The Environmental Sample Processor (ESP): A Device for Detecting Microorganisms *In Situ* Using Molecular Probe Technology

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The Monterey Bay Aquarium Research Institute is a non-profit oceanographic research institution.

The Environmental Sample Processor (ESP): A Device for Detecting Microorganisms *In Situ* Using Molecular Probe Technology

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The National Ocean Service (DOC/NOAA/NOS) is a federal government agency.

1. Abstract

Molecular probes are extremely useful tools for identifying water borne microorganisms and the substances they produce, even when those targets are very dilute and embedded in a taxonomically complex and organic-rich matrix. Application of such technology outside of a laboratory poses many technological challenges, particularly if unattended, real-time synoptic analysis of multiple locations for extended periods of time is desired. The Environmental Sample Processor (ESP) is a novel instrument developed in an effort to overcome these challenges. The ESP collects discrete water samples, concentrates microorganisms and automates application of DNA (or other) molecular probes to enable identification and quantification of particular species captured. The instrument transmits results of DNA probe array-based assays in real-time to a shore-based location for processing, interpretation and dissemination. In addition, the ESP archives discrete samples for nucleic acid, microscopic and toxin analyses for verifying real-time data from the probe arrays as well as facilitating discovery-based analyses in the laboratory. This proposal seeks funds to enhance this on-going instrument development program by constructing and deploying a suite of second generation, internet-accessible ESPs, and by developing real-time toxin detection capabilities. Identification of specific nuisance, harmful or toxic algal species that pose widespread economic concerns and/or are known to negatively impact the health of humans and wildlife is emphasized. Field tests of the ESP will take place in Monterey Bay, California.

2. Table of Partnerships

Partner	Roles and Tasks	Request to NOPP Year 1	Resource share Year 1[1]	Request to NOPP Year 2	Resource share Year 2[2]	Request to NOPP Year 3	Resource share Year 3[2]
C. Scholin and G. Massion, non-profit research (MBARI)	DNA probe array development; field studies of HAB species; ESP engineering	$809,028	$75,068	$894,412	$66,621	$678,552	$69,285
G. Doucette, government (NOS)	Development of toxin detection chemistries for use with the ESP; field studies of HAB species	$83,390	$15,422	$94,680	$16,193	$90,895	$17,003
Ship Time	ESP deployment and field testing			($15,000) included in MBARI request		($15,000) included in MBARI request	
Totals:		**$892,418**	**$90,490**	**$989,092**	**$82,814**	**$769,447**	**$86,288**

[1]Resource share for Year 1 is based on current funding

[2]Resource share for Years 2 and 3 is based on future requests

Part of MBARI's charter is to transfer technology developed here to the greater oceanographic research community. However, MBARI is not positioned to manufacture and provide technical support for equipment in the same manner as a commercial entity would. Thus, MBARI pursues partnerships with commercial companies to disseminate the technology it develops, and it is our intention to license the technology developed under this proposal to an appropriate commercial firm. We intend to pursue this activity in Year 3 when the ESP has been thoroughly evaluated in real world conditions.

3. Relevance to NOPP Objectives

3.1 Support of critical research objectives- observational technique development

Instruments that enable long-term, unattended, *in situ*, application of molecular probes with real-time telemetry of results offer an exceptionally novel approach for detecting water borne microbes as well as the genes they harbor and express. In addition to automating chemical processing of material, such systems must also facilitate collection of relatively large samples so that rare microorganisms (particles) can be captured and made available for identification. In a step toward reaching these goals, scientists and engineers at the Monterey Bay Aquarium Research Institute (MBARI) have collaborated over the past five years to develop a new and innovative device known as the Environmental Sample Processor (ESP, Fig. 1). The capabilities of the existing ESPs and results of field tests of these instruments are detailed below. Although the ESP prototype was designed around the detection of harmful algal bloom (HAB) species as they occur in coastal environments in particular, the concepts embodied in the instrument can be extended to include identification of a diverse array of water borne microorganisms. This proposal represents a collaborative effort between MBARI scientists and engineers along with NOAA/NOS scientists to develop the second generation ESP (2G-ESP) with significantly enhanced processing capabilities and user friendliness as well as decreased lower limits of detection, power consumption, instrument size and cost. One of the long-term objectives of this project is to provide autonomous, molecular probe-based observational capability in 4-D by distributing networks of ESPs on fixed and mobile platforms.

Detection and quantification of specific water borne microorganisms is fundamental to understanding how changes in the physical and chemical oceanographic environment alter the community structure, population dynamics and underlying gene expression of a wide array of prokaryotic and eukaryotic species. With respect to "ecosystem health," only a small fraction of the thousands of microorganisms living in the oceans are known to contribute to widespread economic loss and/or negatively affect the health of humans and wildlife. Harmful algal bloom species and a variety of microbes associated with sewage effluents are good examples of the latter. For such organisms, it is highly desirable to detect and map the distribution of specific species and toxins they may produce well in advance of potential problems they may cause. This demands an integrated network of sampling sites as well as the capability to detect particular organisms at very low concentrations in as near real-time as possible, even when those targets are embedded in complex sample matrixes.

Species-specific molecular probes offer one means of expediting and facilitating the detection and quantification of a wide range of microorganisms found in water samples. However, routine use of this technology outside of a laboratory setting is at present very challenging and in some cases not yet possible, limiting its application for real-time species detection and surveys in the field. Routine, synoptic collection of water samples at multiple locations over extended periods further complicates the application of molecular probe technology in an environmental setting. In stark contrast, many physical, chemical and gross biological properties of the water column can presently be determined in real-time using a variety of airborne, shipboard, moored and mobile autonomous sensor arrays, all of which currently form the backbone of ocean observatory systems. The disparity between our capacity to measure many physical and chemical oceanographic parameters versus our ability to understand the distribution and abundance of specific microorganisms impedes our efforts to more fully characterize a wide range of important biological phenomena, especially monitoring the distribution and forecasting the appearance of harmful organisms in coastal environments.

3.2 Long-term commitment to the proposed research objectives

The design and application of the ESP have been an active area of research at MBARI for over five years, with construction of prototype instruments and preliminary field tests of these devices taking place over the past three years. This progress has been made possible by MBARI's commitment to integrating the talents of scientists and engineers to develop novel technologies for studying the world's oceans.

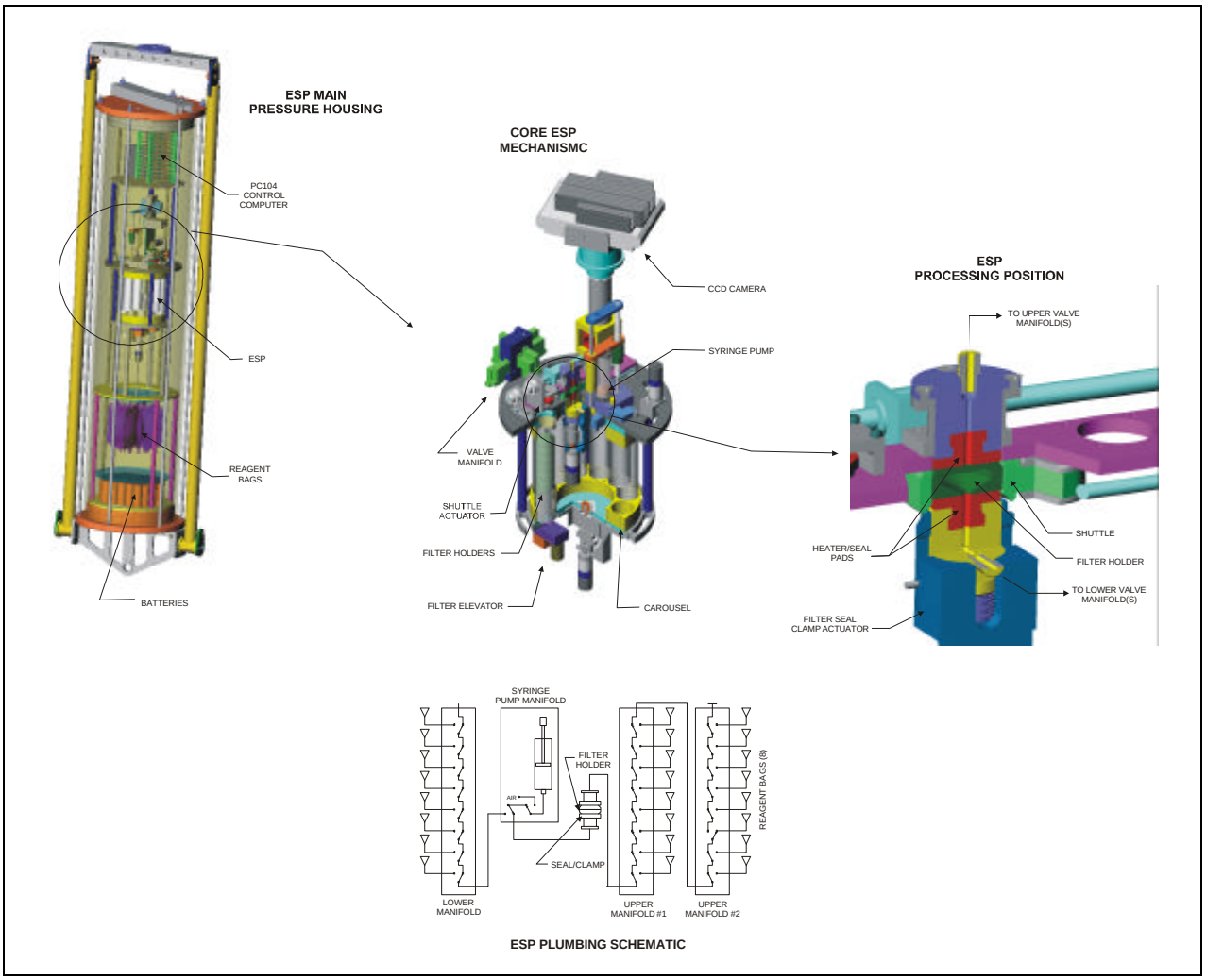

Figure 1. The Environmental Sample Processor (ESP).

The concept driving the development of the ESP rests on the fact that many protocols used to detect organisms or specific molecular "signatures" of interest (e.g., nucleic acids, toxins) require similar sample handling and processing protocols. For example, collection of a sample, concentration of microorganisms (particles) onto user-selected solid supports, chemical processing of material collected, selective adsorption and elution of target analytes using solid phase extraction chemistries, optical detection of reaction products, etc., are all common methodologies used to detect molecules with highly divergent chemical properties. The ESP was built around the idea that by accommodating those kinds of sample manipulations autonomously *in situ*, we would enable a wide range of sample processing methodologies and thereby meet the needs of basic research as well as resource management personnel. The ESP is protected under U.S. Patent Number 6187530.

Present sample processing options automated by the ESP include but are not limited to:

A) preservation of microorganisms (particulate matter) for light and electron microscopy;
B) preservation of microorganisms (particulate matter) for nucleic acid and toxin analyses;
C) application of molecular probes (DNA, antibody) for identification of specific organisms using whole cell formats;

D) application of molecular probes (DNA, antibody) using cell homogenate formats, the latter including nucleic acid extraction and development of custom DNA probe arrays for real-time detection of multiple organisms simultaneously (e.g., Scholin et al. 1997, Miller and Scholin 1998, 2000, Scholin and Anderson 1998, Scholin et al. 1999, 2000).

The chemical processing capabilities of the instrument are being enhanced through collaborations with a number of academic and government laboratories, in particular the NOAA/NOS Marine Biotoxins Laboratory in Charleston, S.C. (Dr. G. Doucette).

3.3 The MBARI/NOAA partnership: Extending the ESP's detection capabilities

Scholin and Doucette have collaborated for over six years to merge the techniques of DNA probe based-identification of HAB species with novel biochemical methods for detecting toxins they produce. A major emphasis of this continuing endeavor is the development of techniques for analyzing field samples in as near real-time as possible. The technique development and field testing efforts have also yielded contributions to our understanding of how HAB species impact marine food webs (e.g., Scholin et al. 2000, 1999; Parsons et al. 1999). Scholin and Doucette have long recognized the need for making synoptic measurements of both species abundance and toxicity at multiple stations routinely in order to more fully understand how distinct physical and chemical oceanographic regimes drive HAB formation/termination and regulate HAB toxicity, which in turn control the extent and severity of negative impacts on food webs. Such trophic effects of HAB toxins range from contamination of fishery resources and their associated public health concerns to mortality events involving marine birds and mammals, including protected and endangered species. Consequently, our collaboration over the past few years has focused increasingly on utilizing the ESP platform as a means of carrying out both species and toxin detection remotely, *in situ*. With respect to this proposal we foresee an equal partnership between scientists at MBARI and NOAA/NOS (Scholin, Doucette, et al.) as well as engineers (Massion et al., MBARI.) to advance development of the ESP system and more rigorously validate its functionality in the field.

3.4 Relevance of ESP to interests of oceanographic community at-large

The ESP is typically deployed along side or directly bundled with other instrument packages such as a conductivity, temperature and depth (CTD) recorder, chlorophyll fluorescence sensor, etc. By integrating data from a network of ESPs with those from sensors characterizing physical and chemical properties of the water column, we aim to generate synoptic views of the distribution and abundance of a variety of target species based on their molecular signatures in an environmentally relevant context, remotely and in real-time.

Many investigators have approached us as to how they might access and use the ESP to facilitate their research. For example, workers at Woods Hole Oceanographic Institution (WHOI), Florida Marine Research Institute (FMRI), NOAA (Seattle, WA and Charleston, SC labs), etc., all have immediate applications of the instrument broadly falling under the heading of emerging coastal ocean observatory programs targeting harmful algal bloom (HAB) species in particular. In all cases, a network of ESPs could potentially provide near real-time species and toxin detection information that would be extremely useful for making predictions as to where and when HAB outbreaks may pose serious concerns. Beyond HAB research and monitoring, the ESP could also be used to enable remote detection of a wide range of water borne microbes or target molecules by "plugging in" a different suite of molecular probes and/or detectors fitted onto the instrument. This concept fits well with the overall objective of developing an integrated ocean observing system.

There are a number of on-going MBARI research programs that compliment and greatly enhance the work proposed here. For example, the biological oceanography (Chavez et al.) and chemical sensor programs (Johnson et al.) provide rich scientific backgrounds and databases against which performance of

the ESP can be judged (see www.mbari.org; click on *Research and Development*, then browse to *Biogeochemistry* and *Chemical Sensor* programs and related topics). In addition, the MBARI chemical sensor group has expressed an interest in exploring the ESP as a tool for collecting suspended sediment samples that might be a source of particulate iron fueling phytoplankton blooms during coastal upwelling events. Lastly, the ESP is also being modified for deep-sea sampling of bacterioplankton and invertebrate larvae (DeLong, Vrijenhoek) under the auspices of grants from the Keck and Packard Foundations. There are many options for reducing the size of the instrument, lowering its power requirement, cost, increasing "user friendliness", etc. However, the MBARI-sponsored development and application of the ESP is limited to in-house prototype development and local deployments (Monterey Bay) only. No internal funding is available to produce new generations of the device or additional copies for use by the oceanographic community at-large.

3.5 Design and operation of the ESP Prototype

The ESP consists of five major subsystems. A carousel stores up to 100 filter holders. A syringe pump is used to draw in seawater samples and dispense the required reagents. A series of custom valve manifolds allows the syringe pump to apply reagents to either the top or bottom ports of the filter holder. A linear shuttle is used to move the filter holders from the carousel to the processing position where the filter holders are sealed in a clamp, thus providing connections to the valve manifolds. The shuttle also moves the filter holders to the optical detection station where probe arrays are imaged using a scientific grade, high resolution CCD camera. The imaging system can support chemiluminescent, fluorescent and colorimetric detection chemistries.

To begin processing a sample, a carousel tube is positioned over the filter elevator and the elevator lifts a filter holder into the shuttle. The shuttle slides the filter holder into the filter seal clamp. Once a filter holder is immobilized, the valve manifolds are set to connect the upper filter holder port to an external port exposed to seawater and the lower filter holder port is connected to the syringe pump. The syringe pump pulls seawater through the filter, concentrating particles (organisms) on the filter medium. The valve manifold and syringe pump are then used to sequentially add reagents to or exchange reagents across the collected material. The target molecules eluted from one filter may be held in the syringe pump while a filter holder with a new filter medium is loaded, and the sample processing protocol continues. For example, sandwich hybridization reactions (Scholin et al., 1998, 1999) require two filters: one for generating a cell homogenate and another for developing DNA probe arrays for detecting particular species. The cell homogenate is held in the syringe pump while the array filter is loaded. The array is imaged after it is developed (see below). Finally, processed or spent filters are unloaded into an empty carousel tube.

Modular valving supports use of a relatively large number of reagents. The filter manifolds are organized as blocks of 8 and can be cascaded for additional capability. In the current format, 16 valves (2 manifold blocks) are connected to the top of the filter clamp and 8 valves (1 manifold block) are connected to the bottom. Additional valves allow the syringe pump to pull reagents "top-to-bottom" across the filter from the upper manifold, or push reagents from the lower manifold "bottom-to-top" across the filter. Waste may be discharged overboard or captured onboard in a dedicated reservoir. Normally closed "fail safe" valves are mounted at the seawater intake and waste ports to minimize the possibility of a failed valve, tube or fitting flooding the housing. The filter holders accommodate a wide variety of filters or chemically absorptive media. A highly accurate and repeatable syringe pump is capable of mixing and dispensing fluid volumes from 10's of µl to 25 ml. The seals used in the filter seal clamp have embedded heater pads that allow for heating to temperatures up to ~100°C at any time during a protocol.

Particular attention has been paid to the manner in which a user controls the actions of the ESP. A simple text based language defines the sequence of steps performed by the instrument. At the lowest level, commands consist of a series of predefined calls such as "LOAD FILTER", "SYRINGE PULL x mL

REAGENT y", "ACQUIRE IMAGE", etc. Sequences of these low-level macros can be written as a subroutine to perform a specific processing protocol (e.g., "WHOLE CELL ARCHIVE","TOXIN ARCHIVE" or "SANDWICH HYBRIDIZATION"). Any number of these subroutine macros can be sequenced in a top level macro that wakes up the instrument at a specific time, performs a sequence of subroutines and puts the instrument to sleep until the next sampling time.

3.6 Field Tests of the benchtop ESP Prototype

Two ESP prototypes have been built at MBARI thus far. One instrument was designed as a laboratory bench top development station; this unit can also be used onboard ships. The second ESP was intended for *in situ*, subsurface, autonomous operation. Examples illustrating the performance of these instruments follow.

The shipboard ESP prototype was deployed in Monterey Bay, August 2000 (Fig. 2). The device was programmed to generate cell homogenates (crude cell lysates) and develop prototype oligonucleotide probe arrays to reveal the presence of several different harmful algal species in near real-time. The same instrument was also used to archive discrete samples for a diverse set of post-deployment processes: whole cell hybridization; domoic acid (toxin) and saxitoxin analyses; and, nucleic acid extraction, PCR amplification of ribosomal RNA genes (rDNA), construction of rDNA clone libraries, sequencing of rDNA clones (data not shown). Here, results summarizing the DNA probe arrays, whole cell probing and toxin analyses emphasize two different toxigenic phytoplankton species: the diatom *Pseudo-nitzschia australis* and the dinoflagellate *Alexandrium catenella.* In the examples shown in Figure 2, neither *P. australis* nor *A. catenella* were detected in samples 1 and 2. In contrast, *P. australis* was detected in samples 3-5 albeit at different concentrations as indicated by the variable intensity associated with the *P. australis*-specific probe. *A. catenella* was detected in sample 5 only.

Samples archived for whole cell (Miller and Scholin, 2000) and toxin receptor binding analyses (see Lefebvre et al. 1999; Powell and Doucette 1999) enabled post-deployment validation of the probe array data collected in real-time. Examples of the whole cell analyses are shown in the lowest series of panels in Figure 2b (1a-5a). Immediately after development of an oligonucleotide array, the ESP was programmed to concentrate and preserve an aliquot of the same sample used to generate the array. Panels 1a and 2a reveal very different phytoplankton communities, neither of which harbor significant numbers of *P. australis* nor *A. catenella*. Both were processed using the universal probe, labeling all species. The sample seen in 1a was collected near a site of upwelling where centric diatoms and heterotrophic dinoflagellates were abundant (ESP1). The sample seen in 2a came from an area where nutrients were depleted, the abundance of phytoplankton much less than that in 1a, and the phytoplankton community was dominated by several species of dinoflagellates as well as very small pennate diatoms (ESP2). In contrast to the first two samples, the probe array seen in panel 3 indicated the presence of *P. australis.* To confirm this prediction, the corresponding, archived whole cell sample was processed using a probe that labels *P. australis* specifically. As predicted, the archived sample did indeed contain this species (panel 3a). Furthermore, the probe array seen in panel 4 indicated *P. australis* was very abundant; whole cell *in situ* hybridization of the corresponding sample also revealed very high numbers of the expected species (panel 4a). The probe array seen in panel 5 predicts the presence of a number of species represented on the grid, in particular both *P. australis* and *A. catenella*. In this case, the archived whole cell sample was processed using a probe specific for *A. catenella* and that species was in fact found embedded within the phytoplankton community (panel 5a). In this same sample, one can also see the faint outline of large pennate diatoms (not label ed by the "A. cat" probe), the likely source of the *P. australis* signal seen on the corresponding probe array.

P. australis and *A. catenella* both produce potent neurotoxins, though different in structure and mode of action (see Hallegraeff et al. 1995). Thus, the oligonucleotide arrays make it possible to not only

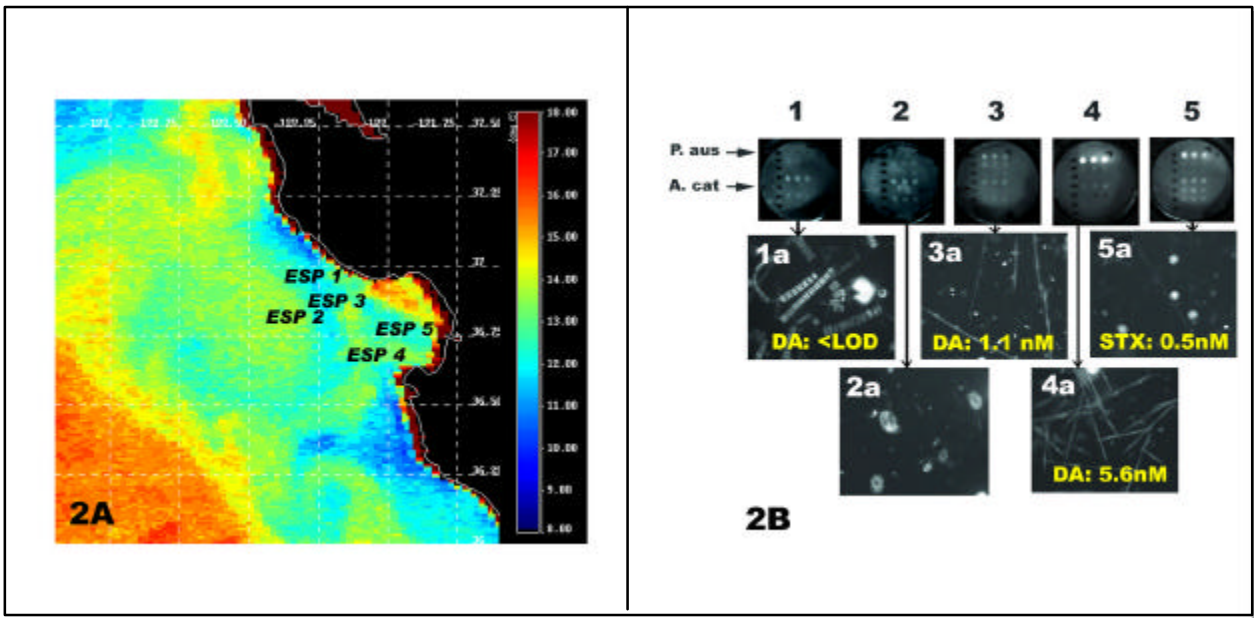

Figure 2. *Examples of data obtained using the shipboard ESP in Monterey Bay, CA. A) Sea surface temperature and locations of sampling stations (ESP 1-5). B) Images of the oligonucleotide probe arrays (middle panel) and micrographs showing results of fluorescent in situ hybridization (bottom panels). Domoic acid (DA) or saxitoxin (STX) activity associated with a sample is printed at the bottom of the micrographs. Locations of probes for P. australis and A. catenella on the arrays, spotted in triplicate, are denoted "P. aus" and A. cat," respectively. To develop an oligonucleotide probe array, the ESP collects 400 ml of sea water, concentrates particles > ~5 µm onto a 25 mm Durapore filter leaving less than 0.1 ml of residual seawater behind, injects 1.5 ml of lysis reagent, heats the sample to ~85°C and the resulting sample homogenate is drawn into the syringe. Using the shuttle and elevators, the sample collection filter is replaced with an oligonucleotide probe array. The sample homogenate is passed over the array, followed by a sequence of reagents that reveal rRNA molecules retained at specific locations on the array grid using the principles of sandwich hybridization (Scholin et al. 1997, 1999). An image of the resulting array is recorded using the CCD camera. For whole cell hybridization, a 50 mL sample was passed through a 3 µm pore size polycarbonate filter and the particles retained were treated with a saline ethanol solution for 1 hour then dried (see Miller and Scholin 2000). The preserved, dried sample was stored onboard the ESP. Following the deployment, the ESP was plumbed with a fresh suite of reagents and programmed to process the archived samples using fluorescent in situ hybridization (Miller and Scholin 2000). Once the hybridization process was completed, the sample filter was recovered, mounted on a slide and viewed using conventional epifluorescence microscopy. In the examples shown here, several different rRNA probes were used: "Univ." (labels rRNA from all organisms), "P. aus" (labels rRNA specific to Pseudo-nitzschia australis), and "A. cat" (labels rRNA specific to Alexandrium catenella). Samples archived for toxin analysis consisted of 750 mL aliquots concentrated onto GF/F filters and dried. See text for further details.*

determine what samples (i.e., specific locations, times) contain what species, but also predict what might contain which corresponding suite of toxins. As a first step towards confirming such predictions, the ESP was programmed to collect an aliquot of the same sample used to generate the probe arrays and used for whole cell archives. The resulting "toxin archive filter" was stored dry onboard the ESP, recovered post-deployment, frozen and shipped to the NOAA Marine Biotoxins Program analytical facility in Charleston, SC. Receptor binding assay results obtained for domoic acid (DA), the toxin produced by *P. australis*, are given in Figure 2. Similar to the whole cell hybridization data, changes in DA levels also reflected trends observed on the probe arrays. In the absence of signal in the "P. aus" channel (panel 1), toxin was not detected, yet when the "P. aus" signal became apparent (panel 3) DA was also detected at a low level. Maximum toxin values coincided with the greatest abundance of *P. australis* as revealed by both the probe array (panel 4) as well as the whole cell hybridization results (panel 4a). As noted above, the PSP

toxin producer, *A. catenella*, was revealed as a component of the phytoplankton community by sandwich hybridization and whole cell probing (panels 5/5a). We were also successful in measuring PSP toxins [as saxitoxin (STX) equivalents by receptor binding assay] associated with particulate material sampled concurrently, thereby demonstrating our ability to detect multiple toxins on archived filters.

As noted earlier, one of our immediate goals is to use the ESP to detect both HAB species and toxins they produce since wide fluctuations are known to occur in toxin synthesis and in the distribution of toxins between particulate and dissolved forms as a function of algal physiological status (Bates et al. 1998, Pan et al. 2001). It is therefore possible to detect algal cells in the apparent absence of any particulate or dissolved toxin they may be capable of producing. In other words, simply the appearance of a potentially toxic algal species provides no information on the associated toxicity (particulate or dissolved) and potential impacts on the local fauna and their consumers (e.g., wildlife, humans). A striking example of variation in cellular toxicity was seen during a *P. australis* bloom event in Monterey Bay, CA (Fig. 3). Concurrent measurements of DA and cell concentrations yielded DA per cell estimates that differed by about 20-fold (JD219 vs. JD241), as well as revealing times when *P. australis* cells did not appear to be producing toxin (e.g., JD255). Clearly, an integrated approach involving detection of both the toxins as well as the organisms that produce them is critical to understanding mechanisms underlying HAB dynamics and for making predictions as to where and when HABs might affect the health of humans and wildlife.

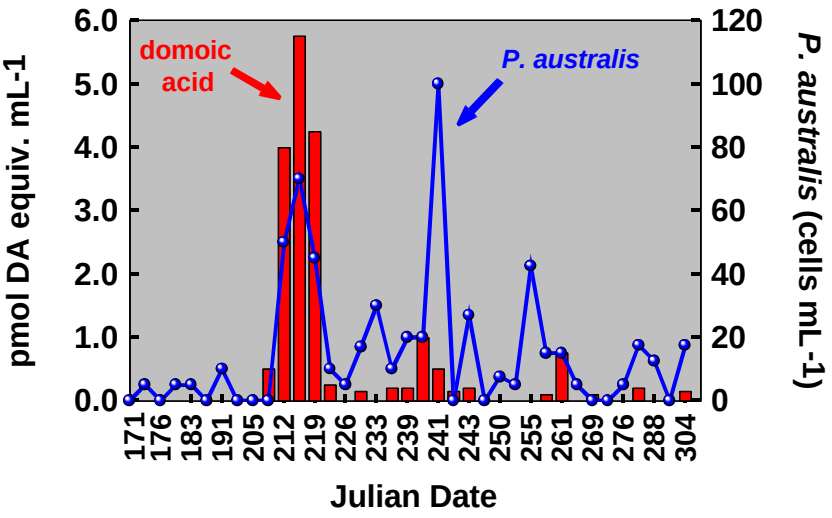

Figure 3. *Time course of Pseudo-nitzschia australis concentration determined by sandwich hybridization and receptor assay-based domoic acid activity in Monterey Bay, CA during 1996 (adapted from Scholin et al. 1999).*

DNA can be extracted from samples collected by the ESP and this material can be used to create gene libraries as a means of exploring and documenting species diversity, etc. We are currently using this approach as means of facilitating sequence discovery, probe development, and for confirming of results of whole cell and cell homogenate-based assays. To demonstrate this capability we used the Qiagen Plant DNA extraction kit to purify DNA from samples that were collected onto the polycarbonate filters and preserved with a saline ethanol solution. The material recovered was used in a PCR reaction to amplify rDNA sequences spanning a contiguous portion of the small to large subunit genes. The resulting PCR amplicons were cloned and subjected to restriction fragment length polymorphism analysis. Sequencing of rDNA clones obtained from samples archived using the ESP is on-going (data not shown). This has revealed an impressive diversity of species, many of which are not typically enumerated in routine phytoplankton counts. By building a set of rDNA reference sequences in conjunction with traditional microscopy-based species identification, we hope to identify key signature sequences that will serve as indicators of community structure. In turn, we will synthesize probes to detect those sequences using the sandwich hybridization technique (Scholin et al., 1999). We will exert specificity of the reactions whenever possible at the

capture (solid phase) step and use "universal" sequences reactive towards all rRNAs as signal probes (liquid phase). This approach could make it possible to develop DNA probe arrays that have a diversity of capture sequences ("detection channels"), all of which share a common set of signal probes – something that is highly desirable logistically. Ultimately, we envision having a variety of different probe array filters targeting particular groups of organisms, some of which will be highly species-specific and offer some quantitative estimate of organisms' abundance while others will be very generic and used to reveal an "index" of community structure.

3.7 First test of the moored, subsurface, remote ESP prototype

The *in situ* ESP was deployed May 2001 in collaboration with Dr. D. Anderson (WHOI) during a Gulf of Maine NOAA ECOHAB-sponsored field expedition. One of the objectives of that program was to follow the onset and fate of a toxigenic *Alexandrium tamarense* bloom, particularly the coupling between the distribution of that species and the prevailing meteorological and oceanographic conditions. *A. tamarense* can be concentrated along shore with the onset of downwelling favorable winds (see Franks and Anderson 1992a, b). We positioned the ESP in an embayment within Casco Bay, ME, where these cells might accumulate under such conditions. The instrument was moored in ~30 m of water with the sample processor module immobilized at 5 m (at low tide) and tethered to a surface float (Figure 4.)

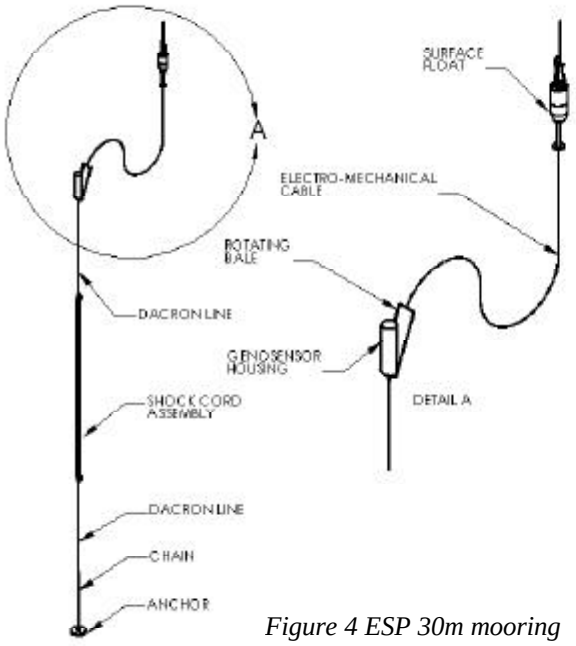

Figure 4 ESP 30m mooring

CTD and chlorophyll fluorescence sensors were attached to the ESP housing; the data obtained from these sensors along with times that the ESP was programmed to sample are shown in Figure 5a. During the deployment, a short burst of downwelling winds was experienced; the effect on the physical properties of the water column was apparent. Following this transition, approximately 1-200 *A. tamarense* per liter were delivered to the mooring site. A series of DNA probe arrays developed subsurface by the ESP illustrates the appearance of that species (Fig. 5b; middle panel). At the same time the ESP was sampling, developing prototype DNA probe arrays and sending its data to shore via radio modem, a ship-based crew used a profiling CTD to characterize the water column and Niskin bottles to collect water samples at a depth and location near that of the ESP mooring. Samples collected manually were then tested for *A. tamarense*, *P. australis* and *P. multiseries/pseudodelicatissima* using a benchtop version of the sandwich hybridization chemistry emulated by the ESP (Scholin et al. 1999). Results of these tests are shown in Figure 5c (lower panel). While *A. tamarense* did accumulate, its concentration was not great enough to warrant public health warnings and shellfish bed closures. Low numbers of *P. australis* also appeared to be present in one of the samples. *P. multiseries/pseudodelicatissima* were not detected in the water autonomously using the ESP nor in the samples collected onboard the ship and processed manually. To our knowledge, this is the first example of autonomous, subsurface, DNA probe-based detection of a HAB species.

Limited tests of the ESP system indicate that it is an innovative and promising new tool that could enable remote, near real-time detection of specific water-borne nuisance, harmful or toxic species that pose economic or health concerns. Moreover, development of instrumentation that allows one to detect

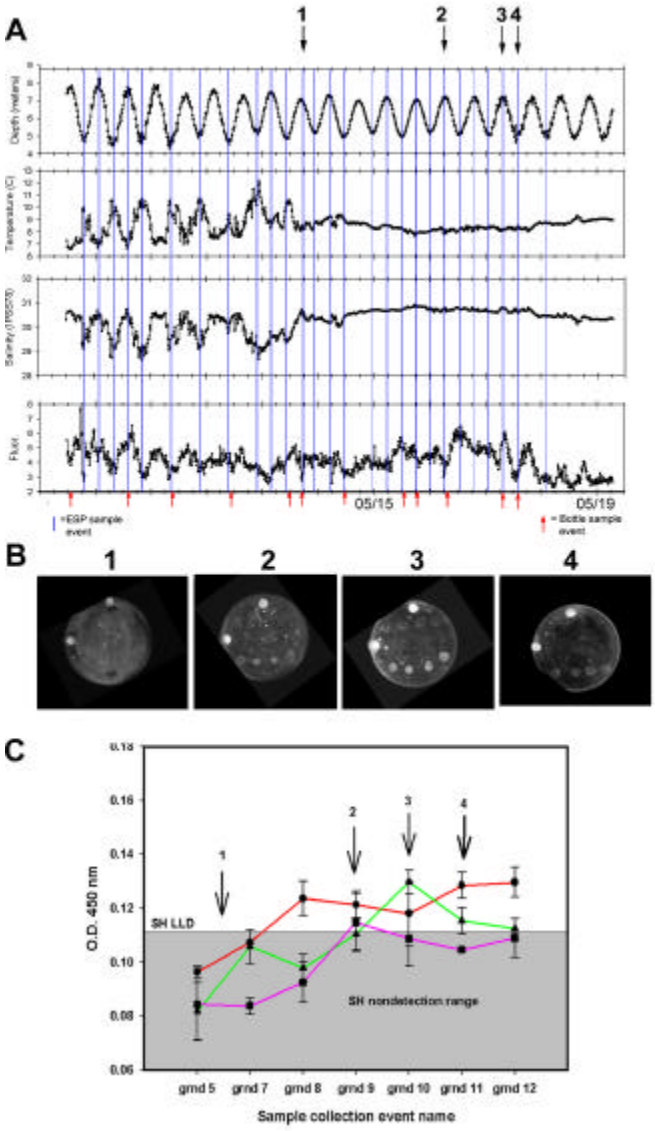

Figure 5. *Examples of data obtained using the in situ ESP in Casco Bay, ME. A) Depth, temperature, salinity and chlorophyll fluorescence recorded onboard the ESP during the deployment. Blue lines show ESP sampling times; red arrows indicate when bottle samples were collected near the ESP mooring; #'s 1-4 correspond to the arrays shown in the middle panel. B) Prototype DNA probe arrays developed onboard the ESP (same method as in Fig. 2); the two bright spots on the top and left of each array are registration marks; probes for P. australis (top), P. multiseries /pseudodelicatissima (middle) and A. tamarense (bottom) are spotted in quadruplicate. C) Results of benchtop sandwich hybridization assays (see Scholin et al. 1999) for bottle samples; probes used are the same as those spotted onto the arrays. The shaded area indicates non-detection range; values above those shaded indicate a positive reaction (i.e., detection of target species).*

pecific, rare microorganisms using molecular probe technology compliments initiatives that employ optical-based sensors that use cells' physical properties to identify species.

4. Project Plan and Anticipated Results

The plan for this project consists of four major elements. The first element is to refine the existing ESP design to make it smaller, more robust, power efficient (etc.), and to incorporate design changes identified by experience gained during previous deployments. The result will be a "second generation ESP" (2G ESP). The 2G ESP California. The second element of the project concentrates on refining production of the DNA probe arrays and expanding the repertoire of automated sample processing protocols, including detection of algal toxins. The third element consists of building two additional 2G ESP units, incorporating any minor design changes identified after deployment of the initial 2G ESP prototype. The fourth element of the project focuses on deployment of these instruments at sites in Monterey Bay as a test of our ability to field a suite of internet accessible ESPs tasked with making synoptic measurements. The suite of ESPs developed would then form an instrument pool that would be made available to the research community for activities sponsored by other funding agencies.

4.1. 2G ESP Design, Testing

The existing ESP has worked successfully on several deployments with extensive support by the original science/engineering design team. Our goal in designing the 2G version (Figure 6) is to produce a system that can be successfully deployed for a variety of applications on a routine basis by a team of trained technicians, thus making it accessible to a much larger group of users than is possible at present. The 2G ESP will build on the same design concepts already validated in the existing units, but incorporate changes that will make it a much more robust, compact and user friendly system. Some of this work, distinct from that proposed here, is already underway at MBARI through funding from the Keck and

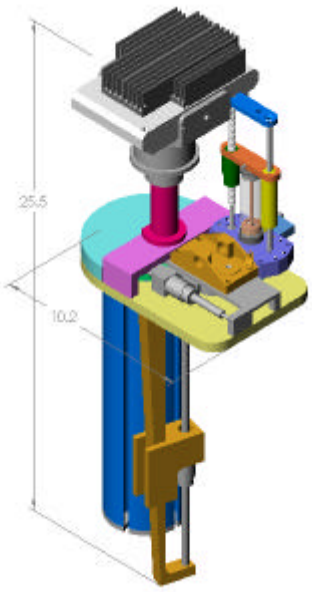

Figure 6. 2G-ESP design concept; dimensions shown are in inches.

Packard Foundations. All engineering design work and fabrication will be carried out at under the supervision of G. Massion and the engineering team at MBARI.

A major design effort undertaken as part of this proposal will reduce the size and complexity of the instrument. Our experience with the ESP to date has generated a clear, prioritized list of modifications to achieve these goals. Fortunately, the basic instrument concept has proven viable and all of these modifications can be characterized as enhancements as opposed to a complete instrument redesign. The following list includes the significant design modifications we will undertake.

1) Modify the physical layout of subsystems to reduce the overall height from 60 inches to a goal of 30 inches.
2) Modify the carousel form factor and mounting mechanism to allow filter holders to be shipped in the carousel and loaded as a unit in the field
3) Replace the current "IV" bag style reagent storage with hard-sided polypropylene/ethylene bottles to simplify loading reagents in the field.
4) Decrease the electronics power consumption. The current ESP electronics are based on off the shelf modules. This approach enabled rapid development of a sea going ESP prototype to prove the instrument concept. Low power and space efficiency issues were not a priority. The current system runs at a minimum of 12 watts continuous during sample processing. With this type of power draw expensive, lithium primary cells were utilized to decrease size and weight. The 2G design will capitalize on our experience gained from the original design and the availability of newer, low power technologies. There are a number of options available which will facilitate a power draw approaching 1/10 the current systems requirements. At these lower power levels less expensive rechargeable battery systems can be employed. A combination of off the shelf computer boards and custom interface boards will be utilized to decrease the physical space requirements of the control electronics. Currently two architectures are proposed. The first uses a single low power processor and the second uses two hierarchically positioned processors. Under this grant, additional architectural options will be evaluated and a design trade-off study will be done to select the most optimal approach based on parameters such as power requirements, form factor, cost, development environments, available software functions, software design time, suitability for commercialization and others.
5) Reposition batteries in a separate housing from the main ESP to allow easier battery changes. Batteries in the existing unit are housed within the ESP pressure housing which makes battery changes problematic.
6) Addition of a second processing station identical to the current station but dedicated to sample collection. We've found that the activities of sample collection and sample processing must be separated physically to prevent formation of interfering precipitates (the background speckling on the arrays shown in Fig 5b). This will also allow a pump to be used for sample collection, which will speed the sample collection process and increase the volumes that can be pumped when

reasonable. The addition of a sample collection pump will also reduce power consumption significantly.

7) Lastly, we will develop a web based graphical user interface (GUI) for the 2G ESP. The current engineering development communication interface uses Unix-based line commands. The transition to a more intuitive GUI will take place incrementally over course of the project. A web interface for the ESP (Figure 7) was always part of the design and we have enough experience at this point to design an effective GUI as one means of disseminating project results and for posting updates on the field expeditions. We envision having a geo-referenced display showing the locations of deployed ESPs; users will be able to access the devices by "clicking" on an instrument node. That interface will allow for data downloads and instrument programming for registered users. Links to general information about the ESP, data from previous deployments, information about the organisms detected, etc., as well as other web sites (e.g., ONR/NOPP, NOAA, WHOI red tide, etc.) directly related to our activities would be available to all that access the site. This concept appears to fit well with the current NOPP objectives.

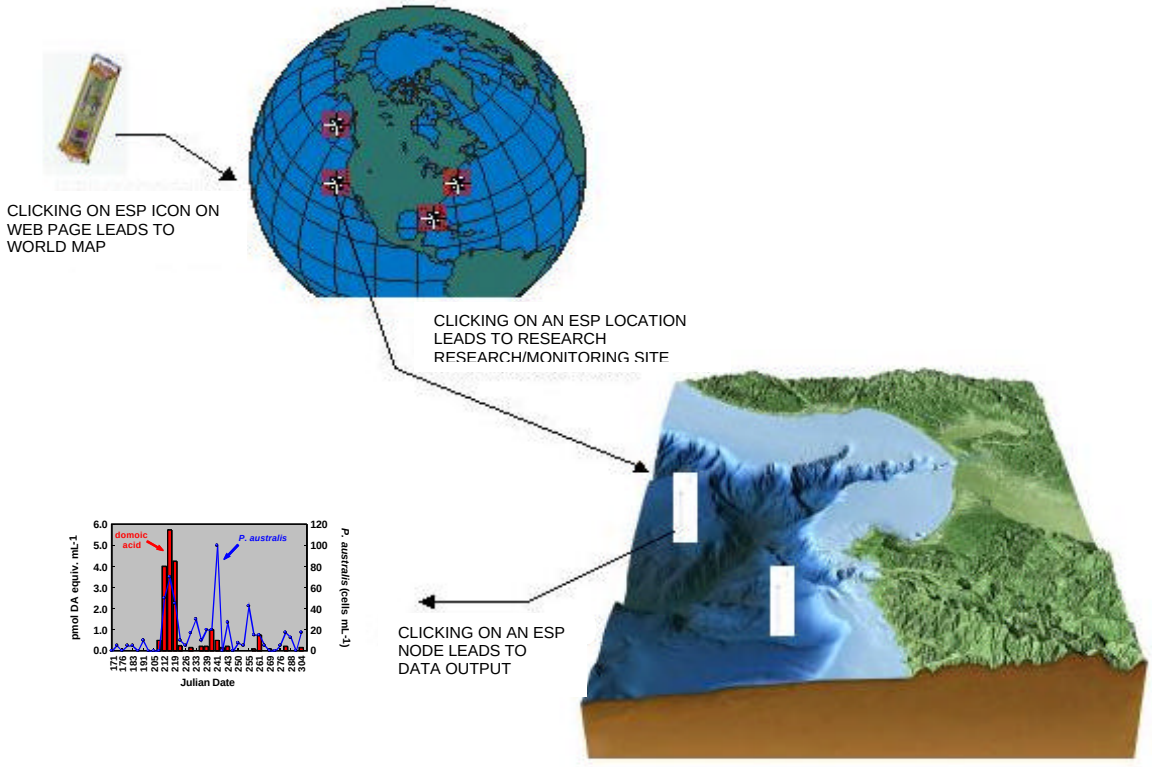

Figure 7. ESP web interface concept.

4.2 Probe array and sample processing protocol development

Scholin's team (MBARI) is currently working under the auspices of an MBARI-sponsored project for the remainder of 2002 to refine manufacturing of DNA probe arrays for use with the ESP. Further development beyond 2002 will require securing additional funding through this project or others. There are a variety of commercially available membranes that can be used as a solid support on which DNA probe arrays are printed. Thus far we have identified four candidates manufactured by the Pall Corporation that are compatible with the ESP's processing capabilities and each of these has a specific set of chemical requirements for immobilizing probes onto the membrane surface and developing the array. In a matrix fashion, we will evaluate the different solid supports, printing requirements, stability of reagents required to develop the various arrays (etc.) to find the best match for long term deployment (e.g., 1 month+) aboard an ESP. We have already gained considerable experience preparing reagents for DNA probe array development and know that all of the reagents except the current enzyme reporting conjugate (see Scholin et al. 1999) exhibit excellent stability (months) even without refrigeration. The signal enzyme used at present is stable for a period of weeks if kept below room temperature. We are currently experimenting with replacing that component with a new conjugate manufactured by Vector Biolabs that is stable for >80 days even when stored at 37^{o}C.

For some organisms (e.g., North American strains of *A. catenella/tamarense*, *P. australis/mulitseries*, *Heterosigma akashiwo*) in particular geographic areas (Monterey Bay, coastal Washington, Puget Sound, Gulf of Maine) we already have suitable probes integrated into the ESP system and preliminary data illustrating their detection potential. Similarly, protocols for archiving samples for whole cell microscopy, nucleic acid and DA analyses are established. These probes and protocols are the focus of an upcoming ESP deployment scheduled for September 2002 in Monterey Bay, CA. For other organisms (e.g., *Gymnodinium/Karenia* species in the Gulf of Mexico, particular *Pseudo-nitzschia* species along the Washington coast, etc.) probes and detection protocols are under development now using the same procedures we have used successfully to date (e.g., Miller and Scholin 2000, Scholin et al. 1999, Tyrrell et al. 2001). As this project progresses we will integrate new probes onto the arrays in an iterative fashion, characterizing different capture and signal probe combinations, thereby providing an opportunity to customize sampling/organism detection capabilities of the ESP to suit specific geographic regions. This flexibility will also allow us to manufacture different arrays that support specific research/monitoring missions in different regions of the world without modifying the ESP hardware significantly.

Doucette's group (NOAA Marine Biotoxins Laboratory) will focus on developing toxin detection protocols for use with the ESP. Previous efforts aimed at obtaining toxin data in concert with HAB species abundance onboard the ESP have relied on archiving cells filtered from replicate water samples. Toxin levels in archived samples were then determined by receptor assay onshore after retrieval of the ESP, followed by estimation of cellular toxicity according to probe-based cell concentrations. This will continue to be our mode of operation for measuring DA levels associated with *Pseudo-nitzschia* populations during the planned deployment of the existing ESP prototype in Monterey Bay during September 2002. Nonetheless, our goal for this project is to acquire the capacity to perform toxin assays autonomously onboard the ESP in a manner analogous to the DNA probe arrays. Efforts during Year 1 will focus on developing toxin assay chemistries and protocols amenable to the remote, autonomous conduct of these tests. This work will involve expanding our current receptor-based emphasis to also include antibody (immunoassay) -based detection of toxins, since the chemistry of the latter class of tests is presently more robust and universally compatible than the former. This compatibility is important in allowing us to take advantage of "multiplexing" with many of the signal generating reagents currently being used with the probe array reporting system (e.g., biotin-streptavidin-HRP), thereby reducing the number of solutions used for only a single process step. In addition, the need to design unique manipulations exclusively for toxin testing will be minimized because the ESP already implements many

procedures used to detect specific molecular "signatures" (e.g., toxin) such as collection of a known sample volume, timed series of reagent exchanges, selective enrichment of target analytes, etc.

Work on the toxin detection component of this project during Year 2 will mirror that being conducted with the probe array assays. Newly developed toxin assay chemistries, formatted on the laboratory bench to be compatible with ESP operational parameters, will be transitioned to a fully functional 2G ESP unit, followed by testing in the lab and subsequent field deployment of successful protocols. Our primary focus will be on determination of DA and brevetoxin associated with toxic *Pseudo-nitzschia* spp. and *Karenia brevis*, respectively, in collaboration with partners working in the appropriate regions. The rationale for this emphasis lies in the fact that both of these toxin groups can be chemically concentrated (if necessary) using existing protocols prior to conducting the assay and both are amenable to receptor- and immunoassay-based detection. The paralytic shellfish poisoning (PSP) toxins produced by *Alexandrium* spp., cannot presently be concentrated on a conventional sorbent and, while detectable by receptor assay, pose a significant challenge to immuno-based detection due problems with antibody cross-reactivity among the numerous toxin congeners potentially present in a sample. We will, nevertheless, be pursuing alternative detection strategies involving other proteins known to bind multiple PSP toxin derivatives (e.g., saxiphilin).

The performance of toxin assays and the data collected in various study regions during Year 2 will be used to guide any necessary modifications to the toxin detection assays prior to the deployment of multiple 2G ESP units in Year 3. It is anticipated that along with the probe array (species detection) data being accessed in near real-time using a web-based GUI, simultaneous data on DA and brevetoxin levels (possibly PSP toxins) will also be available via this interface. By superimposing these two data sets describing cell abundance and toxin level for the same water parcel, calculations of toxin per cell and thus estimates of cellular toxicity will be generated and compared with the chronology of overt signs of toxic HAB impacts (e.g., shellfish toxicity, faunal mortalities, etc.).

4.3 Fabrication of 2G ESPs

During Year 1 the engineering team at MBARI will fabricate the 2G ESP (hardware, software interface, web-based access). A benchtop 2G ESP development station and a single *in situ* 2G ESP will be built, then tested in the lab and field exactly in the same manner that we have done in the past (e.g., Fig. 5, Casco Bay, ME, field test). This unit will be deployed in Monterey Bay during Year 2 to critically evaluate its design performance (Massion, Scholin, Doucette). In this way we will be able to incorporate minor design changes as a result of the lessons learned from an actual deployment before fabricating the remainder of the 2G ESPs required for field tests during the 3rd and final year of the project. Based on successful testing of the 2G ESP unit, an additional 2 copies will be produced during Year 2 to support field studies in Year 3.

4.4 Field Program

The existing first generation (1G) ESP will be deployed in Monterey Bay September 2002 as part of an MBARI-sponsored project (see below). This will give new members of the ESP project team, potentially hired on the basis of this project's funding, an opportunity to work with and deploy the 1G instrument prior to undertaking fabrication of the 2G design. This experience will teach the team how the ESP is used to develop DNA probe arrays for detecting toxic and nontoxic phytoplankton species spanning several different taxonomic classes (diatoms, dinoflagellates, raphidophytes), and potentially one or more species of barnacle (larvae) in collaboration with Vrijenhoek et al. (MBARI). During the same deployment, the ESP will also be used to archive samples for microscopy, phycotoxins and possibly sediment analyses (the latter with Johnson at MBARI). The field effort will involve use of MBARI's R/V *Pt. Lobos* for deploying and recovering the ESP mooring and ground-truth sampling in collaboration with M. Silver et al. (UCSC). This experience will serve as good model for the field tests planned for trials of the 2G instrument in 2003-4.

Progressing from Years 2 to 3, we will work to deploy multiple ESPs simultaneously. Different sites within Monterey Bay will be chosen that reflect distinct physical/chemical regimes (e.g., active area of upwelling vs. "upwelling-shadow" sites). At this point we will have a web-based GUI for accessing raw data with data processing/visualizing protocols built into the GUI. The objective of these experiments will be to evaluate how the distribution of targeted species is affected by location as well as prevailing meteorological/chemical/physical conditions, and how those conditions affect toxicity of *Alexandrium*, *Karenia brevis* and *Pseudo-nitzschia* species in particular.

5. Institutional Capabilities Critical to the Proposed Objectives

MBARI is a non-profit oceanographic research institute dedicated to merging engineering and science concepts to advance development of novel methods, sensors and platforms for studying the world's oceans (see www.mbari.org). In-house facilities and personnel enable the design and fabrication of novel oceanographic research equipment. Sea-going activities are supported by three MBARI-owned and operated research vessels (Zephyr, Pt. Lobos and Western Flyer). Scholin's science laboratory is equipped for phytoplankton culturing, DNA sequencing, DNA probe development and testing using whole cell and cell homogenate techniques. MBARI's research programs in biogeochemical cycling (Chavez, Johnson), chemical sensors (Johnson), and modification of the ESP for deep-sea sampling of bacterioplankton and invertebrate larvae ("Deep ESP", DeLong, Vrijenhoek) all complement the work proposed here. One of Scholin and Massion's long-term objectives is to reduce the form factor and power requirements of the ESP to the extent that it can be readily deployed on fixed and mobile platforms, the latter including drifters and AUVs. The research and development environment of MBARI provides a unique opportunity for realizing those goals.

Doucette and co-workers at the NOAA Marine Biotoxins Program laboratories have collaborated with their partners at MBARI for over six years in the detection of HAB species and their toxins occurring in the Monterey Bay ecosystem. The toxin detection component of this work has incorporated advances in laboratory-based receptor binding assay technologies made within the Biotoxins Program, including analysis of toxin samples collected and archived onboard the current ESP prototype. The funding requested here will support the development of a new suite of remote, automated methods of toxin detection compatible with the operation of the ESP unit, focusing primarily on antibody-based approaches.

6. Vitae of P.I.s

6.1 **CHRISTOPHER ALAN SCHOLIN**

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Professional Work Experience

Monterey Bay Aquarium Research Institute

Associate Scientist III:	12/01 – present
Associate Scientist II:	12/97 – 11/01
Assistant Scientist I:	11/94 – 12/97
Postdoctoral Fellow:	11/92 – 11/94

University of South Carolina, Columbia
Research Assistant Professor
6/86 - 5/87

Education

Massachusetts Institute of Technology - Woods Hole Oceanographic Institution
Joint Program in Biological Oceanography
Field of Study: Application of molecular biological methods to the study of toxic dinoflagellate population biology
Dates Attended: 6/87 - 11/93
Degree: Ph.D. (Dr. D.M. Anderson, thesis supervisor)

Duke University Graduate School
Department of Microbiology and Immunology
Field of Study: Molecular Biology
Dates attended: 8/84 - 5/86
Degree: M.A.

University of California, Santa Barbara
Department of Biology
Fields of Study: Aquatic Biology, Environmental Studies, Microbiology, Molecular Biology
Dates attended: 9/81 - 6/84
Degree: B.A. (Hons.)

University of Missouri, Columbia
Department of Forestry and Wildlife
Field of Study: General Biology
Dates attended: 8/79 - 8/81

Selected Publications from 1996-2002

Scholin, C.A., E. Vrieling, L. Peperzak, L. Rhodes and P. Rublee. 2002. Detection of HAB species using lectin, antibody and DNA probes. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. and Enevoldsen, H.O. [Eds.] *Manual on Harmful Marine Microalgae.* IOC Manuals and Guides, UNESCO (in press)

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.

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Rhodes, L., C. Scholin, I. Garthwaite, A. Haywood and A. Thomas. 1998. Domoic acid-producing *Pseudo-nitzschia* species educed by whole cell DNA probe-based and immunochemical assays. In: Regura, B., Blanko, J., Fernandez, M.L. and Wyatt, T [Eds.], *Harmful Algae*. Xunta de Galicia and Intergovernmental Ocenaographic Commission of UNESCO pp. 274-277.

Miller, P.E. and C.A. Scholin. 1998. Identification and enumeration of cultured and wild *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes and filter-based whole cell hybridization. *Journal of Phycology* **34**: 371-382.

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Scholin, C.A., P. Miller, K. Buck, F. Chavez, P. Harris, P. Haydock, J. Howard and G. Cangelosi. 1997. Detection and quantification of *Pseudo-nitzschia australis* in cultured and natural populations using LSU rRNA-targeted probes. *Limnology and Oceanography* **42**: 1265-1272.

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Vrieling, E., R. Koeman, C. Scholin, P. Scheerman, L. Peperzak, M. Veenhuis and W. Gieskes. 1996. Detection of a domoic acid-producing *Pseudo-nitzschia* species in the Dutch Wadden Sea by electron microscopy and molecular probes. *European Journal of Phycology*.

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Littaker, W., C. Scholin, G.R. Vasta. 2000. *Molecular approaches for identification and environmental detection of Pfiesteria piscicida and Pfiesteria-like dinoflagellates.* Workshop held at the Center of Marine Biotechnology September 1999, Baltimore, MD.

Scholin, C.A., N. Wainwright and G.M. Hallegraeff. 1994. *Feasibility of developing a rapid diagnostic test for toxic dinoflagellates in ships' ballast water*. Report for the Australian Quarantine and Inspection Service.

Selected Workshops and Training Courses

HAB Probe Workshop, MBARI, March 2001 (lab demonstrations/training course)

HAB Tech 2000, Nelson, New Zealand, February 2000 (lab demonstrations)

IOC training course on the taxonomy and biology of harmful marine microalgae, Copenhagen, Denmark, January 1999.

Molecular approaches for identification and environmental detection of *Pfiesteria piscicida* and *Pfiesteria*-like dinoflagellates Baltimore, MD, September 1999.

Workshop on Developing a U. S. Coastal Observing System, Solomons, MD, May 1999.

New Zealand Marine Biotoxins Workshop No. 8. Wellington, New Zealand, November 1997

EPA Workshop on Harmful Algal Blooms, Pensacola Beach, FL, October 1997.

NOAA Assessment of the management and mitigation of harmful algal blooms in coastal waters, Seattle, WA, September 1996.

NATO ASI on the Physiological Ecology of Harmful Algae, Bermuda, May 1996.

Workshop on Molecular Evolution, Woods Hole, MA, August 1995.

The Ecology and Oceanography of Harmful Algal Blooms, Snow Mtn Ranch, CO, August 1994.

Patents

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Tyrrell, J.V., Bergquist, P.R., Bergquist P.L., Scholin, C.A.. Composition and Methods for Dtecting Raphidophytes. US pat pending.

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Education

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1982 M.S. Mechanical Engineering
Emphasis: Oceanographic Engineering
Advisor: Dr. William Lick
Thesis: The entrainment and deposition of fine grained sediments

Work Experience

1980 - 1982	Development Engineer	University of California Santa Barbara
1982 - 1983	Research Engineer	Dynamics Technology Inc.; Torrance, CA
1983 - 1993	Mechanical/Electrical Engineer	SAIC/Maripro; Goleta, CA
1993 - 1995	Chief Engineer	SAIC/Maripro; Goleta, CA
1995 - 1997	Mechanical Engineer	Monterey Bay Aquarium Research Institute
1997 - present	ME Group Supervisor	Monterey Bay Aquarium Research Institute

Selected Publications and Presentations

Scholin, C., G. Massion, E. Mellinger, M. Brown, D. Wright and D. Cline. 1998. The development and application of molecular probes and novel instrumentation for detection of harmful algae. *Ocean Community Conference '98 Proceedings*, Marine Technology Society, Vol. 1 pp. 367-370

NEPTUNE Regional Observatory System Design; D.H. Rodgers, A. Maffei, P.M. Beauchamp, G. Massion, A.D. Chave, T.M McGinnis, S. Gaudet, W.S.D. Wilcock, H. Kirkham; Oceans 2001 Conference, November 6-8, 2001, Honolulu, Hawaii

NEPTUNE Gigabit Ethernet Submarine Cable System; A.R. Maffei, A.D. Chave, G. Massion, S.N. White, J. Bailey, S. Lerner, A. Bradley, D. Yoerger, H. Frazier, R. Buddenberg; Oceans 2001 Conference, November 6-8, 2001, Honolulu, Hawaii

The NEPTUNE Scientific Submarine Cable System; A.D. Chave, H. Kirkham, A.R. Maffei, G. Massion, H. Frazier, A.M. Bradley, S.J. Gaudet, W. Wilcock, D.H. Rodgers, P.M. Beauchamp, J.C. Madden, B.M. Howe; SubOptic 2001, The Fourth International Convention on Undersea Communications, Kyoto, Japan, May 20-24, 2001

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Scholin, C.A., Massion, E.I., Wright, D.K., Cline, D.E., Mellinger, E., Brown, M. 2001. Aquaticautosampler device. US Pat. No. 6187530.

6.3 **GREGORY JOHN DOUCETTE**

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1979 - B.Sc., cum laude. Bowling Green State University, Bowling Green, OH. Major: biology, minor: chemistry.
1982 - M.Sc., Texas A&M University, College Station, TX. Oceanography.
1989 - Ph.D., University of British Columbia, Vancouver, B.C. Botany.

PROFESSIONAL EXPERIENCE:

- Research Oceanographer/Project Leader, NOAA/National Ocean Service, Center for Coastal Environmental Health & Biomolecular Research, Marine Biotoxins Program (Feb. 1999-present).
- Assistant Professor, 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).
- United States National Research Council, Research Associate; Marine Biotoxins Program, Charleston Laboratory, National Marine Fisheries Service. (July 1992-Dec. 1995).
- Postdoctoral Investigator, Woods Hole Oceanographic Institution. Dr. Donald M. Anderson, supervisor (Jan. 1989-June 1992).

NATIONAL/INTERNATIONAL WORKSHOPS AND COMMITTEES (selected):

- NOAA/NMFS Workshop on Marine Biotoxins and Harmful Algae; Charleston, SC (April 1992).
- NSF/COP Workshop on the Ecology and Oceanography of Harmful Algal Blooms; Granby, CO (August 1994).
- UNESCO/SCOR Working Group on the Physiological Ecology of Harmful Algal Blooms; Univ. of Tokyo, Tokyo, Japan (July 1995).
- "Brown Tide Summit," NY Sea Grant Workshop, Ronkonkoma, NY (Oct. 1995).
- NATO Advanced Study Institute on the Ecophysiology of Harmful Algal Blooms; Bermuda Biological Station for Research, Bermuda (May 1996).
- Member, ICES Working Group on Phytoplankton Ecology (1996-present).
- Inter-American Institute for Global Change Research, Workshop on Harmful Algal Blooms; Santiago, Chile (May 1997).
- EPA Harmful Algal Bloom Workshop; Pensacola Beach, FL (Oct. 1997).
- Workshop on Domoic Acid and California Sea Lion Mortality Event, Monterey Bay Aquarium Research Institute, Moss Landing, CA (Aug. 1998).
- Advances in Detection Methods for Fungal and Algal Toxins, Mount Desert Island Biological Laboratory, Salsbury Cove, ME (June 1999).
- National Sea Grant Workshop on the Prevention, Control, and Mitigation of Harmful Algal Blooms, Silver Spring, MD (May 2001).
- Workshop on "Cyanotoxin Detection, Quantitation, and Instrumentation," St. Petersburg, FL (Aug. 2001). Organizing Committee and presenter.
- Workshop on "Biosensors for Harmful Algal Blooms," Solomons, MD (20-22 Mar. 2002). Plenary lecture – "Detection of HAB species, toxins, & toxicities: current approaches & potential for in-situ applications."

INVITED PRESENTATIONS/LECTURES (selected):

- 16th Annual Meeting of Interstate Shellfish Sanitation Conference, Special Session on Marine Biotoxins, San Diego, CA (18-24 July 1998): "Receptor Binding Assays for Marine Biotoxins."

- Coastal Zone '99, San Diego, CA (24-30 July 1999): Session - Science, policy, and environmental decision-making: a focus on harmful algal blooms; Lecture - "Options for prevention, control, and mitigation of harmful algal blooms."
- ICES Annual Science Conference, Stockholm, Sweden (29 Sept.-2 Oct. 1999): Session - Management & Mitigation for Harmful Algae; Keynote Address - "Monitoring & management of harmful algal blooms: a global perspective."
- 1st Symposium on Harmful Marine Algae in the U.S., Woods Hole, MA (5-9 Dec. 2000): West Coast HAB Research - "Approaches to the detection of domoic acid in marine food webs."
- International Society of Exposure Analysis, Charleston, SC (4-8 Nov. 2001): Session: Cyanobacteria in drinking and recreational waters; Lecture: "Automated, *in-situ* detection of harmful algal species and their toxins using the environmental sample processor."

REFEREED PUBLICATIONS (50 total):

Doucette, G.J. & Harrison, P.J. 1990. Some effects of iron and nitrogen stress on the red tide dinoflagellate *Gymnodinium sanguineum. Marine Ecology Progress Series* 62:293-306.

Doucette, G.J. & Harrison, P.J. 1991. Iron and nitrogen nutrition of the red tide dinoflagellate *Gymnodinium sanguineum*. I. Effects of iron depletion and nitrogen source on biochemical composition. *Marine Biology* 110:165-173.

Doucette, G.J. & Harrison, P.J. 1991. Iron and nitrogen nutrition of the red tide dinoflagellate *Gymnodinium sanguineum*. II. Effects of iron depletion and nitrogen source on iron and nitrogen uptake. *Marine Biology* 110:175-182.

Van Dolah, F.M., Finley, E.L., Haynes, B.L., Doucette, G.J., Moeller, P.D. & Ramsdell, J.S. 1994. Development of rapid and sensitive high throughput assays for marine phycotoxins. *Natural Toxins* 2:189-196.

Van Dolah, F.M., Zevotek, N.A., Finley, E.L., Doucette, G.J., Moeller, P.D. & Ramsdell, J.S. 1995. Development of a sensitive, high throughput domoic acid receptor binding assay utilizing microplate scintillation technology. In: Lassus, P., Arzul, G., Erard, E., Gentien, P. & Marcaillou, C. (eds.) *Harmful Marine Algal Blooms*, Lavoisier Science Publ., Paris. pp. 365-370.

Gessner, B.D., Bell, P., Doucette, G.J., Moczydlowski, E., Poli, M.A., Van Dolah, F.M., Hall, S. 1997. Hypertension and identification of toxin in human urine and serum following a cluster of mussel-associated paralytic shellfish poisoning outbreaks. *Toxicon* 35:711-722.

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.

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.

Scholin, C.A., Marin, 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.

Lefebvre, K.A., Powell, C.L., Busman, M., Doucette, G.J., Moeller, P.D.R., Silver, J.B., Miller, P.E., Hughes, M.P., Singaram, S., Silver, M.W. & Tjeerdema, R.S. 1999. Detection of domoic acid in Northern anchovies and California sea lions associated with an unusual mortality event. *Natural Toxins* 7:85-92.

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.

Scholin, C.A., Gulland, F., Doucette, G.J., (22 additional authors). 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* 403:80-4.

Doucette, G.J., Powell, C.L., Do, E.U., Byon, C.Y., Cleves, F. & McClain, S.G. 2000. Evaluation of 11-[^{3}H]-tetrodotoxin use in a heterologous receptor binding assay for PSP toxins. *Toxicon* 38:1465-1474.

Turner, J.T., Doucette, G.J., Powell, C.L., Kulis, D.M., Keafer, B.A. & Anderson, D.M. 2000. Accumulation of red tide toxins in larger size fractions of zooplankton assemblages from Massachusetts Bay, USA. *Marine Ecology Progress Series* 203:95-107.

Pan, Y., Parsons, M.L., Busman, M., Moeller, P.D.R., Dortch, Q., Powell, C.L. & Doucette, G.J. 2001. *Pseudo-nitzschia* sp. cf. *pseudodelicatissima* - a confirmed producer of domoic acid from the northern Gulf of Mexico. *Marine Ecology Progress Series* 220:83-92.

Powell, C.L., Ferdin, M.E., Busman, M., Kvitek, R.G. & Doucette, G.J. 2002. Development of a protocol for determination of domoic acid in the sand crab (*Emerita analoga*): a possible new indicator species. *Toxicon* 40: 481-8.

Tester, P.A., Pan, Y. & Doucette, G.J. In press. Accumulation of domoic acid activity in copepods. In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J. & Lewis, R.J. (eds.) "Harmful Algal Blooms 2000". Proc.9th Int. Conf. Harmful Algal Blooms, IOC-UNESCO, Paris 2002.

Pitcher, G.C., Franco, J.M., Doucette, G.J., Powell, C.L. & Mouton, A. In press. Paralytic shellfish poisoning in the abalone *Haliotis midae* on the west coast of South Africa. *J. Shellfish Res.*

Bargu, S., Powell, C.L., Coale, S.L., Busman, M., Doucette, G.J., & Silver, M.J. Accepted. Krill: a potential vector for domoic acid in marine food webs. *Marine Ecology Progress Series.*

BOOK CHAPTERS (3 total):

Cembella, A.D., Milenokovic, L. Doucette, G.J. & Fernandez, M. 1996. *In vitro* biochemical methods and mammalian bioassays for phycotoxins. In: Hallegraeff, G.M., Anderson, D.M. & Cembella, A.D. (eds.), *Manual on Harmful Marine Microalgae*. IOC-UNESCO, Paris. pp. 177-228.

Cembella, A.D., Doucette, G.J. & Garthwaite, I. In press. *In vitro* assays for phycotoxins. In: Hallegraeff, G.M., Anderson, D.M. & Cembella, A.D. (eds.), *Manual on Harmful Marine Microalgae.* Second Edition. IOC-UNESCO, Paris.

7.1 MBARI Budget - NOPP Proposal		Year One FY2002-03		Year Two FY2003-04		Year Three FY2004-05		Total Request
Salaries/Benefits								
Salaries	**MO**		**MO**		**MO**		**MO**	
Lead Engineer: E. Massion	2	18,846	2	19,600	2	20,384	6	58,830
Lead Scientiest: C Scholin	2	16,814	2	17,487	2	18,185	6	52,486
Electrical Engineer: S Jensen	6	47,850	3	24,882	1.5	12,939	11	85,671
Electrical Tecnician: D. Thompson	6	36,402	9	56,787	2	13,125	17	106,314
Mechanical Engineer: M Brown	12	87,360	12	90,854	12	94,486	36	272,700
Software Engineer: B Roman	12	114,408	12	118,984	12	123,739	36	357,131
Machinist: D Martinez	2	9,793	2	10,185			4	19,978
Science Technician: R Marin							0	0
Subtotal Salary/Wages		**331,473**		**338,779**		**282,858**		**953,110**
Benefits								
Other Professionals @ 49.62 of sal	49.62%	164,477	0 0	168,102	0 0	140,354		472,933
Subtotal Benefits		**164,477**		**168,102**		**140,354**		**472,933**
Total Salary/Benefits		**495,950**		**506,881**		**423,212**		**1,426,043**
Travel:								
Domestic								
US HAB SYM				4,610				4,610
ASLO						5,910		5,910
Subtotal Travel				**4,610**		**5,910**		**10,520**
Materials and Supplies								
2G ESP 1		40,000						40,000
2G ESP 2,3				70,000				70,000
Chem Supplies		7,500		7,500		10,000		25,000
DNA Probe Arrays		10,000		10,000		10,000		30,000
Expendable Plastics		2,500		2,500		2,500		7,500
Microscopy Supplies		1,000		1,000		2,000		4,000
Algae Culturing		1,000		1,000		1,000		3,000
Deployment Expendables				2,500		2,500		5,000
Subtotal Material & Supplies		**62,000**		**94,500**		**28,000**		**184,500**
Other Direct Costs								
ShipTime (No Indirect Costs)				15,000		15,000		30,000
Publications				500		500		1,000
Subtotal Other Direct Costs		**0**		**15,500**		**15,500**		**31,000**
Total Direct Costs		**557,950**		**621,491**		**472,622**		**1,652,063**
Total Indirect Costs (rate: 45%)	45%	**251,078**		**272,921**		**205,930**		**729,929**
Total Direct & Indirect Costs		**809,028**		**894,412**		**678,552**		**2,381,992**

7.1 MBARI CostShare - NOPP Proposal		Year One FY2002-03		Year Two FY2003-04		Year Three FY2004-05		Total Request
Salaries/Benefits								
Salaries	**MO**		**MO**		**MO**		**MO**	
							0	0
Lead Scientiest: C Scholin	2	16,814	2	17,487	2	18,185	6	52,486
Science Technician: R Marin	6	26,000	6	27,040	6	28,122	18	81,162
Subtotal Salary/Wages		**42,814**		**44,527**		**46,307**		**133,648**
Benefits								
Other Professionals @ 49.62 of sal	49.62%	21,244	0 0	22,094	0 0	22,978		66,316
Subtotal Benefits		**21,244**		**22,094**		**22,978**		**66,316**
Total Salary/Benefits		**64,058**		**66,621**		**69,285**		**199,964**
Travel:								
Domestic								
Int'l HAB Mtg		4,110						4,110
Subtotal Travel		**4,110**						**4,110**
Materials and Supplies								
Chem Supplies		2,500						2,500
DNA Probe Arrays		3,000						3,000
Expendable Plastics		800						800
Microscopy Supplies		300						300
Algae Culturing		300						300
Subtotal Materials & Costs		**6,900**						**6,900**
Total Direct Costs		**75,068**		**66,621**		**69,285**		**210,974**
Total Indirect Costs (rate: 45%)	45%							
Total Direct & Indirect Costs		**75,068**		**66,621**		**69,285**		**210,974**

7.2 MBARI (Scholin and Massion) Budget Request Narrative

Much of the budget for this portion of the project is directed towards hiring a team of professional engineers that have the expertise needed to facilitate detailed design of the 2G ESP, fabrication, integration, testing and user training, and that will act in support of field experiments. The same team will be responsible for establishing and maintaining a project web site; an existing MBARI-based server will be used for the latter. For Scholin and Massion, 2 months of salary support is requested in support and management of these activities. Scholin will spend an additional 2 months of time and his research technician (R. Marin) will dedicate 6 months of time as a cost share based on on-going (2002) and future planned ESP projects (2003-5) sponsored by the Packard Foundation. Funds are needed to purchase components required for ESP fabrication and deployment. The latter includes raw materials, onboard computer control, radio modems, cables, etc., as well as mooring components (anchor, chain, rode, surface float). Reagents (chemicals and probes) and disposable plastics (pipette tips, filters, etc.) are required for preparing the DNA probe arrays and testing the instrument, and to offset some of the costs needed for culturing specific target organisms and microscopic analyses to facilitate lab and field testing. Only after the first 2G ESP has been tested successfully (early part of Year 2) will we proceed with construction of replicates (late Year 2). We have included training to transfer the ESP technology to our collaborators in the costs associated with the field deployments. Deployment costs also include expendable materials used for deploying the device, ship time for deployment and recovery of the ESP moorings, and supplies (filters, probes, reagents, batteries) needed to carry out the experiments.

7.3 NOAA Project Budget (Co-PI, G. Doucette)

CATEGORIES	YEAR ONE		YEAR TWO		YEAR THREE		TOTAL PROJECT	
a. Personnel	Federal	Cost-share	Federal	Cost-share	Federal	Cost-share	Federal	Cost-share
Principal Investigator (G. Doucette)		12,191		12,801		13,441		38,433
Postdoctoral Scientist (TBD)	36,000		37,800		39,690		113,490	
b. Fringe Benefits 26.5% of salary (PI) 57.47% of salary + 3% of (total salary + fringe)	22,390	3,231	23,510	3,392	24,685	3,562	70,585	10,185
TOTAL PERSONNEL COSTS	58,390	15,422	61,310	16,193	64,375	17,003	184,075	48,618
c. Travel								
Trip 1 (deployment)			4,420		4,420		8,840	
Trip 2 (deployment)			4,420		4,420		8,840	
Trip 3 (US HAB Sym)			1,530				1,530	
Trip 4 (ASLO mtg.)					2,180		2,180	
TOTAL TRAVEL COSTS			10,370		11,020		21,390	
d. Equipment	0		0		0		0	
TOTAL EQUIPMENT COSTS	0		0		0		0	
e. Supplies								
> Receptor assay suppl. (disposables, reagents)	10,000		8,000		5,000		23,000	
> Toxin conjugate prod. & purification	7,500						7,500	
> Antibody production; & ELISA develop.	7,500		7,500		7,500		22,500	
> Toxin-protein conj. arrays			7,500		5,000		12,500	
TOTAL SUPPLY COSTS	25,000		23,000		17,500		65,500	
f. Contracts	0		0		0		0	
TOTAL CONTRACTUAL COSTS	0		0		0		0	
g. Other Costs	0		0		0		0	
TOTAL OTHER COSTS	0		0		0		0	
h. **TOTAL DIRECT COSTS** (sum of a-g)	83,390	15,422	94,680	16,193	92,895	17,003	270,965	48,618
i. Indirect Costs/Charges 0% of (base)	0	0	0	0	0	0	0	0
TOTAL PROJECT COSTS (sum of h & i)	83,390	15,422	94,680	16,193	90,895	17,003	270,965	48,618
TOTAL REQUESTED FROM NOPP	83,390	0	94,680	0	90,895	0	270,965	0

7.4 NOAA (Doucette) Budget Request Narrative

Co-PI Doucette will dedicate two months per project year to oversee this component of the project; however, as a federal government employee, salary support is not required and this amount is given as in-kind support. Support is requested for a postdoctoral scientist (12 months per project year) to conduct the development and continued refinement of toxin detection chemistries in the laboratory, assist in the integration of toxin detection capabilities into the ESP platform, participate in ESP field deployments, and be responsible for data reduction/management. Funds for travel to MBARI twice during project years 2 and 3 are requested for Doucette and the postdoctoral scientist to meet with co-PIs Scholin and Massion to work out details of integrating toxin detection methods into the ESP platform during laboratory trials, to participate in ESP field deployments, as well as to review and revise toxin detection strategies based on results of the field deployments. The remainder of this component of the project budget is for the purchase of supplies and services needed to develop, conduct, and modify toxin detection assays. These requirements include supplies (primarily disposable plastics, reagents, and toxin) for receptor binding assays and production of cloned receptor preparations, for production and purification of various toxin-protein conjugates to serve as competitors in assays and as immunogens for antibody production. Several conjugates will be produced and tested during optimization of the antibody and ELISA development. Funds will also support production of polyclonal antibodies, development of immunoassay chemistries, and printing of test arrays to be deployed on the ESP platform. These costs are similar for Yrs. 1 and 2, but are reduced in Yr. 3 to include only supplies needed to perform receptor and ELISA-based toxin detection, and reflecting a decreasing dependence on the receptor-based assay.

8. Supporting Information

8.1 P.I.s' current and pending research projects

Investigator: Christopher A. Scholin

Support: X Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Testing and refinement of the environmental sample processor (ESP)
Source of Support: Packard Foundation
Total Award Amount: $324,774 Total Award Period Covered: 1/1/02-12/31/02
Location of Project: Monterey Bay Aquarium Research Institute
Person-Months Per Year Committed to the Project. Cal: 9 Acad: Sumr:

Support: Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: A Remote, Autonomous Sensor for Exploring Oceanic Microbial Diversity
Source of Support: NOAA Ocean Exploration Program
Total Award Amount: $2,571,140 Total Award Period Covered: 5/1/02-4/30/05 (still pending as of 4/10/02)
Location of Project: Monterey Bay Aquarium Research Institute; NOAA/NOS Charleston, SC
Person-Months Per Year Committed to the Project. Cal: 2 Acad: Sumr:

Support: Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: The Environmental Sample Processor (ESP): a device for detecting microorganisms *In Situ* Using Molecular Probe Technology (this proposal)
Source of Support: : NOPP
Total Award Amount: $2,385,778 Total Award Period Covered: 8/1/02-7/31/05
Location of Project: Monterey Bay Aquarium Research Institute
Person-Months Per Year Committed to the Project. Cal: 4 Acad: Sumr:

Investigator: Eugene Massion

Support: X Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: Testing and refinement of the environmental sample processor (ESP)
Source of Support: Packard Foundation
Total Award Amount: $324,774 Total Award Period Covered: 1/1/02-12/31/02
Location of Project: Monterey Bay Aquarium Research Institute
Person-Months Per Year Committed to the Project. Cal: 0.5 Acad: Sumr:

Support: Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: A Remote, Autonomous Sensor for Exploring Oceanic Microbial Diversity
Source of Support: NOAA Ocean Exploration Program
Total Award Amount: $2,571,140 Total Award Period Covered: 5/1/02-4/30/05 (still pending as of 4/10/02)
Location of Project: Monterey Bay Aquarium Research Institute; NOAA/NOS Charleston, SC
Person-Months Per Year Committed to the Project. Cal: 2 Acad: Sumr:

Support: Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: The Environmental Sample Processor (ESP): a device for detecting microorganisms *In Situ* Using Molecular Probe Technology (this proposal)
Source of Support: : NOPP
Total Award Amount: $2,385,778 Total Award Period Covered: 8/1/02-7/31/05
Location of Project: Monterey Bay Aquarium Research Institute
Person-Months Per Year Committed to the Project. Cal: 2 Acad: Sumr:

Investigator: Gregory J. Doucette

Support: X Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: ECOHAB-GOM: the ecology and oceanography of toxic *Alexandrium* blooms in the Gulf of Maine
Source of Support: ECOHAB Inter-Agency Program NSF/NOAA/EPA/ONR
Total Award Amount: $156,633 Total Award Period Covered: 9/1/97-5/31/02
Location of Project: Medical University of South Carolina
Person-Months Per Year Committed to the Cal: .5 Acad: Sumr:

Support: X Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: ECOHAB: Domoic acid in a coastal food web
Source of Support: ECOHAB Inter-Agency Program NSF/NOAA/EPA/ONR
Total Award Amount: $30,360 Total Award Period Covered: 10/1/99-9/31/02
Location of Project: NOAA/NOS/Center for Coastal Environmental Health & Biomolecular Research
Person-Months Per Year Committed to the Cal: 2 Acad: Sumr:

Support: X Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: ECOHAB: Algicidal bacteria targeting *Gymnodinium breve*: population dynamics and killing activitv
Source of Support: : ECOHAB Inter-Agency Program NSF/NOAA/EPA/ONR
Total Award Amount: $408,927 Total Award Period Covered: 9/1/01-8/31/04
Location of Project: NOAA/NOS/Center for Coastal Environmental Health & Biomolecular Research
Person-Months Per Year Committed to the Cal: 2 Acad: Sumr:

Support: ☐ Current X Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: A Remote, Autonomous Sensor for Exploring Oceanic Microbial Diversity
Source of Support: NOAA Office of Ocean Exploration
Total Award Amount: $101,590 Total Award Period Covered: 5/1/02-4/30/05 (still pending as of 4/02)
Location of Project: NOAA/NOS/Center for Coastal Environmental Health & Biomolecular Research; MBARI
Person-Months Per Year Committed to the Cal: 2 Acad: Sumr:

Support: ☐ Current X Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support
Project/Proposal Title: The Environmental Sample Processor (ESP): a device for detecting microorganisms *In Situ* using molecular probe technology (this proposal)
Source of Support: NOPP
Total Award Amount: $270,965 Total Award Period Covered: 8/1/02-7/31/05
Location of Project: NOAA/NOS/Center for Coastal Environmental Health & Biomolecular Research; MBARI
Person-Months Per Year Committed to the Project. Cal: 2 Acad: Sumr:

8.2 Extent of institution participation and support for the program

Over the past decade MBARI as well as the NOAA/NOS Marine Biotoxins Program laboratory have made substantial investments in their infrastructures and staffing that not only make the work proposed here possible, but also offer an exceptionally unique environment that will foster its success. As pointed out earlier, Scholin and Doucette have a long record of collaboration and focussed research programs that form a methodological basis for near real-time species and toxin detection capabilities. Their collaboration with a professional engineering team at MBARI in recent years has made it possible to prototype novel instruments (ESP) that can emulate those methods *in situ*. For the first time, this team is deploying autonomous systems that employ molecular probes as a basis for detecting microorganisms in the ocean. Continued support of this work through NOPP funding will allow us to mature the technology incrementally and make it available to the oceanographic community.

8.3 Names of other agencies receiving the proposal

An earlier version of this proposal was submitted to the NOAA Ocean Exploration Program in November 2001. As of this writing no funding decisions have yet been reached.

9. Bibliography

Bates, S.S. 1998. Ecophysiology and metabolism of ASP toxin production. In: Anderson, D.M., Cembella, A.D. and Hallegraeff, G.M. [Eds.] *Physiological Ecology of Harmful Algal Blooms*. NATO ASI Series, Vol. G 41. Springer-Verlag, Berlin. pp. 405-426.

Franks, P.J.S. and D,M. Anderson. 1992a. Alongshore transport of a toxic phytoplankton bloom in a buoyancy current: *Alexandrium tamarense* in the Gulof of Maine. *Marine Biology* **112**: 153-164.

Franks, P.J.S. and D,M. Anderson. 1992b. Toxic phytoplankton blooms in the southwestern Gulf of Maine: Testing hypotheses of physical control using historical data. *Marine Biology* **112**: 165-174.

Hallegraeff, G.M., D.M. Anderson and A.D. Cembella [Eds.]. *Manual on Harmful Marine Microalgae.* IOC Manuals and Guides No. 33, UNESCO, 1995.

Lefebvre, K.A., Powell, C.L., Busman, M., Doucette, G.J., Moeller, P.D.R., Silver, J.B., Miller, P.E., Hughes, M.P., Singaram, S., Silver, M.W. and Tjeerdema, R.S. 1999. Detection of domoic acid in Northern anchovies and California sea lions associated with an unusual mortality event. *Natural Toxins* 7:85-92.

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CERTIFICATIONS REGARDING DEBARMENT, SUSPENSION AND OTHER RESPONSIBILITY MATTERS; RESTRICTIONS ON LOBBYING; AND DRUG-FREE WORKPLACE REQUIREMENTS

Proposal Title : **The Environmental Sample Processor (ESP): A Device for Detecting Microorganisms *In Situ* Using Molecular Probe Technology**

April 9, 2002
Proposal Number and/or Date

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

ONR Broad Agency Announcement (BAA) #02-001 for Long Range Scientific Projects, published in the Commerce Business Daily, dated 22 AUG 2001.

X Other BAA (list solicitation no., project title, and data of issuance):

Solicitation No.: 02-011 ,
Project Title: Observational Technique Development (sensors and platforms)

National Oceanographic Partnership
Applicants should refer to the regulations cited below to determine the certifications to which they are required to attest. Applicants should also review the instructions for certification requirement under 32 CFR Part 25, "Government-wide Debarment and Suspension (Nonprocurement)"; and 32 CFR Part 28, "New Restrictions on Lobbying"; 32 CFR Part 25, "Government-wide Requirements for Drug-Free Workplace (Grants)". The certifications 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 DEBARMENT, SUSPENSION, AND OTHER RESPONSIBILITY MATTERS--PRIMARY COVERED TRANSACTIONS

(1) The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded by any Federal department or agency;
(b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;
(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and

(d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.
(2) Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

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.

CERTIFICATION REGARDING DRUG-FREE WORKPLACE REQUIREMENTS

Alternate I. (Grantees Other Than Individuals)

(1) The grantee certifies that it will or will continue to provide a drug-free workplace by:
(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;
(b) Establishing an ongoing drug-free awareness program to inform employees about--
(1) The dangers of drug abuse in the workplace;
(2) The grantee's policy of maintaining a drug-free workplace;
(3) Any available drug counseling, rehabilitation, and employee assistance programs; and

(4) the penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will--

(1) Abide by the terms of the statement; and

(2) Notify the employer in writing of his or her conviction for a violation of a criminal drug statute occurring in the workplace no later than five calendar days after such conviction;

(e) Notifying the agency in writing, within ten calendar days after receiving notice under paragraph (d)(2) from an employee or otherwise receiving actual notice of such conviction. Employers of convicted employees must provide notice, including position title, to every grant officer or other designee on whose grant activity the convicted employee was working, unless the Federal agency has designated a central point for the receipt of such notices. Notice shall include the identification number(s) of each affected grant;

(f) Taking one of the following actions, within 30 calendar days of receiving notice under paragraph (d)(2), with respect to any employee who is so convicted--

(1) Taking appropriate personnel action against such employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of 1973, as amended; or

(2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a), (b), (c), (d), (e) and (f).

(2) The grantee may insert in the space provided below the site(s) for the performance of work done in connection with the specific grant:

Place of Performance (Street address, city, county, state, ZIP code)

7700 Sandholdt Road
Moss Landing, CA 95039-9644

Check ____ if there are workplaces on file that are not identified here.

Alternate II. (Grantees Who Are Individuals)

(1) The grantee certifies that:

(a) As a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant;

(b) If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, he or she will report the conviction in writing, within 10 calendar days of the conviction, to every grant office or other designee, unless the Federal agency designates a central point for the receipt of such notices. When notice is made to such a central point, it shall include the identification number(s) of each affected grant.

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:

C. Michael Pinto, CFO & COMPTROLLER Date: 4/9/02