Title: PCMHAB: Implementing the *Karenia* "tricorder" to Improve Red Tide Monitoring and Management in the Gulf of Mexico
Institution: University of South Florida and Florida Fish and Wildlife Conservation Commission
Investigators: John Paul (Lead PI), Katherine Hubbard (Co-PI)
Total proposed budget: $785,026
Budget Period: September 1, 2015 – August 31, 2018

Project Summary

The marine dinoflagellate, *Karenia brevis*, blooms annually in the Gulf of Mexico (GOM) and negatively impacts human and ecosystem health through production of a suite of toxins known as brevetoxins. Blooms of *K. brevis* cause widespread fish kills, and negatively impact human health when toxins become aerosolized along beaches, resulting in respiratory irritation. Brevetoxins also concentrate in shellfish during *K. brevis* blooms, resulting in Neurotoxic Shellfish Poisoning if consumed. Rapid, specific, and accurate quantification of *K. brevis* is needed to monitor waters in shellfish harvesting areas (SHAs) for certain cell thresholds, and to allow more timely warning of bloom conditions in coastal areas. Currently, samples are collected by an extensive phytoplankton monitoring network consisting of volunteers as well as local, county, and state partners. Cells of *K. brevis* are enumerated by phytoplankton analysts, in either fixed or live samples, using light microscopy. Expertise is required to discriminate *K. brevis* from non-toxic but morphologically similar taxa, and samples are processed individually. Samples are not easily enumerated at sea or in most field locations and are shipped to shore-based labs, causing delays in the public access to critical bloom information.

A rapid, sensitive, and specific assay for the detection of *K. brevis* based on nucleic acid amplification technology has been developed and successfully adapted for use with the QuadPyre, a handheld sensor that detects isothermal amplification of nucleic acids using thermoregulated fluorometry. The Overarching Goal of the proposed research is to **develop, demonstrate, and transfer hand-held genetic sensors for *K. brevis* detection to end users that monitor the coastal and estuarine waters of the Gulf of Mexico.** Accordingly, the proprosed PCMHAB research is broken into three specific phases: I-Enhancement of hand-held genetic sensors for *K. brevis* detection through research and development; II-Demonstration and validation of *K. brevis* sensors in field and lab trials; and III- Transfer of technology to end-users and integration of genetic data into HAB observing networks. To advance the utility of the assay, **Phase I** of the project has the specific aims: 1) Simplify the extraction and analysis of high quality RNA from a variety of samples to allow field-based detection and quantification of *K. brevis* cells; 2) Install a second fluorescence channel on the QuadPyre to allow the addition of an internal control (*K. brevis* calibrator molecule) for improved quantification and detection of inhibitors; and 3) Increase utility of QuadPyre through software developments. To demonstrate and validate the assay, **Phase II** aims to: 1) Train end users, primarily state and county monitoring agencies, in the use of QuadPyre; 2) Integrate the QuadPyre into pilot monitoring projects to allow quantitative validation with cell counts conducted by state monitoring agencies. The final phase of the project will enable the transfer of data and technology to end-users, including coastal managers and regional observing networks. Specific aims for **Phase III** include: 1) Provision of technology and/or synthesis of genetic data to end user groups and 2) Integration of genetic data into existing HAB reporting structures in the Gulf of Mexico (e.g. FWC HAB Monitoring; NOAA and GCOOS observing networks).