1. Research category and Sorting Code: ECOHAB and EPA-G2006-STAR-B1

2. Title: ECOHAB: Intraspecific Variation in a Toxin-producing Dinoflagellate

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5. Project Period: 1 July 2006 to 30 June 2009

6. Project Cost: $485,977

7. Project Summary

Toxic dinoflagellates of the genus *Karenia* are a serious economic and public health concern worldwide. The major HAB species in the Gulf of Mexico is *Karenia brevis,* a dinoflagellate that produces a suite of potent neurotoxins (brevetoxins) that can cause fish kills, shellfish toxicity, and respiratory distress in humans. Cell counts alone are not a good predictor of potential toxicity of HABs because the quantity of toxin can vary with species composition, stage of growth, and/or environmental conditions. There also is evidence that variation in cellular toxin content and toxin profiles exist among clones of *K. brevis.* Factors influencing production of brevenal, the naturally occurring antagonist for brevetoxins, among clones of *K. brevis* also are unknown. A more detailed understanding of both genetic diversity and intraspecific toxin composition within and among blooms is needed so that the dynamics and potential potency of toxic dinoflagellate populations can be linked to environmental heterogeneity and change. **Objectives** of this project are as follows: (1) Establish clonal cultures of *K. brevis* isolated during the onset, bloom, and decline of a *Karenia* bloom in order to assess genetic and physiological variability within a bloom population; (2) Determine environmental conditions under which *K. brevis* cells attain maximal potential toxicity by examining variation of toxin content and toxin profiles among clones and how toxin profiles may be altered by perturbations in the environment; (3) Establish indicators/markers linking genetic profiles and intraspecific variation in toxin production in order to predict potency of a bloom. **Approach:** Conduct field sampling in conjunction with the ongoing monitoring program for *Karenia* at the Fish and Wildlife Research Institute (FWRI) in St. Petersburg, Florida. A suite of nuclear-encoded microsatellite markers developed from a *K. brevis* genomic library will be employed as tools to characterize genetic composition of bloom populations. For each clonal isolate established during the course of a bloom event, allele and genotype distributions at 10 microsatellites will form the basis for tests of spatial and temporal (genetic) homogeneity. Bench-scale studies will be performed to evaluate differences in toxin profiles among clones when grown under identical conditions. Experiments with selected clones acclimated to a range of salinities and nutrients in semi-continuous growth and with cultures subjected to rapid changes in growth conditions will be conducted to evaluate effects of environmental conditions on toxin profiles and quantity of brevetoxins and brevenal produced. Data analysis primarily will include tests of spatial and temporal homogeneity (including molecular analysis of variance or AMOVA) of allele (haplotype) and genotype distributions (frequencies). Estimates of haplotype diversity and intrapopulational nucleotide diversity also will be generated. Neighbor-joining topologies of genetic-distance matrices will be used as a means to assess genetic and evolutionary relationships among spatial/temporal samples and to link diversity and structure of isolates of *K. brevis* with the intraspecific variation in toxin production. **Expected Results:** This study will provide critical and much needed information on the variation in toxin composition and production among *K brevis* clones and over the course of a *Karenia* bloom. The database of dinoflagellate microsatellite alleles for the Gulf will be expanded and the extent of diversity in toxin profiles together with genetic profiles will allow development of realistic predictive models. Linking allelic profiles and toxicity will allow prediction of the response of HAB populations to changes in environmental factors. Ultimately, this will result in the capability to predict how environmental factors influence toxicity or potency of a *Karenia* bloom.

Keywords: *marine, ecological effects, genetic polymorphism, genetics, toxin, indicator*