ABSTRACT

1. Research Category (ECOHAB: Ecology of Harmful Algal Blooms); Funding Opportunity Number: EPA-G2006-STAR-B1
2. Title: Engineering Upgrades and Field Trials of the Autonomous Microbial Genosensor
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4. Institution: College of Marine Science, University of South Florida, 140 Seventh Ave. S., St. Petersburg, FL 33701; Center for Ocean Technology, University of South Florida, 140 Seventh Ave. S., St. Petersburg, FL 33701
5. Project Period: 9/1/06-8/31/09
6. Project Costs:
7. Project Summary

Harmful algal blooms can be major catastrophes in terms of economic losses, aquatic organism mortalities, and deleterious impacts on human health. To predict onset of harmful algal blooms, monitor their severity, and to accurately determine their termination, rapid, reliable, and accurate methods are needed to detect HAB species. A major goal is to incorporate rapid and accurate detection methods into ocean observing systems. We have used the ribulose-1,5-biphosphate carboxylase/oxygenase large subunit gene (*rbcL*) as a molecular tag to detect *K. brevis* in a prior ECOHAB-funded project. We developed an assay that uses the novel Nucleic Acid Sequence-Based Amplification (NASBA) and molecular beacon technology. NASBA amplification, which is isothermal, is more amenable to field assays and autonomous platforms than PCR, which requires thermal cycling. With prior funding from ONR and NSF, we have incorporated our NASBA-based detection technology into the Autonomous Microbial Genosensor (AMG), the first sensor buoy to perform nucleic acid amplification to detect harmful algae. Based upon our experience with this system we would now like to improve the AMG with several engineering upgrades and embark on a series of field deployments to fully test this system. Our objectives are to: ***1. To upgrade the current AMG to a dual channel detection system and other improvements 2. To reduce overall system size and weight by optimizing packaging of the fluidic management system and pressure vessel 3. To build a second AMG unit 4. To determine performance of both units through a series of field deployments***. For Objective 1, we will install a second fluorescence channel in the AMG to enable detection of an internal control for quantitation and determination of performance. Alternatively, the second channel can enable detection of a second target species or a different gene (ie. a *K. brevis* PKS gene). Objective 2 aims to decrease the overall size and weight of the AMG to facilitate easy deployment. Construction of a second AMG (Objective 3) will enable simultaneous deployment and data collection from two sites, which is the main goal of Objective 4. We will manually sample during operation modes of the AMG during field deployments to ensure proper performance, and simultaneous samples will be microscopically counted for *K. brevis.* The outcome of this research will be an autonomous RNA amplification platform capable of detecting and providing quantitative information on *K. brevis* populations in near real time. The system will be targeted toward *Karenia brevis* but with simple modification should be able to target any HAB species. This proposal coincides with the NOAA agency interests described in the RFP: “Development of new methods for measuring HAB cells and toxins, especially those

that can be used in observing systems or provide enhanced monitoring capability are especially encouraged".

8. Supplemental Keywords: Biological buoys