Identifying Toxigenic Algae Using RNA-Based Molecular Technologies

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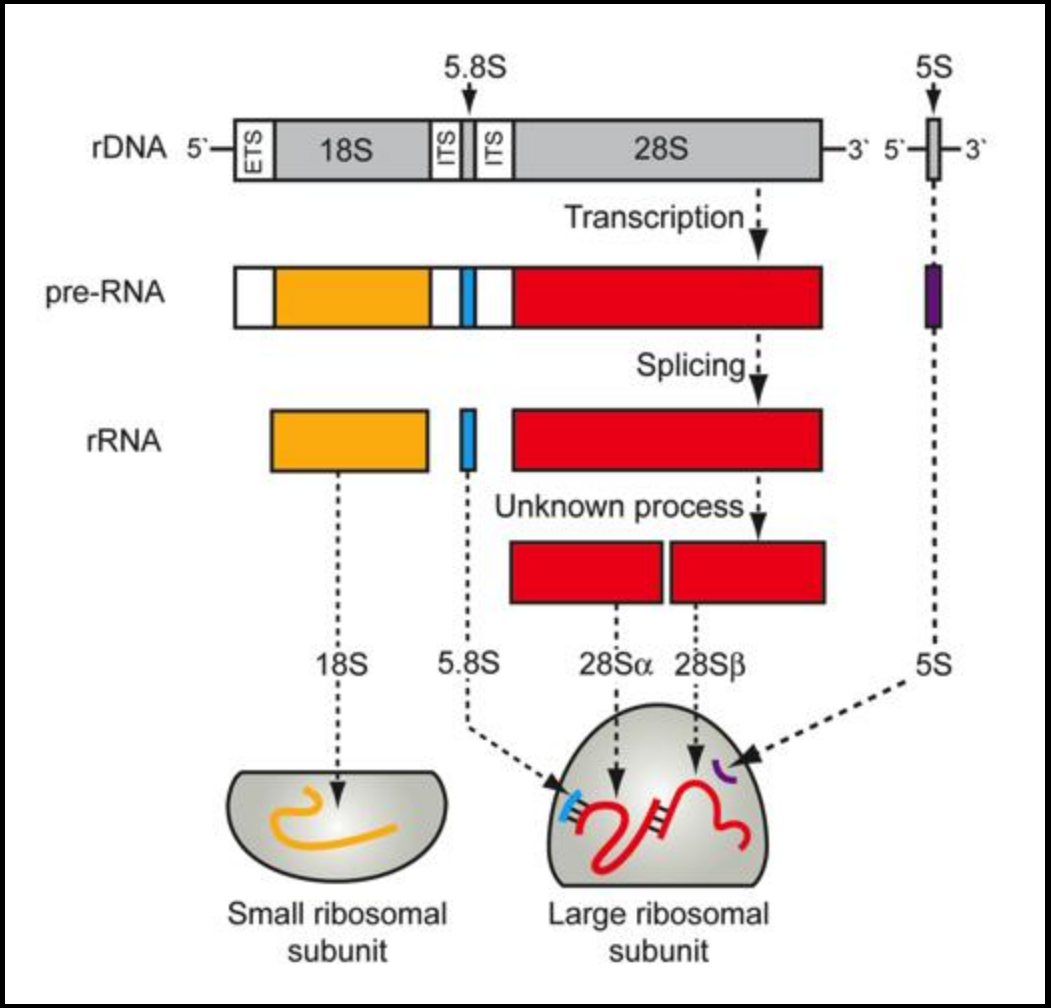
Advantages of Quantitative Molecular Technologies

- Microscopy is time-consuming and many species look alike
- Molecular approaches often faster, enable species or gene-specific ID and quantification, ‘early warnings’
- Examples: DNA - polymerase chain reaction (PCR), rRNA - sandwich hybridization assay (SHA) for species, protein-based (ELISAs and others) for toxin

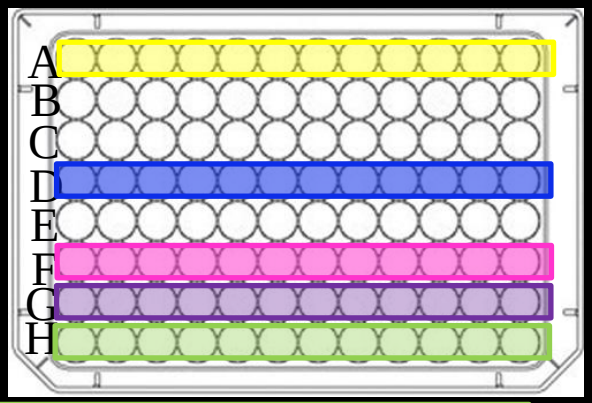
Why rRNA?

- High numbers in cell
- Species-specific sequences
- Characterizes live organisms
- Transcribed as single operon

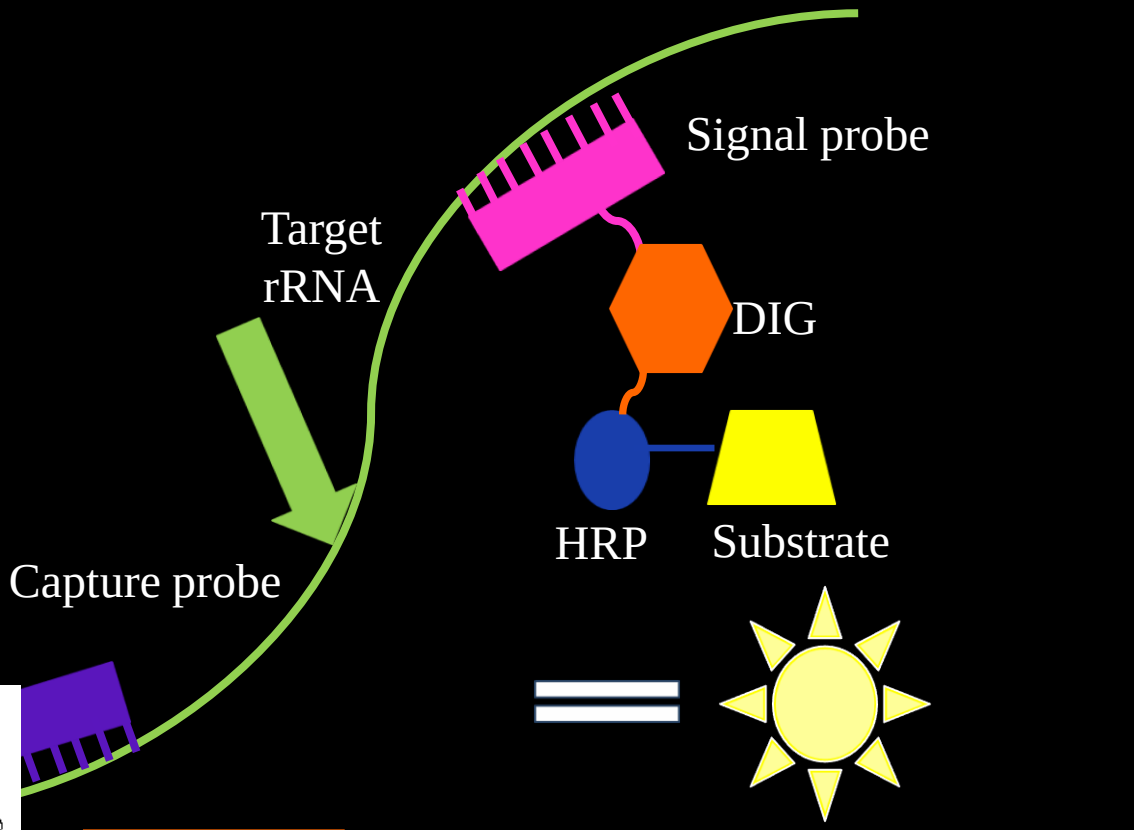


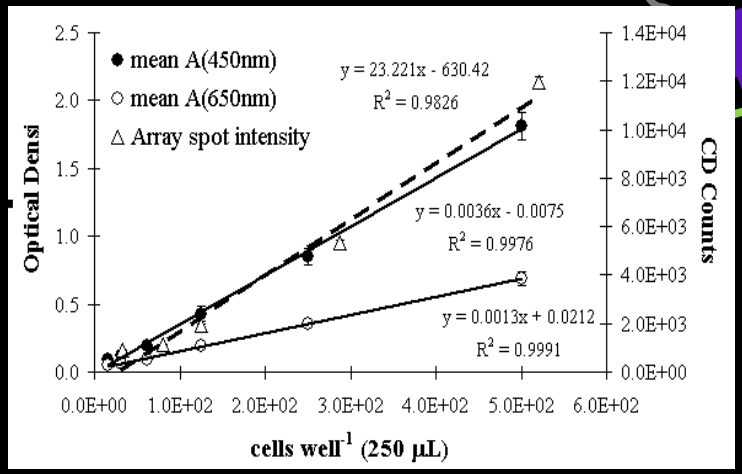
(Winnebeck et al. 2010)

Sandwich Hybridization Assay (SHA)



>1 Species together (PN, Alex, etc.)





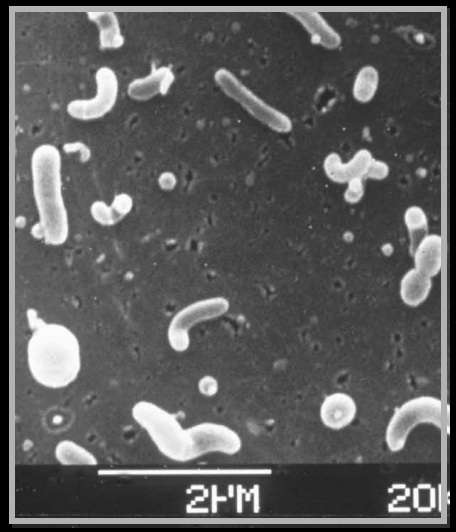
Advantages

Rapid (~1 hr); multiplex (up to 12 rxns); species or group-specific IDs; cost-effective

Scholin et al. 1999; Goffredi et al. 2005; Greenfield et al. 2006, 2008; Doll et al. 2014, Main et al. 2014, 2018

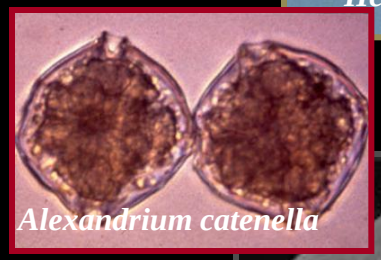
Examples of Target Organisms

Marine Microbes

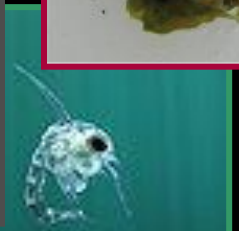


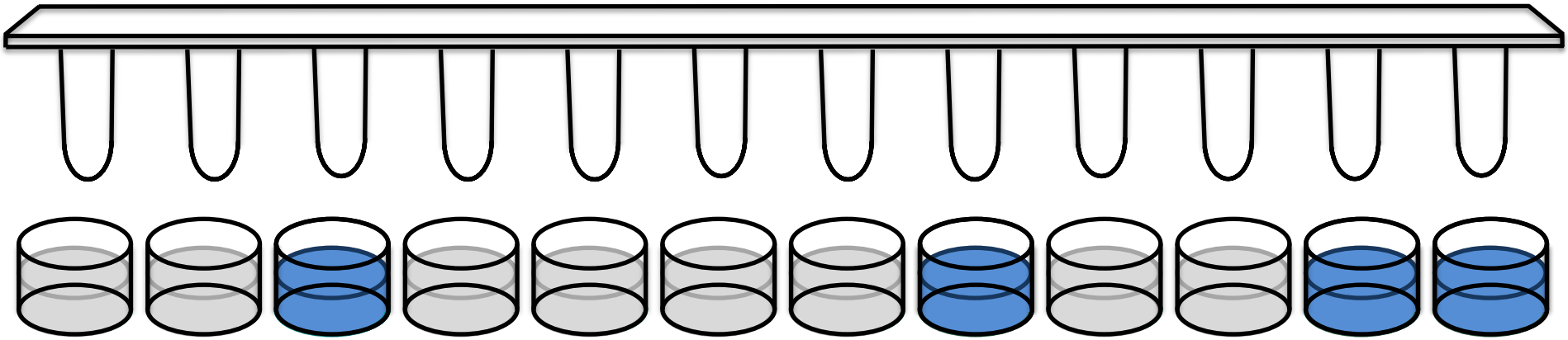
Roseobacter
Cytophaga
SAR86
Pelagibacter
Picophytoplankton
Marine Group I/II Archaea
Marine Delta
OM60/KTC1119
S-oxidizing symbionts

Harmful Algae



Invertebrate Larvae

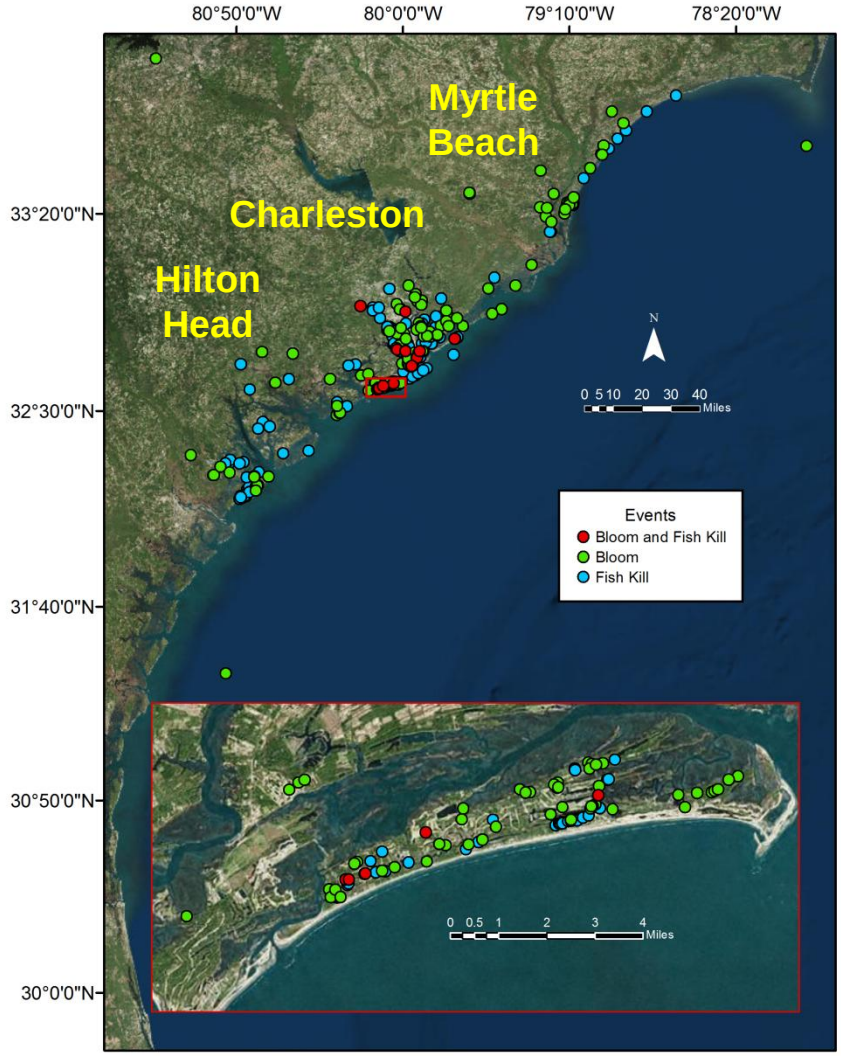




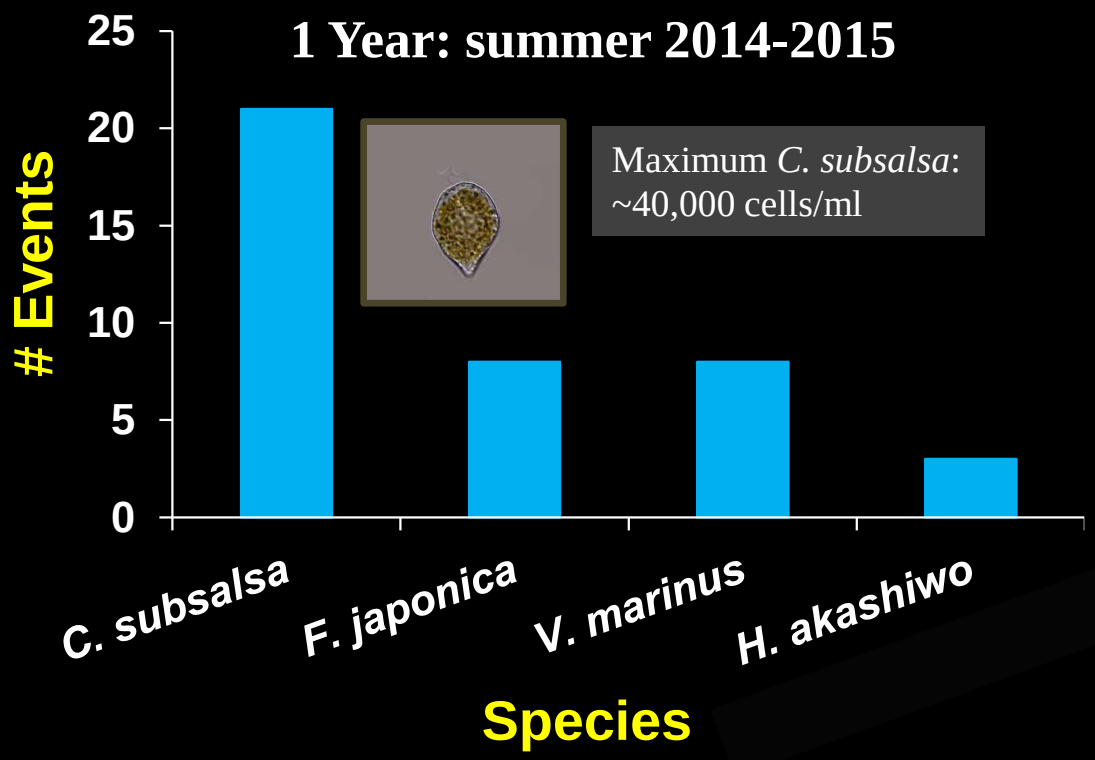

Affirm
PROCESSOR
MicroProbe
CORPORATION
ON/OFF
Process Completed
RUN
HELP
30.0
30.0
OMEGA

Ideal for Detecting Multiple HABs

- Example: Coastal SC
- **1,300+** events since 2001
  - ~430 FKs, **1 in 4 HAB-related**
  - Raphidophytes & cyanobacteria are most HABs
- Primarily urban regions



Multiple Causative HABs



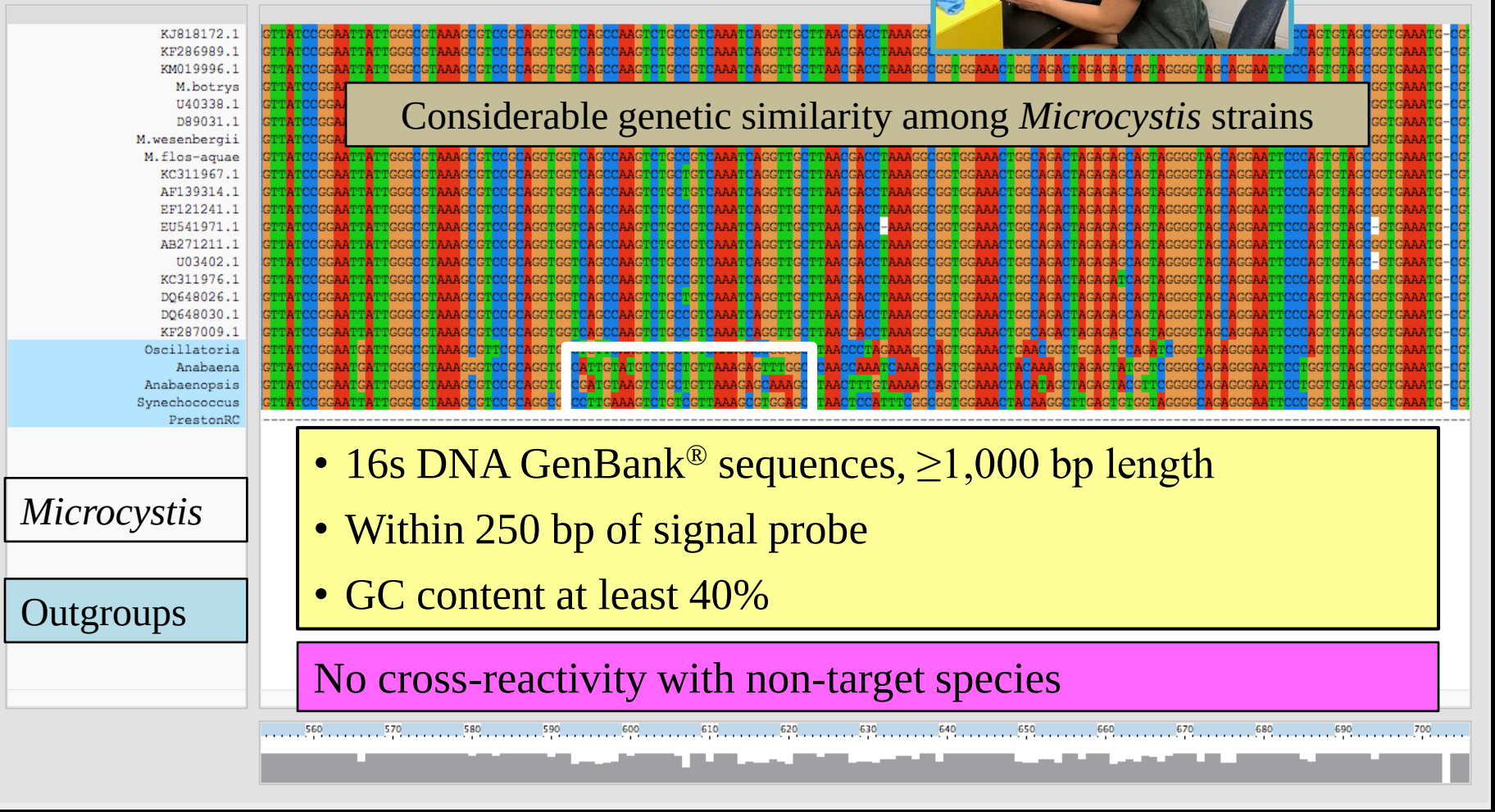
- Most common bloom and fish kill species = ***Chattonella subsalsa*** (~30%)
- Raphidophytes ~41% combined bloom + FK
- Next = cyanobacteria (~55% blooms)
- Remainder = *Pseudo-nitzschia*, dinoflagellates, euglenas, others

SHA applications developed for many of those species

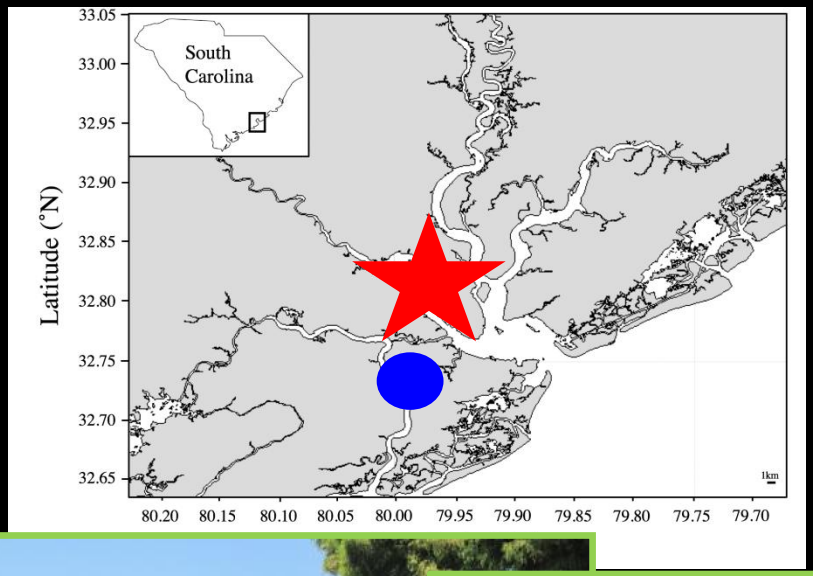
New SHA for *Microcystis* spp.

Cyanobacteria: largest #HABs worldwide; *Microcystis* is the most common genus. Enhances early warnings for blooms to safeguard public health, prediction, and management

Capture Probe Design

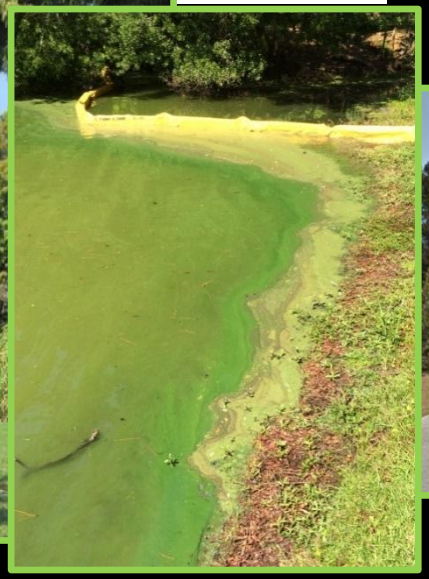


Field Sampling

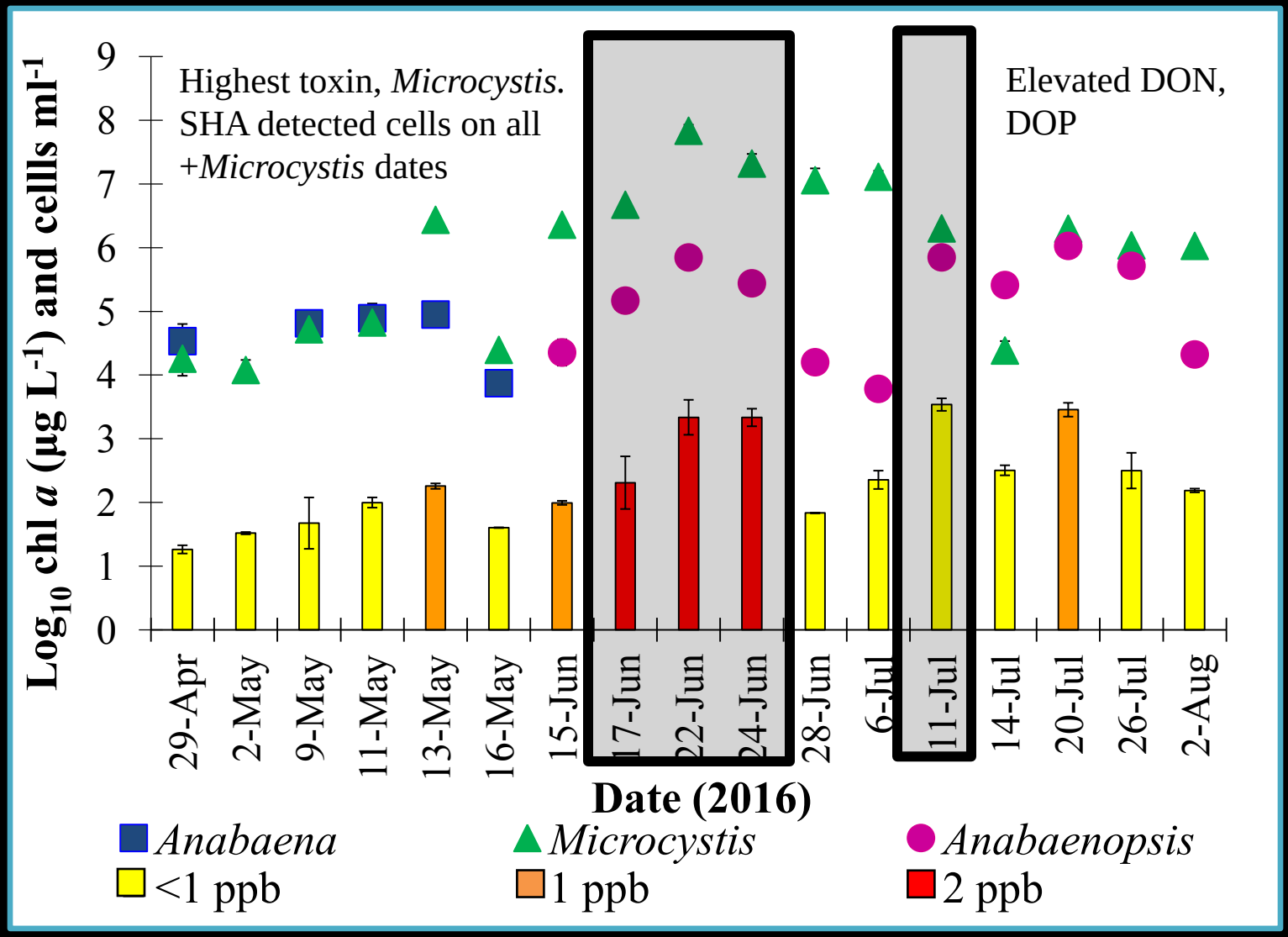


- Southeast among the most rapidly growing regions
- >21,000 stormwater ponds
- Shallow, high residence times, stagnate, accumulate nutrients

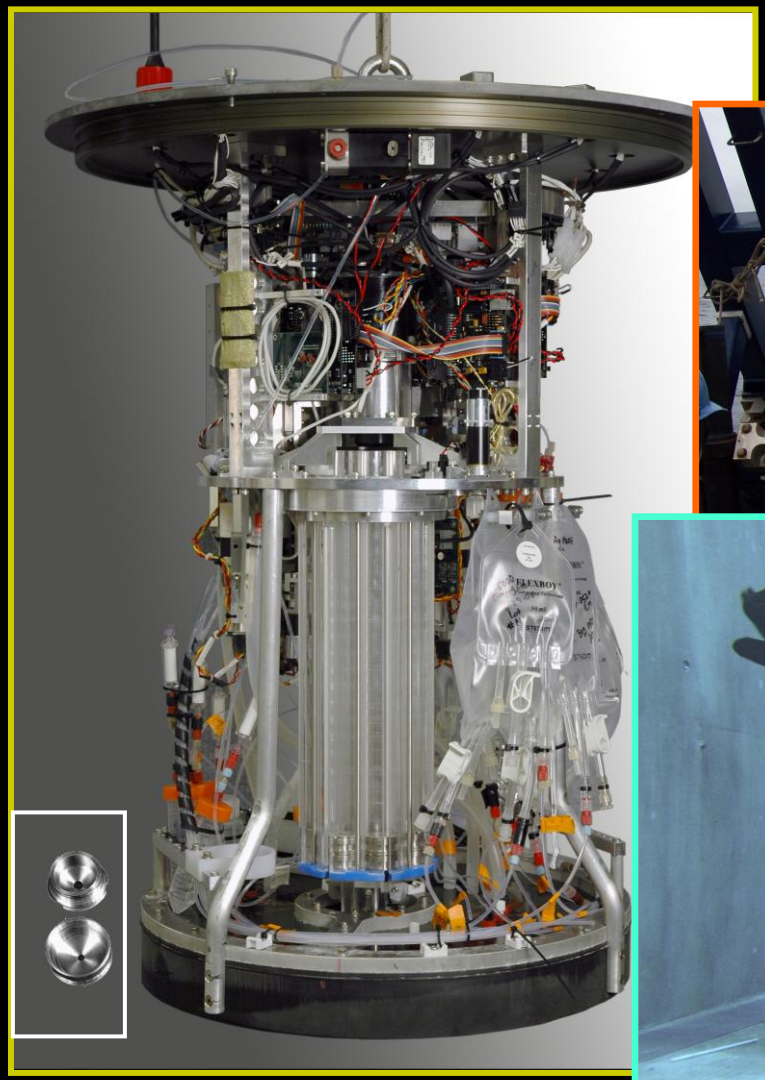
Numerous HABs and fish kills, high likelihood of public contact.
55% of these HABs are cyanos!

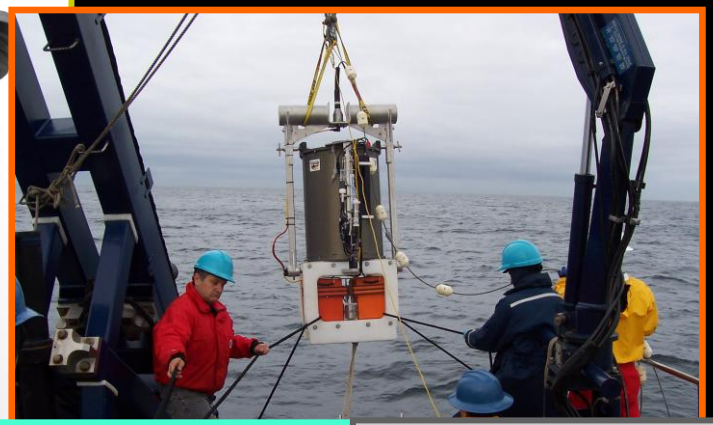


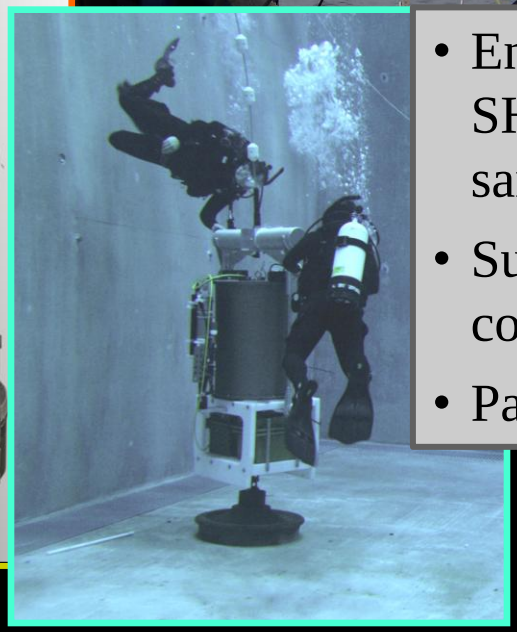
Field Sampling



Upscaling Temporal Resolution: Environmental Sample Processor (ESP)





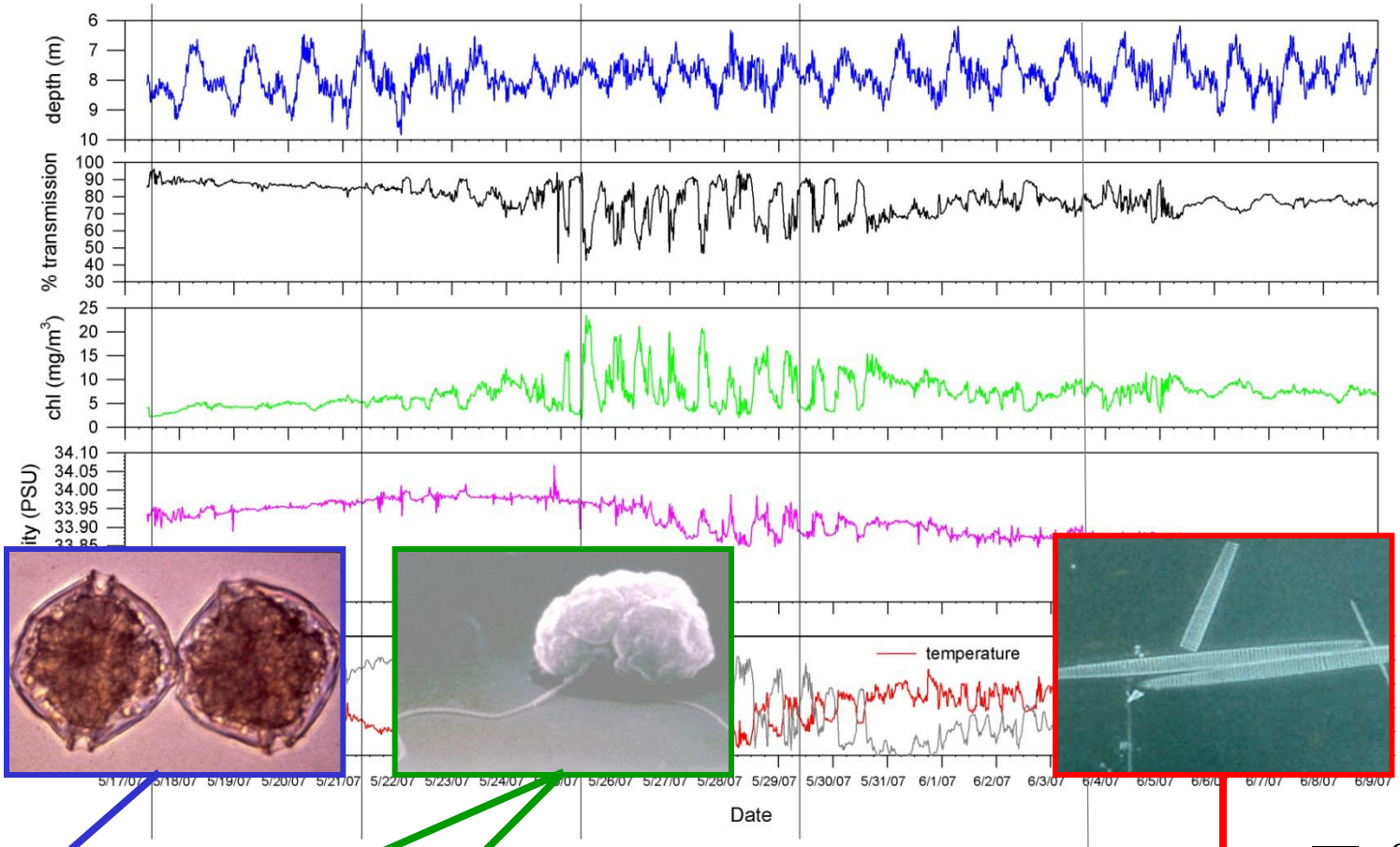


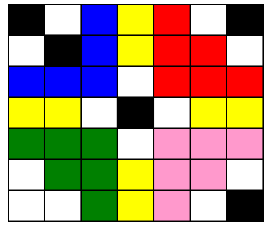
- Enables near-real time *in situ* SHA and protein microarrays, sample archival, qPCR
- Surface, mid- and 4K depth configurations, AUV
- Partner with other sensors

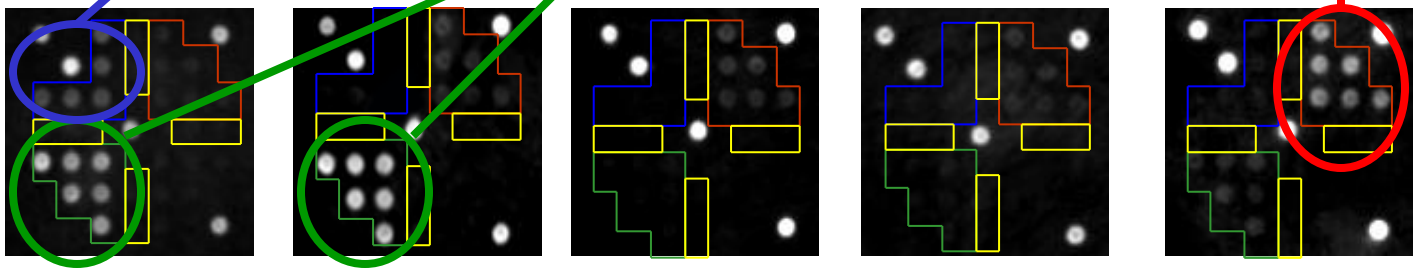
ESP Field Deployment

Monterey Bay, CA
May 17-June 11, 2007

In situ Detection of Harmful Algae







May 17 | May 21 | May 25 | May 29 | June 4

1L Sample Volume

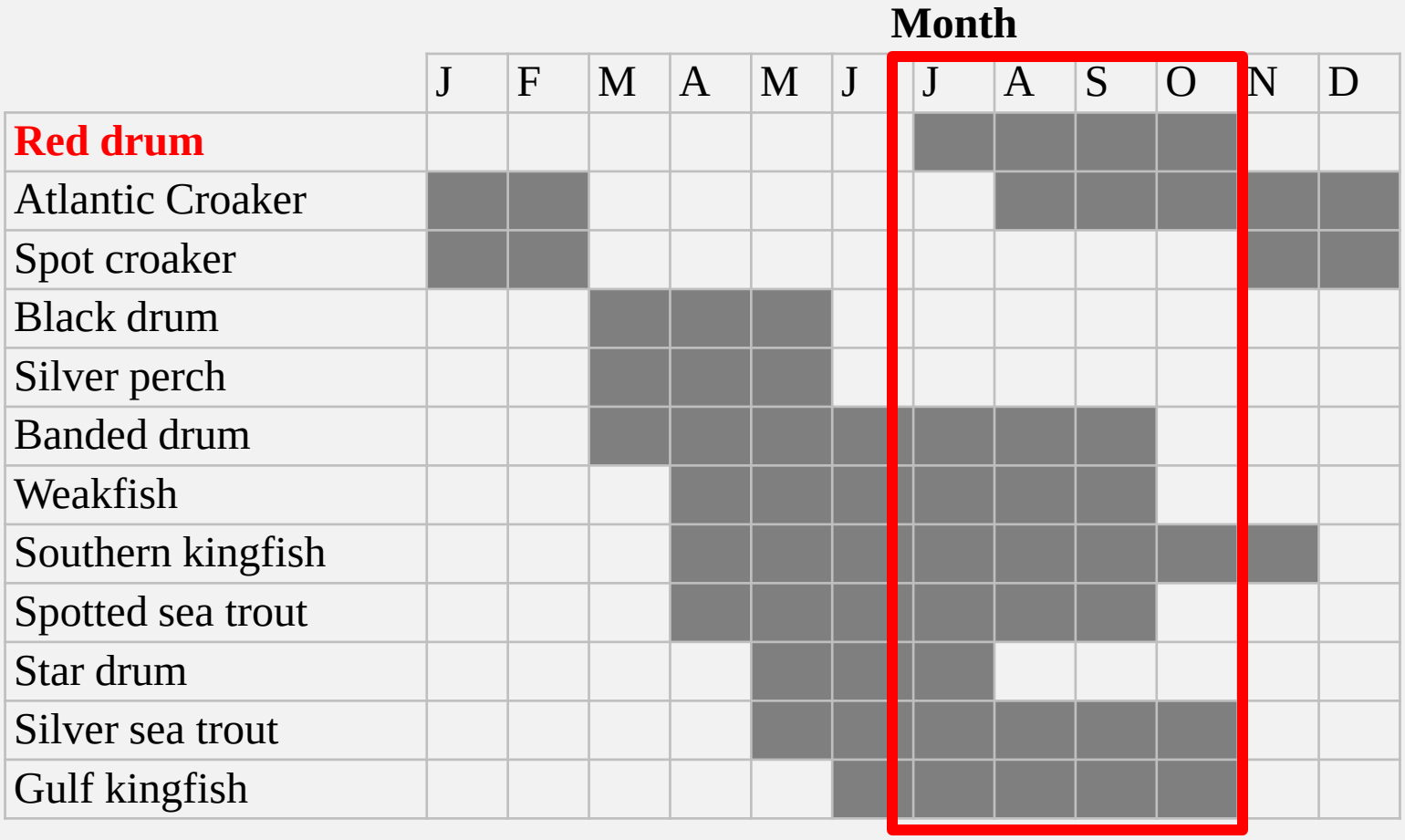
- control
- *Alexandrium tamarense/catenella*
- *Pseudo-nitzschia multiseries*
- *P. pseudodelicatissima/ multiseries*
- *Heterosigma akashiwo*
- *P. australis*

Greenfield et al. 2006, 2008 *L&O: Methods*

Adaptable for non-HAB taxa

?

Sciaenid Spawning in SC Rivers and Estuaries



SHA and qPCR

Similarities:

- Concentrating a sample
- Lysing cell membranes
- Using DNA probes to identify sequences
- Quantification of genetic material

Differences:

	SHA	**qPCR**
NA Extraction?	No	Yes
Detection mode	Direct	Amplified product
Genetic target	Large subunit rRNA	DNA
Quantification	Absorbance	Fluorescence emission

MERHAB: Methods 'Bake Off'

Study organism:
Heterosigma akashiwo

- Globally-distributed euryhaline HAB: causes fish kills and declining water quality
- Validated SHA and qPCR methods
- Low global diversity in non-chloroplast genome

- Sedgewick rafter as "gold standard" (Godhe et al. 2007)
- 9 counts per sample collection

- Multiple filters with specified cell number
- Flash-frozen (N_2)

- Add lysis buffer
- Heat, combine lysate, filter

Same homogenate: qPCR and SHA (96-well plate)

Calibration and Preservation

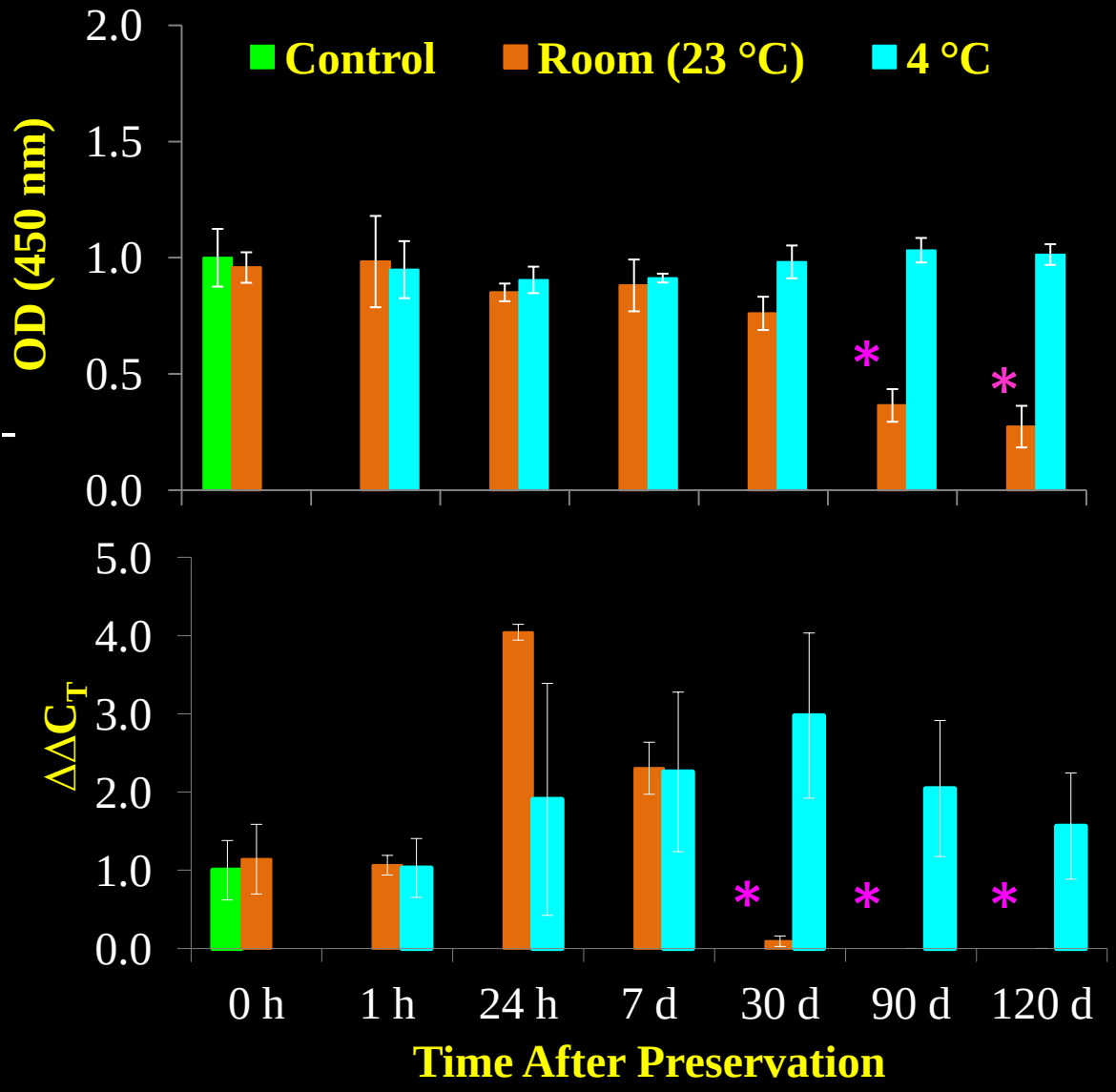
Calibration

- Geographically distinct *H. akashiwo* strains exhibited variability, but it was minor

- SHA and qPCR were nearly identical; SHA had higher pre-bloom sensitivity, qPCR had wider range (pre-dilution)

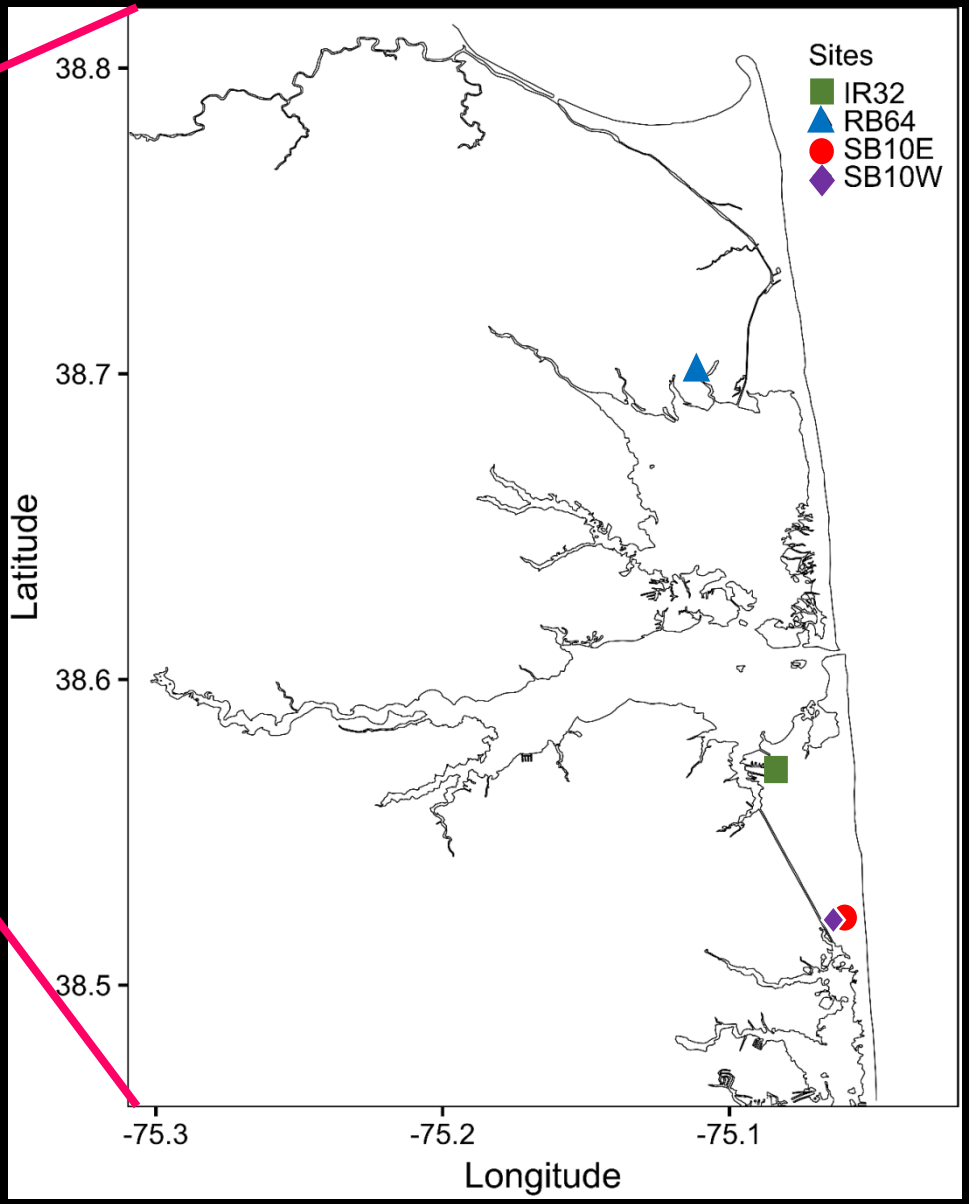
Preservation

- T and assay type influenced quantification



Doll et al. 2014, L&O Methods

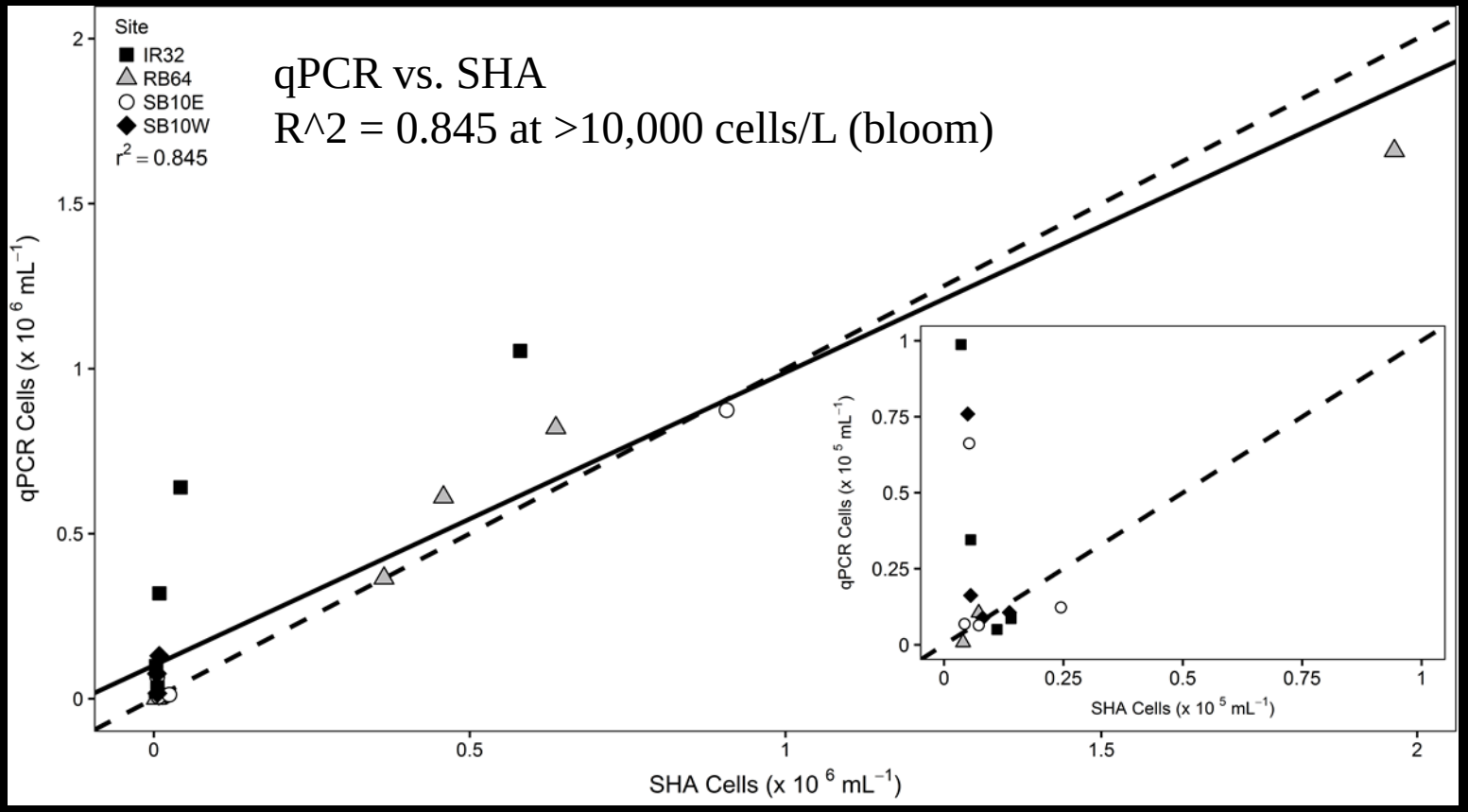
Bloom Assessment and Prediction



- Delaware Inland Bays (DIBs), May-Aug 2015
- Max: 3 x 10^6 cells/L, 18-May; 1 x 10^6 cells/L 8-June; both Rehoboth Bay (RB64)

Main et al. 2018, J. Applied Phycology

Bloom Assessment and Prediction

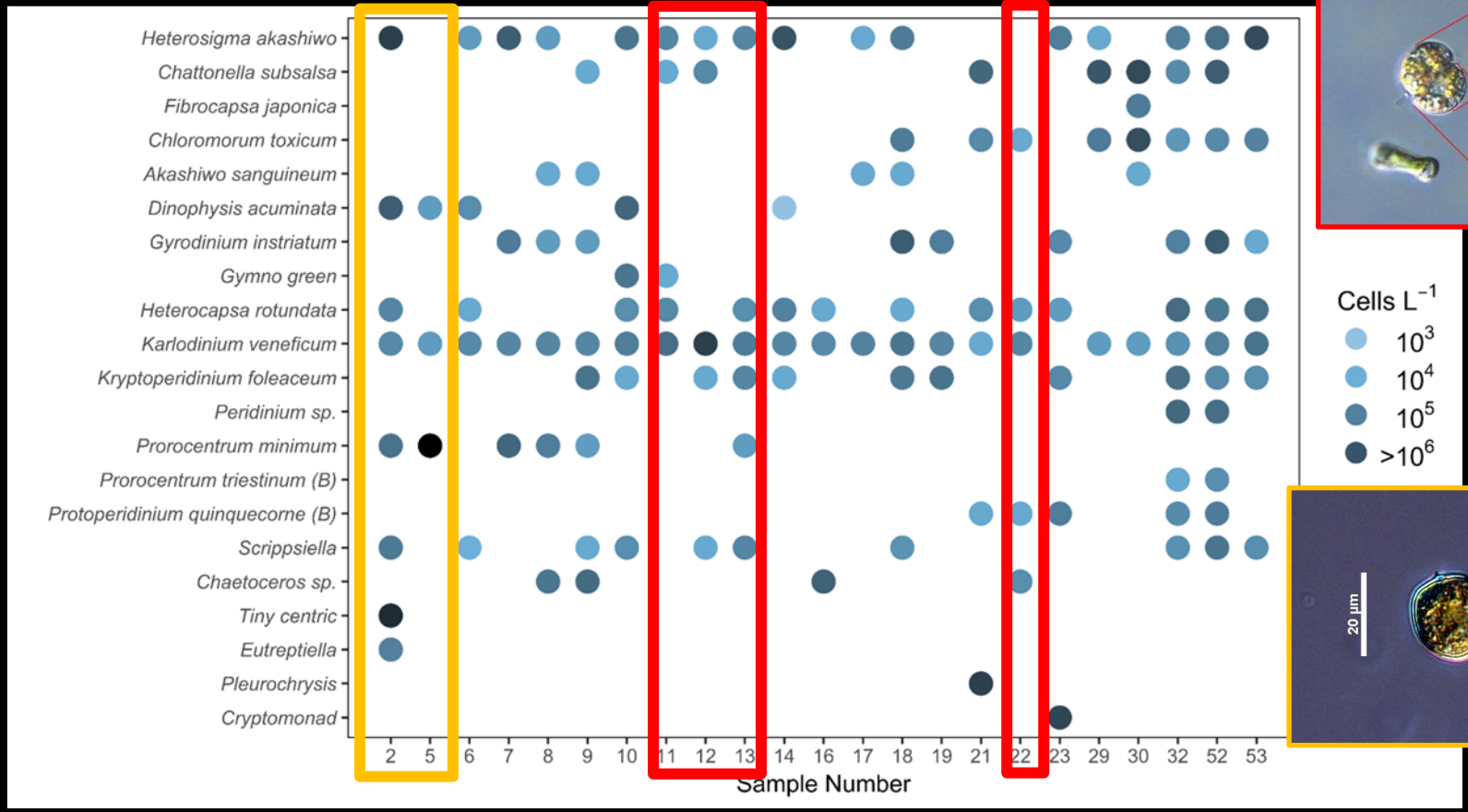


Great agreement at bloom concentrations – but…below and unlike lab findings – qPCR ***overestimated*** *H. akashiwo*. **WHY?**

Main et al. 2018, J. Applied Phycology

Nutrients and co-occurring phytoplankton

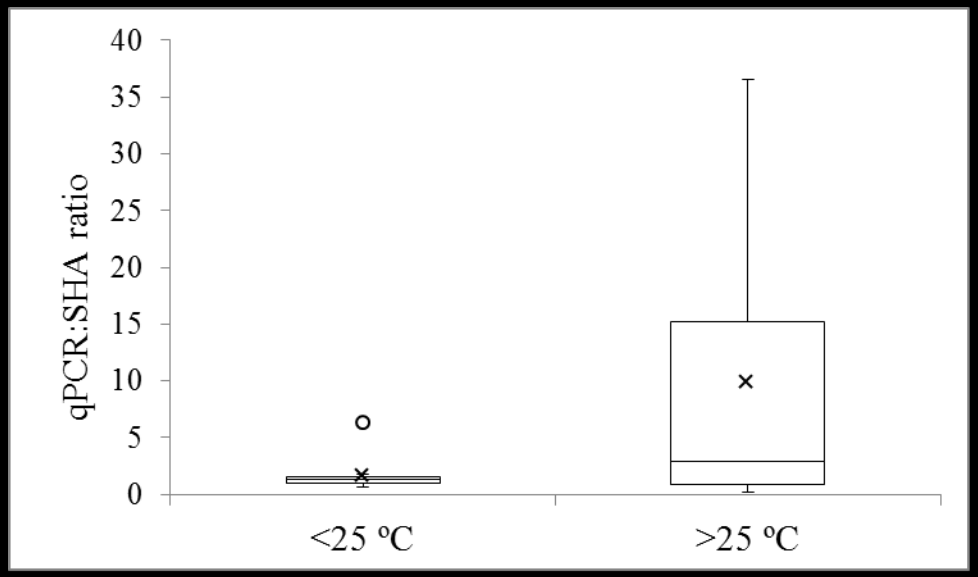
H. akashiwo abundances had no real pattern associated with N-form but positively and significantly ($p < 0.01$-0.001) correlated with Si and P

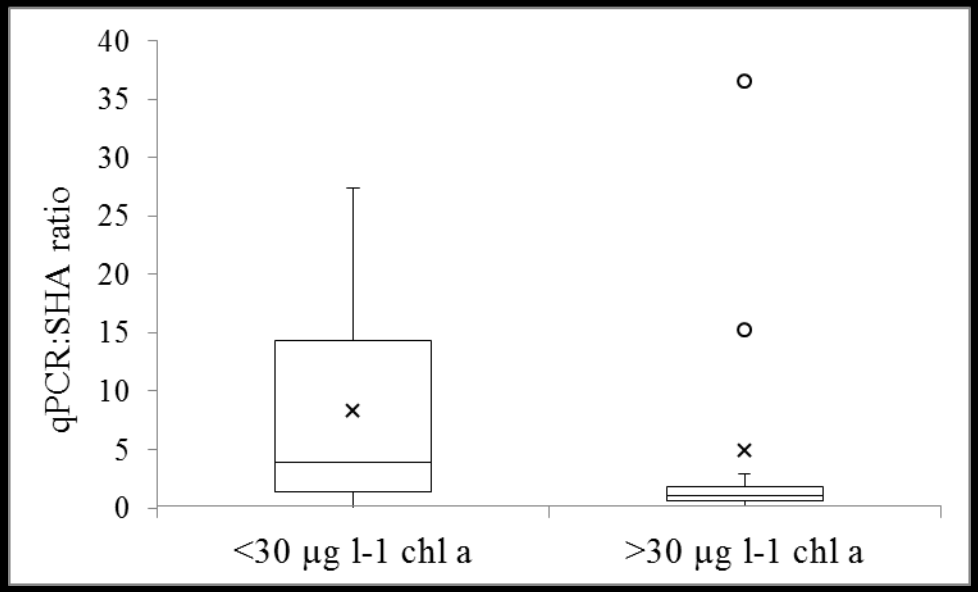


qPCR:SHA elevated at high *Karlodinium veneficum* (red) lower at high *Prorocentrum minimum* (orange); overall concentrations did not correlate with *H. akashiwo*.

Main et al. 2018, J. Applied Phycology

T and phytoplankton biomass



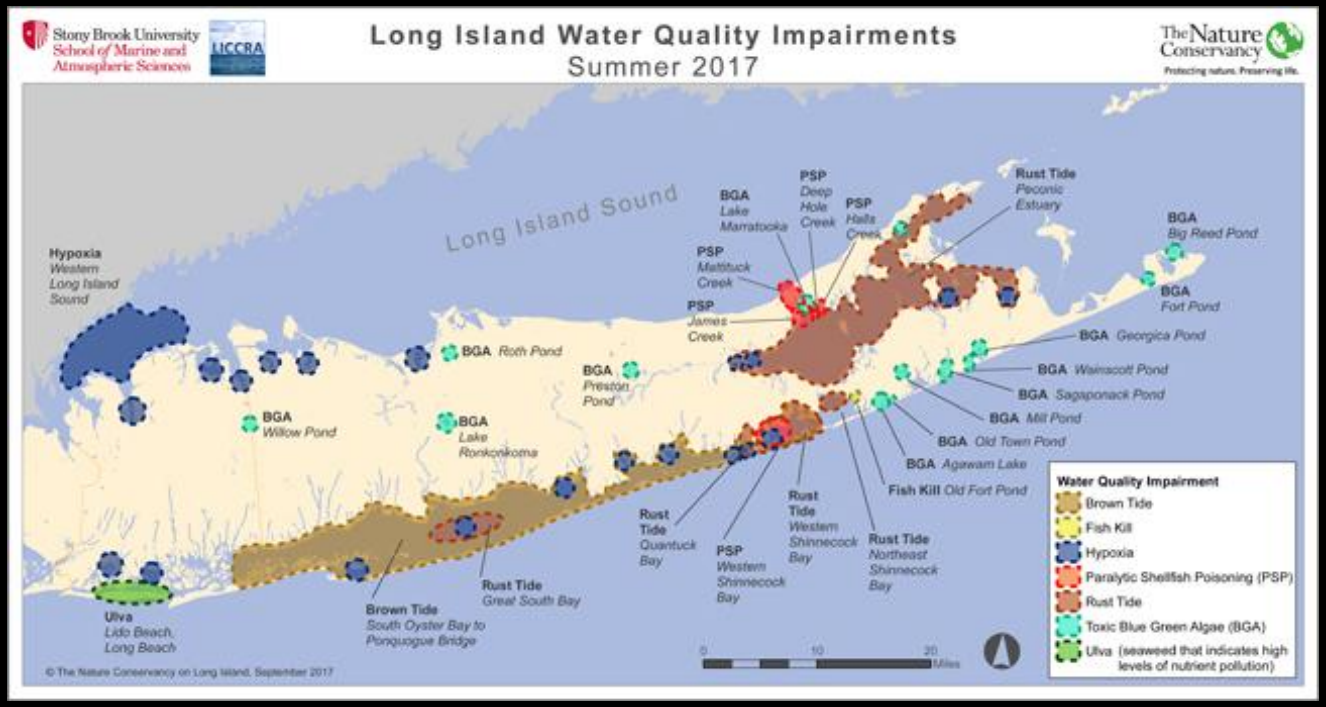


- Strong agreement between methods <25 ºC
- Most *H. akashiwo* blooms occur in this T-range, suggesting thermal stress
- Greater agreement >30 µg/L Chl *a*, consistent with Handy et al. (2005) showing *greater qPCR accuracy with mixed communities*
- Outliers >30 µg/L were during late blooms – *cell senescence?*

Main et al. 2018, J. Applied Phycology

Regional Applications

- Multiple regional HAB species and shellfish toxins
- Recent *Pseudo-nitzschia* blooms
- Active toxin surveillance New England and NY areas
- Several SHA protocols (*Alexandrium*, *Margalefidinium* [*Cochlodinium*], etc



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ASRC link: http://environment.asrc.cuny.edu/people/dianne-greenfield/