Project Number: 901204

Project Start: Jan. 1, 2012[1] **Project Duration (in years): Year 4 of 5 [1]**

Please indicate if proposal includes requests for any of the following (mark all that apply).

☐	☐	☐	☐	☐
Ship Time	**ROV**	**AUV**	**Wave Glider**	**Mini-ROV**

Please indicate what type of project is being proposed (mark only one).

☐	☐	X	☐	☐
Feasibility/Scoping [Less than 100 days effort]	**Development[2]**	**Research**	**Infrastructure[2]**	**Education**

☐	☐
Time Series	**Institute Initiative**

Project Manager[3]: Jim Birch

Principal Investigator: Chris Scholin

Lead Scientists: Roman Marin, Chris Preston, Bill Ussler, Holly Bowers

Lead Engineer: N/A

Project Title: Enhancing detection chemistries for investigating microbial ecology

[1] This date and number should remain the same for the duration of the project. If your project start date for 2015 is not January 1, please indicate the start date in the first cell of the milestone chart.
[2] Development and infrastructure projects must complete the risk matrix (see item #5 at the end of the proposal form).
[3] Not all projects will require all four titles to be filled in. Please indicate individuals only for those titles that are appropriate for your project.

1) ABSTRACT (new projects only)

N/A

2) PROPOSAL

a) Project Overview

Molecular analytical analyses are commonly employed to assess the presence, abundance and activity of wide variety of marine organisms. Our overall goal is to enable greater use of such methods by developing the means to autonomously collect, preserve and process discrete samples in situ. Achieving this overall goal fundamentally rests on two different types of activities: methods development, and instrumentation engineering. This proposal concerns the former – devising ways to collect a range of microbes, phytoplankton, small invertebrates, and environmental DNA (eDNA), and interrogate that material using a variety of molecular analytical procedures in both laboratory and field settings. Work associated with the instrumentation development effort is captured separately under the SURF Center proposal (901205). When combined, the methodological and instrumentation advances contribute to creating a class of device known as "ecogenomic sensors"; the Environmental Sample Processor (ESP) exemplifies this concept (Scholin 2013).

In past years under the auspices of this project, we developed ways to collect and process samples using DNA and antibody probe chemistries (e.g., Harvey et al. 2012, Ryan et al. 2011, Preston et al. 2011, Doucette et al. 2009). We also pioneered methods for preserving samples under ambient conditions to enable high fidelity laboratory analyses currently not practical to deploy on fully autonomous platforms (e.g., DNA and RNA sequencing - Ottesen et al. 2011, 2013, 2014; proteomics – Saito et al. 2011).

While we have made considerable progress in advancing new systems for studying marine microorganisms in situ, and for returning samples for laboratory analyses, there nevertheless remains much work to refine methodologies for effecting sample acquisition and processing in ways that are consistent with what ocean-going platforms support in terms of payload size, capacity and deployment durations. Given a small space, no temperature control, low power, and minimal volume for expendable reagents, etc., what techniques can we envision that will allow us to maximize our ability to utilize cutting edge molecular analytical chemistries and sample preservation capabilities? Overcoming these issues is the focus of this project; applications of the 2G ESP, and development of the 3G ESP/LRAUV, provide specific science use cases and requirements specifications that guide the work associated with this proposal.

b) Continuing Projects

Current status of project and results to date

For 2014 we proposed to concentrate on four areas as listed below; a brief progress report for each follows:

- Functional testing of the 3G ESP sample collection and processing cartridges
- Analysis of data/samples collected from the 2013 deep-sea ESP (D-ESP) MARS deployment
- Analysis of samples collected from the MARS microbial incubation experiment
- Studies of *Pseudo-nitzschia* species that may play a role in harmful algal bloom outbreaks in California waters (continuation of Holly Bowers' postdoctoral work).

Functional testing of the 3G ESP sample collection and processing cartridges

The 3G ESP system is designed around a single-use sample collection and processing cartridge. The current design allows up to 60 cartridges to be loaded onto a rotating valve assembly that can be fitted to the LRAUV (Fig. 1). Each cartridge can carry electronics/logic, sample collection media, and reagents. Applying reagents to collected material in a timed sequence is achieved by rotating the cartridge to a position where actuators are located. Under the auspices of the SURF Center (901205), we have worked with a private consulting company, Accel Biotech (http://www.accelbiotech.com/site/), to develop the cartridges.

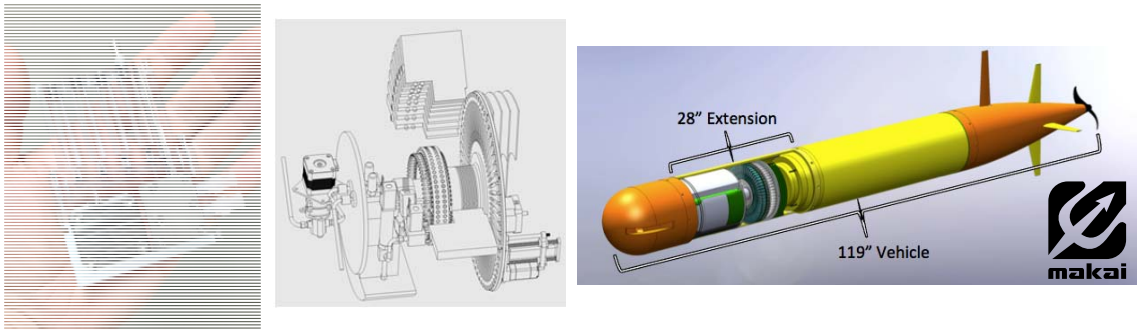

Figure 1. Elements of the 3G ESP/LRAUV system: (left) prototype, single-use cartridge that contains sample collection media and processing reagents; (middle) solid model showing a cartridge attached to a circular valve assembly; (right) the 3G solid model assembly fitted into a 12-inch diameter by 28-inch housing in the forward space of the LRAUV. The resulting system will have the capacity to collect and process up to 60 samples while ranging freely in the ocean over days-weeks to 300 meters depth.

Once a sample has been acquired using a cartridge, it can either be preserved for later laboratory analyses or analyzed immediately using one or more "analytical modules" (devices downstream of the sampler) to detect target substances in situ. This has led to the development of two types of cartridges: an archival cartridge (AC) that is only used to preserve material, and a "lyse-n-go" cartridge (LGC) that is used for more complex protocols that prepare a sample for immediate downstream analysis.

Thus far, our major focus has been on evaluating methods related to use of the AC, particularly with respect to material/reagent compatibility, sample concentration, reagent delivery, fluid flow, and testing to determine how well samples have been preserved for DNA/RNA-based analyses. The latter has been done in conjunction with Ed DeLong at MIT/UH as a part of our NSF MRI project (see SURF Center proposal for more information). We have solved a number of problems, and now have a beta version of the AC that will be tested throughout the remainder of this year. The work on the AC has provided insights into the design of the LGC that will be used to create an extract that will be

passed to a surface plasmon resonance (SPR) or digital PCR (dPCR) device for detecting specific metabolites or nucleic acids; development of the SPR and dPCR modules is sponsored by external grants from NSF and the Moore Foundation, respectively.

Methods development work associated with the LGC revolves around the use of new, commercially available enzyme cocktails that dissolve cell material, thus freeing DNA/RNA and other metabolites for direct analysis (see http://www.zygem.com). If these enzyme cocktails prove successful, we can greatly simplify and speed up sample processing relative to the standard extraction protocols we use for both lab and 2G ESP-based applications. Consequently, we are investing a significant amount of time evaluating the use of this sample handling methodology. Preliminary tests show it is compatible with both SPR and dPCR detection chemistries, it shows promise in preparing organisms from marine samples, and the key reagent has thus far proven to be stable for 147 days when held at room temperature (longest time point evaluated to date). These attributes also make it an ideal candidate for use with the 3G ESP system. This August our collaborators will deliver the SPR and dPCR analytical modules to MBARI; we will then begin to delve into the methodological details of achieving end-to-end sample processing –from collection to real-time analysis– in the context of a working 3G ESP prototype.

Analysis of data/samples collected from this year's deep-sea ESP (D-ESP) MARS deployment

The D-ESP was deployed at MARS for over eight months during 2013. Over that time we collected 24 samples that were analyzed in situ, in real time, as well as preserved aliquots of the same material for later laboratory analyses. Real-time analyses employed in this study included use of DNA probe arrays for detecting 35 unique ribosomal RNA (rRNA) sequences (Figure 2), and quantitative PCR (qPCR) for estimating the abundance of particular genes [ammonium oxidizing Archeae "a" and "b" genes (AOAa, AOAb); Group 1 Archeae (G1Ar); deep-sea RuBisCO (CbbM – carbon fixation); *Pseudo-nitzschia spp.* (PseudG – marine diatom found in surface waters]. Real-time data from contextual sensors was used to trigger D-ESP sampling events. In this way, we were able to collect and analyze material found throughout a wide range of temperature, salinity, and oxygen conditions observed at MARS. Our findings show that the MARS site is dynamic: it is replete with an active population of microbes that are capable of carbon fixation and ammonia oxidation, and we detected inputs of photic-zone-derived sources of carbon.

This year we have been working to analyze the preserved samples using a DNA analysis technique known as 16S-tag sequencing (Caporaso et al 2012; ISME J. 6: 1621–1624) to reveal variations in microbial community structure that cannot be observed using the probe arrays. However, the published method uses sequencing primers that do not detect many oceanic microbes. We therefore redesigned the primers to ensure all known Monterey Bay microbial groups are included, and tested those with samples recovered from a previous D-ESP deployment. Initial results indicate that greater microbial diversity is observed using the modified primers. We also found that two species of bacteria appear to have colonized (biofouled) the D-ESP sampler; when processing the sequence data, sequences recovered from these organisms can be eliminated to reveal the natural microbial community. Based on these findings, we are now ready to examine the archived samples collected during the 2013 deployment and will do so before the end of this year. A manuscript describing the outcomes of this work is in preparation.

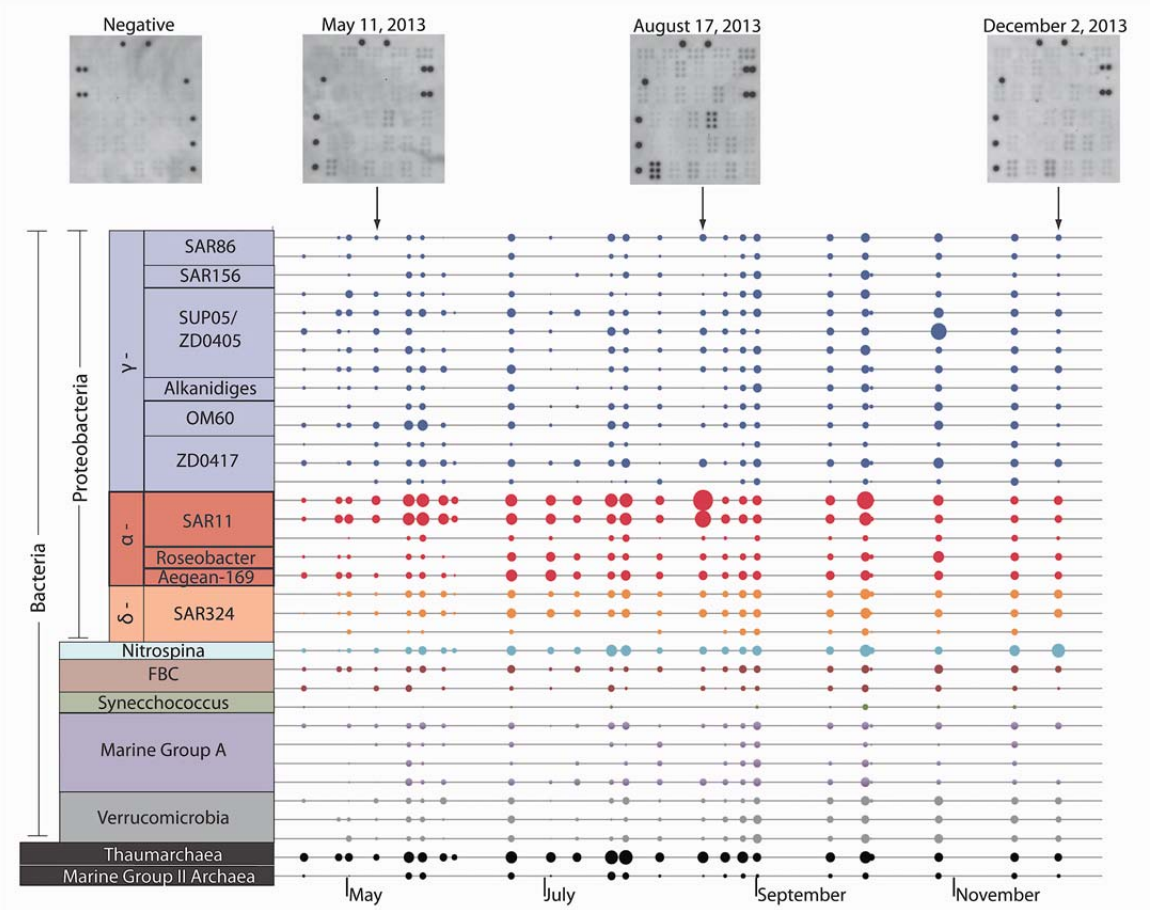

Figure 2. Example showing rRNA-targeted probe arrays (top) collected using the D-ESP before (left, filtered seawater negative control) and during the MARS deployment (dated). The arrays consist of 35 unique rDNA probes for various microbial groups as shown at left. The signal intensity from those probes is shown as a function of time, with increasing spot size indicating greater signal. The spots visible on the control array are controls for orienting the array (Preston *et al.*, in prep.).

Analysis of samples collected from the MARS microbial incubation experiment

During 2013 we devised a prototype in situ incubation system to facilitate investigations of microbial-mediated biogeochemical cycles in the deep-sea. The initial test of that system centered on respiration of oxygen versus nitrate in the context of oxygen minimum zones (OMZs). Projections of a warming ocean and an expansion of OMZs lead to predictions that microbial community structure and metabolic function will change, greatly contributing to enhanced oxygen-depletion in the meso-pelagic and a shift in the biogeochemistry of OMZs towards a more chemically reduced state. We are attempting to use the *in situ* microbial incubation system to test this theory by manipulating the nitrate/oxygen ratio in a controlled fashion. The field component of this investigation was completed in January 2014.

Samples from the incubation device were preserved in situ using a previously described fixative (Feike et al. 2012; *ISME J.* 6:461-470), but in our hands this caused a precipitate to form making immediate sample filtration/processing impossible. However, several days post sample recovery the precipitate dissipated and we were able to collect material using a standard filtration method; preliminary analysis of two of those samples show that we can recover DNA and RNA which is key to evaluating the results of the incubation experiment. Nevertheless, the impacts of the post-fix precipitate and prolonged sample hold times are not yet known.

At this point we are targeting analysis of two nitrate reductase genes (*nar*G and *nap*A) that marine bacteria use to respire nitrate as an alternative to oxygen. We have recovered these genes from representative water column samples obtained at MARS, and sequencing of that material is currently underway to provide the basis for development of assays for quantifying both the presence of those genes and their levels of expression in material collected from the incubation experiment. If results of those analyses suggest a switch in microbial respiration from oxygen to nitrate as a result of altering the oxygen/nitrate ratio, then we will be in a position to formulate a more robust *in situ* microbial incubation experimental plan for 2015-2016.

Studies of Pseudo-nitzschia species that may play a role in harmful algal bloom outbreaks in California waters (continuation of Holly Bowers' postdoctoral work)

Of the ~44 described species of diatoms in the genus *Pseudo-nitzschia,* only a handful are known to produce domoic acid (DA). During three separate ECOHAB/CANON field experiments, we have used sampling capabilities afforded by the ESP, Dorado Gulpers, and traditional CTD bottle casts to identify *Pseudo-nitzschia* species as well as DA in the field as a step toward better elucidating which species are contributing to DA outbreaks in California waters.

Live samples have been used to establish a collection of *Pseudo-nitzschia* cultures (n=~1500). From this we have identified twelve different species, including two potential variants of a single species, *P. delicatissima*, based on rRNA gene sequencing. A subset of the collection has been tested for DA, and representatives of each species have been assessed for cross-reactivity with probes currently used on the ESP and in the laboratory. Testing has revealed putative DA production in at least one species previously described as non-toxic, and two species cross-react with probes for other species. Figure 3 depicts the level of diversity encountered on the San Pedro shelf region during our 2014 deployment. These assessments are critical in furthering our knowledge about niches for key phytoplankton species in an effort to improve monitoring strategies and design relevant ecological studies.

During 2013-2014, we also devised a scanning electron microscopy (SEM) protocol for retrieving *Pseudo-nitzschia* frustules from spent sample collection filters used on the ESP. This method allows us to directly correlate species presence with other in situ testing, such as DA detection, post-deployment. We are in the process of using this technique to analyze filters retrieved from ESPs deployed during the ECOHAB 2013 mission in San Pedro to determine what species were associated with DA that was detected in situ. In addition, we used the same method to examine sample filters recovered from the 2013 MARS D-ESP deployment to verify the presence of *Pseudo-nitzschia* frustules associated with real-time detection of that organism using a PCR-based assay that deployed as an element of the MARS mission (Figure 4).

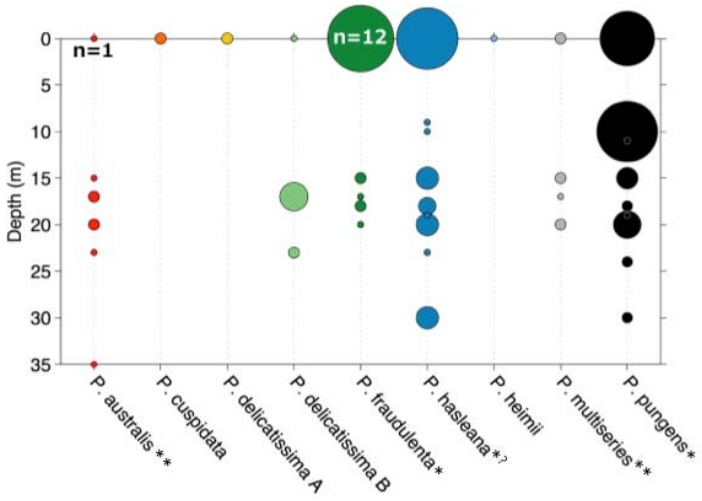

Figure 3. Plot depicting *Pseudo-nitzschia* diversity and depth in the water column, based on clonal cultures isolated from CTD casts during the spring 2014 ECOHAB mission to the San Pedro shelf. **=species that have tested positive for DA, *=species that have tested weakly positive for DA, *?=species with unconfirmed DA production.

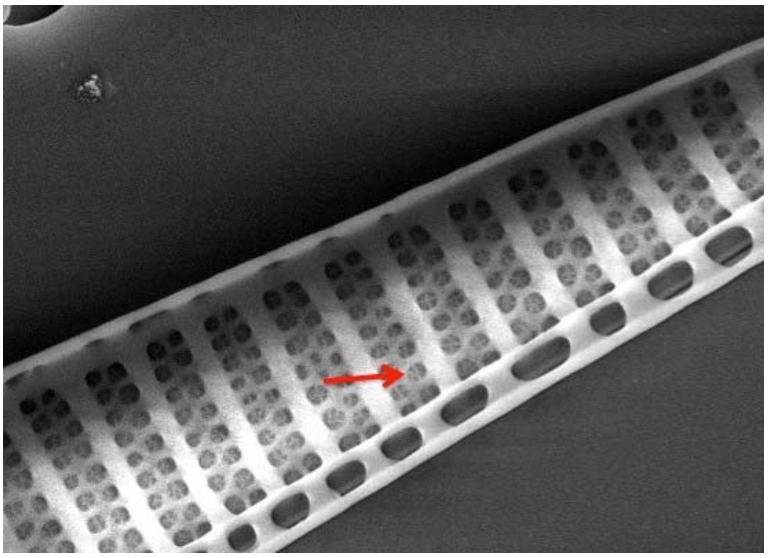

Figure 4. *Pseudo-nitzschia* frustule recovered from a sample filter from the 2013 MARS deployment of the D-ESP. During the deployment, the D-ESP detected a weak pulse of *Pseudo-nitzschia*, presumably from material that originated in surface waters and sank to the MARS site. However, that signal was based only on DNA. Frustules recovered from the same sample filter allow for use of established morphometric analyses, like poroid pattern and structure (red arrow), to provide definitive (i.e., accepted) species identification. The combined DNA and morphological analyses prove that the D-ESP did in fact detect *Pseudo-nitzschia* at MARS.

Does it relate to the Technology Roadmap? If so, please describe how.
(See http://www.mbari.org/about/TechnologyRoadmap.pdf.)

Yes. This project contributes directly to the *Taking the Laboratory into the Ocean*, and *Advancing a Persistent Presence* engineering initiatives, particularly in response to Problem Statements #'s 1, 2, 3, 6, and 8. In our case, both engineering push and science pull have helped to shape development of methods needed for identifying organisms and assessing their activities using molecular probe technology – most specifically through the development and application of ecogenomic sensors/samplers as summarized in the Project Overview section of this proposal.

Significant changes from previous project proposal

None.

Publications and manuscripts based on the results of this project from 2012 to present (papers overlap with those listed in 901205 since they involve both probe chemistry and instrument develop)

Ottesen, E.A., C.R. Young, S.M. Gifford, J.M. Eppley, R. Marin III, S.C. Schuster, C.A. Scholin, and E.F. DeLong. 2014. Multispecies diel transcriptional oscillations in open ocean heterotrophic bacterial assemblages. *Science*, 345 (6193), 207-212. [DOI:10.1126/science.1252476]

Robidart, J.C., Church, M.J., Ryan, J.P., Ascani, F., Wilson, S.T., Bombar, D., Marin, R III, Richards, K.J., Karl, D.M., Scholin, C.A., Zehr, J.P. 2014. Ecogenomic sensor reveals controls on N2-fixing microorganisms in the North Pacific Ocean. *The ISME Journal* **8,** 1175–1185 (doi:10.1038/ismej.2013.244)

Brewer, P.G., Hofmann, A.F, Peltzer, E.T., and Ussler, W., III. 2014. Evaluating microbial chemical choices: The ocean chemistry basis for the competition between use of O_2 or NO_3^- as an electron acceptor. *Deep Sea Research I,* 87:35-42.

Ottesen, E.A., C.R. Young, J.M. Eppley, J.P. Ryan, F.P. Chavez, C.A. Scholin, E.F. DeLong. 2013. Rhythm and synchrony of gene expression among sympatric marine microbial populations. *Proc. Natl. Acad. Sci.,* 110 (6): 488-497. (doi/10.1073/pnas.1222099110)

Pargett, D., S. Jensen, B. Roman, C. Preston, W. Ussler, P. Girguis, R. Marin III, J. Birch, C. Scholin. Deep Water Instrument for Microbial Identification, Quantification, and Archiving. 2013. Oceans. MTS/IEEE Conference Publications

Ussler, W. III, C. Preston, P. Tavormina, D. Pargett, S. Jensen, B. Roman, R. Marin III, S. Shah, P.R. Girguis, J.M. Birch, V. Orphan, C. Scholin, 2013. Autonomous application of quantitative PCR in the deep sea: *In situ* surveys of aerobic methanotrophs using the deep-sea environmental processor. *Environmental Science and Technology* 47:9339-9346 (doi/10.1021/es4023199).

Scholin C.A. 2013. Ecogenomic Sensors. In: Levin S.A. (ed.) Encyclopedia of Biodiversity, second edition, Volume 2, pp. 690-700. Waltham, MA: Academic Press.

Harvey, J.B.C, J.P. Ryan, R. Marin III, C.M. Preston, N. Alvarado, C.A. Scholin, R.C. Vrijenhoek. 2012. Robotic sampling, in situ monitoring and molecular detection of marine zooplankton. *Journal of Experimental Marine Biology and Ecology* 413: 60–70

Manuscripts in review and preparation

Olins, H.C., Rogers, D.R., Vidoudez, C., Preston, C.M., Ussler III, W., Pargett, D.M., Jensen, S.M., Roman, B., Scholin, C., Birch, J., and Girguis, P.R. To be submitted to *The ISME journal*. Diverse transcriptomic profiles reveal striking differences in microbial activity among diffuse hydrothermal flows with a vent field.

Rogers, D.R., Olins, H.,Vidoudez, C., Preston, C.M., Ussler III, W., Pargett, D.M., Jensen, S.M., Roman, B., Scholin, C., Birch, J., and Girguis, P.R. To be submitted to *Proc. Natl. Acad. Sci. In situ* RNA preservation and co-registered geochemistry at a diffuse flow hydrothermal vent reveal spatial separation of active carbon-fixation pathways along a geochemical gradient.

Seegers, B.N., Birch, J.M., Marin, R. III, Scholin, C.A., Caron, D.A., Seubert, E.L., Howard, M.D.A., Roberston, G.L., and Jones, B.H. **(in review)**. Subsurface seeding of surface harmful algal blooms observed through the integration of autonomous gliders, moored Environmental Sample Processors, and satellite remote sensing in Southern California. *Limnol. Oceanog.*

Varaljay, V.A., Robidart, J., Preston, C.M, Gifford, S.M. , Durham B.P., Ryan, J.P., Marin III, R., Kiene, R.P., Zehr, J.P., Scholin, C.A. and Moran, M.A. Gene Regulation Controlling Volatile Sulfur Flux from the Ocean. Submitted to Science: Rejected; being reworked for different journal

Yamahara K.M.*, Demir-Hilton E.*, Preston C.M., Marin III R., Pargett D., Roman B., Jensen S., Birch J., Boehm A.B., Scholin C.A. To be submitted to *Environmental Science and Technology*. Simultaneous monitoring of fecal indicators and harmful algae using an in-situ autonomous sensor. * co-first authors

c) New and Continuing Projects: Plans for 2015

Research Activities

For 2015 we propose to focus on four areas:

1) Testing/evaluation of methods proposed for 3G ESP sample collection and analysis
2) Complete analysis of samples collected from the 2013 D-ESP MARS deployment
3) Evaluate samples recovered from the 2013 MARS microbial incubation experiment: determine if the experiment was successful and conduct a scoping effort for a possible follow-on experiment in 2016
4) Complete studies of *Pseudo-nitzschia* species that may play a role in harmful algal bloom outbreaks in California waters (conclusion of Holly Bowers' postdoctoral work).

1) Testing/evaluation of methods proposed for 3G ESP sample collection and analysis

We will continue to focus on developing and refining protocols for the AC and LGC cartridges. Tests of the AC will focus on storage of the archived samples in time frames and under conditions that mimic 3G ESP/LRAUV deployments to insure that target analytes (specifically DNA and mRNA) are stabilized and can be recovered quantitatively as dictated by established science use-case scenarios. Work with the LGC will concentrate on use of the Zygem sample homogenization scheme using SPR and dPCR analytical modules. We will evaluate the utility and fidelity of the sample preparation and detection chemistries using a variety of environmental samples and cultures. In all cases, tests of the new assays will be compared against currently accepted laboratory-based, analytical "gold standards".

2) Complete analysis of samples collected from the 2013 D-ESP MARS deployment

The majority of samples recovered from the MARS 2013 deployment should be processed by the end of 2014. In 2015, we will complete analysis of the entire D-ESP data set and prepare a manuscript for publication likely to either *Deep-Sea Research* or *Environmental Microbiology*. This work will represent the first seasonal microbial community description of MARS, and document the first long-term, D-ESP deployment.

3) *Evaluate samples recovered from the 2013 MARS microbial incubation experiment: determine if the experiment was successful and conduct a scoping effort for a possible follow on experiment in 2016*

The immediate goal of the *in situ* incubation experiments is to determine if alteration of the oxygen/nitrate ratio results in the predicted shift of microbes respiring nitrate over oxygen as the ratio of those two terminal electron acceptors changes in favor of nitrate. To that end we will complete analysis of the existing samples, though note that problems with sample preservation may compromise results of the experiment and potentially complicate its interpretation. Nevertheless, development of the assays needed for sample analysis will help guide a potential future effort should results warrant future attempts of repeating the experiment.

We will revisit options for preserving samples *in situ* since high fidelity recovery of gene expression patterns is essential for evaluating the predicted microbial respiration cascade in response to ocean warming. Development of *in situ* methods for preserving mRNA on particulate filters to obtain levels of gene expression is already an active research activity within the ESP group and by other investigators (e.g., Craig Taylor at WHOI). There are two fundamental approaches to solving this problem: (a) pump a water sample through a filter and then apply the preservative (currently used by the ESP and a system manufactured by McLane Research); or (b) add the preservative to the sample prior to filtration. There are advantages and disadvantages to either approach; we need to work through these systematically in the laboratory to determine if it is feasible to address them in the context of a controlled experiment at MARS in 2016.

4) Complete studies of Pseudo-nitzschia species that may play a role in harmful algal bloom outbreaks in California waters (conclusion of Holly Bowers' postdoctoral work).

Holly Bowers' MBARI postdoctoral fellowship ends September 2014; she will continue her work through 2015 under the auspices of the NOAA ECOHAB grant. Summer/Fall 2014 will be used to re-design current probe(s) for detecting *Pseudo-nitzschia* on various platforms (ESP, benchtop SHA, whole cell hybridization). Field samples for validating the new probes will be collected from Monterey Bay as part of a field campaign led by Moss Landing Marine Laboratories in Fall 2014. These probes will be incorporated into the planned ECOHAB/CANON field study in Spring 2015. Uncovering diversity of *Pseudo-nitzschia* species found along the California coast will continue through continued culturing and analysis of environmental samples. We have established an informal collaboration with Dr. Nina Lundholm, a leading taxonomist on *Pseudo-nitzschia,* at the Natural History Museum of Denmark, University of Copenhagen. She has been instrumental in confirming species identification from SEM images, and we will work closely with her to identify species in our culture collection as well as those from spent ESP filters (etc.). This material will aid Dr. Lundholm's work in assessing cryptic diversity in certain *Pseudo-nitzschia* species complexes that occur globally. We have also developed a collaboration with Dr. Kate Hubbard, a *Pseudo-nitzschia* researcher with the

Florida Fish and Wildlife Conservation Commission. She has refined a genetic fingerprinting technique that uncovers diversity of *Pseudo-nitzschia* in complex environmental samples. This technique has been used to assess diversity of *Pseudo-nitzschia* species found in the Gulf of Maine, Gulf of Mexico, and Pacific Northwest. Archived samples we hold from 2013, 2014 and 2015 field studies will allow us to expand this effort and realize a larger regional understanding of *Pseudo-nitzschia* diversity. Particular attention will be given to samples collected by the Dorado/Gulper as it transected through chlorophyll patches during our ECOHAB/CANON field experiments. Supporting hydrological data will allow us to extensively characterize features in which different *Pseudo-nitzschia* species are found. Information gleaned from pure cultures will allow us to ground truth DNA fingerprinting results, and help refine the technique. As part of MLML's Fall 2014 field campaign, we aim to collect samples through any frontal zones (if present) to further assess *Pseudo-nitzschia* diversity. We will continue to work closely with Dr. Hubbard as both laboratories uncover cryptic species in order to identify/develop probe sets for detecting *Pseudo-nitzschia* species on global and/or regional scales. Finally, we will complete the SEM work on filters recovered from the D-ESP MARS mission as well as those from previous ECOHAB studies. Data from all analyses will be synthesized into the regional context of *Pseudo-nitzschia* identification from the past ~20 years (data generated from MBARI, UCSC, MLML).

Engineering/technology development

N/A – all engineering developments are captured under project # 901205 (SURF Center)

Field programs (detail ship requirements/deployments/diver requirements)

N/A – all field activities are captured primarily under the SURF Center (# 901205) and CANON (#901009) projects. However, the expendable supplies associated with non-grant funded field operations are included in this budget request; other supplies needed for lab operations are also included in external grants that make up the SURF Center.

Specific deliverables by the end of 2015

- Validated protocol for DNA and mRNA preservation and storage that support laboratory omics studies using the AC and LGC's.
- Development of sample homogenization protocol that is compatible with the LGC for downstream detection of bacterioplankton, HAB species, DA, and microbial source tracking (water quality) indicators using the 3G ESP's SPR and dPCR analytical modules.
- Submit a manuscript detailing results of the D-ESP MARS deployments
- Complete analysis of samples from the 2013-2014 MARS in situ incubation experiment; present results at a scientific meeting and prepare manuscript for publication if warranted
- Initiate a scoping study for a possible *in situ* incubation experiment at MARS in 2016

- Prepare a manuscript describing the SEM protocol for identification of *Pseudo-nitzschia* frustules from spent ESP filters (this will either be a stand-alone techniques paper or presented within the context of the overall MARS deployment paper)

- Prepare manuscripts detailing results of Holly Bowers' postdoctoral work

Budget justification

The total budget request is $131,122.

This request reflects our past experience in developing and applying molecular analytical methods. As in previous years, core supplies are those necessary for laboratory-based, exploratory studies as well as for participation in field campaigns not covered by external grants. As discussed in the proposal, significant effort will be given to investigate/validate sample processing methods that will inform 3G engineering development. We will also process and analyze samples from *in situ* microbial incubation experiments, as well as samples archived from previous field campaigns. Sequence information (NGS and Sanger Sequencing) from *in situ* microbial incubations, archived samples from field campaigns, and newly isolated *Pseudo-nitzchia* spp. will be used to characterize the communities and to develop new qPCR and SHA assays.

To accomplish these activities, and to support general lab operations, funds are needed to purchase the following broad categories of supplies:

- reagents for ESP deployments and laboratory SHA ($31K);
- materials for producing custom ESP probe arrays ($7.5K);
- DNA and RNA purification kits ($5.55K),
- reagents for sequencing prep and sequencing ($9.5K)
- reagents for incubation experiments ($4.05K)
- primers and probes for existing and new, whole cell, fluorescence, qPCR, and sandwich hybridization assays, as well as ARISA analysis on samples ($16.5K).
- phytoplankton and bacteria culture, and microscopy supplies ($5K);
- consumable plastics (pipette tips, filter flasks, filters, etc. $9.5K);
- PCR reagents ($17K);
- general use laboratory reagents ($2.1K);

We are also requesting funds for Matlab maintenance fees ($750).

As in previous years, we ask for funds to cover basic maintenance and repair of important laboratory equipment; service contracts for our array printer ($9K), dishwasher and laminar flow hood ($2K), and the StepOnePlus qPCR machine ($1.2K), as well as filter replacements for our Millipore water system ($2.8K).

In addition, we have requested funds to attend conferences to present data from previous field campaigns and report on chemistries used to inform G3 ESP development. Ussler would like to attend the general American Society of Microbiology (ASM) meeting in New Orleans, LA. Preston would

like to attend the Deep Sea Biology Symposium in Aveiro, Portugal. Marin would like to attend the US HAB meeting in Long Beach, CA. Dues, registration fees, and travel/lodging for these three meetings are estimated at $7,672.

Project team

Chris Scholin, Jim Birch, Bill Ussler, Chris Preston, Roman Marin, Holly Bowers (Holly is supported through external grants).

Project lab space

Scholin probe chemistry lab (A-249); ESP technology transfer project lab (B-125); 3G ESP development lab (A-244; shared project lab space).

Data availability outside project team, uniqueness

Results of this work are primarily made available through peer-reviewed publications and through technology transfer/collaborations.

Export Regulation
Does the project team anticipate any foreign collaborations or international travel?

We continue collaborating with Theirry Baussant of IRIS (International Research Institute of Stavangar) in Norway. In 2015 we will be visiting IRIS with an ESP for laboratory testing of target sequences at/near the site of a possible future deployment. International travel will result on account of these activities, but as far as we are aware, there is nothing of a nature subject to ITAR or EAR restrictions.

We also anticipate travel to Ireland for a project team meeting related to the NSF EAGER grant.

Does the project team plan or anticipate export controlled purchases, shipments, or transfers of items (e.g., research instruments, proprietary data, hand-carried tools or samples) outside the U.S. temporarily or permanently?

One 2G ESP and accompanying support materials will be shipped to Norway in Fall, 2015.

Monterey Bay National Marine Sanctuary SESAs (General map)
Projects will need to indicate whether or not work will be conducted within one of the Monterey Bay National Marine Sanctuary's Ecologically Significant Areas (SESAs).

No work in 2015 will be conducted within a SESA as defined as seafloor; we may operate in water columns above a SESA (TBD).