Routine Rapid Detection of *Heterosigma* in Natural Samples Using DNA Probes

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OUTLINE

- Background
- Sandwich hybridization, what is it and how does it work?
- Field Data
- Alternative platforms – automated detection

Studies of *Heterosigma akashiwo*

- Distribution
- Abundance
- Ecology
- Impacts

Require identification and enumeration of cells acquired from a variety of locations over a sustained period of time

IMPEDIMENTS TO SPACIAL AND TEMPORAL OBSERVATIONS OF *Heterosigma akashiwo*

- Accurate identification and enumeration requires live sample and a microscopist trained to identify the variety of morpho-types that *Heterosigma* may take.

- Fixing samples for transportation and storage for later analysis is problematic do to the deformation and/or destruction of delicate *Heterosigma* cells by the fixation techniques.

Overcoming Impediments to *Heterosigma* observations

In the past 30 years, advances in molecular technologies primarily targeted for the biomedical field have been adapted to increase the speed of detection for a variety of marine and freshwater organisms.

Challenges to adapting molecular assays to marine and freshwater environments

- Most biomedical molecular assays typically require return of samples to a specially equipped laboratory

- Natural samples contain wide diversity of non-target organisms that create complex sampling matrixes

- Sample preparation can be time consuming, complex and expensive

What a good molecular assay would be

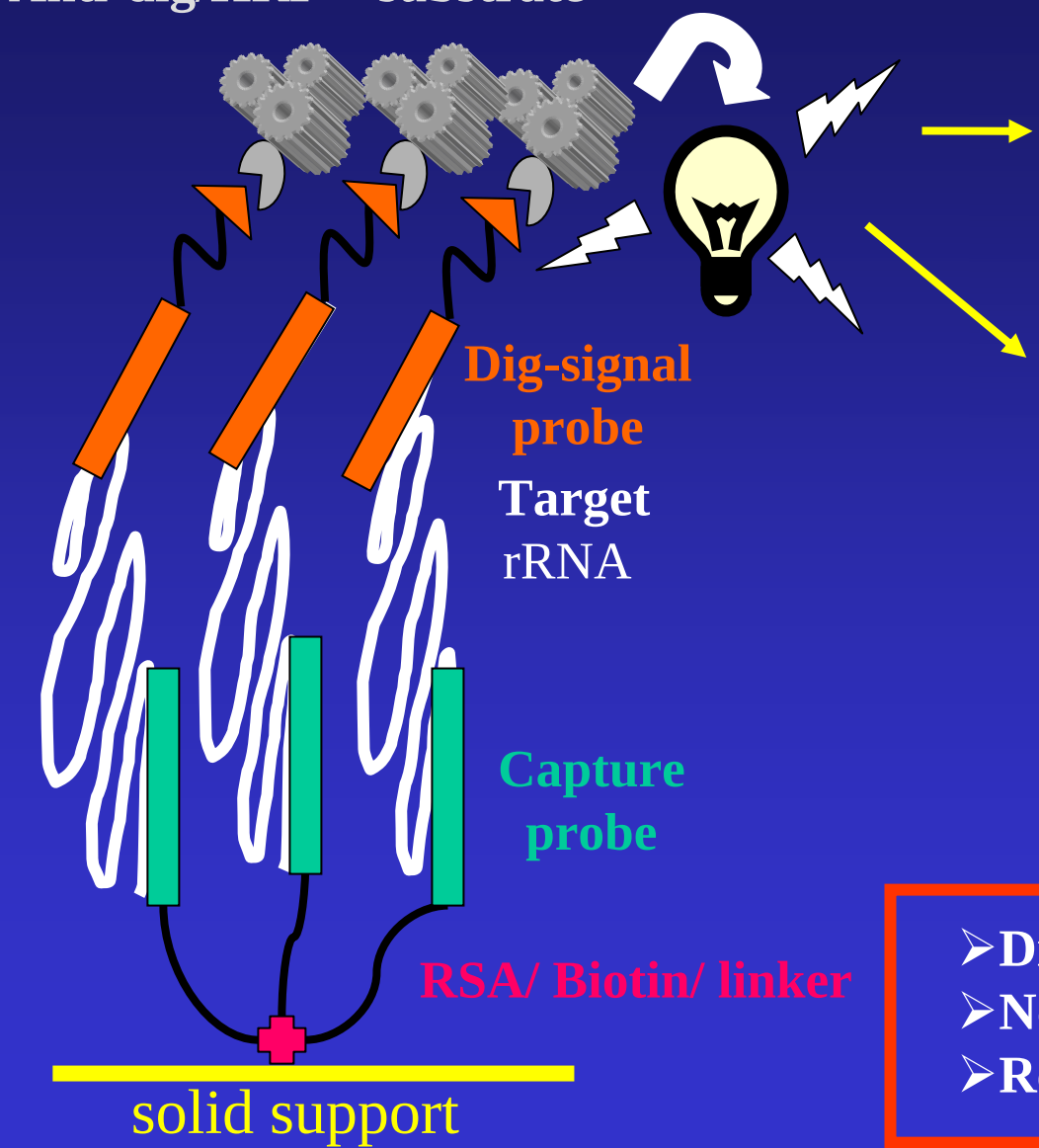
- It should be fast
- It should provide reliable data
- It should be adaptable to detect a variety of organisms
- It should be easy to use
- It should be portable
- It should be inexpensive
- It should be easily obtainable

Direct Detection

In 1991 a paper by Van Ness and Chen described a method to detect human pathogens directly from a crude homogenate

- The assay used short oligonucliotides (12 – 50 mers) in a chaotrope-based hybridization solution
- Probes were attached to a solid support
- Hybridizations take place at near RT (21-30^{o}C)
- Hybridization reactions take place in minutes not hours
- Minimal sample handling, semi-automated processing

SHA Chemistry



Imaged array

Photo courtesy of A. Haywood

Verification by matching 96-well bench run

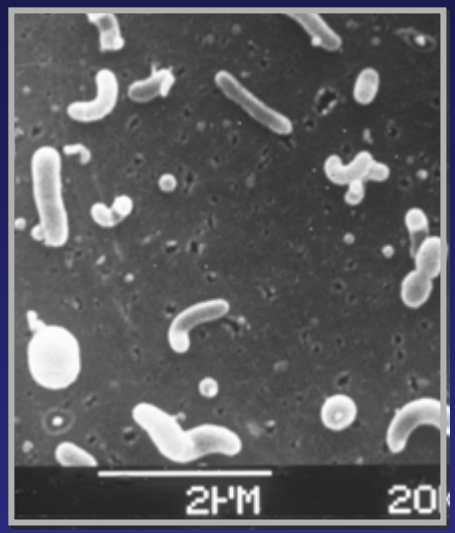
array spot intensity ∝ absorbance (450 nm)

- **Direct capture of target sequence**
- **No purification required**
- **Reagents stable at room temp**

Scholin et al. 1996, 1999; Goffredi et al. 2005; Greenfield et al. 2006

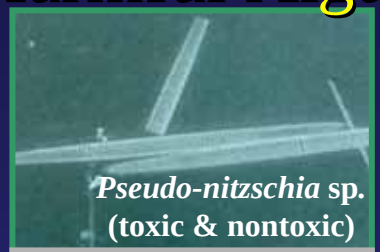
Target Organisms

Marine Microbes



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

Harmful Algae


***Pseudo-nitzschia* sp.**
(toxic & nontoxic)


Heterosigma akashiwo
(& other raphidophytes)


Alexandrium tamarense/ catenella

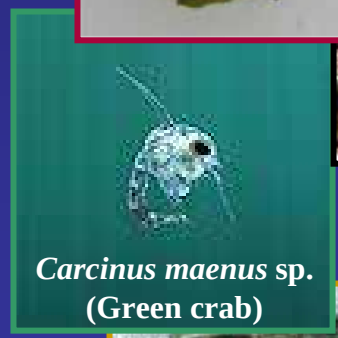

***Karenia* sp.**

Invertebrate Larvae


Balanus glandula
(Acorn barnacle)


***Mytilus* sp.**
(Shore mussels)


Osedax


***Carcinus maenus* sp.**
(Green crab)


Polychaete

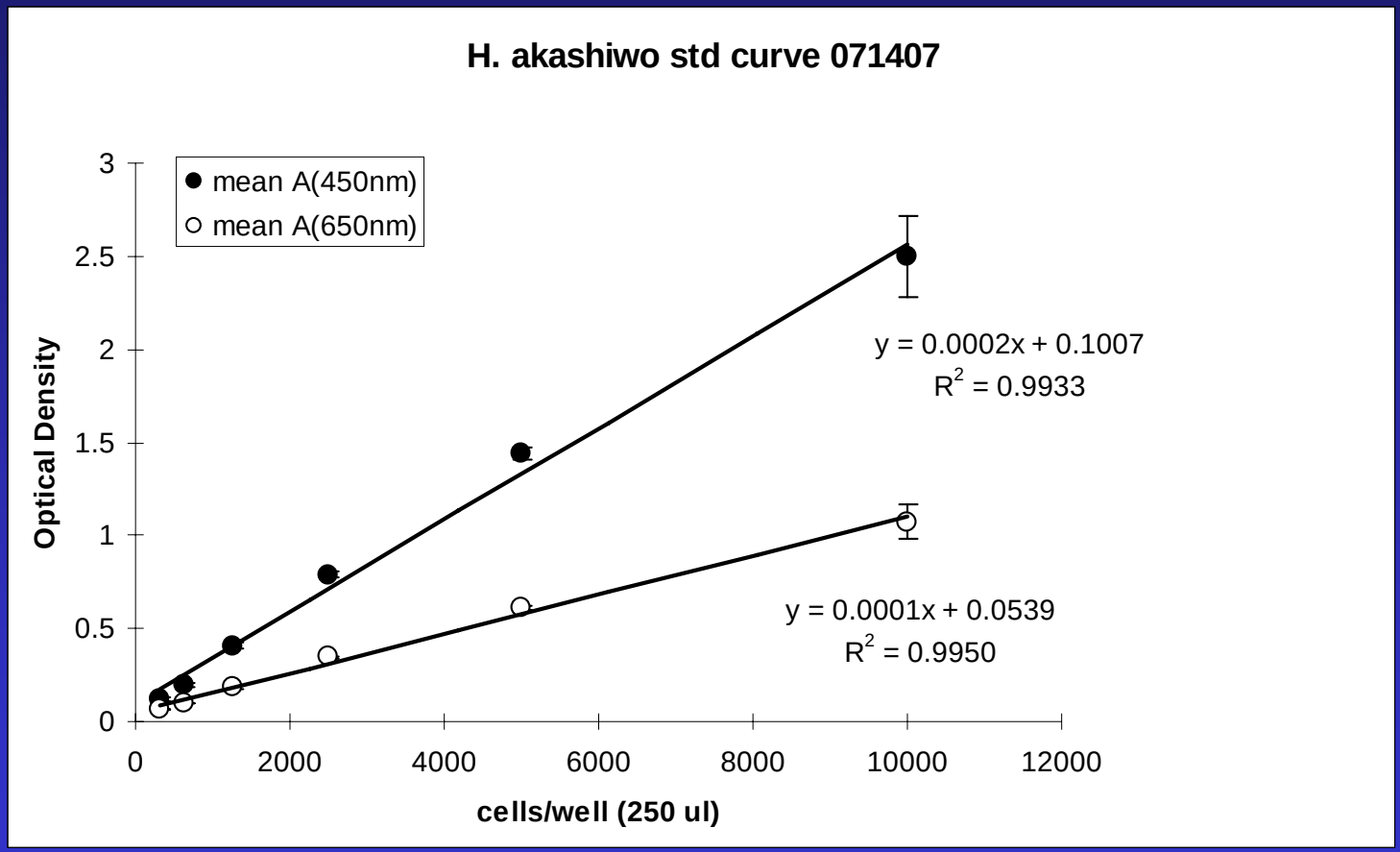
In 2001 John V. Tyrell et al. developed the SHA for the detection of *Heterosigma akashiwo* and other raphidophytes in marine samples using the 96-well microplate format

- Results in 1 hour
- 8 samples per run per instrument

In May 2004, New Zealand accredited the SHA for rapid identification and enumeration of *Heterosigma akashiwo* and other HAB species.

(International Accreditation New Zealand: ISO 17025)

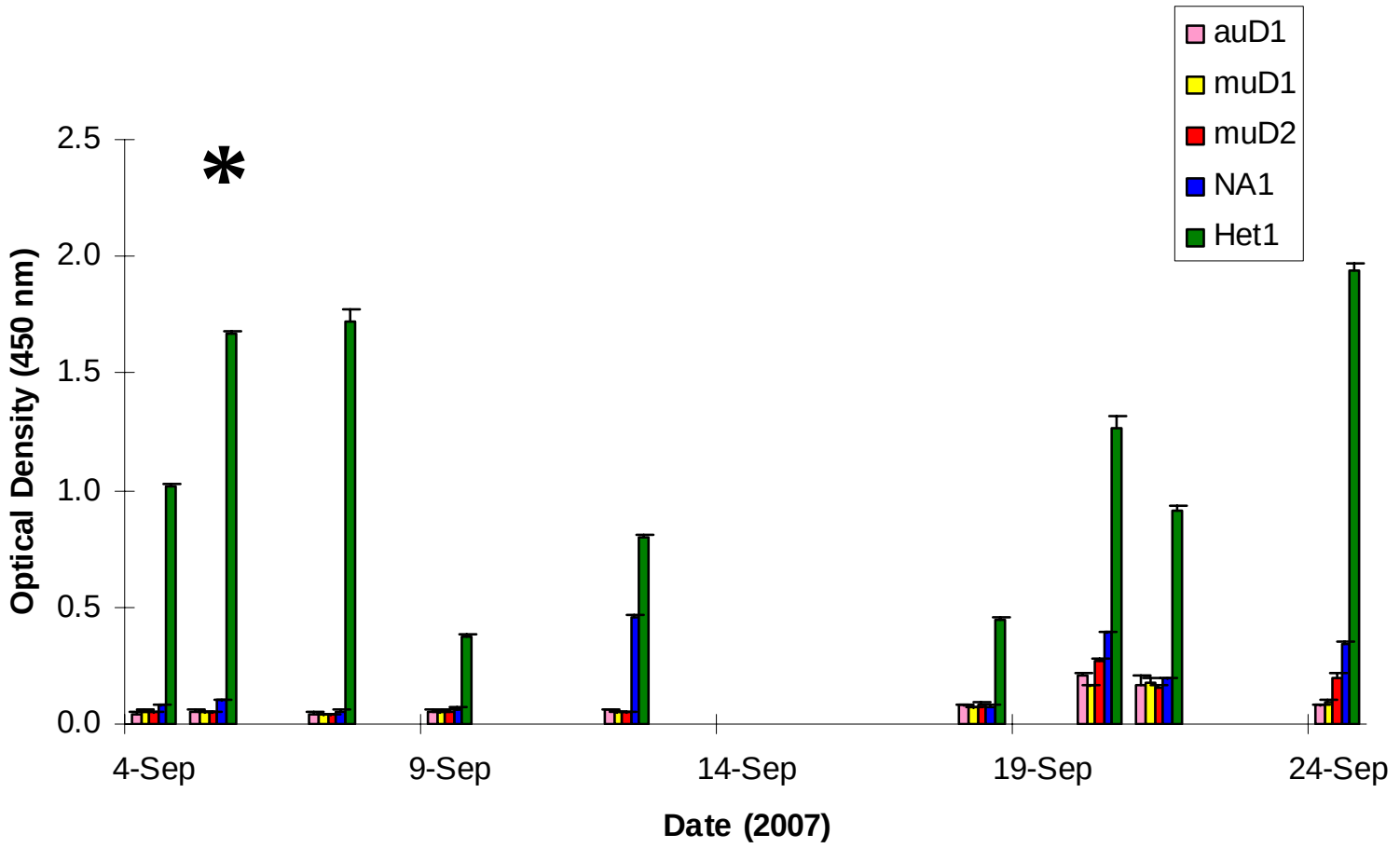
Response of Sandwich Hybridization assay



Unpublished data Dianne Greenfield

- Response of the SHA system is linear
- The SHA system is very sensitive and can detect *Heterosigma* far below action levels
- Tests and experience show the assay to be insensitive to wide range of sample matrixes, including clay that might be used to mitigate effects of blooms
  - Tyrrell et al., 2001 *Harmful Algae* **1**:205-214.
- SHA assay has been validated using PCR
  - O'Halloran et al. *Harmful Algae* **5**: 124-132.

2007 Monterey Bay California field samples collected during ESP Network Deployment



unpublished data Dianna Greenfield

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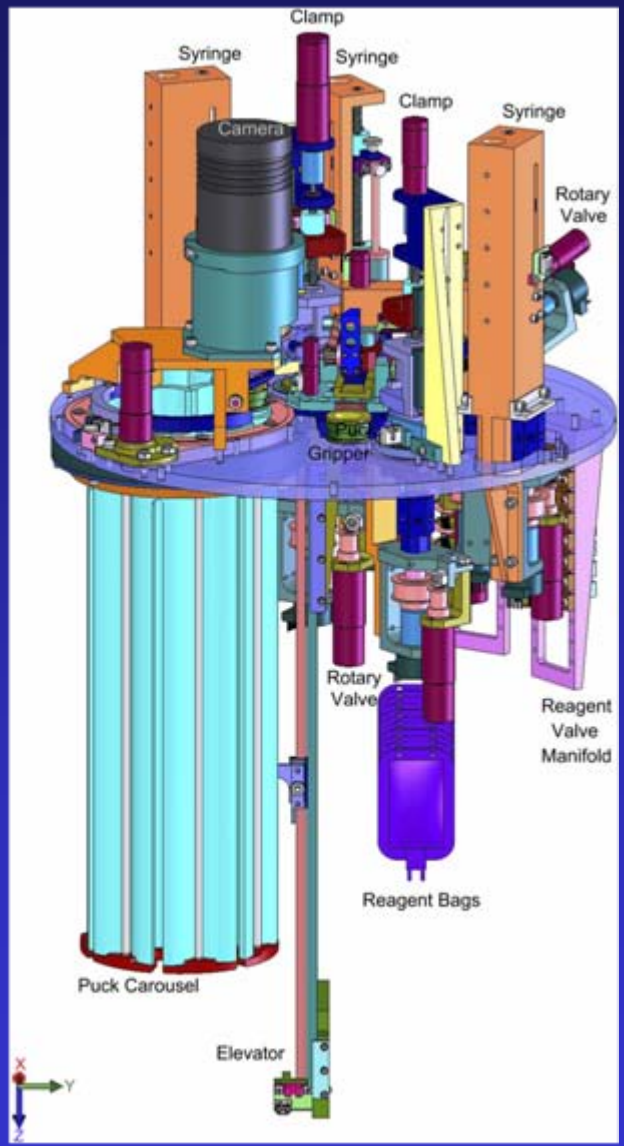
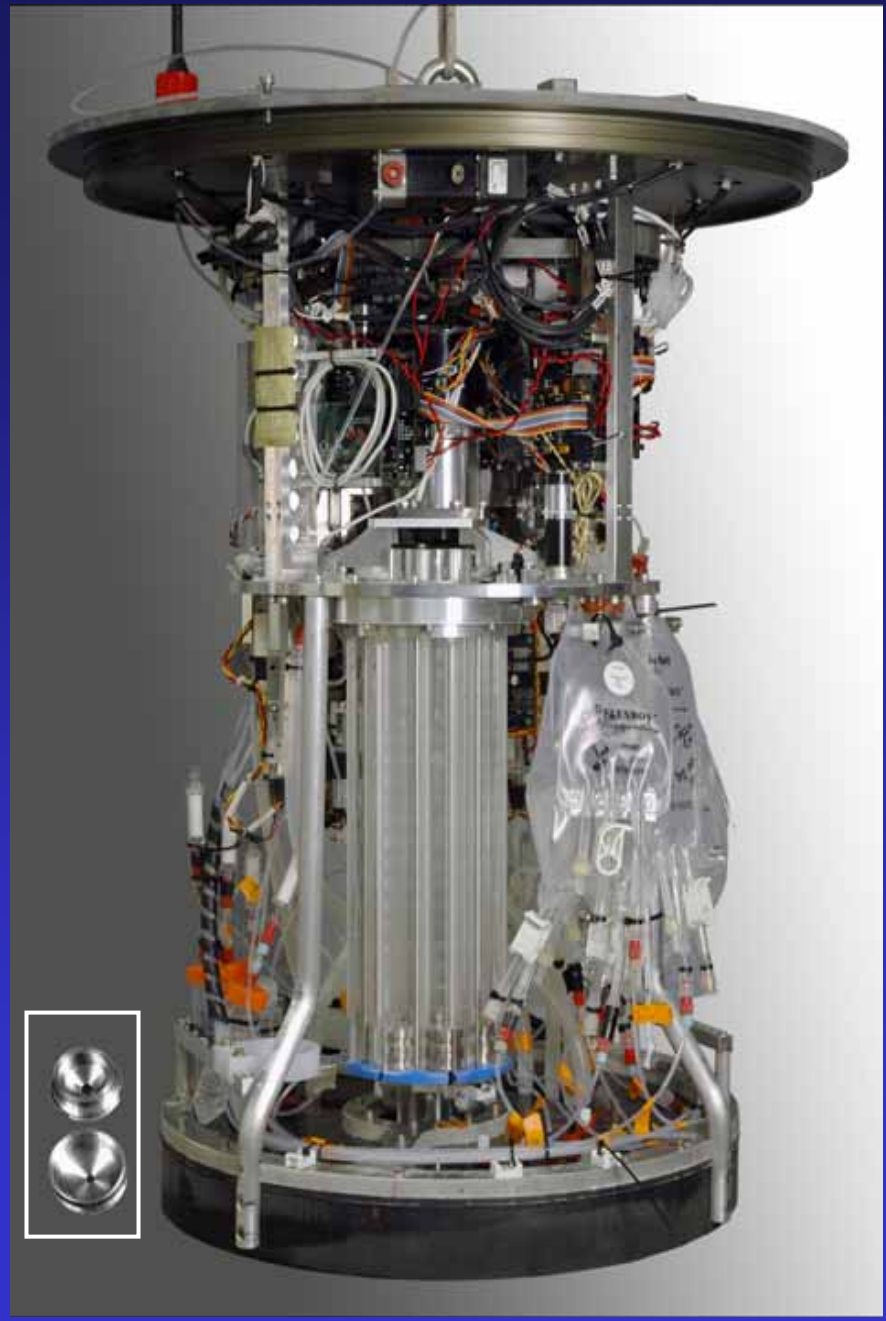
The SHA detection scheme has been fully automated using the Environmental Sample Processor (ESP)


Clamp
Syringe
Syringe
Clamp
Syringe
Camera
Rotary Valve
Gripper
Rotary Valve
Reagent Valve Manifold
Reagent Bags
Puck Carousel
Elevator
X
Y
Z

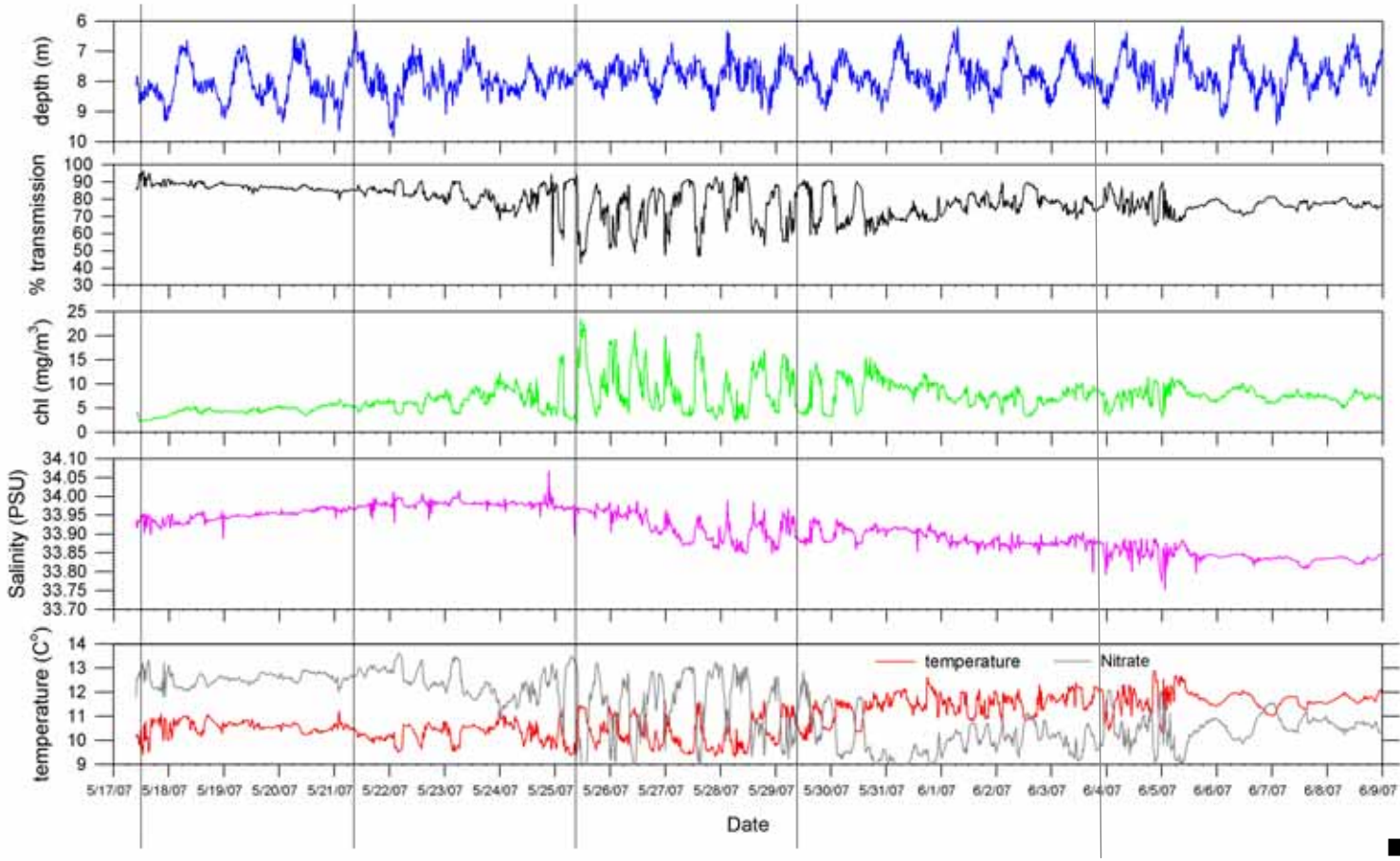


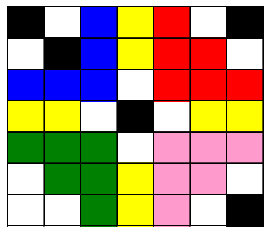
ESP configured for shallow water deployment (50m)

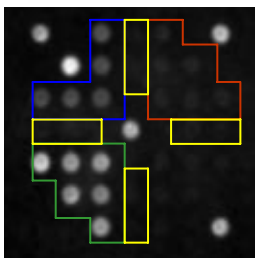
ESP Field Deployment

Monterey Bay, CA
May 17-June 11, 2007

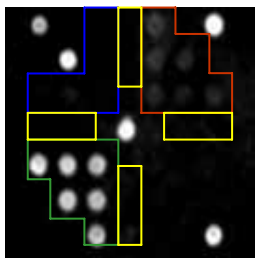
In situ Detection of Harmful Algae



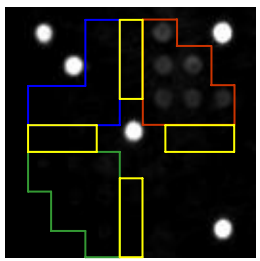




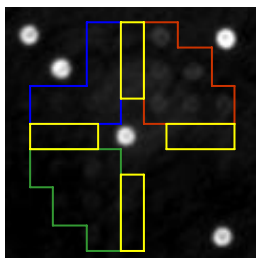
May 17, 2007
1000 ml



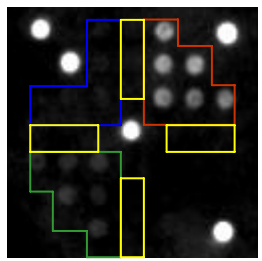
May 21, 2007
1000 ml



May 25, 2007
1000 ml



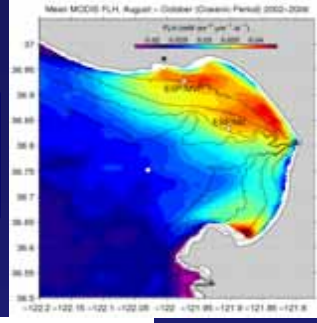
May 29, 2007
1000 ml

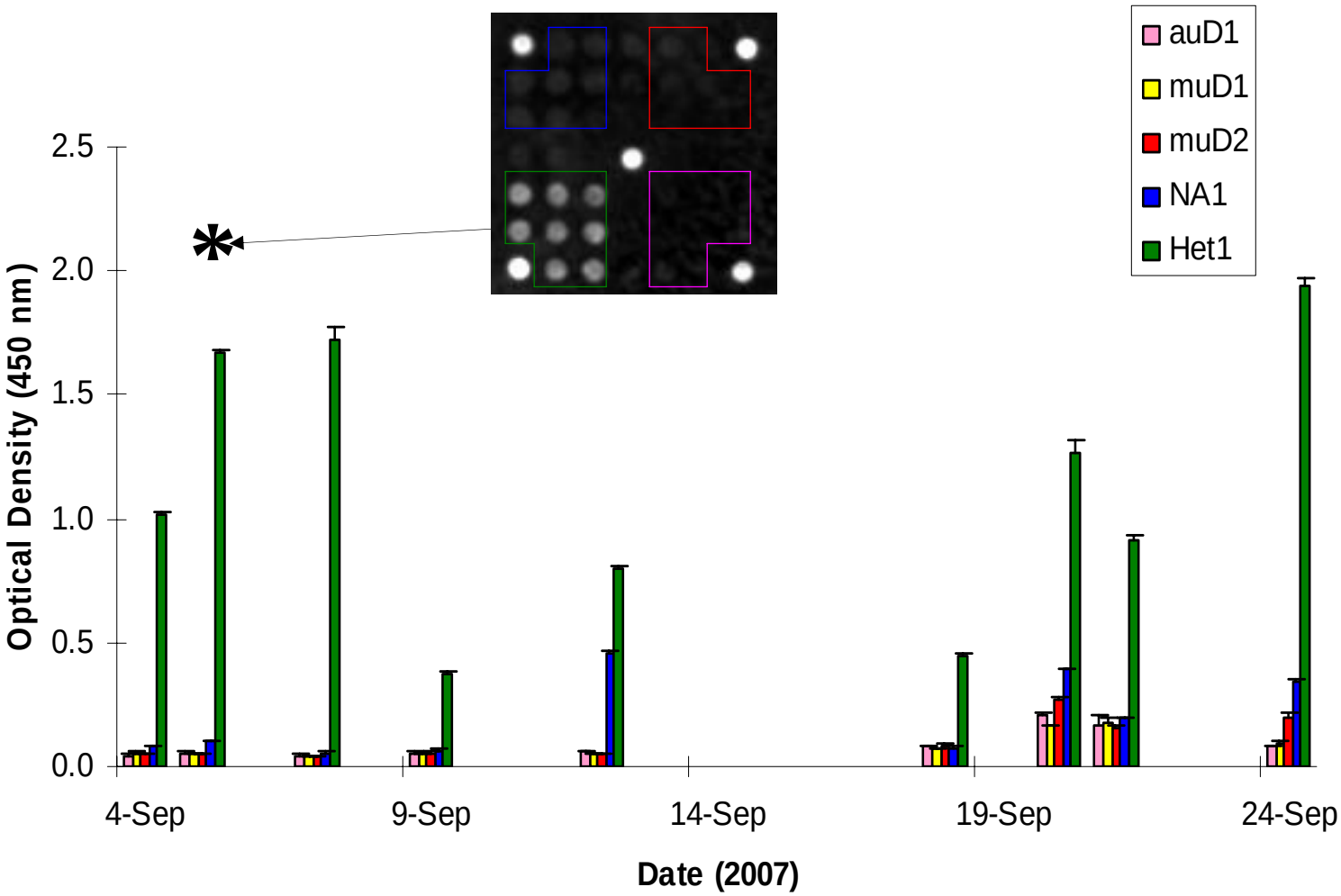


June 4, 2007
1000 ml

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

2007 ESP – Network Deployment





unpublished data Dianne Greenfield

Some Conclusions

- The SHA system as been shown to be a rapid, reliable, method to identify and estimate abundance of *Heterosigma* species in natural samples

- The SHA system can detect *Heterosigma akashiwo* and a variety of other HAB spp. at low concentrations

- The system is easy to operate with minimal sample manipulation

- The SHA systems direct capture of target is insensitive to bio-mass loading

- Biasing of results from amplification is not an issue with the SHA system

- SHA can provide useful data for resource managers and researchers

- Changes in target organisms can be correlated with contextual data and give us the ‘big picture’ of the dance between the organisms and their environment.