed to carry out assays at prescribed dates/times, and contextual sensors were programmed to sample several times per hour. Manuscripts that document these milestones are in preparation.

[2] This project is sponsored primarily by NASA with the aim of developing the ESP system for year-long deployments at cold seep and hot vents to study the changes in microbial community structure in response to changes in seismic activity, fluid flow, and water chemistry (etc.).

B.1c *Specificity, sensitivity, and calibration of the ESP.* A major focus of our efforts has been the simultaneous detection of various organisms in a single sample utilizing rRNA-targeted DNA probe array chemistry (Greenfield et al. 2006, and refs. therein). Probes are used in both a 96-well plate (benchtop) and flow through membrane formats. The SHA employs two probes: capture and signal. Capture probes are immobilized onto a solid support and capture target sequences from solution. After washing, one or more signal probes are applied, and then colorimetric or chemiluminescent reporting ensues. To date we have devised rRNA-targeted probes for a variety of harmful and toxic algae, including species of *Alexandrium*, *Pseudo-nitzschia*, *Heterosigma*, *Chattonella*, *Fibrocapsa*, *Karenia*, *Karlodinium* and *Cochlodinium* (e.g., Scholin et al. 2003, Haywood et al. in press, Mikulski et al. in press). In New Zealand, both the FISH and SHA methods have gained international accreditation and are used to regulate shellfish harvests (e.g., Ayers et al. 2005 and refs. therein). Recent progress made on assays for invertebrates (Goffredi et al. 2006), including the invasive European green crab (Jones et al. in press), and marine bacteria (Paul et al. 2007, Preston in prep.) offer opportunities for engaging a wide range of investigators. We have demonstrated the sensitivity of the SHA to be on the order of 0.5nM for rRNA transcribed from cloned DNA and low pM/high fM for oligonucleotides up to ~50b in length. Both cloned sequences and oligonucleotides can be used for calibration (see below) and specificity testing.

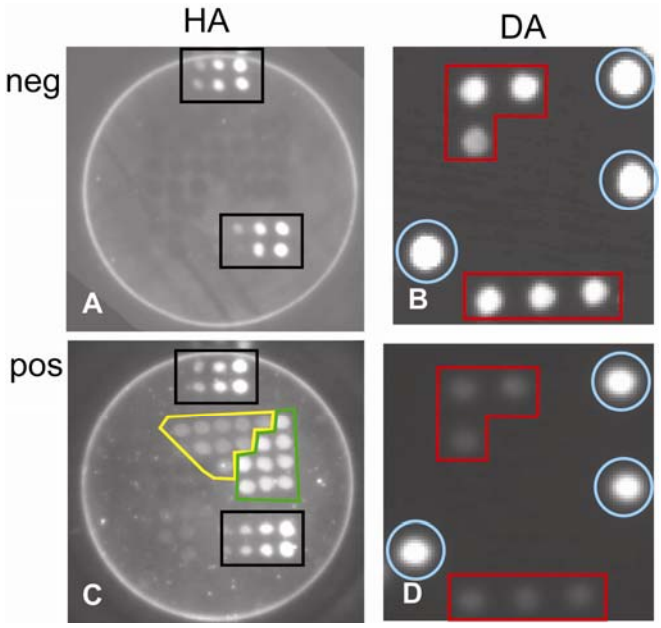

Figure 3. In situ detection of *Pseudo-nitzschia* spp. and domoic acid (DA) in Monterey Bay March 2006 using the ESP. A) DNA array with probes for *Pseudo-nitzschia australis, P. multiseries / pseudodelicatisima, Alexandrium catenella, and Heterosigma akashiwo* (HAB array). Only control spots (black rectangles) are visible. Targeted species are below detection level. B) Protein array for DA, corresponds to (A). Blue ringed spots are IgG controls confirming consistency of detection chemistry. Spots in red rectangles are DA conjugate. Maximum intensity in DA conjugate spots indicates DA levels are below detection limit, whereas decreasing spot intensity indicates presence of DA (competitive ELISA). C) HAB array from a later time indicates the presence of *P. australis* (yellow bounded spots) and *P. multiseries / psuedodelicatissima* (green bounded spots), both of which can produce DA. D) DA array that corresponds to (C); the DA conjugate spots are considerably dimmed, indicating the presence of this toxin (from Doucette et al. and Greenfield et al., in prep; see also Appendix 1).

Work to date indicates that reagents utilized by the ESP are stable at room temperature for at least 6 months. Additional reagent and array stability tests are planned for 2008 targeting working temperatures of 2-30°C. Samples archived using our current method are stable for ~2mos; there are options for extending this further. The SHA also provided reasonable, near real-time estimates of target species abundance for *A. fundyense* in the GOM during a 10-day shipboard survey. While early SHA results were at times variable compared to whole cell (WC) assays (Anderson et al. 2005), more recent comparisons of SHA data generally agreed quite well with WC tests ($R^2 = 0.7$) and led to an early warning for impending outbreaks of shellfish toxicity along the Maine coast in 2004 (Fig. 4). When reagent quality is assured, we are thus

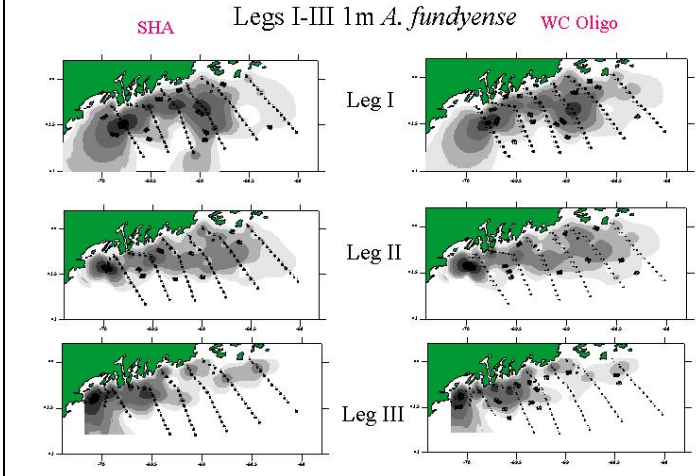

Figure 4. Contours of *A. fundyense* abundance along the coast of western Maine during the onset of shellfish toxicity in 2004. Stations were initially sampled during Leg I (top) and revisited during Legs II (mid) and III (bottom). The left panels show the *A. fundyense* estimates using a 96-well plate format for SHA, while the right panels show the same samples analyzed using the whole-cell (WC) oligo-nucleotide microscope method. Very similar distribution patterns were observed with either format during all 3 legs as the toxic cells were transported along the coast near Casco Bay. This data confirmed that SHA probe chemistry is valid for GOM *A. fundyense* populations.

confident that SHA chemistry is reliable for targeting *A. fundyense* populations in the GOM. These efforts have also underscored the different needs of researchers and resource managers (e.g., Matweyou et al. 2004, Tyrell et al. 2001, 2002, Anderson et al. 2005, Haywood et al. in press). Understanding the needs of different user groups will be critical to commercializing the ESP and its detection chemistries.

Relevant to this proposal, Greenfield et al. (2006 and in prep.) have recently demonstrated that the ESP arrays yield results that are directly comparable to the benchtop, 96-well plate version of the assay. For both formats, signal intensities are linearly correlated with the number of cells collected. From this relationship, one can derive estimates of the abundance of particular species in cultured and natural samples. The extent to which cellular rRNA content changes in response to environmental fluctuations is not yet fully understood (e.g., Anderson et al. 1999). Consequently, we view results of the SHA as providing approximate, not precise, cell density estimates. Nevertheless, our work to date illustrates we are capable of sensing particular species at low abundance, at concentrations that are relevant to both scientists and resource managers. For example, the data shown in Fig. 2 illustrate detection of several well-known HAB species at levels well below those requiring a management response. These same species cause harm in many regions of the world.

To ensure that false signals from the ESP are minimized, we have advanced methods for calibrating the SHA using 'linkers' (oligonucleotides containing complements of capture and signal probes). Linkers provide a chemically defined reference for quality assurance testing of reagents and probes used in the assay. They can be fielded with the instrument to facilitate remote calibration if desired. We also employ capture probes on the printed arrays that are complementary to signal probes used in developing that array. These probes always return a positive reaction when an array is developed and thus serve as an internal control (see Figs. 2, 3). We are currently exploring use of these chemistry controls as quantitative measures of array performance (i.e. as a calibration reference for normalizing potential inter-array variability).

The ESP is now at a stage where it is feasible to initiate technology transfer to a limited number of groups with needs for remote in-water sensing capability. Currently, MBARI is constructing three copies of the instrument with funding from NSF/MBARI. The primary goal is to leverage those instruments to initiate technology transfer to users in research and resource management positions with HABs serving as one major application of the ESP demonstration pathway towards commercialization. Those funds, however, are not sufficient to enact an actual transfer

of complete ESP systems. Instead we are restricted to lab studies and limited, local (Monterey Bay) field demonstration projects. Here we propose to work with members of the Doucette (NOAA/NOS Marine Biotoxins Program), Anderson and McGillicuddy labs (WHOI) to evaluate the ESP and its utility for studying *A. fundyense* blooms in the GOM. The GOM provides an ideal field site for this given the recurrent toxic blooms and close ties between researchers and managers charged with managing HAB outbreaks.

B.1d *HABs in the Gulf of Maine and the Nauset Marsh System: testing the ESP in a protected shoreline location and in open coastal waters.* The GOM, including the coastlines of its three bordering New England states (Maine, New Hampshire, and Massachusetts), is a vast region with extensive shellfish resources, large portions of which are frequently contaminated with PSP toxins produced by *A. fundyense*. As a result, state-run monitoring programs are in operation on an annual basis, making closure and re-opening decisions on the basis of shellfish toxicity measurements. Several *A. fundyense* habitats occur in the region including isolated, shallow embayments with easy access for sampling, and more challenging open, coastal waters of the gulf (Anderson, 1997). Both of these environments are ideal locations for demonstration and transfer of the ESP technology since both experience recurrent harvesting closures. Due to the HAB threat, the GOM is the site of a planned NOS operational forecasting system for HAB events that will begin in 2009. It is also the site of a major 5-year NOAA funded regional project called GOMTOX (see below) that can provide large-scale contextual data on *A. fundyense* dynamics to supplement ESP observations. There is a clear need for continuous *in situ* measurements to support management decisions in the GOM (see support letters, Appendix 2).

The Nauset Marsh System (NMS) on Cape Cod is a shallow, protected *A. fundyense* "hot spot" that frequently produces intense toxic blooms that are readily sampled from shore. Blooms within NMS are localized phenomena due to the restrictive nature of the Nauset inlet (connecting to the open ocean) and reduced exchange within the salt ponds of the system (Anderson and Stolzenbach, 1985). In particular, Salt Pond is an extremity of the NMS where flushing is weak (Aubrey et al. 1997), allowing toxic cells to accumulate to high population densities. PSP toxicity has occurred in Salt Pond in 14 of the last 15 years. An increasing trend in PSP incidence has highlighted the need for increased water quality monitoring of HABs and other pathogens within the NMS. The Anderson laboratory maintains a long history of collaboration with state and local shellfish managers as part of continuing *A. fundyense* research there. We will thus have access to a shore-based facility that can house the ESP in close proximity to Salt Pond, allowing us to configure the instrument for land- or dock-based operation, an application of the instrument that opens up significant commercial markets due to the needs for water quality testing worldwide. Concurrent with this proposed project, a pending Sea Grant project led by PI Anderson will sample intensely during a spring/neap tidal cycle in the NMS, providing measurements of *A. fundyense* abundance in the system to complement the proposed ESP observations. Therefore, Salt Pond is an ideal site for the initial ESP testing and demonstration.

In contrast, large-scale blooms in the coastal waters of the GOM are more challenging, requiring extensive resources and moderately-sized vessels to deploy and service instruments. Toxic blooms (Fig. 4, 5) have been the focus of intense investigation through ECOHAB-GOM– a regional program designed to characterize toxic outbreaks in the GOM (Anderson et al. 2005a). Another multi-investigator, 5-year observational and modeling program called GOMTOX is currently funded to extend that work. Several conceptual models of *A. fundyense* bloom dynamics within the Maine Coastal Current System (MCC; Anderson et al. 2005, McGillicuddy et al. 2005) have been formulated, and these allow us to identify key branch points in the *A. fundyense* transport pathways (see arrows in Fig. 6). Thus, the coastal GOM is a well-studied

region for which we have a deep underlying understanding of the annually recurrent bloom dynamics and coupling to outbreaks of PSP toxicity.

Of the many possible locations for ESP deployment along the Gulf of Maine transport pathway, the Casco Bay region of the western Maine coast (Fig 6) has been selected for multiple reasons. It is one of the most intense "hot spots" with respect to the frequency and duration of PSP toxicity outbreaks along the open coast. Indeed, it was a successful demo site for the 1G ESP in 2001 (Scholin et al., in press). However, the most intense blooms likely originate in the adjacent or "upstream" coastal waters (Keafer et al. 2005). Mussel bags hung on offshore moorings revealed toxicity 1-2 weeks before the inshore inter-tidal shellfish within Casco Bay became toxic. Placement of an ESP in the offshore waters near the Bay would detect the incoming populations before they impact the coast. This is of obvious management value. Therefore, the coastal waters offshore of Casco Bay have been selected as a test site to evaluate the performance of the sensor package in an unprotected, open coastal environment.

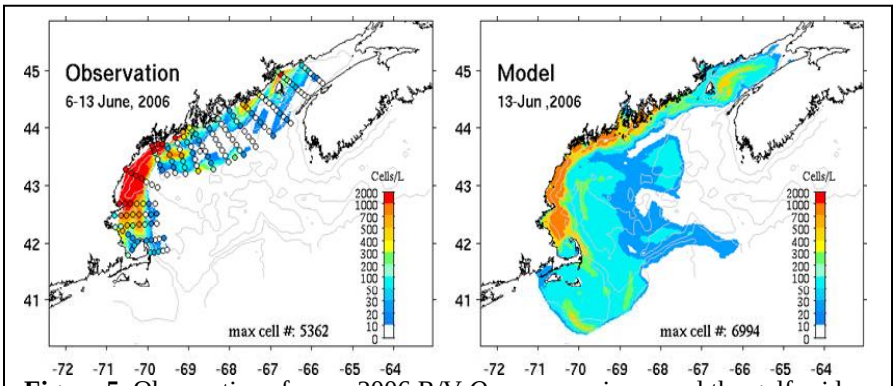

Figure 5. Observations from a 2006 R/V *Oceanus* cruise reveal the gulf-wide distribution of *Alexandrium fundyense* with highest abundances found in the western GOM (left). The modeled bloom (right) shows a similar spatial distribution. Open circles denote station locations.

Another motivation for this choice is that a hierarchy of hydrodynamic and physical-biological models has been applied to various aspects of *A. fundyense* blooms in this region. Early studies focused on the spatial disconnect between inshore *A. fundyense* blooms and offshore cyst beds (McGillicuddy et al., 2003; Hetland et al., 2002). As additional observations became available, model sophistication improved considerably, both in terms of regional-scale models based on climatology as well as limited-area hindcast and nowcast/forecast models (McGillicuddy et al. 2005; Stock et al., 2005, 2007; He et al., 2005). The most recent implementation of the coupled physical-biological nowcast/forecast model is based on the Regional Ocean Modeling System (ROMS) - a state-of-the-art, free-surface primitive equation ocean circulation model (Schepetkin and McWilliams, 2005). ROMS has been configured for the northeastern US coastal ocean (He et al., submitted), in a doubly-nested configuration embedded in a ~10km resolution operational model of the North Atlantic (http://hycom.rsmas.miami.edu/dataserver). The ROMS elements consist of shelf-scale model spanning from Cape Hatteras to Nova Scotia, and a high-resolution GOM ROMS, covering the coastal regions from Nantucket to the Bay of Fundy at ~1-km resolution.

The high-resolution GOM ROMS is an ideal framework for a coupled physical-biological model of *A. fundyense* dynamics. Current formulation of the biological model follows Stock et al. (2005) and McGillicuddy et al. (2005) and includes germination, growth, mortality, and nutrient limitation. Currently, near-real-time quasi-operational nowcasts and forecasts are made using observed cyst distributions, cyst germination and vegetative cell growth rates, and continuous real time river flow and hydrographic data from USGS, GOMOOS and NOAA moorings. Figure 5 shows an example of the general agreement between 2006 cruise observations of cell

abundance in the GOM and output from our physical/biological model for the same time. The model was initiated in early March and agreed well with the regional cell abundance throughout the event. Note that a major closure of offshore waters for harvesting of surf clams, ocean quahogs, and whole scallops was enforced by federal officials, in part because of the offshore information provided by the model. Model output is supplied to state and federal resource managers on a weekly basis to aid in management decisions. It has proved to be extremely valuable, as otherwise they are faced with closure and re-opening decisions on the basis of widely scattered shellfish testing alongshore, with no knowledge of the spatial extent of the bloom, particularly in offshore waters. Model output from 2005, 2006, and 2007 is available on the world-wide-web (http://omgrhe.meas.ncsu.edu/Redtide/). This model will serve as the basis for the NOS HAB forecasting system that will become operational in 2009, as described in the supporting letter from R. Stumpf (Appendix 2). In the longer term, multiple ESPs deployed at critical branch points in the MCC flow will provide data that can be assimilated into the model to provide more accurate HAB forecasts for the region. This information will assist managers, regulators, and industry to improve the utilization of shellfish resources threatened by PSP, and to enforce appropriate safeguards for human health.

B.1e *ESP commercialization status.* Commercialization of the ESP remains a challenge at this time. Key reasons are that few instruments are available for testing and costs of producing additional custom-made instruments in low numbers by the development team are high. In addition, the instrument embodies a unique mix of robotics, molecular biology and ocean engineering that is unfamiliar to many potential industry partners. Moreover, we lack an established track record validating the performance of the instrument in different environments and in the hands of a broad user base. In short, the ESP is in the proverbial "technology transfer chasm" between a few functional prototypes being operated by the development team and the desired goal of a stable, commercialized product suitable for use by the masses.

Consequently, we propose here a focused project to bridge that chasm by constructing and placing complete ESP instruments at selected government and academic institutions. Those devices will be used to demonstrate detection of HAB species and toxins in laboratory, estuarine, and open coastal settings in support of ongoing regional HAB monitoring activities. In so doing, we aim to prove the utility of the ESP system and to spur opportunities for its commercialization. Commercial success will require being able to supply end-users with an ocean-capable instrument and varied, expendable, molecular analytical supplies. We believe that mix can be achieved by establishing a consortium of academic and industry partners. With support from NOPP, we will explore that possibility by including representatives from McLane Research Laboratory and Applied Biosystems as participants in this project to provide input relevant for commoditizing the ESP system (see letters of collaboration from ABI and McLane, Appendix 2).

B.2 Project Plan

Development of HAB sensors, observing systems, models to detect and forecast the trajectory of harmful algal blooms for all U.S. coasts, and the transfer these capabilities to managers are recognized national priorities (e.g., NCCOS Strategic Plan FY 2005- FY 2009). Towards meeting those needs, the major objectives of this project are to: 1) construct two copies of the ESP and transfer those instruments to federal and academic partners; 2) demonstrate *in situ* detection of harmful HAB species and toxins in protected and open ocean regimes of the Northeast Atlantic; 3) integrate data products derived from the ESP into the Gulf of Maine regional HAB monitoring network; and 4) explore options for commercializing the ESP technology. To achieve these goals, five major tasks have been identified.

Task 1. *Production of ESPs and technology transfer from MBARI to NOAA and WHOI.* During year-1 MBARI will procure parts for and construct two core ESPs and transfer those machines to the Doucette and Anderson laboratories. Ahead of delivery, project participants will learn how to operate the ESP within a laboratory setting, perform relevant molecular assays, and participate in a deployment (Monterey Bay) for training purposes. Quality controlled reagents and expendable supplies will be produced at MBARI and shipped to partner institutions. By the beginning of year-2 MBARI, will complete a dedicated mooring and telemetry system for one core ESP, and will transfer that to WHOI as well. MBARI personnel will participate in field deployments in Salt Pond and in the open waters of the GOM in years-2&3, and provide technical support for laboratory and field trials throughout the duration of the project.

Task 2. *Laboratory studies of specificity, sensitivity and precision.* After transferal of the instruments, project participants will gain hands-on experience with the ESP in their own labs. Initial experiments will focus on running standard calibration series using synthetic, cultured and natural samples analyzed with both laboratory-based and ESP methodologies. Dr. Doucette and members of his team are already familiar with operating the ESP and conducting cELISA assays (see Appendix 1) and the SHA (Mikulski et al. in press). The Anderson lab is also very familiar with the SHA chemistry in the 96-well plate format for use in the laboratory and on ships. They require training to operate the ESP in their own lab and to field it in the GOM. They own two SHA processors, various epifluorescent microscopes for WC assays, and other necessary equipment.

Through inter-lab comparisons we will assess the lower limit of detection and response of the ESP for local strains of *Alexandrium fundyense*. Cultures of *A. fundyense* and its close relative *A. catenella* from Monterey Bay are available from the Anderson and Scholin labs. Available SHA probes react with both species equally (Scholin 1998). Results from the ESP will be compared to other methods using the same SHA chemistries and WC microscopic assays (Anderson et al. 2005). This task will include establishing reproducible standard curves with blanks. Local field samples containing various background cell concentrations will be spiked with *A. fundyense* cells and bench tested. Finally, archived field samples stored in liquid nitrogen from previous blooms will also be tested and compared to WC counts derived from replicate samples.

Task 3. *Field testing in different application settings.* After hands-on bench testing and familiarity with the ESP technology has been achieved by project participants, the two ESPs delivered for this project will be deployed in the field in year-2 & 3 to demonstrate their reliability, identify areas for improvement, and to validate their utility within a management framework (Fig. 6). The initial field deployment (year-2; spring 2010) will be in Salt Pond. Additionally, the IOOS program has funded the PIs to build another ESP scheduled for delivery and deployment during 2010 at a highly-instrumented, open coastal site (Buoy B) where additional experience will be gained in that highly challenging environment. During year-3 (spring, summer 2011), the deployments will become more complex with three ESP moorings deployed simultaneously within the open coastal waters of the GOM. The ESP obtained with IOOS funding will supplement an ESP array within a critical HAB region along the coast of Maine, but IOOS support for that effort is only for a single year, and for a very short deployment that will end in 2010, so funds are requested here to keep those moored observations intact during 2011 (the final year of this project).

The first ESP built and delivered to WHOI will be deployed spring 2010 at Salt Pond within the NMS on Cape Cod, a shallow, estuary with annually recurrent *A. fundyense* blooms that cause annual shellfish closures. The ease of access for frequent ground truthing and line-of-sight

communications makes this location especially advantageous for the first deployment and initial field trials. The specific details of the site and frequency of sampling for the ESP will be chosen and adjusted in consultation with our management partners at the Massachusetts Division of Marine Fisheries and local shellfish constables (see attached letters). To demonstrate the utility in this shallow system, the ESP will be housed in an existing shore-based shed with a "sipper" tube extending into the adjacent waters, simulating a dock-mounted test platform. To ground truth the surrounding waters, weekly sampling (using SHA and/or WC analysis) will be performed near the "sipper" tube and within the adjacent waters of Salt Pond during the April-June bloom period. Note that this ESP demonstration will benefit from an ongoing NOAA Sea Grant funded project to co-PI Anderson that will be focused on *A. fundyense* bloom dynamics within the NMS. In particular, several intense surveys of the NMS are planned during a two-week spring/neap tidal cycle to observe rapid changes in the *A. fundyense* abundance within the marsh system. The ESP will be embedded into that effort with its sample frequency increased during that time. Since cell counts will be taken on a frequent basis for the Sea Grant project, we will obtain valuable, high frequency data against which the concurrent ESP cell abundance estimates can be compared. This will provide much-needed validation, and will demonstrate the ESP's flexibility to change sampling times and frequencies to adjust to the needs of managers and researchers. A protocol for near-real time information exchange from the ESP data stream and other supporting information to shellfish managers will be also established via a password-protected website.

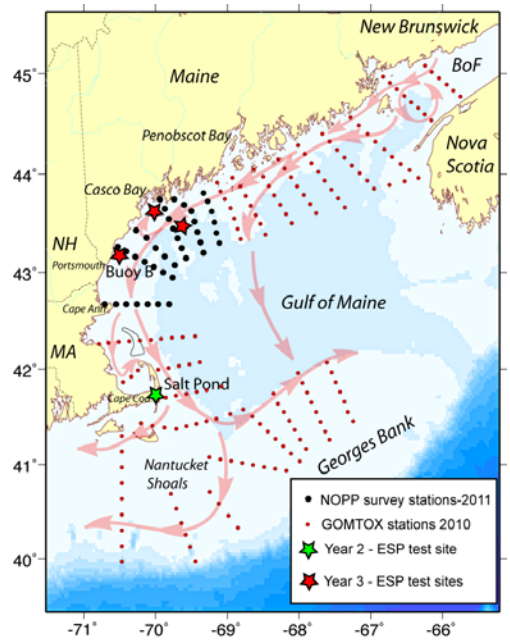

Figure 6. ESP deployment locations and adjacent ground-truthing stations for year-2 & 3. The year-2 ESP deployment is proposed for Salt Pond on Cape Cod, a protected embayment with isolated intense blooms. A separate IOOS-built ESP will be deployed at GoMOOS Buoy B, a test of the performance in open waters. Year-3 ESP deployments will be located in an array in the open waters of the Gulf of Maine near Casco Bay (2 ESPs) and near Buoy B (one IOOS-built ESP). Survey stations for validation of the ESPs are shown in black and encompass a region surrounding the ESPs. Stations to be occupied during a 5-year complementary GOMTOX program are shown as red dots with the red arrows denoting major *A.fundyense* transport pathways.

Also for year-2, limited IOOS funds will support only a single deployment and recovery in the coastal ocean at Buoy B (Fig. 6). To revisit the Buoy B site, we request 6 days of ship time on the R/V Tioga (WHOI) during year-2 to cover two trips to Buoy B for instrument re-deployment and ground-truth sampling near the mooring. The R/V Tioga is a very capable coastal research vessel (60ft) with required lift capabilities from the A-frame, dedicated CTD/rosette, current profilers, and an underway hydrographic data collection system. Additional opportunistic *A. fundyense* sampling will be done by Ru Morrison (University of New Hampshire) as part of a monthly transect near Buoy B (funded by IOOS) with the samples shipped to WHOI for analysis.

In the subsequent field year (year-3; spring 2011), operation of multiple ESPs in open waters of the GOM will be demonstrated to local GOM managers, the larger global HAB and observatory communities, and our commercial partners. Casco Bay and the western Maine coastline is an ideal region for this demonstration since blooms along that stretch of coast are annually recurring with shellfish toxicity detected every year since 1972. The blooms occur

along this coast in the spring/early summer so deployment during this time will undoubtedly yield positive ESP results with insight into the spatial and temporal patterns of the *A. fundyense* population detected by an array of ESPs. Using experience gained from Salt Pond and the IOOS deployment at Buoy B, we propose to deploy the 2 ESPs in the Casco Bay region of the Maine coast to form the basis of an ESP array (Fig. 6; also see Fig. 5 for typical bloom distribution within the area of the proposed array). Two ESPs will allow the detection of coastal blooms transported from the adjacent offshore waters onto shellfish beds within Casco Bay, an area of intense shellfish and phytoplankton monitoring by the state (see L. Mercer support letter). One or more of these instruments (ideally all three) will be configured with toxin detection capabilities (in addition to cell detection) through the efforts of co-PI Doucette (see Appendix 1). MBARI will loan one of its moorings so that the NOAA unit can be deployed. Since IOOS support ends in 2010, funds are also requested to maintain the IOOS ESP at the Buoy B site providing the 3rd ESP in the array further downstream in the coastal flow. Specific ESP site locations will be adjusted in discussions with state shellfish and water quality managers. Those decisions will be supported by modeled experiments to determine the best locations to capture bloom events (see Task 4 below).

In support of year-3 field operations we propose three short cruises using a UNOLS class vessel (R/V Oceanus or R/V Endeavor) for initial deployment, servicing, final recovery, and ground-truthing of the ESP array. The initial deployment cruise in April and the recovery cruise in July will require five days each. A mooring turnaround in June will demonstrate the ability to handle multiple ESPs at sea. This will require eight days of UNOLS time to conduct a hydrographic survey in concert with recovery and redeployment of the three ESPs. The instruments will routinely sample at a frequency of about every other day for 45-60 days (45-60 days before and 45 days after the turnaround=90-120 days total) with higher frequencies programmed during initial deployment and recovery to increase the number of samples for verification when the ship is nearby. In addition to the mooring operations, ship time will be used for ESP field validation using CTD/Niskin bottles to collect samples and hydrographic data at each ESP site and in the surrounding waters of the western GOM. Thus, three snapshots of *A. fundyense* distribution and abundance upstream and downstream of the moorings during each cruise will provide the large-scale perspective needed to interpret the variability of the time-series ESP data.

Task 4. *Assimilation of ESP data into nowcast/forecast models.* Currently, our *Alexandrium* modeling effort is an academic activity that is unlikely to be sustained as such. Recognizing this, and acknowledging the accuracy and value of our model, the NOS is committed to implementing an operational red tide forecasting system for the GOM beginning in 2009, and has funds in their budget for the NOAA personnel and other costs of this regional service that will utilize existing models and observational infrastructure. Details of this plan are contained in a support letter from R. Stumpf, the lead scientist for transitioning research capabilities into operational forecasts. The critical need for the ESP system in this planned forecasting system is evident in his statement "*I cannot emphasize enough how adding HAB ESP sensors to this observing system will aid in assuring accurate and reliable forecasts of HABs*".

In the meantime, we will work to demonstrate the manner in which ESP data should be collected and disseminated for use in forecasting models. Assimilation of ESP data into our models is one of the most urgent of our development efforts. As part of the IOOS project, co-PI McGillicuddy, will lead the modeling team to conduct observing system simulation experiments (OSSEs) to optimize ESP mooring locations, and to assimilate data from a single deployment in 2010. This leverage from IOOS obviates the need for a modeling budget in the first two years of this project. However, deployment of three ESPs in 2011 (after IOOS as ended) will constitute a major

increase in ESP data flow. Our year three modeling budget reflects the personnel time required to help collect samples at sea for validation, process the data and assimilate it into the nowcast/forecast system using the ROMS adjoint model that has now become part of the standard software distribution (http://marine.rutgers.edu/po/index.php?model=roms).

Task 5. *Develop a strategy for commercializing the ESP*. As outlined above in section B.1c, the suite of available assays that can run on the ESP is growing and applications are now envisioned in a variety of regions around the world. With development of the microfluidics and real-time PCR module (under NSF OCE-314222; see B.3), inquiries from those developing 'analytical modules' as well as those interested in deploying PCR assays in a remote sensory context are mounting and demand for the ESP is increasing rapidly. In fact, Scholin has written four letters of support for researchers who are responding to this NOPP solicitation, each developing "analytical modules" or "contextual sensors" that they hope to bundle with the ESP for field investigations in the near future.

Placing the ESP in a commercial market, however, requires finding manufacturers with expertise in robotics, *in situ* oceanographic sensors, molecular biology and custom reagent manufacturing capabilities. This combination has proven elusive thus far. Those considered have either proven inappropriate for technical reasons, or desire a "ready-to-manufacture" product suite that is still one step beyond our current status. All require more detailed information about potential market size and production volumes, and/or require assurances that great profit will follow product launch in a very short period of time (e.g., one group wanted a 100:1 return on investment within 3 years). Hence our strategy here is to look beyond a single partner and involve multiple industry representatives with expertise in oceanographic sensors (McLane Research Laboratories) and molecular biology (Applied Biosystems Inc.) as observers throughout the duration of this study so that an effective, realistic assessment of the commercial potential of the ESP is achieved (see letters in Appendix 2). We also expect to receive valuable advice and guidance about possible modifications to the ESP that might facilitate broader applications during commercialization. Indeed, our plans to deploy an ESP on shore during year-1 is intended to demonstrate the instrument's applicability for water quality analyses in freshwater, estuarine and ocean systems, with instruments located on docks or in small structures immediately adjacent to the water being tested. This is a huge market that might lead to commercialization, compared to the oceanographic sensor market that may not be perceived as large enough for some companies. In this manner we aim to build strategic, beta testing partnerships representing both end-users and potential commercial producers of this technology. This partnership will serve to prove the utility of the ESP system, help identify issues that must be resolved prior to commercial release of the instrument. To familiarize McLane and ABI with the ESP and its operational and support requirements, representatives from both companies will visit MBARI and participate in the training workshop that will be convened in year-1. Non-disclosure agreements (NDAs) will be established and details concerning production of the ESP hardware and custom reagents will be shared among project participants. Thereafter, over the course of year-2 & 3, we will derive estimates for what a "production model" ESP will cost and devise a list of accessories, expendable reagents and customer services required for supporting instruments sold commercially. If deemed necessary, a consultant may be contracted to help assess the market for the ESP and/or provide assistance developing a business plan. By year-3, if not sooner, we will have completed our assessment and be in a position to commoditize the ESP system.

B.3 Leveraged activities

Applications of the ESP involving the microfluidic interface and long-term deep-sea operations represent other user groups who would benefit from commercialization of the instrument under

this program. Analytical modules place a requirement on the ESP to prepare complex environmental samples for highly refined reactions (e.g., PCR, microarrays) and dispense reagents on a microfluidic scale. For that reason we are building a microfluidic interface for the ESP, a take-off point for distributing samples and reagents to one or more detection modules. Current development at MBARI is focused on supporting a reusable solid phase extraction column for purifying nucleic acids and a real-time PCR module built by Lawrence Livermore National Laboratory. This same sample handling and distribution block could support a variety of analytical devices that may emerge in the future. We expect this to run as a standalone unit as well for laboratory testing, and our objective is to produce several copies for use with a restricted number of core ESPs. In support of deep-sea operations we are developing a 4000m-rated ESP system. That device will be tested on the MARS cable node (http://www.mbari.org/mars/) late 2008/early 2009. Grants from NSF, NASA and MBARI are supporting those activities.

Importantly, as noted above, several on-going observation and modeling projects in the GOM provide a unique context for ESP measurements, with considerable cost savings accruing to the project proposed here. These include a Sea Grant project in the NMS that will examine *A. fundyense* dynamics of that isolated system and its exchange with the coastal ocean. *Alexandrium* transport pathways and shellfish toxicity patterns will be a subject of investigation in GOMTOX, a regional research program focused on *A. fundyense* dynamics in the GOM that will run concurrently with the ESP testing proposed herein (see Fig. 6). As stated above, IOOS has funded one ESP build and deployment in 2010 with modeling efforts as one part of the development of NERACOOS (Northeastern Regional Coastal Ocean Observing System). The Buoy B location will be maintained with sensors for (e.g. temperature, salinity, currents) that will support the ESP measurements.

C. Project Schedule and Milestones (see Appendix 1 for details concerning algal toxin work):

C.1 Year-1 – September ‘08 to August ‘09

- Fabricate two core ESPs, transfer to NOAA and WHOI.
- Fabricate one surface mooring for the ESP.
- Train project participants how to use the ESP.
- Establish NDAs with industry partners and begin assessing ESP market potential
- Validate function of the ESPs in partner labs.
- Refine the existing DA assay; initiate saxitoxin assay development (see Appendix 1).

C.2 Year-2 September ‘09 to August ‘10

- Conduct field validation studies at two different sites.
  - Deploy NOPP ESP at Salt Pond for initial training and testing of dockside application.
  - Deploy IOOS-funded ESP at Buoy B; NOPP support will help faciliate ground-truthing performance of the open ocean mooring.
  - McLane and ABI serve as observers to activities at WHOI and Salt Pond.
- Validate performance of the ESP using SHA and WC methodologies (and PCR if available/desired).
- Initiate field tests of the saxitoxin assay and corresponding lab validation trials.
- Assimilate ESP data with modeled products for presentation to local resource managers (supported by IOOS).

C.2 Year-3 September ‘10 to August ‘11

- Deploy an array of three ESP moorings in the GOM (this requires three UNOLS survey cruises for deployment, servicing, recovery).
  - McLane and ABI serve as observers as in year-2

- Analyze SHA/WC and archived ESP samples for field validation.
- Deploy saxitoxin assay on one or more ESP units.
- Assimilate ESP data with modeled products for presentation to local resource managers (NOPP support).
- Finalize plan for commercializing the ESP.

D. Assertion of Data Rights

The PIs on this proposal pledge to make data available as per policy developed by the U.S. GLOBEC program (http://globec.whoi.edu/globec-dir/data-acknowledgement-policy.html).

E. Deliverables

- Construction and transfer of two ESPs for use by government and academic labs ('09)
- Deployment of the ESP in different regimes (protected embayment, open coastal/offshore; '10).
- Field an array of three ESP moorings to provide near real-time detection of HAB species and toxins they produce ('11).
- Assimilation of ESP data into regional models ('10-'11).
- Provision of data products for resource managers and the public at-large ('10-'11).
- Evaluation of the commercial potential of the ESP in collaboration with MacLean and ABI (complete by or before end of year-3).
- Results of this work submitted for peer-reviewed publications, posted on websites, and presented at meetings and workshops as appropriate.

F. Management Approach

The overall development and experimental evaluation of the ESP will be managed by the P.I., Chris Scholin, in conference with members of the ESP engineering team at MBARI and co-PIs Anderson, McGillicuddy, Keafer and Doucette. Members of the project team will communicate regularly by e-mail and phone during the duration of the study. Annual meetings will take place before, during or after seasonal field deployments. Lead investigators from each institution will prepare annual progress and financial reports as required. Results will be disseminated via publications, meetings and workshops. Doucette will lead the toxin work; D. Anderson will lead lab studies at WHOI; Keafer, Anderson and McGillicudy will lead field investigations in Salt Pond and the GOM, and McGillicuddy will lead modeling efforts.

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Roman, B., C. Scholin, S. Jensen, E. Massion, R, Marin III, C. Preston, D. Greenfield, W. Jones, and K. Wheeler. 2007. Controlling a Robotic Marine Water Sampler with the Ruby Scripting Language. *Journal of American Laboratory Automation* **12**: 56-61.

Scholin, C.A. Morphological, genetic and biogeographic relationships of *Alexandrium tamarense, A. catenella* and *A. fundyense*. 1998. In: Anderson, D.M., Hallegraeff, G.M. and Cembella A.D. [Eds.], *The Physiological Ecology of Harmful Algal Blooms.* NATO Advanced Study Institute Series. Springer-Verlag, Heidelberg. pp 13-27.

Scholin, C.A., E. Vrieling, L. Peperzak, L. Rhodes and P. Rublee. 2003. Detection of HAB species using lectin, antibody and DNA probes. In: G.M. Hallegraeff, D.M. Anderson A.D. Cembella (eds), *Manual on Harmful Marine Microalgae,* pp. 131-64. Paris, Intergovernmental Oceanographic Commission, UNESCO. (Vol. 11. Second Edition).

Scholin, C. S. Jensen, B. Roman, E. Massion, R. Marin, C. Preston, D. Greenfield, W. Jones, K. Wheeler. 2006. The Environmental Sample Processor (ESP) – An Autonomous Robotic Device for Detecting Microorganisms Remotely using Molecular Probe Technology. Proceedings, OCEANS 2006 MTS/IEEE Conference. Boston, MA. Marine Technology Society, Columbia, MD.

Scholin, C.A., G.J. Doucette, and A.D. Cembella. In press. Prospects for developing automated systems for in situ detection of harmful algae and their toxins. In: M. Babin, C.S. Roesler and J.J. Cullen (eds.) *Real-Time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Blooms*, pp 413-462, UNESCO Publishing, Paris, France. [Note, this publication has been 'in press' for several years due to problems with the publisher; it is, unfortunately, out of our control. Page proofs were approved earlier this year (2007).]

Shchepetkin, A., McWilliams, J.C., 2005. The Regional Oceanic Modeling System: A split explicit, free-surface, topography-following-coordinate ocean model. Ocean Modelling 9, 347-404.

Stock, C.A., McGillicuddy, D.J., Anderson, D.M., Solow, A.R., Signell, R.P., 2007. A comparative modeling study of blooms of toxic dinoflagellate Alexandrium fundyense in the western Gulf of Maine in 1993 and 1994. Continental Shelf Research 27, 2486-2512.

Stock, C.A., McGillicuddy, D.J., Solow, A.R., Anderson, D.M., 2005. Evaluating hypotheses for the initiation and development of *Alexandrium fundyense* blooms in the western Gulf of Maine using a coupled physical-biological model. Deep-Sea Research II 52 (19-21), 2715-2744.

Tyrrell, J.V., C.A. Scholin, P.R. Berguist and P.L. Berguist. 2001. Detection and enumeration of *Heterosigma akashiwo* and *Fibrocapsa japonica* (Raphidophyceae) using rRNA-targeted oligonucleotide probes. *Phycologia* **40**: 457-467.

Tyrrell, J.V., L.B. Connell and C.A. Scholin. 2002. Monitoring for *Heterosigma akashiwo* using a sandwich hybridization assay. *Harmful Algae* **1**:205-214.

H. Curriculum Vitae

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Education

1979 –81, University of Missouri, Columbia, Department of Forestry and Wildlife
1984 B.A. (Hons.), University of California, Santa Barbara, Department of Biology
1986 M.A., Duke University Graduate School, Department of Microbiology and Immunology
1992 Ph.D., Massachusetts Institute of Technology - Woods Hole Oceanographic Institution Joint Program in Biological Oceanography
1992-94, Postdoctoral Fellow Monterey Bay Aquarium Research Institute (MBARI)

Professional Work Experience

1986–87, University of South Carolina, Columbia, Research Assistant Professor
1994–97, MBARI**,** Assistant Scientist I
1997–2001, MBARI**,** Associate Scientist II
2001–2006, MBARI**,** Associate Scientist III
July 2005–present, Chair, MBARI Research Division
2006–present, MBARI**,** Senior Scientist IV

Current Committee Service

External advisory committe for the University of Miami's NSF-NIEHS Oceans & Human Health Center (http://www.rsmas.miami.edu/groups/ohh/)

Selected Recent Peer Reviewed Publications

W.J. Jones, C. Preston, R. Marin III, C. Scholin, R. Vrijenhoek A Robotic Molecular Method for in situ Detection of Marine Invertebrate Larvae. *Molecular Eclology Notes* (in press).

John Paul, Chris Scholin, Ger van den Engh, Mary Jane Perry. 2007. In situ Instrumentation. *Oceanography* **20**: 58-66.

Scholin, C.A., G.J. Doucette, and A.D. Cembella. In press. Prospects for developing automated systems for in situ detection of harmful algae and their toxins. In: M. Babin, C.S. Roesler and J.J. Cullen (eds.) *Real-Time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Blooms*, pp 413-462, UNESCO Publishing, Paris, France.

Roman, B., C. Scholin, S. Jensen, E. Massion, R, Marin III, C. Preston, D. Greenfield, W. Jones, and K. Wheeler. 2007. Controlling a Robotic Marine Water Sampler with the Ruby Scripting Language. *Journal of American Laboratory Automation* **12**: 56-61.

Greenfield, D.I., R. Marin III, S. Jensen, E. Massion, B. Roman, J. Feldman, C. Scholin. 2006 Application of the Environmental Sample Processor (ESP) methodology for quantifying *Pseudo-nitzschia australis* using ribosomal RNA-targeted probes in sandwich and fluorescent *in situ* hybridization. *Limnology and Oceanography: Methods* **4**: 426-435.

Metfies, K., K. Töbe, C. Scholin, and L.K. Medlin. 2006. Laboratory and field applications of ribosomal RNA probes to aid the detection and monitoring of harmful algae. In: *Ecology of Harmful Algae* (Granéli, E., and Turner, J.T. eds), pp. 311-325. Springer Verlag, Berlin, Heidelberg, New York.

Lundholm, N., Ø. Moestrup, Y. Kotaki, C. Scholin, P. Miller. 2006. Inter- and intraspecific variation of the *Pseudo-nitzschia delicatissima*-complex (Bacillariophyceae) illustrated by rRNA probes, morphological data and phylogenetic analyses identification of *P. decipiens* and *P. dolorosa* spp. Nov. *Journal of Phycology* **42**: 464-481.

Goffredi, S.K., W. Jones, C. Scholin, R. Marin, S. Hallam, R.C. Vrijenhoek. 2006. Molecular detection of marine larvae. *Marine Biotechnology* **8**: 149-160.

Ayers K, Rhodes L, Tyrrell J, Gladstone M, Scholin C. 2005. International accreditation of sandwich hybridisation assay format DNA probes for micro-algae. *New Zealand J. Marine and Freshwater Res.* **39**: 1225–1231.

Anderson, D.M., D.M. Kulis, B.A. Keafer, K.E. Gribble, R.Marin and C.A. Scholin. 2005. Identification and enumeration of *Alexandrium spp.* from the Gulf of Maine using molecular probes. *Deep-Sea Research II* 52: 2467-2490.

Babin, M., J.J. Cullen, C.S. Roesler, P.L. Donaghay, G.J. Doucette, M. Kahru, M.R. Lewis, C.A. Scholin, M.E. Sieracki, and H.M. Sosik. 2005. New approaches and technologies for observing harmful algal blooms. *Oceanography* **18**: 210-227.

Ryan, J.P., H.M. Dierssen, R.M. Kudela, C.A. Scholin, K. S. Johnson, J.M. Sullivan, A.M. Fisher, E.V., Rienecker, P.R. McEnany, and F.P. Chavez. 2005. Coastal Ocean Physics and red tides: an example from Monterey Bay, California. *Oceanography* **18**: 246-255.

Matweyou, J.A., D.A. Stockwell, C.A. Scholin, S. Hall, V.L. Trainer, J.D. Ray, T.E. Whitledge, A.R. Childers, F.G. Plumley. 2004. Use of Alexandrium rRNA targeted probes to predict PSP events on Kodiak Island, Alaska. In: K. A. Steidinger, J. H. Landsberg, C. R. Tomas and G. A. Vargo (eds.) *Harmful Algae 2002*, pp. 267-269. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO. St. Petersburg, Florida, USA.

Scholin, C.A., F. Gulland, G. Doucette and others. 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* **403**: 80-84.

Scholin, C., R, Marin, P. Miller, G. Doucette, C. Powell, J. Howard., P. Haydock and J. Ray. 1999. Application of DNA probes and a receptor binding assay for detection of *Pseudo-nitzschia* (Bacillariophyceae) species and domoic acid activity in cultured and natural samples. *Journal of Phycology* **35**: 1356-1367.

Selected Contribution

Scholin, C. S. Jensen, B. Roman, E. Massion, R. Marin, C. Preston, D. Greenfield, W. Jones, K. Wheeler. 2006. The Environmental Sample Processor (ESP) – An Autonomous Robotic Device for Detecting Microorganisms Remotely using Molecular Probe Technology. Proceedings, OCEANS 2006 MTS/IEEE Conference. Boston, MA. Marine Technology Society, Columbia, MD.

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B.S., Mechanical Engineering, Massachusetts Institute of Technology, 1970
M.S., Civil Engineering, Massachusetts Institute of Technology, 1976
Ph.D., Aquatic Sciences, Department of Civil Engineering, MIT, 1977

Director, Coastal Ocean Institute, Woods Hole Oceanographic Institution, 2004-present
Senior Scientist, Woods Hole Oceanographic Institution, 1991-present
Associate Scientist, Woods Hole Oceanographic Institution, 1983-1991; tenure 1987
Assistant Scientist, Woods Hole Oceanographic Institution, 1979-1983
Postdoctoral Investigator, Woods Hole Oceanographic Institution, 1978-1979
Instructor, Massachusetts Institute of Technology Department of Civil Engineering, 1978

PROFESSIONAL SOCIETIES:

American Society of Limnology and Oceanography
Phycological Society of America
International Society for the Study of Harmful Algae (Member, ISSHA Council)
The Oceanography Society

SELECTED COMMITTEES AND DISTINCTIONS

Recipient, Stanley W. Watson Chair for Excellence in Oceanography (1993)
Recipient, NOAA Environmental Hero Award (1999)
Recipient, ISSHA's Yasumoto Lifetime Achievement Award (2006)
Bruun Memorial Medal, Bruun Memorial Lecture. IOC Assembly, Paris, France, 2005
Director, NATO ASI: The Physiological Ecology of Harmful Algal Blooms (1996)
Director, U.S. National Office on Marine Biotoxins and Harmful Algal Blooms
Member, Scientific Steering Committee, GEOHAB (1998-2003)
Scientific Advisor, U.S. Delegation to the IOC/FAO Intergovernmental Panel on Harmful Algal Blooms (1992-present)
Testimony before the Subcommittee on Oceans and Fisheries of the Committee on Commerce, Science, and Transportation, United States Senate 105th Congress, Second Session, May 20, 1998
Testimony for US Commission on Ocean Policy, 2002; prepared White Paper on Harmful Algal Blooms for inclusion in the Commission's report "Oceans and Human Health"
Testimony before the Committee on Science, Subcommittee on Environment, Technology and Standards, U.S. House of Representatives Hearing on the "Harmful Algal Bloom and Hypoxia Research Amendments Act of 2003", March 13, 2003
Briefing before the House Science Committee, "Coastal Nutrient Pollution and Harmful Algal Blooms", September 24, 2003
Briefing before New England congressional staffers, The 2005 New England red tide", June 14, 2005
Congressional briefings, "Harmful Algal Blooms (Red Tides) and Ocean Health", House of Representatives, and U.S. Senate, June 23, 2005

Testimony before the Subcommittee on Fisheries Conservation, Wildlife and Oceans, U.S. House of Representatives Hearing on H.R. 1856, the Harmful Algal Bloom and Hypoxia Research Amendments Act of 2003, February 26, 2004.

RELEVANT PUBLICATIONS
(Publications selected from over 230 authored or co-authored publications)

2005 Anderson, D.M., B.A. Keafer, D.J. McGillicuddy, M.J. Mickelson, K.E. Keay, P.S. Libby, J.P. Manning, C.A. Mayo, D.K. Whittaker, J.M. Hickey, R. He, D.R. Lynch, and K.W. Smith. Initial observations of the 2005 *Alexandrium fundyense* bloom in southern New England: General patterns and mechanisms. Deep-Sea Res. II 52(19-21): 2856-2876.

2005 Anderson, D.M., C.A. Stock, B.A. Keafer, A. Bronzino Nelson, B. Thompson, D.J. McGillicuddy, M. Keller, P.A. Matrai, and J. Martin. *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep-Sea Res. II 52(19-21): 2522-2542.

2005 Anderson, D.M., B.A. Keafer, W.R. Geyer, R.P. Signell, and T.C. Loder. Toxic *Alexandrium* blooms in the western Gulf of Maine: The plume advection hypothesis revisited. Limnol. Oceanogr. 50(1): 328-345.

1997 Anderson, D. M. Bloom dynamics of toxic *Alexandrium* species in the northeastern United States. Limnol. & Oceanogr. 42: 1009-1022.

1994 Anderson, D. M., D. M. Kulis, G. J. Doucette, J. C. Gallager and E. Balech. Biogeography of toxic dinoflagellates in the genus *Alexandrium* from the northeast United States and Canada as determined by morphology, bioluminescence, toxin composition, and mating compatibility. Mar. Biol. 120: 467-478.

OTHER SIGNIFICANT PUBLICATIONS

2005 Anderson, D.M., D.M. Kulis, B.A. Keafer, K.E. Gribble, R. Marin, and C.A. Scholin. Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using molecular probes. Deep-Sea Res. II 52(19-21): 2467-2490.

2005 Keafer, B.A., J.H. Churchill, D.J. McGillicuddy, and D.M. Anderson. Bloom development and transport of toxic *Alexandrium fundyense* populations within a coastal plume in the Gulf of Maine. Deep-Sea Res. II 52(19-21): 2674-2697.

2005 McGillicuddy, D.J., Jr., D.M. Anderson, D.R. Lynch, and D.W. Townsend. Mechanisms regulating large-scale seasonal fluctuations in *Alexandrium fundyense* populations in the Gulf of Maine: Results from a physical-biological model. Deep-Sea Res. II 52(19-21): 2698-2714.

2005 Stock, C.A., D.J. McGillicuddy, A.R. Solow, D. M. Anderson. Evaluating hypotheses for the initiation and development of *Alexandrium fundyense* blooms in the western Gulf of Maine using a coupled physical-biological model. Deep-Sea Res. II 52(19-21): 2715-2744.

2003 McGillicuddy, D.J., Jr., R.P. Signell, C.A. Stock, B.A. Keafer, M.D. Keller, R.D. Hetland, and D.M. Anderson. A mechanism for offshore initiation of harmful algal blooms in the coastal Gulf of Maine. J. Plankt. Res. 25(9): 1131-1138.

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EDUCATION:

Bowling Green State University	Major: Biology	B.Sc. cum laude/1979
Texas A&M University	Major: Oceanography	M.Sc./1982
University of British Columbia	Major: Botany	Ph.D./1989
Woods Hole Oceanographic Institution	Harmful Algal Blooms	Postdoctoral/1989-1992
National Marine Fisheries Service	Marine Biotoxins	Postdoctoral/1992-1995

PROFESSIONAL EXPERIENCE:

- Research Oceanographer/Project Leader, NOAA/National Ocean Service, Center for Coastal Environmental Health & Biomolecular Research, Marine Biotoxins Program (Feb. 1999-present).
- Faculty Member, Marine Biomedical and Environmental Sciences Program/College of Graduate Studies, Medical University of South Carolina (Jan. 1996-present).
- Adjunct Faculty Member, Graduate Faculty of the University, Marine Biology Program, University of Charleston (Oct. 1992-present).
- Project Leader, Marine Biotoxins Program, Charleston Laboratory, National Marine Fisheries Service (Jan. 1996-Jan. 1999).

NATIONAL/INTERNATIONAL WORKSHOPS AND COMMITTEES (selected since 2002):

- Workshop on "Biosensors for Harmful Algal Blooms," Alliance for Coastal Technologies, Solomons, MD (Mar. 2002). Plenary lecture – "Detection of HAB species, toxins, & toxicities: current approaches & potential for *in-situ* applications."
- Workshop on "Real Time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Blooms," Villefranche-sur-mer, France (June 2003). Plenary lecture - "Biosensors & toxin detection."
- Member, Council of the International Society for the Study of Harmful Algae (ISSHA) (2003-present).
- Technical Cooperation Expert, International Atomic Energy Agency; Project: Application of Nuclear Techniques to Address Harmful Algal Bloom Impacts in the Benguela Region (2002-continuing).
- Workshop on U.S. National Plan for Algal Toxins and Harmful Algal Blooms, Charleston, SC (March 2004).
- Member, AOAC Task Force on Algal Toxins (2004-continuing).
- Member, International Advisory Board, EC BioCop Project on New Technologies to Screen Multiple Contaminants in Foods (2005-continuing).
- Member, U.S. National HAB Committee (July 2007-June 2010).
- Workshop on "National Scientific Research, Development, Demonstration, and Technology Transfer Plan on Reducing Impacts from HABs", Woods Hole, MA (June 2007).
- Workshop on "National Scientific Research, Development, Demonstration, and Technology Transfer Plan on Reducing Impacts from HABs", Woods Hole, MA (25-28 June 2007).

FIVE MOST RELEVANT REFEREED PUBLICATIONS (80 total):

- Doucette, G.J., Logan, M.M., Ramsdell, J.S., Van Dolah, F.M. 1997. Development and preliminary validation of a microtiter plate-based receptor binding assay for paralytic shellfish poison toxins. *Toxicon* 35:625-636.
- Scholin, C.A., Marine, R. III, Miller, P.E., Doucette, G.J., Powell, C.L., Haydock, P., Howard, J., Ray, J. 1999. DNA probes and a receptor binding assay for detection of *Pseudo-nitzschia* (Bacillariophyceae) species and domoic acid activity in cultured and natural samples. *Journal of Phycology* 35:1356-67.
- Babin, M., Cullen, J.J., Roesler, C.S., Donaghay, P.L., Doucette, G.J., Kahru, M., Lewis, M.R., Scholin, C.A., Sieracki, M.E., Sosik, H.M. 2005. New approaches and technologies for observing harmful algal blooms. *Oceanography* 18:210-227.
- Doucette, G.J., Turner, J.T., Powell, C.L., Keafer, B.A., Anderson, D.M. 2005. ECOHAB-Gulf of Maine. Trophic accumulation of PSP toxins in zooplankton during *Alexandrium fundyense* blooms in Casco Bay, Gulf of Maine, April – June, 1998. I. Toxin levels in *A. fundyense* and zooplankton size fractions. *Deep-Sea Research II* 52:2764-2783.
- Campbell, K., Stewart, L.D., Doucette, G.J., Fodey, T.L., Haughey, S.A., Vilariño, N., Kawatsu, K., Elliott, C.T. 2007. An assessment of specific binding proteins suitable for the detection of paralytic shellfish poisons using optical biosensor technology. *Analytical Chemistry* 79:5906-5914.

FIVE RELATED REFEREED PUBLICATIONS:

- Powell, C.L., Doucette, G.J. 1999. A receptor binding assay for paralytic shellfish poisoning toxins: recent advances and applications. *Natural Toxins* 7:393-400.
- Parsons, M.L, Scholin, C.A., Miller, P.E., Doucette, G.J., Powell, C.L., Fryxell, G.A., Dortch, Q., Soniat, T. 1999. *Pseudo-nitzschia* species (Bacillariophyceae) in Louisiana coastal waters: molecular probe field trials, genetic variability, and domoic acid analyses. *Journal of Phycology* 35:1368-78.
- Pitcher, G.C., Franco, J.M., Doucette, G.J., Powell, C.L., Mouton, A. 2001. Paralytic shellfish poisoning in the abalone *Haliotis midae* on the west coast of South Africa. *Journal of Shellfish Research* 20:895-904.
- Ruberu, S.R., Liu, Y.-G., Wong, C.T., Perera, S.K., Langlois, G.W., Doucette, G.J., Powell, C.L. 2003. A receptor binding assay for paralytic shellfish poisoning toxins: optimization and interlaboratory comparison. *Journal of the Association of Official Analytical Chemists* 86:737-745.
- Doucette, G.J., Cembella, A.D., Martin, J.L., Michaud, J., Cole, T.V.N., Rolland, R.M. 2006. PSP toxins in north Atlantic right whales (*Eubalaena glacialis*) and their zooplankton prey in the Bay of Fundy, Canada. *Marine Ecology Progress Series* 306:303-313.

MOST RELEVANT BOOK CHAPTERS (9 total):

- Cembella, A.D., Doucette, G.J., Garthwaite, I. 2003. *In vitro* assays for phycotoxins. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (eds.), *Manual on Harmful Marine Microalgae*. Second Edition. Monographs on Oceanographic Methodology, 11. IOC-UNESCO, Paris. pp. 297-345.
- Doucette, G.J., Maneiro, I., Riveiro, I., Svensen, C. 2006. Phycotoxin pathways in aquatic food webs: transfer, accumulation and degradation. In: Granéli, E., Turner, J.T. (eds.) *Ecology of Harmful Algae*. Springer-Verlag, Heidelberg. pp. 283-295.
- Scholin, C.A., Doucette, G.J., Cembella, A.D. In press. Prospects for developing automated systems for *in situ* detection of harmful algae and their toxins. In: Babin, M., Cullen, J., Roessler, C. (eds.), *Real time coastal observing systems for ecosystem dynamics and harmful algal blooms*. Monographs on Oceanographic Methodologies, Vol. 10. Paris: Intergovernmental Oceanographic Commission of UNESCO.

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B.S. Biology, Virginia Polytechnic Institute and State University. 1977
Graduate study, College of William and Mary/
Virginia Institute of Marine Science, 1980-1983
Research Associate, Woods Hole Oceanographic Institution, 1992-present
Research Assistant, Woods Hole Oceanographic Institution, 1983-1992
Laboratory Technician, Virginia Institute of Marine Science, 1977-1983

Selected Experience at Sea as Chief Scientist:

2007	R/V Tioga	GOMTOX cruises in Mass Bay-deploy/recover McLane samplers
2006	R/V Oceanus	GOMTOX cyst survey in the Gulf of Maine
2005	R/V Tioga	Six emergency 2-day cruises during 2005 southern NE bloom
2005	R/V Oceanus	Eleven day cruise – Gulf of Maine cyst survey
2004	R/V Cape Hatteras	Eleven day cruise – ECOHAB cyst project - Gulf of Maine
2001	R/V Cape Hatteras	Two 10-day cruises in Gulf of Maine – ECOHAB-GOM
2000	R/V Gulf Challenger	Eight 2-3 days cruises in Casco Bay – ECOHAB-GOM
1999	R/V Gulf Challenger	Three 2 day cruises in Mass Bay – Sea Grant/MWRA
1998	R/V Gulf Challenger	Eleven 2-3 day cruises - ECOHAB-GOM
1997	R/V Endeavor	ECOHAB cyst survey in the Gulf of Maine
1996	R/V Gulf Challenger	Four 2 day cruises in Mass Bay. Toxin transfer in the Food Web
1994	R/V ARGO Maine	Five Cruises. Transport of *Alexandrium* in the Gulf of Maine

RELEVANT PUBLICATIONS

2005 Keafer, B.A., J.H. Churchill, D.J. McGillicuddy, and D.M. Anderson. Bloom development and transport of toxic *Alexandrium fundyense* populations within a coastal plume in the Gulf of Maine. Deep-Sea Res. II 52(19-21): 2674-2697.

2005 Keafer, B.A., J.H. Churchill, and D.M. Anderson. Blooms of the toxic dinoflagellate, *Alexandrium fundyense*, in the Casco Bay region of the western Gulf of Maine: Advection from offshore source populations and interactions with the Kennebec River plume. Deep-Sea Res. II 52(19-21): 2631-2655.

2005 Anderson, D.M., B.A. Keafer, D.J. McGillicuddy, M.J. Mickelson, K.E. Keay, P.S. Libby, J.P. Manning, C.A. Mayo, D.K. Whittaker, J.M. Hickey, R. He, D.R. Lynch, and K.W. Smith. Initial observations of the 2005 *Alexandrium fundyense* bloom in southern New England: General patterns and mechanisms. Deep-Sea Res. II 52(19-21): 2856-2876.

2005 Anderson, D.M., B.A. Keafer, W.R. Geyer, R.P. Signell, and T.C. Loder. Toxic *Alexandrium* blooms in the western Gulf of Maine: The plume advection hypothesis revisited. Limnol. Oceanogr. 50(1): 328-345.

2005 Anderson, D.M., C.A. Stock, B.A. Keafer, A. Bronzino Nelson, B. Thompson, D.J. McGillicuddy, M. Keller, P.A. Matrai, and J. Martin. *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep-Sea Res. II 52(19-21): 2522-2542.

OTHER SIGNIFICANT PUBLICATIONS

2005 Turner, J.T., G.J. Doucette, B.A. Keafer, D.M. Anderson. Trophic accumulation of PSP toxins in zooplankton during *Alexandrium fundyense* blooms in Casco Bay, Gulf of Maine, April - June, 1998. II. Zooplankton abundance and size-fractionated community composition. Deep-Sea Res. II 52(19-21): 2784-2800.

2003 Franks, P.J.S. and B.A. Keafer. 2003. Sampling techniques and strategies for coastal phytoplankton blooms. pp. 51-76, in: Hallegraeff, G.M., D.M. Anderson, and A.D. Cembella (Eds.), *Manual on Harmful Marine Microalgae*, UNESCO, Paris.

1993 Keafer, B.A. and D.M. Anderson. The use of remotely-sensed sea surface temperatures in studies of *Alexandrium tamarense* bloom dynamics. Smayda, T.J. and Shimizu, Y. (eds.) in *Toxic Phytoplankton Blooms in the Sea.* Elsevier Science Publishers. 763-768.

1992 Keafer, B.A., K.O. Buessler, and D.M. Anderson. Burial of living dinoflagellate cysts in estuarine and nearshore sediments. Marine Micropaleontology 20: 147-161.

1987 Anderson, D.M. and B.A. Keafer. An endogenous annual clock in the toxic marine dinoflagellate, *Gonyaulax tamarensis.* Nature 172: 89-107.

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Education:

	1987	B.A., *cum laude*, Engineering Sciences, Harvard College
	1989	M.S., Applied Physics, Harvard University
	1993	Ph.D., Earth and Planetary Sciences, Harvard University

Professional Experience:

1987-1990	Graduate Fellowship, Office of Naval Research
1989	Visiting Scientist, Institut Für Meereskunde, Kiel, Germany
1990-1993	Research Assistant, Harvard University
1993-1995	Modeling Fellowship, University Corporation for Atmospheric Research
1993-1995	Postdoctoral Scholarship, Woods Hole Oceanographic Institution
1995-1999	Assistant Scientist, Woods Hole Oceanographic Institution
1999-2007	Associate Scientist (tenure in 2003), Woods Hole Oceanographic Institution
2007-present	Senior Scientist, Woods Hole Oceanographic Institution

Honors and Awards:

1987	Office of Naval Research Graduate Fellowship
1993	Woods Hole Oceanographic Institution Postdoctoral Scholarship
1993	UCAR Postdoctoral Fellowship in Ocean Modeling
1998	Office of Naval Research Young Investigator Award
2000	Lindeman Award, American Society of Limnology and Oceanography
2003	AGU Editors' Citation for Excellence in Refereeing

Research Interests:

Influence of physical forcing on planktonic ecosystems and elemental cycling; mesoscale ocean dynamics; primary production; coastal circulation; zooplankton population dynamics; harmful algal blooms; numerical modeling and data assimilation.

Ph.D. Advisors: Allan R. Robinson, Harvard University
James J. McCarthy, Harvard University

Postdoctoral Advisor: Kenneth H. Brink, Woods Hole Oceanographic Institution

Professional Activities:

1994-2002	Scientific Steering Committee, U.S. Joint Global Ocean Flux Study
1996-1999	NSCAT Science Team
1997-present	NASA Earth Observing System Interdisciplinary Science Working Group
1998	Participant and white paper author in NSF decadal planning activity for Biological Oceanography (OEUVRE)
1999-2001	Organizing Committee, Ecological Determinants of Ocean Carbon Cycling
1999-2002	National Ocean Partnership Program Committee on Oceanographic Information Technology Infrastructure
1999-2002	Organizing Committee, U.S. Surface Ocean - Lower Atmosphere Study
2000-present	Ocean Vector Winds Science Team

2000-2001	Editorial Advisory Board, Encyclopedia of Ocean Sciences
2001-2003	NSF Ocean Carbon Cycle Research Program Committee
2004-present	Deputy Director, Woods Hole Center for Oceans and Human Health
2004-present	Scientific Steering Committee, U.S. Global Ecosystem Dynamics (Globec)
2004-present	Editorial board, *Dynamics of Atmospheres and Oceans*

Relevant Publications:

1. McGillicuddy, D.J., Signell, R.P., Stock, C.A., Keafer, B.A., Keller, M.D., Hetland, R.D. and D.M. Anderson, 2003. A mechanism for offshore initiation of harmful algal blooms in the coastal Gulf of Maine. *Journal of Plankton Research*, 25(9), 1131-1138.
2. McGillicuddy, D.J., Anderson, D.M., Lynch, D.R. and D.W. Townsend, 2005. Mechanisms regulating the large-scale seasonal fluctuations in *Alexandrium fundyense* populations in the Gulf of Maine: results from a physical-biological model. *Deep-Sea Research II*, 52, 2698-2714.
3. Stock, C.A., McGillicuddy, D.J., Solow, A.R. and D.M. Anderson, 2005. Evaluating hypotheses for initiation and development of *Alexandrium fundyense* blooms in the western Gulf of Maine using a coupled physical-biological model. *Deep-Sea Research II,* 52, 2715-1744.
4. He, R., McGillicuddy, D.J., Lynch, D.R., Smith, K.W., Stock, C.A. and J.P. Manning, 2005. Data assimilative hindcast of the Gulf of Maine coastal circulation. *Journal of Geophysical Research*, 110, C10011, doi:10.1029/2004JC002807.
5. McGillicuddy, D.J., Anderson, D.M., Solow, A.R., and D.W. Townsend, 2005. Interannual variability of *Alexandrium fundyense* abundance and shellfish toxicity in the Gulf of Maine. *Deep-Sea Research II,* 52, 2843-2855.

Other Significant Publications:

6. McGillicuddy, D.J., Kosnyrev, V.K., Ryan, J.P. and J.A. Yoder, 2001a. Covariation of mesoscale ocean color and sea surface temperature patterns in the Sargasso Sea. *Deep-Sea Research II*, 48, 1823-1836.
7. McGillicuddy, D.J. and A. Bucklin, 2002. Intermingling of two *Pseudocalanus* species on Georges Bank. *Journal of Marine Research*, 60, 583-604.
8. McGillicuddy, D.J., Anderson, L.A., Doney, S.C., and M.E. Maltrud, 2003. Eddy-driven sources and sinks of nutrients in the upper ocean: results from a 0.1 degree resolution model of the North Atlantic. *Global Biogeochemical Cycles*, 17(2), 1035, doi:10.1029/2002GB001987.
9. Davis, C.S**.** and McGillicuddy, D.J.**,** 2006. Transatlantic Abundance of the N_2-Fixing Colonial Cyanobacterium *Trichodesmium*. *Science*, 312, 1517-1520.
10. McGillicuddy, D.J., Anderson, L.A., Bates, N.R., Bibby, T., Buesseler, K.O., Carlson, C.A., Davis, C.S., Ewart, C., Falkowski, P.G., Goldthwait, S.A., Hansell, D.A., Jenkins, W.J., Johnson, R., Kosnyrev, V.K., Ledwell, J.R., Li, Q.P., Siegel, D.A. and D.K. Steinberg, 2007. Eddy/Wind Interactions Stimulate Extraordinary Mid-Ocean Plankton Blooms. *Science*, 316, 1021-1026.

I. Ship Use Request

For year-2, limited IOOS funds will support only a single deployment and recovery in the coastal ocean at Buoy B (Fig. 6). To revisit the Buoy B site, we request 6 days of ship time on the R/V Tioga (WHOI) during year-2 to cover two trips to Buoy B for instrument deployment and ground-truth sampling near the mooring. The R/V Tioga is a very capable coastal research vessel (60ft) with required lift capabilities from the A-frame, dedicated CTD/rosette, current profilers, and an underway hydrographic data collection system (see http://www.whoi.edu/page.do?pid=8159).

A total of 18 days of UNOLS time spread over 3 cruises is requested to accomplish the ESP array deployment during spring/summer of 2011(year-3 of this project). UNOLS ship requests for these cruises follow:

UNOLS Ship Time Request Form - Section ONE

UNOLS Request ID #: 20071204120032GY
Version #: 004
Last Modified: 2007/12/04 19:30 EST
Date Issued: 2007/12/04 19:34 EST

P.I. Name Last: Scholin First: Christopher MI: A

Institution: Monterey Bay Aquarium Research Institute (MBARI)
Address: 7700 Sandholdt Road
Moss Landing, CA 95039

Research vessel required for:
_ Ancillary Only
X Principal Use
_ No Ship Required
_ Long Range Planning Document

Phone: (831) 775-1779 Fax: (831) 775-1620 Email: scholin@mbari.org

Co P.I. Name	Institution	Co P.I. Name	Institution
Donald Anderson	Woods Hole Oceanographic Institution	Dennis McGillicuddy	Woods Hole Oceanographic Institution

Proposal Title:

Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployments, Technology Transfer, and Commercial Evaluation - ONR Program Point of Contact Dr. James E. Eckmann NOPP

Large Program Name: NOPP
If Other, specify:

Research Purpose: Instrumentation
If Other, specify: Multi-discipline as well: Detection of HABs for resource management

New Proposal? Y
Funded Grant? N
Institutional Proposal #:
Agency Proposal #:

Renewal? N
Grant #:

Agency Submitted to: ONR
Amount Requested: 1,527,404
Start Date: 09/01/08
End Date: 09/31/11

Foreign EEZ? N
Area(s) of Operation: NA6
Lat/Long:
Begin: 41N 70W
End: 44N 68W

Year	Ship(s) Requested (Name or Size)	# Science Days Req.	Optimum Dates	Alternate Dates
2011	Oceanus	5	April 26-30, 2011	May 3-7, 2011
2011	Oceanus	8	June 2-9, 2011	June 7-14, 2011
2011	Oceanus	5	July 12-16, 2011	July 19-23, 2011

Total Science & Ship Days Needed: 18

Number in Science Party: 18

PORTS
Start: Woods Hole
Intermediate:
End: Woods Hole

Equipment Required:

X Vans
_ Dynamic Positioning
_ Helicopter Operation
_ P-Code GPS
_ Multibeam
_ MCS
_ SCS
_ Alvin
_ ROV
_ ABE
_ 680 Cond.

Other Special Equipment; Comments:

sleeping Van is required to berth one crew to recover, reset, and redeploy ESP instrument in addition to science crew to sample the surrounding Gulf of Maine waters for validation of the instrument--perference would be the R/V Oceanus that has this extra berthing capability; staging out of Woods Hole in also preferable; requested dates are critical to the success of this project due to the timing of HAB events in the western Gulf of Maine
==========

Appendix 1: Statement of Work (Co-Investigator G. Doucette only, NOAA/NOS)

This statement of work outlines the tasks to be performed under the direction of Co-Investigator Dr. Gregory Doucette, representing the Federal entity DOC/NOAA/National Ocean Service. This work constitutes an integrated, coordinated element of the proposal being submitted by Principal Investigator Dr. Christopher Scholin of the Monterey Bay Aquarium Research Institute, and Co-Investigators Drs. Donald Anderson and Dennis McGillicuddy of the Woods Hole Oceanographic Institute. The primary contributions of this component will be the laboratory-based refinement and calibration of an assay for domoic acid (DA) formatted previously for the Environmental Sample Processor (ESP) platform, as well as the initial development and testing of a new assay for the PSP toxin class. Essential to achieving these aims will be delivery of a core ESP instrument from MBARI to Dr. Doucette's laboratory, as described in the main body of this proposal.

The ESP-formatted DA assay was designed and developed as part of a previous NOPP-funded project (NSF OCE-0314089). A protocol for the extraction of DA from phytoplankton samples acquired by the ESP (> 90% recovery) was also developed as part of this work. The DA assay employs a competitive ELISA (cELISA) approach and utilizes a membrane-based protein array with the same chemiluminescence reporting system as used in the LSU rRNA probe-based assay for HAB species detection, thereby permitting 'multiplexing' of several reagents. Briefly, a toxin extract produced onboard the ESP (generally representing material concentrated from 500 to 1000 mL of seawater) is combined with a DA-specific antibody and exposed to a membrane array spotted with a DA-protein conjugate; the toxin in solution competes with the toxin-protein conjugate immobilized on the array for the antibody available in solution; antibody bound to the array (inversely proportional to the toxin concentration in the sample extract) is visualized with a chemiluminescent signal that is captured by an onboard CCD camera (see Fig. 2 in main proposal). The DA assay has undergone several successful test deployments on the ESP in Monterey Bay, CA during 2006 and 2007, representing the first ever autonomous, sub-surface detection of a phycotoxin (manuscript in prep.).

The next critical steps needed to move the DA assay towards a more 'operational' status are further refinement of the assay to minimize changes in response over one to two month deployments, as well as extensive calibration to critically establish assay performance parameters and reproducibility. This labor-intensive effort will require constant access to a core ESP instrument in our laboratory. To date the majority of our assay development work has been carried out using a 'hand-held' assembly consisting of an ESP 'puck' (i.e., membrane array holder) and syringes, which is not capable of accurately reproducing the precise microfluidics of the ESP instrument. Two attempts at calibrating the DA assay on the core ESP unit, using a non-toxic *Pseudo-nitzschia* extract spiked with certified DA reference material (NRC/IMB, Halifax, NS, Canada) to account for matrix effects, have been conducted during visits to Dr. Scholin's

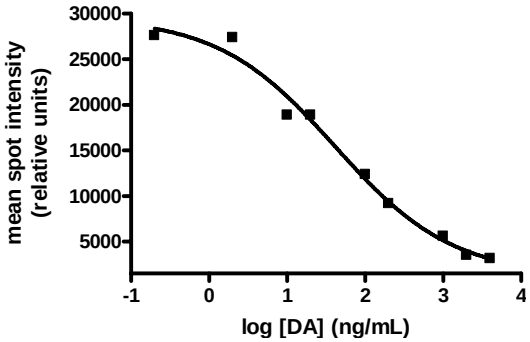

Fig. 1. Calibration curve for DA cELISA conducted onboard core ESP; EC50 (i.e., half-maximal assay response) is ~ 40 ng/mL.

laboratory at MBARI in 2006 and 2007. The most recent results (Fig. 1) indicate that this assay will permit detection of 0.2 ng DA/mL of seawater for a 0.5 L sample collected by the ESP, which is sufficiently sensitive to detect DA levels far below what is generally considered problematic or 'bloom' conditions. However, this result represents a single calibration, whereas it is essential to generate numerous curves to properly characterize assay reproducibility. Moreover, additional, iterative testing of modified assay reagents is required to further refine and assess the long-term stability of the DA test.

A second, equally important focus of our group during this project will be to initiate development and testing of an analogous assay for detection of PSP toxins onboard the ESP. This is especially relevant for ESP deployments planned for the western Gulf of Maine region, where PSP toxin-producing *Alexandrium* spp. will be targeted by the ESP arrays for HAB species detection. All of the information obtained during development and refinement of the DA cELISA for the ESP in terms of array spotting, reagent stability and reactivity, antibody/antigen ratios, etc. will be extremely valuable in designing the PSP toxin assay. We currently have direct access to antibodies against the PSP toxins, as well as saxitoxin-protein conjugates, through a collaborator on another project aimed at developing a surface plasmon resonance (SPR)-based detection method for this toxin class. These fully characterized, custom reagents will be provided at a minimal cost to the project. In fact, the DA antibody and DA-protein conjugate used in the ESP DA assay are currently employed in an SPR-based DA detection method run routinely in our laboratory (formatted as a cELISA; Traynor et al., 2006). We are confident that adopting this same strategy for development of the PSP toxin assay will bring a high probability for success. We should note that the PSP antibodies currently in hand show reduced cross-reactivity with the N-1 hydroxy saxitoxin derivatives (i.e., NEO, GTX1/4, C3/4); however, several parallel efforts outside the scope of this project are being pursued to address this issue and any antibodies developed will be freely available for the work proposed herein.

A primary component of proposal Year 1 essential for carrying out much of the work outlined above will be delivery of a core ESP instrument to our laboratory near the end of the year. Prior to this, our efforts during Year 1 will continue employ the hand-held apparatus described above and used for much of our assay development thus far; however, materials will be sent periodically to Dr. Scholin's laboratory for testing/evaluation on their available core ESP units. We can, to a certain extent, continue to refine the DA cELISA and, importantly, importantly, initiate the concurrent development of the PSP toxin extraction method and ESP-formatted assay using this hand-held device. We will also plan a visit to MBARI during Year 1 for training on laboratory-based operation of the core ESP (we do have some experience already from our two previous visits to perform assay calibrations) so as to minimize down-time following delivery of the instrument. We will likely field at least several DA arrays during the planned ESP training deployment in the western Gulf of Maine in cooperation with the NERACOOS Program (Buoy B). Additional ESP deployments will be conducted in Monterey Bay by Dr. Scholin during the Year 1 time frame (but apart from the current proposal) and we will take advantage of these opportunities to continue our field-based testing of the DA assay.

With the planned delivery of the core ESP instrument to our laboratory by the start of Year 2, much of our focus will turn to the intensive refinement, evaluation, and calibration of the DA cELISA on this unit. We have considerable experience (over 15 years) with the development and

validation of *in vitro* assays for algal toxins and the work required for this process will now be possible with constant access to an ESP instrument. We also expect that following our Year 1 effort, at least a preliminary version of the PSP toxin assay will also be ready for initial formatting/testing on the core ESP. Continued development of this assay should proceed with far greater efficiency given both the availability of an ESP instrument (as compared to use of the hand-held version) and the knowledge gained from our work on the analogous DA cELISA. In addition, we will plan to perform initial field tests of the PSP toxins assay during proposed test deployments of the ESP in the western Gulf of Maine (i.e., Salt Pond, MA; NERACOOS/Buoy B).

Work during Year 3 will shift exclusively to the PSP toxin assay, with our aim being to establish a functional assay for the planned field work in the western Gulf of Maine, which will involve the simultaneous deployment of three ESP instruments. Based on what is learned from the Year 2 deployment, we expect that additional refinements to reagents may be necessary to address the issue of assay stability over extended periods in the field. In addition, efforts will be focused on the rigorous calibration of this assay and establishing assay performance parameters, as will have been carried out for the DA assay during Years 1 and 2.

References

Traynor, I.M., Plumpton, L., Fodey, T.L., Higgins, C., Elliott, C.T. 2006. Immunobiosensor detection of domoic acid as a screening test in bivalve molluscs: comparison with liquid chromatography-based analysis. J. AOAC Int. 89, 868-872.

Appendix 2: Letters of Collaboration and Support

McLANE
RESEARCH LABORATORIES, INC

Falmouth Technology Park
121 Bernard E. St. Jean Drive, East Falmouth, Massachusetts 02536 USA
Telephone: (508) 495-4000 • Fax: (508) 495-3333
E-Mail: mclane@mclanelabs.com

November 30, 2007
Dr. Chris Scholin
Monterey Bay Aquarium Research Institute (MBARI)
7700 Sandholdt Rd.
Moss Landing, CA 95039-0628

RE: ONR-BAA-07-040 Topics 3A and 3B.2.

Dear Chris:

McLane Research Laboratories Inc is pleased to support further development and commercialization of the technology described in your proposal to ONR-BAA-07-040 Topics 3A and 3B.2. As an oceanographic equipment manufacturing company with a 25-year history of successful deployments of time-series *in situ* pumps, valves, and filter sampling systems, we look forward to observing the detailed manufacturing process and deployment requirements of the next generation Environmental Sample Processor (ESP).

In addition, we expect that our close proximity to and prior successful collaborations with Woods Hole Oceanographic Institution will be beneficial to the project. As an industry partner during this next phase of sampler development, we expect that we will be able to combine our existing knowledge and experience in the oceanographic sampler market to improve the prospects for future commercialization of the ESP.

We look forward to working together on this project.

Sincerely,

Michael Mathewson
General Manager

850 Lincoln Centre Drive
Foster City, CA 94404

November 30, 2007

Dr. Chris Scholin
Monterey Bay Aquarium Research Institute (MBARI)
7700 Sandholdt Rd.
Moss Landing, CA 95039-0628

RE: ONR-BAA-07-040 Topics 3A and 3B.2.

Dear Chris:
Applied Biosystems is very pleased that you and your team are advancing the use of molecular biological analysis for detecting and characterizing waterborne organisms. This represents a very promising application of nucleic acid detection technology and is highly relevant to harmful algal bloom research and monitoring. Your work towards use of real-time PCR in the environmental surveillance arena will certainly extend that concept further and broaden applications of this technology among a diverse user base.

Based on our discussions thus far, we believe that Applied Biosystems has commercial offerings including instrumentation, reagents and software that could add value to the research and technology transfer you have proposed in your response to ONR-BAA-07-040 Topics 3A and 3B.2. Specifically, we believe the following offerings are currently relevant to your pursuits.

TaqMan® Environmental Master Mix 2.0
TaqMan® Exogenous Internal Positive Control Reagents
AB 7500 Real-Time PCR System

In addition to these offerings, we have a world-class design and development pipeline for real-time PCR assays. This pipeline has produced commercial testing kits for various pathogens including E. coli O157:H7, Listeria monocytogenes, Salmonella enterica, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter sakazakii, Influenza virus, Bacillus anthracis, Yersinia pestis, Francisella tularensis, and Brucella species. We are confident that this pipeline could be employed to help deliver tests for many of the species that you mention in your proposal and others that are relevant in the environmental monitoring domain.

Aside from technical support and collaborative opportunities, I look forward to exploring options for commercialization of the ESP technology with you and the project team. This project represents an excellent opportunity for my team to learn more about the end-users of this new system, and how AB might participate in providing equipment and reagents for this emerging and rapidly growing market.

Lastly, I would like to highlight that Applied Biosystems owns rights to a broad portfolio of intellectual property related to the real-time detection and analysis of genetic material. Our licensing department is available to meet with you and your research partners to assess whether items in this portfolio might contribute to your success. We recognize the global importance of advancing remote in-water sensing technologies for water quality monitoring, water resource management and the ocean sciences. We look forward to continuing to work with you and your team.

Best regards,

Chris Melançon
Director, BioSecurity Business

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Ocean Service, N/SCI1
Silver Spring MD 20910

December 3, 2007

Dr. Donald M. Anderson
Senior Scientist
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543

Dear Don,

I am writing in support of your proposal to the NOPP program: "Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployments, Technology Transfer, and Commercial Evaluation".

NOAA has implemented an operational capability for forecasting of Harmful Algal Blooms in the Gulf of Mexico. We are moving forward with plans to expand this capability through a set of regional operational forecast capabilities, specifically including the Gulf of Maine, and I am lead scientist for the transition of research capabilities into the operational forecasts. The Gulf of Maine is a priority region for implementation of this capability because of the significant and annual HAB impacts and because of the significant progress in research. Critical to the success of an operational forecast system are moorings that provide the necessary data on the presence of HAB cells (along with important oceanographic properties) at critical locations. The GoMOOS moorings can provide the critical information where the HAB transport is greatest. I cannot emphasis enough how adding HAB ESP sensors to this observing system will aid in assuring accurate and reliable operational forecasts of HABs. An operational system will be closely integrated with the local fisheries management community and depend on the GoMOOS and regional research community.

I strongly endorse your proposal and look forward to coordination with your group on HAB monitoring and forecasting efforts in the Gulf of Maine.

Sincerely,

Richard P. Stumpf, Ph.D.
Oceanographer

STATE OF MAINE
DEPARTMENT OF
MARINE RESOURCES
MARINE RESOURCES LABORATORY
P.O. BOX 8, 194 MCKOWN POINT RD
W. BOOTHBAY HARBOR, MAINE 04575-0008

JOHN ELIAS BALDACCI
GOVERNOR

GEORGE D. LAPOINTE
COMMISSIONER

November 30, 2007

Dr. Donald M. Anderson
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543

Dear Don:

I am happy to support the NOPP proposal, "Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployments, Technology Transfer, and Commercial Evaluation", you are submitting with MBARI. As a member of the Board of Directors of the Gulf of Maine Ocean Observing System (GoMOOS) and Director of the Maine Department of Marine Resources (DMR) research and monitoring programs, I fully support this collaborative effort to support ocean observing, improve our capability to monitor and understand harmful algal blooms (HABs), and manage our living marine resources.

Data from the GoMOOS buoy array have been invaluable to Maine DMR in helping us to better understand circulation patterns in the Gulf of Maine, the effects of temperature on molting of lobsters, movements of shrimp, and predictions of *Alexandrium* distribution during the 2005 red tide event. However, none of those buoys contain sensors to indicate the presence of HAB species in the region. Utilizing ESP buoys just offshore of Casco Bay, Maine and at the GoMOOS B site in an array to obtain near real-time estimates of *Alexandrium* abundance would be extremely useful information to our resource managers since that region of the coast is the first to experience shellfish toxicity each spring.

I fully support the development of tools and products that will support our stock assessment and management activities in the Northeast. If you need supporting information on shellfish toxicity along the Maine coast to compare with the ESP measurements, please let us know. Good luck with your proposal.

Sincerely,

Linda P. Mercer

Linda P. Mercer, Director
Bureau of Resource Management

http://www.Maine.gov/dmr

PHONE: (207) 633-9500 FAX: (207) 633-9579

Commonwealth of Massachusetts
Division of Marine Fisheries
251 Causeway Street, Suite 400
Boston, Massachusetts 02114
(617)626-1520
fax (617)626-1509

Paul J. Diodati
Director

Deval Patrick
Governor
Ian A. Bowles
Secretary

November 30, 2007

Dr. Donald M. Anderson
Biology Department, MS #32
Woods Hole Oceanographic Institution
Woods Hole, MA 02543

Dear Don:

I am pleased to learn that you are submitting a proposal with MBARI to build and deploy Environmental Sample Processors (ESPs) for eventual deployment off Casco Bay, Maine, Portsmouth, New Hampshire and Salt Pond, Massachusetts. Information on cell populations in each of these areas is very important to my group at the Massachusetts Division of Marine Fisheries as we are responsible for monitoring shellfish toxicity for the whole commonwealth. As you know, *Alexandrium* populations are transported down the coast from western Maine in the spring, so detection of populations off Casco Bay, Maine and Portsmouth, NH (GoMOOS Buoy B) would put us on alert for transport to our northern coastline and into Massachusetts Bay. A separate deployment in Salt Pond within the Nauset Estuarine System on Cape Cod is extremely beneficial to our management needs since it is an isolated system with its own dynamic that is not well understood. The Nauset system has experienced intense red tides almost every year over the last ten years requiring closure to shellfishing with subsequent hardship to those that depend on that resource for their income. Near real-time data on *Alexandrium* populations in this area would also prove very useful to us at the state level as well as to local resource managers.

Our long history of close collaboration with your laboratory (20+years) has been especially beneficial since during your previous research, you have been able to provide the underlying understanding of bloom dynamics in the region along with timely data on *Alexandrium* populations in the Gulf of Maine and Salt Pond areas that helped with management decisions. This was extremely critical during the historic red tide of 2005 where we were able to learn about the location and abundance of these populations, particularly in close proximity to productive Massachusetts' shellfish beds. Some of those beds were closed based on your timely *Alexandrium* estimates. However, this involved a tremendous effort on your part as well as considerable funding for emergency shiptime and personnel. The ESP technology will potentially remedy that problem since a well-placed ESP coupled with large-scale bloom modeling efforts will provide data and information that your group cannot possibly provide on a continual basis. I believe ESPs deployed at strategic locations in the GOM could have detected that 2005 bloom. Therefore, MA DMF strongly supports your efforts to automate this process through the use of continuous samplers in the field with the data flowing into a network of available products useful for daily management of the shellfish resources.

For our part, we will gladly continue to share our weekly shellfish toxicity data with you, most especially during the times that the ESPs are in the water so you have a comparison of your ESP and other ground-truth *Alexandrium* estimates with the resultant toxicity levels. I am very excited to see significant progress being made towards an automated observing system for HABs in the Gulf of Maine.

Good luck with your proposal - we look forward to continuing our close collaboration with you.

Sincerely,

J. Michael Hickey
Chief Shellfish Biologist

c: Paul Diodati, Director
Dan McKiernan, Deputy Director
Dave Whittaker, Senior Marine Fisheries Biologist

VOLUME 2: COST PROPOSAL

ONR BAA ANNOUNCEMENT # ONR-BAA-07-040

Title: Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployment, Technology Transfer, and Commercial Evaluation

Prime Offeror:

Monterey Bay Aquarium Research Institute
Moss Landing, California

Subrecipients:

Woods Hole Oceanographic Institution
Woods Hole, Massachusetts

NOAA National Ocean Service
Charleston, South Carolina

Technical Contact:
Dr. Christopher Scholin
7700 Sandholdt Road
Moss Landing, CA 95039
831 775 1700(ph) 831 775 1620 (fax)
Scholin@mbari.org

Administrative Contact:
Patrice A. Carroll
7700 Sandholdt Road
Moss Landing, CA 95039
831 775 1700(ph) 831 775 1620 (fax)
grants@mbari.org

Duration of effort: 1 September 2008 – 31 August 2011

Funds Requested: Y1- $503,921 Y2 - $510,266 Y3 - $513,216 Total: $1,527,404

Employer ID: 77-0150850
Type of Applicant: Research Institute, Private, Non Profit 501(c)(3) Organization
Submitted to CFDA Number: 12.300

Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployments, Technology Transfer, and Commercial Evaluation

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NON – UNOLS SHIP USE

For year-2, limited IOOS funds will support only a single deployment and recovery in the coastal ocean at Buoy B (Vol.1, Fig. 6). To revisit the Buoy B site, we request 6 days of ship time on the R/V Tioga (WHOI) during year-2 to cover two trips to Buoy B for instrument deployment and ground-truth sampling near the mooring. Costs for that total $14.K, 6 days at $2,400 per day.

The R/V Tioga is a very capable coastal research vessel (60ft) with required lift capabilities from the A-frame, dedicated CTD/rosette, current profilers, and an underway hydrographic data collection system (see http://www.whoi.edu/page.do?pid=8159).

TABLE OF PARTNERSHIPS- MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

Principal Investigator	Institution	FY 08 funds Requested	FY 09 funds Requested	FY10 funds Requested	TOTAL
C. Scholin Lead PI	MBARI (Non-profit)	$475,797	$287,107	$248,761	$1,011,666
D. Anderson B. Keafer D. McGillicuddy Co-Pis	WHOI (Non-Profit)	$28,124	$223,159	$264,455	$515,738
G. Doucette Co-PI	NOAA/NOS (Government)	$2,000	$49,380	$49,899	$101,279
C. Melancon Industry Rep	Applied Biosystems Inc. **(Business)**	$0	$0	$0	$0
C. Mathewson Industry Rep	McLean Research Laboratories Inc. **(Business)**	$0	$0	$0	$0

Budget

MBARI - SUMMARY BUDGET	Yr 1		Yr 2		Yr 3		Yr 1-3	
SALARIES/WAGES	# Months	2008 -2009	# Months	2009 - 2010	# Months	2010 - 2011	# Months	Total
Scholin - Principal Investigator	1.00		1.00		1.00		3.00	
Massion - Sr. Mechanical Engineer	1.00		1.00		0.50		2.50	
Jensen - Electrical Engineer	1.00		1.00		1.00		3.00	
Roman - Software Engineer	1.00		1.00		1.00		3.00	
Everlove - Mechatronics Engineer	3.00		6.00		4.00		13.00	
Schramm - Software Engineer	1.50		1.00		1.00		3.50	
Machinist	2.00						2.00	
Total Reg Salary		**91,638**		**99,044**		**82,446**	**30.00**	**417,886**
TOTAL SALARY & WAGES		91,638		99,044		82,446	30.00	417,886
BENEFITS (Rate-Reg) 53.00%		48,568		52,493		43,696		144,758
TOTAL BENEFITS		48,568		52,493		43,696		144,758
TOTAL SAL & BEN		**140,206**		**151,537**		**126,142**	**0.00**	**562,644**
EQUIPMENT Includes CIP Equip items >$5K								
1 Pressure housing		13,250						13,250
2 CTD/fluorometer/OBS/O		10,000						10,000
Subtotal Equipment		**23,250**						**23,250**
CIP EQUIPMENT - Fabrication								
Outside Service 2x 40K for mfg parts for ESP fab		80,000						80,000
COTS parts 2x 40K for ESP fab		80,000						80,000
COTS and mfg parts for ESP mooring		40,000						40,000
Subtotal CIP Equipment		**200,000**						**200,000**
SUBAWARD								
Subaward: WHOI		28,124		223,159		264,455		515,738
Subtotal Subaward		**28,124**		**223,159**		**264,455**		**515,738**
SUPPLIES: Instrument Spares		0		5,000		5,000		10,000
Mooring Hdwr/maint/spares				10,000		10,000		20,000
Expendable reagents for ESP probe chemistry		10,000		15,000		15,000		25,000
Subtotal Supplies		**10,000**		**30,000**		**30,000**		**70,000**
TRAVEL Domestic Travel: $300 air, $500 hotel, $200 meals,								
$200 rental car/truck = $1.2K per trip								0
Yr 1 - 2 to scope site, Yr2&3 3 to deps/turnarounds		3,600		3,600		3,600		
Subtotal Travel		**3,600**		**3,600**		**3,600**		**10,800**
SHIPPING/POSTAGE								
Shipping		5,000		5,000		5,000		15,000
Subtotal Shipping		**5,000**		**5,000**		**5,000**		**15,000**
Total Direct Costs		**410,180**		**413,296**		**429,197**		**1,252,674**
Modified Total Direct Costs Base		**183,806**		**190,137**		**164,742**		**538,686**
Total Indirect Costs Rate: 51%		**93,741**		**96,970**		**84,019**		**274,730**
TOTAL DIRECT & INDIRECT COSTS		**503,921**		**510,266**		**513,216**		**1,527,404**

Budget

MBARI - INSTITUTIONAL BUDGET	Yr 1		Yr 2		Yr 3		Yr 1-3	
SALARIES/WAGES	# Months	2008 -2009	# Months	2009 - 2010	# Months	2010 - 2011	# Months	Total
Scholin - Principal Investigator	1.00		1.00		1.00		3.00	
Massion - Sr. Mechanical Engineer	1.00		1.00		0.50		2.50	
Jensen - Electrical Engineer	1.00		1.00		1.00		3.00	
Roman - Software Engineer	1.00		1.00		1.00		3.00	
Everlove - Mechatronics Engineer	3.00		6.00		4.00		13.00	
Schramm - Software Engineer	1.50		1.00		1.00		3.50	
Machinist	2.00						2.00	
Total Reg Salary		**91,638**		**99,044**		**82,446**	**30.00**	**273,128**
TOTAL SALARY & WAGES		**91,638**		**99,044**		**82,446**	**30.00**	**273,128**
BENEFITS (Rate-Reg) 53.00%		48,568		52,493		43,696		144,758
TOTAL BENEFITS		**48,568**		**52,493**		**43,696**		**144,758**
TOTAL SAL & BEN		**140,206**		**151,537**		**126,142**	**0.00**	**417,886**
EQUIPMENT Includes CIP Equip items >$5K								
1 Pressure housing		13,250						13,250
2 CTD/fluorometer/OBS/O		10,000						10,000
Subtotal Equipment		**23,250**						**23,250**
CIP EQUIPMENT - Fabrication								
Outside Service 2x 40K for mfg parts for ESP fab		80,000						80,000
COTS parts 2x 40K for ESP fab		80,000						80,000
COTS and mfg parts for ESP mooring		40,000						40,000
Subtotal CIP Equipment		**200,000**						**200,000**
SUPPLIES: Instrument Spares		0		5,000		5,000		10,000
Mooring Hdwr/maint/spares				10,000		10,000		20,000
Expendable reagents for ESP probe chemistry		10,000		15,000		15,000		25,000
Subtotal Supplies		**10,000**		**30,000**		**30,000**		**70,000**
TRAVEL Domestic Travel: $300 air, $500 hotel, $200 meals, $200 rental car/truck = $1.2K per trip								0
Yr 1 - 2 to scope site, Yr2&3 3 to deps/turnarounds		3,600		3,600		3,600		
Subtotal Travel		**3,600**		**3,600**		**3,600**		**10,800**
SHIPPING/POSTAGE								
Shipping		5,000		5,000		5,000		15,000
Subtotal Shipping		**5,000**		**5,000**		**5,000**		**15,000**
Total Direct Costs		**382,056**		**190,137**		**164,742**		**736,936**
Modified Total Direct Costs Base		**183,806**		**190,137**		**164,742**		**538,686**
Total Indirect Costs Rate: 51%		**93,741**		**96,970**		**84,019**		**274,730**
TOTAL DIRECT & INDIRECT COSTS		**475,797**		**287,107**		**248,761**		**1,011,666**

MBARI Statement of Work

Year 1 September 2008-August 2009

- Procure parts for and fabricate two core Environmental Sample Processors (ESPs); calibrate and conduct instrument acceptance tests at MBARI.
- Convene ESP operations training workshop at MBARI for project participants. This will include instruction on laboratory use, set-up, programming, data management, basic service, preparation for deployment, at-sea deployment/recovery of the ESP.
- Install core ESPs at the NOAA/NOS Marine Biotoxins Laboratory (Doucette) and WHOI (Anderson lab), and provide a dedicated, custom-configured laptop for operating the ESP and processing data. Installation of the ESP requires travel to partner institutions for instrument set-up and preliminary testing to confirm the machines are operating properly.
- Produce custom probe arrays and coordinate production of quality-assured expendable reagents for use at MBARI, NOAA and WHOI (this requires moderate scale production runs, documenting QA/QC protocols, creating a tracking scheme for reagent lots, etc.).
- Coordinate inter-lab calibration experiments using standardized operating procedures to evaluate instrument performance against natural and synthetic samples.
- Fabricate one coastal mooring for ESP surface water operations (includes data telemetry, CTD package, 50m-rated pressure housing, power system, subsurface platform, rode anchor and shipping container). Deliver to WHOI ahead of year-2 field operations.
- Coordinate formation of industry partnerships; initiate opportunities for commercializing the ESP.

Year 2 September 2009-August 2010

- Produce quality-assured custom probe arrays and expendable reagents for use at MBARI, NOAA and WHOI in support of ESP operations. Coordinate inter-lab calibration experiments and logistics in preparation for Salt Pond deployment spring/summer 2010 (see Vol. 1).
- Deploy ESP at Salt Pond, establish data telemetry system. This includes travel to WHOI to assist with pre-deployment instrument configuration and remote operation, and to establish procedures data interpretation and dissemination.
- Participate in field and laboratory studies to validate performance of the ESP as described in Vol. 1.
- Provide support for integrating new toxin assays devised by Doucette's team for use on the ESP (see Vol. 1 or 2, Appendix 1).
- Summarize results for publication in peer-reviewed journals.
- Continue formation of industry partnerships and efforts to commercialize the ESP. Work towards definition of instrumentation products, accessories, expendable reagents, production costs, required customer support, pricing structure, and market assessment.

Year 3 September 2010-August 2011

- Produce and quality-assure custom probe arrays and expendable reagents for use at MBARI, NOAA and WHOI in support of ESP operations. Coordinate inter-lab calibration experiments and logistics in preparation for fielding three ESPs April-June 2011.
- Deploy an array of three ESPs in the Gulf of Maine (see Vol. 1), establish data telemetry, management and dissemination system. This requires travel to WHOI to assist with instrument configuration, deployment/servicing/recovery, and remote operation.
- Participate in cruises and laboratory work to validate performance of the ESP.
- Summarize results for publication in peer-reviewed journals.
- Complete plan for commoditizing the ESP system.

Declarations

MBARI will retain ownership of the ESPs and the mooring for fielding the instrument throughout the duration of this project. By the end of year-1, equipment constructed will be formally transferred to NOAA/NOS Marine Biotoxins Laboratory and WHOI and remain in receiving labs' custody for the duration of this project. During year-3, both ESPs produced under the terms of this proposal will be used for field operations in the Gulf of Maine. At the end of this project, all custom made hardware will be returned to MBARI in lieu of any future agreement to the contrary.

In the event the instrument malfunctions or is damaged, MBARI will service, repair or replace the equipment at its discretion.

NOAA and WHOI acknowledge that the ESP is an experimental device for research purposes only, and as such will not hold MBARI liable for any damages that may be incurred as a result of using the instrument or its mooring in the laboratory or field.

MBARI Budget Justification

Personnel

C. Scholin, P.I., will oversee project planning and implementation in collaboration with co-I's Doucette, Anderson, Keafer, and McGillicuddy. He will also oversee activities at MBARI to fabricate, test, ship to and install equipment at partner institutions in concert with the MBARI engineering and science support team. He will spend 1 mo per year on this project.

Roman Marin III, Senior Research Specialist in Scholin's lab, will participate up to 6mos per year at no cost to this project. Mr. Marin will be responsible for production of custom reagents and arrays, support inter-lab calibration experiments and participate in field and laboratory studies as required.

The engineering team consists of Gene Massion (Mechanical Engineer), Scott Jensen (Electrical Engineer), Brent Roman (Software Engineer; ESP operation), Cheri Everlove (Mechatronics Engineer), Rich Schramm (ESP data management, GUI) and a machinist (TBD). Gene Massion will devote 1 mo time in year-1 &2, 0.5 mo. in year-3. He will be responsible for configuring moorings/dockside installations and contribute to marine operations. Scott Jensen is the lead engineer for the core ESP and will devote 1 mo time to this project each year. He will be responsible for overall operational integrity of the instruments and engineering matters related to field deployments and laboratory operations. He will participate in the training workshop, as well as off-site visits to NOAA and WHOI as required for installing equipment in partner labs and instrument deployment/recoveries. Brent Roman will be responsible for configuring the ESPs and dedicated laptops, and will devote 1 mo time to this project each year. He will provide support for integrating new toxins assays that will come from the Doucette lab, and will provide support for lab and field operations. Cheri Everlove will be responsible for procuring parts to build the ESPs and mooring, undertake assembly/documentation of the instruments and mooring, calibrate the instruments, participate in the user workshop, and will travel to partner institutions as required for laboratory and field operations support. She will devote 3 mo in year-1, 6 mo in year-2, and 4 mo in year-3. Rich Schramm will oversee the data management (DM)/user interface (UI) application that project participants will use throughout this project. Development of the DM/UI application is currently sponsored by MBARI and will reach operational status in 2008. This system will allow users to configure the ESP for extended missions and manage the data (and associated metadata) that will stem from those deployments. As the lead on the DM/UI effort, he will work with project participants to establish standards for sharing ESP data and data products via web access, and will participate in off-site laboratory and field trials as required. He will devote to this project 1.5 mo in year-1 and 1 mo each in year-2 & 3. Finally, 2 mo of machinist time (from the MBARI pool of machinists) is requested in year-1 for assisting with custom fabrication of ESP instrument and mooring components.

Capital Equipment

Funds are requested to purchase a 50m-rated pressure housing for the ESP ($13.25K) and CTD package ($10K) for outfitting the ESP mooring that will be deployed in outlaying years of the project. The CTD/fluorometer/transmissometer (or OBS) will be bundled with the ESP to provide contextual environmental data during field deployments. This equipment is essential to ESP field operations.

Fabricated Equipment

Both commercial-off-the-shelf (COTS) and custom manufactured parts (MP) are needed to construct the core ESPs and mooring. Based on a production run of several units, and assuming some minor re-work that may be required during the assembly (e.g. particular COTS part that is no longer available, changes to all lead-free electronics, processor upgrades, etc) as well as purchase of an initial set of spares that are compliant with this production run, cost of COTS and MP are estimated as follows:

Type of part	ESP instrument	ESP mooring
COTS	$40K	$30K
MP	$40K	$10K

Over time we expect costs of the ESP and mooring to decline significantly as production volumes increase and the design matures with larger scale production in mind.

Supplies

Funds are requested for all materials used to produce custom probe arrays, reagents and cultured and synthetic samples for inter-lab calibration (DNA probes, expendable reagents, expendable plastics, culture supplies, probe array membrane support, filters, etc.). Supplies total $10K in year-1 and $15K each for year-2 & 3. In addition to science supplies, we request $5K each in year-2 & 3 for instrument spares (electronic components, syringes, valves, cables, etc). Finally, we request $10K each in year-2 & 3 for spares/equipment maintenance to support field operations (batteries, custom fabrication for dockside deployment, mooring spares, telemetry system, batteries, etc. – all COTS or MP components that could be lost or damaged under normal operating conditions and would require immediate replacement during field trials/cruises to avoid interruption of ESP service).

Travel

A total of $3.6K per year is requested for travel to partner institutions to install/service equipment at partner labs (year-1), deploy/recover ESPs (year-2 & 3), participate in laboratory and field trials, and to attend annual project meetings, relevant workshops or scientific meetings. Each trip is estimated as being multi-day with expenses roughly as follows: $300 airfare, $500 hotel, $200 meals, $200 car/truck rental. In practice, each trip may be more or less than the assumed $1.2K per person per trip depending on the specifics of what is required of the project team. We expect persons from MBARI will participate in all field expeditions, and it may be necessary for either science or engineer personnel to travel to partner institutions in the event problems arise and can not be solved via phone or e-mail exchange.

Shipping

Shipping expenses are estimated at $5k per year. This includes charges to transport samples, reagents, instrument and spares, mooring equipment, etc. It may be necessary to ship the ESPs or components therein between partner institutions and MBARI for upgrade, repair or replacement as the study progresses. In some cases, shipments will require overnight service (e.g., some reagents or samples that require temperature control).

Indirect charges

All charges associated with labor, fringe benefits, travel, and non-capital expenses are subject to MBARI's federally negotiated indirect cost rate of 49% MTDC. Total pre-production for materials is $223.25K. Of that amount, $13.25K is for a 50m-rated pressure housing for the ESP and is considered capital equipment. Capital equipment also includes a dedicated CTD package ($10K) for the mooring. No indirect costs are applied to capital equipment (i.e., greater than 5K).

MBARI's Indirect Rate Agreement is established with NSF. The current Rate Agreement is dated September 4, 2007. Subrecipient WHOI's rate Agreement is established with ONR on a Salary and Fringe Benefit base, as described on the following page. NOAA does not include indirect costs in the total budget as it is a federal entity.

Budget

WHOI	Yr 1		Yr 2		Yr 3		Yr 1-3	
SALARIES/WAGES	# Months	2008 -2009	# Months	2009 - 2010	# Months	2010 - 2011	# Months	Total
Anderson - Co Principal Investigator	0.00	0	2.00		1.00		3.00	0
McGillicuddy - Co Principal Investigator	0.00	0	0.00		0.53		0.53	0
Anderson - Sr. Scientist	0.00		2.00		1.00		3.00	0
Keafer - Res Associate	0.78		4.00		2.75		7.53	0
2 Other Professionals	1.00		3.64		8.71		13.35	
TOTAL SAL & BEN		**11,065**		**92,592**		**122,251**	**0.00**	**225,908**
SUPPLIES:								
Laboratory supplies		4000		7,000		15,000		26,000
Subtotal Supplies		**4,000**		**7,000**		**15,000**		**26,000**
TRAVEL								
Domestic		3,205		3,822		2,481		9,508
Subtotal Travel		**3,205**		**3,822**		**2,481**		**9,508**
OTHER DIRECT COSTS								
Guest Student Stipends				16,200				
Computer Services & other						1,187		1,187
Shipping/Postage				1,682		1,561		3,243
Insurance				5,000		13,500		18,500
Tioga =- full day				14,400				14,400
Subtotal Shipping		**0**		**37,282**		**16,248**		**53,530**
Total Direct Costs		**18,270**		**140,696**		**155,980**		**314,946**
Laboratory Overhead		**6,453**		**54,000**		**71,034**		**131,487**
General & Administrative		3,401		28,463		37,441		69,305
Total Indirect Costs		**9,854**		**82,463**		**108,475**		**200,792**
TOTAL DIRECT & INDIRECT COSTS		**28,124**		**223,159**		**264,455**		**515,738**

Cumulative Budget

	Approximate Labor Months			
	09/01/08 08/31/09	**09/01/09 08/31/10**	**09/01/10 08/31/11**	**Total**
A. Senior Personnel				
1. DONALD M. ANDERSON, Co-PI	0.00	2.00	1.00	3.00
2. BRUCE A. KEAFER, Co-PI	0.78	4.00	2.75	7.53
3. DENNIS J. MCGILLICUDDY, Co-PI	0.00	0.00	0.53	0.53
B. Other Personnel				
2. Other Professionals	1.00	3.64	8.71	13.35
C. Total Salaries & Benefits	**$11,065**	**$92,592**	**$122,251**	**$225,908**
E. Travel				
1. Domestic	3,205	3,822	2,481	9,508
Total Travel	**3,205**	**3,822**	**2,481**	**9,508**
G. Other Direct Costs				
1. Materials and Supplies	4,000	7,000	15,000	26,000
4. Computer Svc	0	0	660	660
6. Other	0	37,282	15,588	52,870
Total Other Direct Costs	**4,000**	**44,282**	**31,248**	**79,530**
H. Total Direct Costs	**$18,270**	**$140,696**	**$155,980**	**$314,946**
I. Indirect Costs				
1. Laboratory Overhead	6,453	54,000	71,034	131,487
2. General & Administrative	3,401	28,463	37,441	69,305
Total Indirect Costs	**9,854**	**82,463**	**108,475**	**200,792**
J. Total Direct and Indirect Costs	**$28,124**	**$223,159**	**$264,455**	**$515,738**
L. Amount of this Request	**$28,124**	**$223,159**	**$264,455**	**$515,738**

Anderson component

	Approximate Labor Months			
	09/01/08 08/31/09	**09/01/09 08/31/10**	**09/01/10 08/31/11**	**Total**
A. Senior Personnel				
1 . D. ANDERSON, Senior Sci	0.00	2.00	1.00	3.00
2 . B. KEAFER, Res Assoc III	0.78	4.00	2.75 *	7.53
B. Other Personnel				
2. Other Professionals				
J. KLEINDINST, Ctr Admin II	0.00	0.00	0.50	0.50
K. NORTON, Res Asst II	1.00	3.64	2.50*	7.14
C. Total Salaries & Benefits	**$11,065**	**$92,592**	**$69,638**	**$173,295**
E. Travel (see detail)				
1. Domestic	3,205	3,822	2,481	9,508
Total Travel	**3,205**	**3,822**	**2,481**	**9,508**
G. Other Direct Costs (see detail)				
1. Materials and Supplies	4,000	7,000	15,000	26,000
6. Other	0	37,282	15,061	52,343
Total Other Direct Costs	**4,000**	**44,282**	**30,061**	**78,343**
H. Total Direct Costs	**$18,270**	**$140,696**	**$102,180**	**$261,146**
I. Indirect Costs				
1. Laboratory Overhead	6,453	54,000	40,350	100,803
2. General & Administrative	3,401	28,463	21,268	53,132
Total Indirect Costs	**9,854**	**82,463**	**61,618**	**153,935**
J. Total Direct & Indirect Costs	**$28,124**	**$223,159**	**$163,798**	**$415,081**
L. Amount of this Request	**$28,124**	**$223,159**	**$163,798**	**$415,081**

Travel Detail

Period 1 09/01/08 - 08/31/09

1. Domestic

trip to MBARI (Boston, MA to Monterey, CA)

Lodging	Nightly Rate (inc. Tax)	1 Rooms	14 Nights	@125	1,750	
Airfare	Round Trip	1 Tickets		@600	600	
Ground	General Grnd Transport.	1		@225	225	
Per Diem	Inside Continental US	1 People	14 Days	@45	630	
				Trip Total:	3,205	

Total Domestic Travel - Period 1 **3,205**

Period 2 09/01/09 - 08/31/10

1. Domestic

Scientific meeting (Boston, MA to San Francisco, CA)

Lodging	Nightly Rate (inc. Tax)	1 Rooms	6 Nights	@125	750	
Misc. Expens	Meeting Registration	1 People		@400	400	
Airfare	Round Trip	1 Tickets		@600	600	
Ground	General Grnd Transport.	1		@200	200	
Per Diem	Inside Continental US	1 People	6 Days	@45	270	
				Trip Total:	2,220	

Salt Pond field work (Woods Hole, MA to Eastham, MA)

Ground	Mileage	10		@53	530	
Per Diem	Inside Continental US	1 People	10 Days	@12	120	
				Trip Total:	650	

NH field work (Woods Hole, MA to Portsmouth, NH)

Lodging	Nightly Rate (inc. Tax)	2 Rooms	2 Nights	@125	500	
Ground	Mileage	2		@136	272	
Per Diem	Inside Continental US	2 People	2 Days	@45	180	
				Trip Total:	952	

Total Domestic Travel - Period 2 **3,822**

Period 3 09/01/10 - 08/31/11

1. Domestic

Scientific meeting (Boston, MA to San Francisco, CA)

Lodging	Nightly Rate (inc. Tax)	1 Rooms	6 Nights	@125	750	
Misc. Expens	Meeting Registration	1 People		@400	400	
Airfare	Round Trip	1 Tickets		@600	600	
Ground	General Grnd Transport.	1		@200	200	
Per Diem	Inside Continental US	1 People	6 Days	@45	270	
				Trip Total:	2,220	

Narragansett field work (Woods Hole, MA to Narragansett, RI)

Ground	Mileage	3		@87	261	
				Trip Total:	261	

Total Domestic Travel - Period 3 **2,481**

Other Direct Costs - Detail

	09/01/08 08/31/09	09/01/09 08/31/10	09/01/10 08/31/11	Total
G. Other Direct Costs				
1. Materials and Supplies				
a. Laboratory Supplies	4,000	7,000	15,000	26,000
Total Materials and Supplies	**4,000**	**7,000**	**15,000**	**26,000**
6. Other				
a. Guest Student Stipend	0	16,200	0	16,200
b. Insurance	0	500	0	500
c. Over the Side Insurance	0	4,500	13,500	18,000
d. Shipping & Postage	0	1,682	1,561	3,243
e. Tioga - full day	0	14,400	0	14,400
Total Other	**0**	**37,282**	**15,061**	**52,343**
Total Other Direct Costs	**4,000**	**44,282**	**30,061**	**78,343**

McGillicuddy component

	Approximate Labor Months			
	09/01/08 08/31/09	**09/01/09 08/31/10**	**09/01/10 08/31/11**	**Total**
A. Senior Personnel				
1 . D. ANDERSON, Senior Sci	0.00	0.00	0.00	0.00
2 . D. MCGILLICUDDY, Senior Sci	0.00	0.00	0.53	0.53
B. Other Personnel				
2. Other Professionals				
K. SMITH, Res Assoc III	0.00	0.00	5.71	5.71
C. Total Salaries & Benefits	**$0**	**$0**	**$52,613**	**$52,613**
G. Other Direct Costs (see detail)				
4. Computer Svc	0	0	660	660
6. Other	0	0	527	527
Total Other Direct Costs	**0**	**0**	**1,187**	**1,187**
H. Total Direct Costs	**$0**	**$0**	**$53,800**	**$53,800**
I. Indirect Costs				
1. Laboratory Overhead	0	0	30,684	30,684
2. General & Administrative	0	0	16,173	16,173
Total Indirect Costs	**0**	**0**	**46,857**	**46,857**
J. Total Direct & Indirect Costs	**$0**	**$0**	**$100,657**	**$100,657**
L. Amount of this Request	**$0**	**$0**	**$100,657**	**$100,657**

Other Direct Costs - Detail

	09/01/08 08/31/09	09/01/09 08/31/10	09/01/10 08/31/11	Total
G. Other Direct Costs				
4. Computer Svc				
a. Technical Assistance (CIS)	0	0	660	660
Total Computer Svc	**0**	**0**	**660**	**660**
6. Other				
a. Communications	0	0	225	225
b. Photocopying	0	0	225	225
c. Shipping & Postage	0	0	77	77
Total Other	**0**	**0**	**527**	**527**
Total Other Direct Costs	**0**	**0**	**1,187**	**1,187**

Statement of Work – WHOI

Year 1

WHOI personnel will travel to MBARI and participate in a Monterey Bay, CA deployment of an ESP for training purposes. Following training, we will make standards and perform relevant molecular assays for future validation studies at WHOI. We will also collect field samples during blooms in Salt Pond, Eastham, MA and the Gulf of Maine (GOM) and archive these samples for later ESP testing.

Year 2

After delivery of the first NOPP ESP from MBARI to WHOI, the Anderson laboratory will bench test and operate the unit with additional training and oversight from MBARI. Initial experiments will focus on running standard calibration series using synthetic, cultured and natural samples analyzed with both laboratory-based (96 well plate SHA and whole-cell formats) and ESP methodologies.

The initial ESP field deployment will be in Salt Pond, with ground truthing conducted on a weekly basis during the bloom season. We will also maintain the IOOS-funded ESP deployed at the Buoy B site in the Gulf of Maine. This will entail additional ground truthing and servicing not provided by IOOS funding. McLane Research will be invited to participate in these laboratory and field activities for potential commercialization.

Year 3

Work will require preparation and deployment of three ESPs within the open coastal waters of the GOM. Three cruises are scheduled - one for initial deployment; one for turn-around of all three ESPs; and one for the final recovery of the instruments. A UNOLS request has been submitted for these three cruises (total request 18 days). Near real-time data will be analyzed and compared with samples from these cruises to validate ESP performance.

In addition to the major cruise effort, assimilation of ESP data into our models will also be a significant effort in this year as all three ESPs begin reporting data. McGillicuddy will lead the modeling team to process the data and assimilate it into the nowcast/forecast system using the ROMS adjoint model. Assimilated data products will be distributed to our resource management partners on a timely basis.

Budget Justification - WHOI

Personnel: D.M. Anderson, PI, will be responsible for project planning and coordination of the Gulf of Maine and Salt Pond components. B. Keafer, co-PI, will assist with project planning and execution, and will organize and conduct bench testing, field operations to deploy/recover/ground truth the ESP, and *A. fundyense* analyses of the samples and ESP data stream. K. Norton, Research Assistant, will assist with all lab and field-testing of ESP including participation in cruises and whole cell and SHA sample processing. J. Kleindinst, Administrator, will provide support in year three, assisting with reports and publications.

In year three, co-PI D.J. McGillicuddy will oversee the effort to assimilate ESP data into the near-real-time quasi-operational forecast model of *A. fundyense* currently operating in the region[1]. The actual hands-on work will be carried out by Research Associate K.W. Smith. Mr. Smith is an expert in data assimilation techniques, and is familiar with the ROMS-based adjoint methodology. Smith will be responsible for processing the ESP data, preparing it for assimilation into the model, executing data-assimilative simulations, and visualizing the results. McGillicuddy will oversee the design of the numerical experiments and participate in the analysis of the results.

The Anderson laboratory has established a successful collaboration with Northeastern University in which coop students come to the lab for six-month periods, gaining knowledge and experience by working on a variety of research topics. To assist with this project, we are budgeting for two students in year two (see Other Costs below).

Travel. In year one, we are requesting funds for B. Keafer to travel to MBARI for training prior to ESP deployments. In years two and three, we have budgeted travel for field work: year two includes ten trips to Salt Pond (Eastham, MA) and two trips to Portsmouth, NH; year three includes three trips to Narragansett, RI to load and unload gear for the cruises. (Although we are requesting R/V *Oceanus* time in year three, we are budgeting a modest amount of travel to RI in the event that our shiptime is assigned on the R/V *Endeavor*.) In years two and three, we are requesting funds for one PI to attend a national scientific meeting (such as ASLO) to present results of this project.

Materials and supplies. We are requesting funds for the whole cell and SHA sample collection and processing. Supplies needed include: oligo-nucleotide probes, SHA plates, microscope supplies, various sample containers, filters, and 1 Dewar for Liquid N_2 sample storage.

Other Direct Costs. Over-the-side insurance will be needed for one ESP deployment in year two, and three in year three. Inland marine insurance will also be needed for one ESP unit in year two, which will be located at Salt Pond, Eastham, MA. Shiptime on the R/V *Tioga* (WHOI vessel) is also requested – 6 days in year 2. Funds for shipping in years two and three are needed to cover the cost of shipping samples between MBARI, NOAA-NOS and WHOI for intercalibration. We also anticipate some samples from 'ships of opportunity' and are budgeting funds to cover the cost of shipping samples to us. In year three, modest funds are budgeted for communications and photocopying as well as technical assistance from the WHOI computer center. Funds are requested in year two to support stipend costs for two Northeastern University coop (undergraduate students) for six months each. This project will provide diverse training to co-op students. The Northeastern University cooperative program has proved to be an excellent source of good students for other projects that are ongoing in this laboratory. For a modest cost, we are able to provide a significant learning experience to students who would otherwise not be exposed to oceanography.

Budget Information: The Woods Hole Oceanographic Institution calculates overhead rates (both Laboratory Costs and General & Administrative Costs) as a percent of total direct salaries

[1] http://omgrhe.meas.ncsu.edu/Redtide/Redtide_07/

and benefits, as allowed by OMB Circular A-122. Direct salaries exclude overtime-premium pay. A proposed labor month is equal to 152 hours or 1824 hours annually versus 2080 hours (40 hours/week for 52 weeks). The difference is for vacations, holidays, sick time, and other paid absences, which are included in Employee Benefits.

The rates included in the proposal are negotiated with ONR or they are estimates. When estimated rates are finalized, costs will be in accordance with the rate agreement.

With the assistance of Pricewaterhouse Coopers, WHOI undertook a study to determine what our 1997 combined F&A overhead rate would be, if we used the MTDC method used by most Universities, which are subject to OMB Circular A-21. We repeated the study and determined that our rate for 2004 would be 51.31%--lower than most research institutions and research-intensive universities.

VOLUME 2: COST PROPOSAL

APPENDIX 1. FEDERAL ENTITY – NOAA/NATIONAL OCEAN SERVICE

COST DETAIL

Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployments, Technology Transfer, and Commercial Evaluation

Co-Investigator Dr. Gregory J. Doucette
Marine Biotoxins Program
Center for Coastal Environmental Health & Biomolecular Research
NOAA/National Ocean Service
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This proposal is submitted pursuant to the Broad Agency Announcement ONR-BAA-07-040, dated 21 August 2007, entitled "National Oceanographic Partnership Program (NOPP) and Interagency Committee on Ocean Science and Resource Management Integration (ICOSRMI)," Topic 3.

Funding Requested: $101,279

Period of Performance: May 1, 2008 to April 30, 2011

Gregory J. Doucette
Co-Investigator
Marine Biotoxins Program
NOAA/NOS/NCCOS/CCEHBR

Geoffrey I. Scott
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The National Ocean Service (DOC/NOAA/NOS) is a federal government agency.
Employer ID (EIN or TIN): 52-08216908

Budget

NOS/NOAA		Yr 1		Yr 2		Yr 3		Yr 1-3	
SALARIES/WAGES		# Months	2008	# Months	2009	# Months	2010	# Months	Total
	G. Doucette	2.00	0	2.00	0	2.00	0	6.00	0
	C. Mikulski			4.00	19,600	4.00	20,580	8.00	40,180
	Total Salary & Wages		**0**		**19,600**		**20,580**		**40,180**
	Total Benefits 55% of salary		0		10,780		11,319		22,099
	TOTAL SALARY & BENEFITS		**0**		**30,380**		**31,899**		**62,279**
SUPPLIES	Expendable Reagents (includes custom antibodies				13,000		7,500		20,500
	and protein conjugates for domoic acid and saxitoxin)								
	Protein arrayer maintenance				6,000		6,000		12,000
	Subtotal Supplies		**0**		**19,000**		**13,500**		**32,500**
TRAVEL	Yr. 1: 1 wk to MBARI - ESP training/assay calibration		2,000						2,000
	Yr. 2: 2 trips (1wk/ea) to WHOI - ESP deployment/recovery				3,000				3,000
	Yr. 3: 3 trips (1wk/ea) to WHOI - ESP deployment/						4,500		4,500
	turnaround/recovery								
	Subtotal Travel		**2,000**		**0**		**4,500**		**6,500**
Total Direct Costs			**2,000**		**49,380**		**49,899**		**101,279**
Total Indirect Costs	**Rate:** **0%**		**0**		**0**		**0**		**0**
TOTAL DIRECT & INDIRECT COSTS			**2,000**		**49,380**		**49,899**		**101,279**

Statement of Work – NOAA/ NOS FEDERAL ENTITY

This statement of work outlines the tasks to be performed under the direction of Co-Investigator Dr. Gregory Doucette, representing the Federal entity DOC/NOAA/National Ocean Service. This work constitutes an integrated, coordinated element of the proposal being submitted by Principal Investigator Dr. Christopher Scholin of the Monterey Bay Aquarium Research Institute, and Co-Investigators Drs. Donald Anderson and Dennis McGillicuddy of the Woods Hole Oceanographic Institute. The primary contributions of this component will be the laboratory-based refinement and calibration of an assay for domoic acid (DA) formatted previously for the Environmental Sample Processor (ESP) platform, as well as the initial development and testing of a new assay for the PSP toxin class. Essential to achieving these aims will be delivery of a core ESP instrument from MBARI to Dr. Doucette's laboratory, as described in the main body of this proposal.

The ESP-formatted DA assay was designed and developed as part of a previous NOPP-funded project (NSF OCE-0314089). A protocol for the extraction of DA from phytoplankton samples acquired by the ESP (> 90% recovery) was also developed as part of this work. The DA assay employs a competitive ELISA (cELISA) approach and utilizes a membrane-based protein array with the same chemiluminescence reporting system as used in the LSU rRNA probe-based assay for HAB species detection, thereby permitting 'multiplexing' of several reagents. Briefly, a toxin extract produced onboard the ESP (generally representing material concentrated from 500 to 1000 mL of seawater) is combined with a DA-specific antibody and exposed to a membrane array spotted with a DA-protein conjugate; the toxin in solution competes with the toxin-protein conjugate immobilized on the array for the antibody available in solution; antibody bound to the array (inversely proportional to the toxin concentration in the sample extract) is visualized with a chemiluminescent signal that is captured by an onboard CCD camera (see Fig. 2 in main proposal). The DA assay has undergone several successful test deployments on the ESP in Monterey Bay, CA during 2006 and 2007, representing the first ever autonomous, sub-surface detection of a phycotoxin (manuscript in prep.).

The next critical steps needed to move the DA assay towards a more 'operational' status are further refinement of the assay to minimize changes in response over one to two month deployments, as well as extensive calibration to critically establish assay performance parameters and reproducibility. This labor-intensive effort will require constant access to a core ESP instrument in our laboratory. To date the majority of our assay development work has been carried out using a 'hand-held' assembly consisting of an ESP 'puck' (i.e., membrane array holder) and syringes, which is not capable of accurately reproducing the precise microfluidics of the ESP instrument. Two attempts at calibrating the DA assay on the core ESP unit, using a non-toxic *Pseudo-nitzschia* extract spiked with certified DA reference material (NRC/IMB, Halifax, NS, Canada) to account for matrix effects, have been conducted during visits to Dr. Scholin's

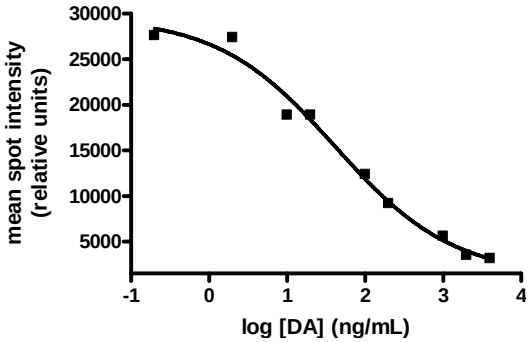

Fig. 1. Calibration curve for DA cELISA conducted onboard core ESP; EC50 (i.e., half-maximal assay response) is ~ 40 ng/mL.

laboratory at MBARI in 2006 and 2007. The most recent results (Fig. 1) indicate that this assay will permit detection of 0.2 ng DA/mL of seawater for a 0.5 L sample collected by the ESP, which is sufficiently sensitive to detect DA levels far below what is generally considered problematic or 'bloom' conditions. However, this result represents a single calibration, whereas it is essential to generate numerous curves to properly characterize assay reproducibility. Moreover, additional, iterative testing of modified assay reagents is required to further refine and assess the long-term stability of the DA test.

A second, equally important focus of our group during this project will be to initiate development and testing of an analogous assay for detection of PSP toxins onboard the ESP. This is especially relevant for ESP deployments planned for the western Gulf of Maine region, where PSP toxin-producing *Alexandrium* spp. will be targeted by the ESP arrays for HAB species detection. All of the information obtained during development and refinement of the DA cELISA for the ESP in terms of array spotting, reagent stability and reactivity, antibody/antigen ratios, etc. will be extremely valuable in designing the PSP toxin assay. We currently have direct access to antibodies against the PSP toxins, as well as saxitoxin-protein conjugates, through a collaborator on another project aimed at developing a surface plasmon resonance (SPR)-based detection method for this toxin class. These fully characterized, custom reagents will be provided at a minimal cost to the project. In fact, the DA antibody and DA-protein conjugate used in the ESP DA assay are currently employed in an SPR-based DA detection method run routinely in our laboratory (formatted as a cELISA; Traynor et al., 2006). We are confident that adopting this same strategy for development of the PSP toxin assay will bring a high probability for success. We should note that the PSP antibodies currently in hand show reduced cross-reactivity with the N-1 hydroxy saxitoxin derivatives (i.e., NEO, GTX1/4, C3/4); however, several parallel efforts outside the scope of this project are being pursued to address this issue and any antibodies developed will be freely available for the work proposed herein.

A primary component of proposal Year 1 essential for carrying out much of the work outlined above will be delivery of a core ESP instrument to our laboratory near the end of the year. Prior to this, our efforts during Year 1 will continue employ the hand-held apparatus described above and used for much of our assay development thus far; however, materials will be sent periodically to Dr. Scholin's laboratory for testing/evaluation on their available core ESP units. We can, to a certain extent, continue to refine the DA cELISA and, importantly, importantly, initiate the concurrent development of the PSP toxin extraction method and ESP-formatted assay using this hand-held device. We will also plan a visit to MBARI during Year 1 for training on laboratory-based operation of the core ESP (we do have some experience already from our two previous visits to perform assay calibrations) so as to minimize down-time following delivery of the instrument. We will likely field at least several DA arrays during the planned ESP training deployment in the western Gulf of Maine in cooperation with the NERACOOS Program (Buoy B). Additional ESP deployments will be conducted in Monterey Bay by Dr. Scholin during the Year 1 time frame (but apart from the current proposal) and we will take advantage of these opportunities to continue our field-based testing of the DA assay.

With the planned delivery of the core ESP instrument to our laboratory by the start of Year 2, much of our focus will turn to the intensive refinement, evaluation, and calibration of the DA cELISA on this unit. We have considerable experience (over 15 years) with the development and

validation of *in vitro* assays for algal toxins and the work required for this process will now be possible with constant access to an ESP instrument. We also expect that following our Year 1 effort, at least a preliminary version of the PSP toxin assay will also be ready for initial formatting/testing on the core ESP. Continued development of this assay should proceed with far greater efficiency given both the availability of an ESP instrument (as compared to use of the hand-held version) and the knowledge gained from our work on the analogous DA cELISA. In addition, we will plan to perform initial field tests of the PSP toxins assay during proposed test deployments of the ESP in the western Gulf of Maine (i.e., Salt Pond, MA; NERACOOS/Buoy B).

Work during Year 3 will shift exclusively to the PSP toxin assay, with our aim being to establish a functional assay for the planned field work in the western Gulf of Maine, which will involve the simultaneous deployment of three ESP instruments. Based on what is learned from the Year 2 deployment, we expect that additional refinements to reagents may be necessary to address the issue of assay stability over extended periods in the field. In addition, efforts will be focused on the rigorous calibration of this assay and establishing assay performance parameters, as will have been carried out for the DA assay during Years 1 and 2.

References

Traynor, I.M., Plumpton, L., Fodey, T.L., Higgins, C., Elliott, C.T. 2006. Immunobiosensor detection of domoic acid as a screening test in bivalve molluscs: comparison with liquid chromatography-based analysis. J. AOAC Int. 89, 868-872.

Budget Justification

Support for the NOAA/NOS portion of this project involving detection of domoic acid and PSP toxins onboard the ESP, including personnel, supplies, and travel, is being requested as follows: Yr. 1: $2,000; Yr. 2: $49,380; Yr. 3: $49,899.

Personnel

G. Doucette will dedicate two months per year to this component of the project - as a federal government employee, salary support is not required and is given as in-kind support. He will be responsible for application of the ESP for detection of domoic acid and PSP toxins by overseeing the assembly, quality control and provision of all dedicated reagents/supplies employed for this assay as well as the processing and evaluation of data generated. All activities will be coordinated closely with PI Dr. Christopher Scholin (MBARI) and Co-Investigators Drs. Anderson and McGillicuddy (WHOI) who will be responsible collectively for logistical components associated with configuration, deployment, and operation of the ESP.

Salary support is requested for C. Mikulski (Biol. Res. Tech.) at four months per project year during Years 2 and 3. She will be responsible for preparation, testing, and final quality assurance of all reagents/supplies (e.g., toxin arrays) required for conduct of the domoic acid and PSP toxin assays onboard the ESP. She will prepare for and carry out all ESP instrument/assay calibrations

ahead of all planned deployments in cooperation with the MBARI and WHOI groups. She will also be responsible for processing/initial evaluation of all raw toxin data generated by the ESP.

Supplies

Supplies requested for deployment of the domoic acid and PSP toxin assays onboard the ESP are as follows: Expendable Reagents - all commercially available chemicals, toxin certified reference standards, filters, and expendable plastics; custom DA antibody/DA-protein conjugate and custom saxitoxin antibodies/saxitoxin-protein conjugates are included in the Expendable Reagents category at a combined cost of $7,500 charged in Year 2 and $2,000 charged in Year 2 (PSP assay reagents only); Protein arrayer maintenance - parts and service for annual maintenance of Biodot protein arrayer used to print DA and saxitoxin arrays.

Travel

Support is requested for travel of one person from NOAA (Charleston, SC) to MBARI (Moss Landing, CA) for one week during Year 1 to participate in ESP training and to conduct DA assay calibration (this will take place prior to the delivery of a core ESP unit to NOAA). During Years 2 and 3, support is requested for travel of one person from NOAA to WHOI (Woods Hole, MA) to participate in ESP field deployments. In Yr. 2, this will involve two trips of one week duration for ESP deployment and recovery; in Yr. 3 three trips of one week each will be required for ESP deployment, turnaround, and recovery. The individual making the trips to WHOI in Yrs. 2 and 3 will also participate in the associated survey cruises as part of these deployments.

CERTIFICATION REGARDING RESTRICTIONS ON LOBBYING
(Proposals Greater than $100,000)

Proposal Title

Applications of the Environmental Sample Processor (ESP) to Harmful Algal Bloom Management: Field Deployments, Technology Transfer, and Commercial Evaluation

Proposal Number and/or Date 10 Dec 2007

The above referenced proposal was submitted in response to: (select one)

_____ The ONR Broad Agency Announcement (BAA) #08-001, as published in FedBizOpps on 5 Sep 07.

__X__ Other BAA (List Solicitation No., Project Title, and Date of Issuance):

ONR BAA-07-040

Applicants should review the instructions for certification requirement under 32 CFR Part 28, "New Restrictions on Lobbying." 32 CFR Part 25, The certification shall be treated as a material representation of fact upon which reliance will be placed when the Office of Naval Research determines to award the covered transaction, grant, or cooperative agreement.

CERTIFICATION REGARDING LOBBYING ACTIVITIES

The following certification applies only to actions exceeding $100,000:

Section 1352, Title 31, U.S.C. (PL 101-121, Section 319) entitled "Limitation on use of appropriated funds to influence certain Federal contracting and financial transactions."

(1) No Federal appropriated funds have been paid or will be paid by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 31, U.S.C. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

As the duly authorized representative of the applicant, I hereby make the above certifications on behalf of the applicant.

Applicant Institution:

MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

Taxpayer Identification Number (TIN):

77-0150580

Printed Name, Title and Signature of Authorized Representative: X

Patrice A Carroll

PATRICE A. CARROLL, SR. GRANTS SPECIALIST

Date: 05 DEC 2007