Implementing the *Karenia* "tricorder" to Improve Red Tide Monitoring and Management in the Gulf of Mexico

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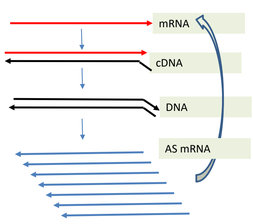
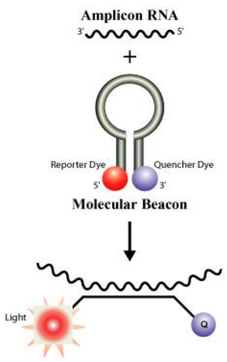
Quantifying *Karenia brevis* with genetic sensors

Red tides in Florida coastal waters (principally *Karenia brevis* blooms) can threaten human health and cause millions of dollars lost in tourism, agriculture, seafood, and leisure industries. Currently, state detection and enumeration of *K. brevis* employs light microscopy to differentiate this toxic alga from closely related non-toxic and less toxic species (in 100-150 samples weekly), and shellfish harvest areas are closed once concentrations reach or exceed 5000 cells L^{-1}. The overarching goal of our research is to develop, demonstrate, and transfer hand-held genetic sensors for timely, on-site *K. brevis* detection to end users, including citizens that monitor the coastal and estuarine waters of the Gulf of Mexico. Given the system performance specification—to detect and quantify *K. brevis* across at least three orders of magnitude with a detection limit of < 10 cells—we first evaluated a NASBA assay specific to *K. brevis* on three low-cost genetic platforms (USF QuadPyre, the Douglass Scientific AmpliFire™ and the Biomeme two[3]), and chose the AmpliFire™ based upon reproducibility and durability. Lab trials also indicated that cells, RNA, and DNA standards corresponded well, with the latter being the most stable and therefore amenable to inclusion in field kits. The lab validations informed several field trials in Southwest Florida that targeted different stages of a *K. brevis* bloom in 2016, as well as a 3-month study of *K. brevis* abundance in Bayboro Harbor, St. Petersburg, FL. These field validations of *K. brevis* sensors indicated a high fidelity between microscope counts and those determined via the handheld sensors (R-squared = 0.96), and provided valuable opportunities for training end-users. As the technology is further transferred and integrated within HAB observing networks, data from the handheld sensors will be uploaded to GCOOS for automated processing and calculation of *K. brevis* cell abundance, as well as data visualization, for end-users (FDEP, FDACS, FWC).

FIND Technology: FLUORESCENT ISOTHERMAL NASBA DETECTION

NASBA Simplified





NASBA is a nucleic acid amplification protocol that targets gene-Specific RNA using 3 enzymes at 41ºC (isothermal amplification).
Target specific RNA is detected by molecular beacons, fluorescent oligonucleotides that bind only to target RNA sequences

Evaluating an array of "tricorder" instruments resulted in the selection of the AmpliFire platform

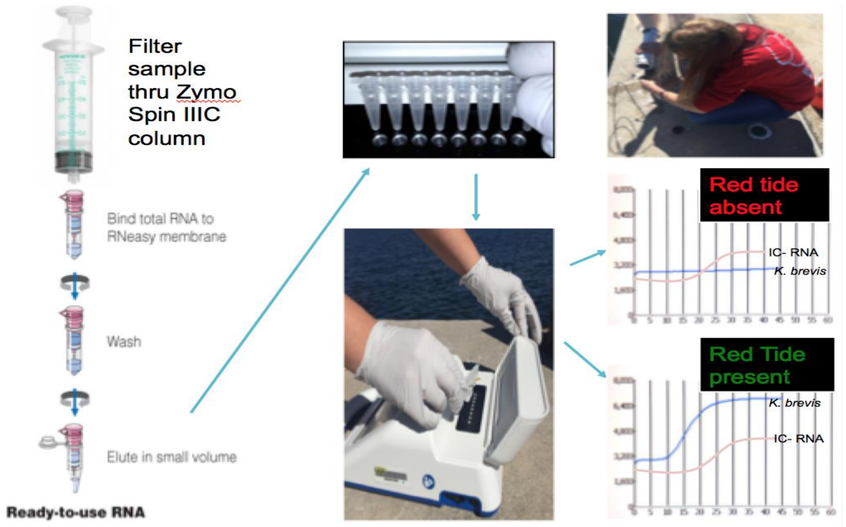
"It's Life, Jim, but not as we know it…"

Hand-held genetic analyzers (AKA "Tricorders")

EVALUATION OF HANDHELD GENETIC SENSORS

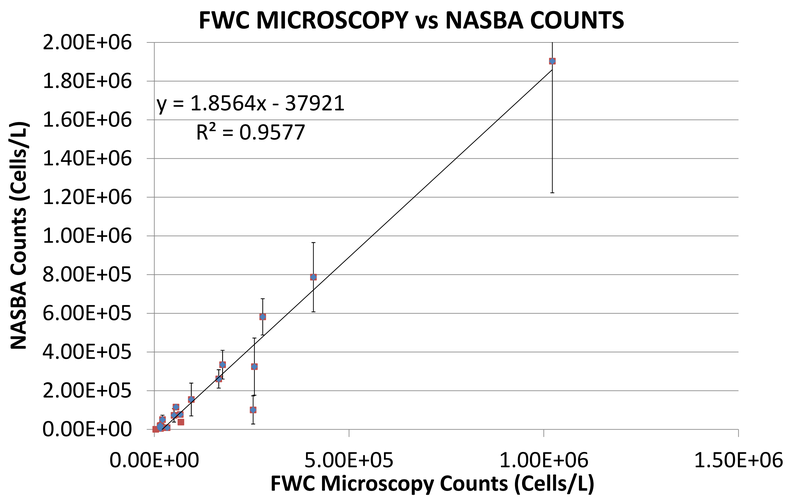
Name	Manu-facturer	No. of Samples	Color Channels	ISO or PCR	Data Security	Precision	Est. Cost	Availability
A. Quadpyre	USF	4	1	ISO	External Laptop	Good	$2,500.00	No
B. Tricorder	Starfleet Command	1	1	???	Shipboard Computer	N/A	N/A	Distant Future
C. AmpliFire	Douglass Scientific	8	3	ISO	High	Excellent	$8,995.00	2 Weeks
D. Two-3	Biomeme	3	2	ISO or PCR	All data goes to Biomeme	Poor	$4,500.00	Unknown

In situ processing delivers near real-time results



Phytoplankton cells are collected and 2.5mL to 25mL of sample is filtered onto nucleic acid purification columns using a simple field manifold with a hand pump; RNA is purified in the field and NASBA is performed using the AmpliFire.

In situ analysis shows a close correlation between light microscopy and NASBA enumerations

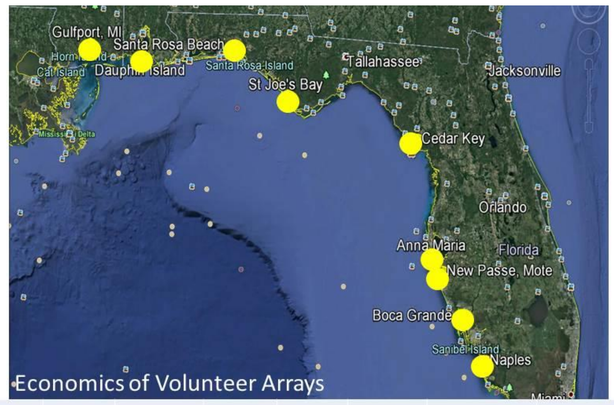


Nineteen samples from Tampa Bay environments were analyzed from 2016-2017 by FWC microscopy counts and NASBA counts. A relatively tight correlation (R^2=0.96) indicated that NASBA could be an acceptable replacement for microscopy counts.

Volunteers are trained in NASBA workshops

Image provided by J. Wickham

Putative nodes for a volunteer network array in Florida



	Hardware	Deployment	Total	Cost for 10 units	NASBA assay	1000 assays	Total
Amplifire	$8,995	$50	$9,045	$90,450	$25	$25,000	$115,450

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