A BUOY-BASED IMAGING SYSTEM FOR CONTINUOUS MONITORING OF THE TOXIC DINOFLAGELLATE *Karenia brevis* IN THE GULF OF MEXICO

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A new optical detection system, the submersible FlowCAM (FluidImaging Technologies, Inc.) has been modified to optimize detection of *Karenia brevis*, the toxic dinoflagellate responsible for fish kills in the Gulf of Mexico. We are developing a buoy-based *in situ* continuous monitoring system capable of detecting *K. brevis* in real-time. Preliminary laboratory tests to validate automatic counting and to optimize data retrieval of captured images of *Karenia brevis* will be presented. Ultimately, the FlowCAM will be tested in conjunction with the existing Texas Automated Buoy System (TABS) and modeling program as a tool for early warning of harmful algal bloom events in the Gulf of Mexico. Continuous measurements of cell abundance, nutrient and oxygen concentrations, temperature, salinity and currents will be linked directly to the existing TABS web site for real-time display along with phytoplankton images.

DETERMINATION OF OPTIMAL BIOTIN-LABELED PNA CAPTURE PROBE TO STREPT-AVIDIN BINDING RATIOS

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Identification of harmful algal species directly from environmental samples is a rapidly growing field. Water quality managers, researchers and public health officials require timely and accurate detection of harmful species in a cost effective manner. Therefore, there is a need for improved methods capable of rapid on-site analysis. Emerging techniques utilized peptide nucleic acid (PNA) instead of DNA as probes. PNAs are DNA mimics that bind to RNA or DNA targets as do DNA, but they possess unique hybridization qualities demonstrating enhanced sensitivity and broad hybridization conditions (Nielsen & Egholm 1999).

These experiments are a first step in incorporating PNAs to the standard sandwich hybridization assay (SHA) currently in use for harmful algae (Scholin et al 1996). In the SHA a biotin-labeled capture probe is bound to a solid support through a streptavidin (SA) -biotin linkage. This solid support is then cycled through wells containing target molecules, signal probe, washes and color development reagents. This set of experiments was designed to optimize one of the initial steps in the SHA, that of SA binding to the biotin-labeled capture probe. Early work found an optimal SA to biotin-labeled probe ratio was 1.2:1, with a standard probe concentration of 400ngml^{-1} and a streptavidin concentration of 2.5µg ml^{-1}. To determine the optimal biotin-labeled probe to SA ratio for biotin-labeled PNA probes two sets of experiments were designed. The first kept the probe concentration constant (400µg ml^{-1}) and varied the SA concentration (1.25µg ml^{-1}, 2.5µg ml^{-1}, 5µg ml^{-1}, and 10µg ml^{-1}) (Fig 1) and the other kept the SA concentration constant (2.5µg ml^{-1}) while changing the probe concentration (1.5µg ml^{-1}, 1µg ml^{-1}, 0.5µg ml^{-1}, .0.25µg ml^{-1}) (Fig 2). The target molecule was large subunit ribosomal RNA extracted from pre-frozen (-80^{o} C) *Alexandrium tamarense* cells (isolate NWFSC 405). Target cell concentration in these experiments was 1000 cells per reaction, signal probe concentrations were kept at 300ng ml^{-1} and reactions were run in triplicate.

These data show that the optimal concentration of SA for 400ng ml^{-1} capture probe is 2.5µg ml^{-1} and the optimal capture probe concentration for 2.5µg ml^{-1} SA is 500ng ml^{-1} (or 0.5µg ml^{-1}). This ratio is similar to that found for SA binding to a DNA based biotin-labeled capture probe of 1.2:1.

Fig.1 Vary SA

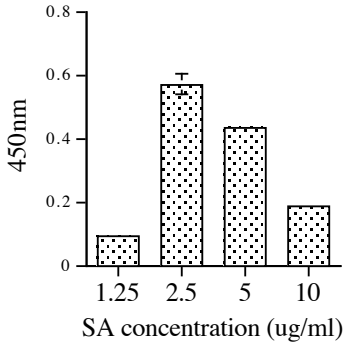

Fig. 2 Vary Probe

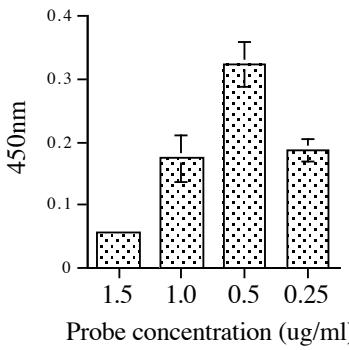

Table 1 PNA probe sequence 5' to 3'

Capture probe	GTG CAA CAC TCC CAC C
Signal probe	GTC CTT TTC ATA TTT CCC

Figure 1 and 2.
Both SA (fig 1) and capture probe (fig 2) concentrations were varied in a standard SHA format using frozen *Alexandrium* cells as target.

References:

Nielsen, P. E. and Egholm, M., Eds. 1999. Peptide Nucleic Acids; Protocols and Applications. Norfolk, England, UK, Horizon Press.

Scholin, C.A., Buck, K.R., Britschgi, T., Cangelosi, G., Chavez, F.P. 1996. Identification of *Pseudo-nitzschia australis* (Bacillariophyceae) using rRNA-targeted probes in whole cell and sandwich hybridization formats. Phycologia 35, 190-197.

THE CYST TRANSCRIPTOME: COMPREHENSIVE ANALYSIS OF GENE EXPRESSION PATTERNS IN *Pfiesteria piscicida* LONG-TERM RESTING CYSTS

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Following a bloom, the toxic dinoflagellate, *Pfiesteria piscicida*, encysts and quickly leaves the water column. The cysts are then poised to respond to conditions that directly motivate excystment into toxic or non-toxic swimming zoospores. The cellular responses to environmental conditions that trigger life stage transitions in *Pfiesteria* are currently unknown. It can be assumed, however, that the load of viable *Pfiesteria* cysts in the sediment at any given time may directly determine the dynamics and success of a future bloom. In addition, repeated minor blooms in a restricted area over many seasons could charge the sediment for a more significant bloom several seasons later. Using molecular techniques, we can establish the organism's distribution in sediments and thus its potential for future blooms. DNA-based techniques, however, are not a reliable indication of cyst viability. Because gene transcripts are more labile than DNA, the presence of specific transcripts may be used as a proxy for cyst viability. Using Serial Analysis of Gene Expression (SAGE), we prepared gene expression libraries of nontoxic zoospore and long-term resting cyst life stages of *P. piscicida*. This technique provides a catalog of gene transcripts that is both comprehensive and quantitative. By comparing the SAGE libraries, we are able to identify transcripts that are specific to each life stage.

Here, we present our investigation of gene expression patterns in long-term resting cysts of *Pfiesteria piscicida*. The SAGE library provides us with an unprecedented opportunity to examine cellular activities and mechanisms involved in the formation, maintenance and termination of the cyst life stage in response to environmental conditions. In addition, several transcripts have been identified that may be used as molecular markers for cyst viability in sediment samples. When combined with DNA-based techniques, these markers will allow us to assess both the distribution and viability of *Pfiesteria* cysts in sediment samples.

THE DEVELOPMENT AND APPLICATION OF A QUANTITATIVE PCR ASSAY FOR *Alexandrium fundyense* FROM THE GULF OF MAINE

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Toxic dinoflagellates of the genus *Alexandrium* are responsible for seasonal harmful algal blooms (HAB) in New England coastal waters and other temperate environments. Members of this genus produce saxitoxin that can result in paralytic shellfish poisoning (PSP) along the coastlines of the US, Canada, and many other countries. Even low cell densities of *Alexandrium fundyense* can result in PSP with serious illness, or death, if humans or other consumers ingest sufficient contaminated shellfish. Observations of *A. fundyense* and PSP outbreaks in the northeastern US indicate that populations accumulate in a variety of oceanographic habitats and there is considerable interest in the development and application of monitoring tools that will allow better prediction and study of toxic, but low density populations, in these environments.

To expand the tools available for *A. fundyense* detection and monitoring we developed a PCR-based assay that is specific, quantitative, and appears to be useful for high throughput screening of *A. fundyense* cell density. Primers were designed to amplify a 174bp region of the large subunit ribosomal RNA gene (LSU). These primers specifically amplify target *A. fundyense* strains and not *A. ostenfeldii*, *A. andersonii*, or toxic dinoflagellates from other genera that we tested. With this primer set we used SYBR green to quantitatively amplify the LSU product from surface field samples during a June 2003 cruise in the Gulf of Maine. Cell density was calculated by comparing the amplification in field samples against a standard curve built from an *A. fundyense* Gulf of Maine isolate in culture. In the field samples *A. fundyense* abundance, as calculated with the PCR-based assay, was in the same range as the cell density calculated with other methods. Cloning and sequencing of the amplification product from several stations revealed it to be from *A. fundyense*.

With this PCR-based approach to assessing *A. fundyense* cell density we are able to screen 26 samples in triplicate at one time. We believe this method shows great promise for continued research and monitoring efforts that need estimates of cell number.

ASSESSMENT OF BIO-OPTICAL MODELS DURING TWO EAST COAST ALGAL BLOOMS: CELL SIZE DEPENDENCE

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Recently there has been increased interest in the remote detection of harmful algal blooms (HABs), given that they are often associated with significant changes in water color. Questions have arisen, however, regarding the applicability of established optical models to extreme algal blooms, as algorithms are generally developed and tested during conditions of low to moderate chlorophyll (Chl) concentrations and polydispersed particle distributions, conditions atypical of blooms. We assessed the application of bio-optical models during two intense algal blooms along the east coast of the US: 1) a bloom of the dinoflagellate *Prorocentrum micans* in West Boothbay Harbor, Maine and 2) a brown tide of the pelagophyte *Aureococcus anophagefferens* in Quantuck Bay, New York. In particular, we investigated: 1) a spectral chlorophyll-specific absorption (a*) model based upon Chl concentration (Bricaud et al. 1995), 2) an inherent optical property (IOP) inversion model to estimate particle size distributions from particulate beam attenuation (Boss et al. 2001), and 3) an apparent optical property (AOP) reflectance inversion model to estimate phytoplankton absorption spectra (Roesler and Perry 1995).

In situ conditions during each bloom were characterized by high Chl concentrations (up to 494 and 40 $\mu g\ L^{-1}$, respectively) and particle size ranges that did not display the Junge-like distributions typically encountered in the ocean. Particle area and volume distributions exhibited a modal diameter of 23 μm and 2 μm during the *P. micans* and *A. anophagefferens* blooms, respectively. Even with extremely high Chl, measured a* coefficients indicated algal photoacclimation, yet measurements of Chl alone were not sufficient to model spectral phytoplankton absorption for either algal species. The IOP model was robust despite violation of key assumptions regarding the particle size distributions. However, limitations exist for the interpretation of particle size slopes predicted from spectral attenuation slopes. For a large cell such as *P. micans*, the inversion model accurately predicts the size distribution of non-bloom particles. However, for blooms of small cells like *A. anophagefferens*, the model accurately estimates the entire particle size distribution including the bloom-forming cells. This is because the beam attenuation is preferentially sensitive to small particles. The reflectance inversion model was robust, predicting phytoplankton absorption to within 10% of the measured values for each bloom. Success of certain optical parameters and relationships during blooms suggests that some existing optical models may be used for detecting/studying algal blooms, although for some models the size of the cells causing the bloom may have to be considered for accurate interpretation. From continued studies during blooms, regional-specific and/or species-specific sets of appropriate optical techniques can be determined thereby improving our capabilities for optical HAB detection and the development of early warning systems.

MONITORING NUTRITIONAL PHYSIOLOGY IN HARMFUL ALGAE: ASSESSMENTS OF ENZYMATIC ACTIVITY AS A MEASURE OF ORGANIC NUTRIENT HYDROLYSIS

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Dissolved organic forms of nitrogen (DON), such as amino sugars, and phosphorus (DOP), such as phosphomonoesters, can influence growth, bloom dynamics, and toxin production in harmful algae. DON and DOP may be difficult nutrient pools to exploit and often have to be hydrolyzed before they can be taken up or used by the cell for growth. We can assess potential DON and DOP utilization by harmful algal species through measures of enzymatic activity. Specifically, our study is focused on two major enzymes, acetylglucosaminidase and alkaline phosphatase. Acetylglucosaminidase is used to break down amino sugars and alkaline phosphatase is typically able to hydrolyze a variety of phosphomonoesters.

Classical enzyme assays, such as the bulk assay for alkaline phosphatase, have been used to investigate DOP hydrolysis. However, these assays are not able to determine the enzymatic activity of specific phytoplankton species and are confounded by the fairly ubiquitous nature of these enzymes in many phytoplankton and heterotrophic bacteria which may regulate their activity in subtly different manners. Consequently, we have targeted our study to those enzymes where cell-specific assays may be possible. Here we utilize two enzyme substrates from Molecular Probes Inc. that are soluble and colorless when unreacted, but insoluble and fluorescent when reacted. Thus, they can precipitate and fluorescently tag individual phytoplankton cells that have specific enzyme activities. This technology is called enzyme labeled fluorescence (ELF).

We are screening harmful algae for acetylglucosaminidase and alkaline phosphatase activity. Preliminary data from members of the genus *Alexandrium* indicates that *A. catenella* expresses acetylglucosaminidase activity when grown on a variety of nitrogen sources. In related work with other harmful species, such as the dinoflagellate *Gymnodinium catenatum,* we have identified alkaline phosphatase activity when grown under phosphate deplete conditions. With these initial screenings, it appears that we can detect both acetylglucosaminidase and alkaline phosphatase activity using ELF substrates. With the use of ELF we are hopeful that field populations can be screened for these activities, thus indicating potential DON and DOP utilization in specific harmful species.

RADIOLABELLED SAXITOXIN FOR RECEPTOR ASSAYS: DEVELOPMENT OF IMPROVED LABELING STRATEGIES

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Due to their response spectrum to the various saxitoxins, assays based on the sodium channel receptor site provide the most reliable estimate of paralytic shellfish poison toxicity in seafood samples. Receptor binding assays, using appropriately labeled toxin, offer the most practical option to the mouse bioassay, but the appropriate labeling of the toxin presents a challenge. At present, the employment of the receptor binding assay is constrained by uncertainties in the availability of exchange labeled saxitoxin, which has been the basis of previous work. Current efforts are focused on both improving the technique for exchange labeling, to assure a reliable supply, and on developing alternative labeled toxins.

USE OF THE RAPID LATERAL FLOW IMMUNOCHROMATOGRAPHIC TESTS FOR PSP AND ASP IN PHYTOPLANKTON APPLICATIONS

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The Jellett Rapid Tests have been used in many countries for detection of PSP and ASP toxins in shellfish tissue. These data have been published (Jellett et al. 2002, Toxicon 40: 1407-1425). Recently the Rapid Tests have been applied to the detection of PSP and ASP toxins in phytoplankton from culture and in seawater. Approximately 20 types of toxigenic dinoflagellates (*Alexandrium* spp., *Gymnodinium catenatum, Pyrodinium bahamense* var. *compressum*) in culture were positive in the Rapid Test for PSP and in the HPLC, while 7 strains of non-toxigenic *Alexandrium* and several strains of co-occurring non-toxigenic dinoflagellates were negative. Similarly, ASP toxins in *Pseudonitzschia* spp. cultures were detected using the Rapid Test for ASP.

Semi-quantitation using serial dilutions of the samples showed similar results to HPLC quantitation. Phytoplankton were prepared by collection of the cells using filtration and then transfer of the cell material into 0.1N acetic acid solution. The limit of detection was about 2ng toxin of ASP or PSP toxin in the sample. The Rapid Tests were then used to detect PSP and ASP in the field. A toxigenic bloom of *Pseudonitzschia seriata* was detected using field sampling over one summer in Nova Scotia and toxin was confirmed using LC-MS. Net tows containing *Pseudonitzschia* and/or *Alexandrium* spp. from Nova Scotia, the Gulf of Maine and coastal Washington State, USA, were also found to be positive in both the Rapid Tests and by analytical methods. Samples found to be negative using analytical chemistry were also negative in the Rapid Tests. Toxin per cell determines how many cells can be detected in the Rapid Tests, but it was possible to detect as few as 50 cells of a toxic strain of *Alexandrium* sp. (AL18B).

MAPPING *Karenia brevis* BLOOMS UTILIZING AUTOMATED OPTICAL DISCRIMINATION

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In the waters off western Florida, blooms of the toxic dinoflagellate *Karenia brevis (=Gymnodinium breve)* (Davis) have occurred nearly annually (K. Steidinger, pers. comm.) and the geographic extent of the blooms appears to have increased in recent years. When undetected, these blooms resulted in contamination of commercial seafood production, disruption of recreational fishing enterprises, and despondent tourists. Economic impact studies showed a loss to Florida of nearly 20 million dollars from a bloom in 1971 and more than 15 million dollars from a 1973-74 bloom. There is a critical need to detect and track these toxic algal blooms over ecologically relevant spatial/temporal scales. To do this requires long-term monitoring efforts which can delineate the frequency and distribution of both phytoplankton and HABs.

Satellite and airborne remote sensing are beginning to show promise for the detection and tracking surface expressions of these HABs, but subsurface events remain problematic (Stumpf and Culver, pers. comm.). An approach has been developed to detect *Karenia brevis* blooms through analysis of absorbance spectra, measured by *in situ* instrumentation. The classification technique relies on fourth-derivative analysis of particle absorbance spectra. When applied to natural, mixed populations of phytoplankton in the eastern Gulf of Mexico the fourth-derivative discrimination technique showed a significant, linear relationship between the derivative spectrum-based similarity index and the fraction of chlorophyll biomass contributed by *K. brevis*.

This presentation will be a compilation of results from four ECOHAB-Florida cruises and five local surveys utilizing this approach over the past six years. These results demonstrate the utility of the technique to mapping the distribution of *Karenia brevis* bloom patches over the central west coast of Florida. New modes of deployment that are underway, including fixed moorings and autonomous underwater vehicles, will be presented and results discussed. This technique, applied in these deployment modes, in conjunction with new molecular-based species-detection technology form the core of an automated detection and tracking network being developed along the west coast of Florida.

APPLICATION OF CULTURED NEURONAL NETWORKS FOR IDENTIFICATION OF TOXIC ALGAE

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On-line, near-real time detection systems for harmful algal species and their toxins is a rapidly emerging field aimed at forecasting bloom development, persistence, and toxicity as well as providing data to facilitate rapid and more effective responses to harmful algal blooms. Recently the Naval Research Laboratory demonstrated that the neuron-based biosensor, which relies on the use of cultured neuronal networks grown over microelectrode arrays (MEA) is capable of detecting the concentration-dependent alterations in extracellular action potentials or spikes from the network upon introduction of several purified marine toxins to recording media. We have now sought to determine whether the neuronal networks could detect these toxins directly in the seawater growth medium of the toxic algae.

Spinal cord and frontal cortex tissue were prepared from embryonic mice and cells were seeded onto 64 electrode MEAs. All recordings were performed at 37˚ C with media/toxin perfusions at flow rate of 0.75 ml/min.

Our results demonstrated that neuronal networks could be used for analysis of algal samples lysed directly in the seawater growth medium of the toxic algae *Alexandrium fundyense* and *Karenia brevis*. The MEA responded to signals characteristic of each toxin class from these cultures, but not from non-toxic isolates of the same algal genus. This successful trial provided evidence that the prototype MEA has the capacity to detect toxins associated with cells of toxic algal species and has the potential for monitoring toxin levels during harmful algal blooms.

USE OF ITS-SPECIFIC POLYMERASE CHAIN REACTION (PCR) ASSAYS TO IDENTIFY PHYTOPLANKTON SPECIES IN COMPLEX NATURAL ASSEMBLAGES

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Pfiesteria piscicida, *Karenia brevis* and other potentially harmful microalgae frequently co-occur with morphologically similar species that cannot be distinguished using light microscopy. The inability to distinguish harmful from non-harmful species can make accurate assessment of public health threats based solely on light microscopy difficult. Over the past ten years molecular assays have been developed that allow unambiguous detection of harmful species in natural assemblages. This study specifically evaluated whether unique sequences in the ribosomal internal transcribed spacer regions (ITS1 and ITS2) could be used to develop PCR assays capable of detecting *Pfiesteria piscicida* and related co-occurring species (Litaker et al., 2003). The ITS regions were selected because they are more variable than the flanking small subunit (SSU) or large subunit (LSU) ribosomal RNA genes and more likely to contain species-specific sequences. Sequencing of the ITS regions revealed unique oligonucleotide primer binding sites for *Pfiesteria piscicida*, *Pfiesteria shumwayae*, Florida "Lucy" species, two cryptoperidiniopsoid species, "H/V14" and "PLO21," and the estuarine mixotroph, *Karlodinium micrum*. These unique primer sites were used to develop species-specific PCR assays with a minimum sensitivity of 100 cells in a 100 mL sample (1 cell mL^{-1}). The assays were then used to successfully detect *P. piscicida* and related PLOs in field samples.

Each PCR assay included positive, negative, blank extraction, and spiked DNA controls. The positive controls consisted of amplifying a known amount of purified target DNA. This control ensured that the PCR reagents were properly assembled and that the DNA *Taq* polymerase was functional. The negative control reactions lacked DNA and served to identify if reagent stocks had been contaminated with target DNA, or if cross-contamination of target DNA had occurred during reaction preparation. The blank DNA extraction controls consisted of processing unused filters using the standard DNA extraction procedure. This allowed identification of cross-contamination during the DNA isolation step. The spiked controls involved adding the same quantity of target DNA as used for the positive control reactions to a random subset of extracted DNA reactions. Reduction of PCR amplification in the spiked control reactions relative to the positive control reactions indicated PCR inhibition. The most common causes of PCR inhibition include the carry over of cations, carbohydrates or phenolics (humic compounds) that adversely affect the function and specificity of DNA polymerases. Modification of the PCR reaction pH by compounds extracted with the DNA can also inhibit PCR reactions or cause production of nonspecific amplification products. Omission of these crucial controls can lead to misinterpretation of the field PCR results. Additional details of assay development including field sample collection, DNA purification, primer design, and PCR optimization of will be discussed.

References:

R. W. Litaker, M. W. Vandersea, S. R. Kibler, K. S. Reece, N. A. Stokes, K. Steidinger, D. F. Millie, B. J. Bendis, R. M. Pigg, and P. A. Tester. 2003. Identification of *Pfiesteria piscicida* (Dinophyceae) and *Pfiesteria*-like organisms using ITS-specific PCR assays. Journal of Phycology 39: 754-761.

GENE EXPRESSION PROFILING IN MOUSE BRAIN AFTER DOMOIC ACID EXPOSURE

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Domoic acid (DA) is a rigid analog of the neurotransmitter glutamate and a potent agonist of the kainate subtype of the glutamate receptor. Persistent activation of these receptor subtypes results in rapid excitotoxicity, which leads to calcium dependent cell death and neuronal lesions in areas of the brain where glutaminergic pathways are heavily concentrated. To better understand the mechanisms and pathways involved in response to toxic levels of DA in mammals, we have employed microarrays to study global gene expression in the mouse brain. Adult ICR mice were subjected to an IP, LD50 dose (4mg/kg) of DA while controls received PBS. Mice were sacrificed and immediately dissected at 30, 60 and 240 minute time points. Agilent catalog 20K feature mouse oligo arrays were run in triplicate for each time point, comparing a different experimental mouse against a pooled control for each time point, totaling nine arrays. Using Acuity v3.1 software, the arrays were filtered for quality based on signal intensity and signal to noise ratio. The remaining features from the replicate arrays were averaged and used for K-means and hierarchical clustering. Approximately 2-2.5% of all the genes represented on the array showed a significant change in expression compared to controls. Using selected genes, array expression data was additionally verified through RT-PCR. Fos, a calcium dependent immediate early response gene, was one of the highest up-regulated genes at both 30 and 60 minutes, which is consistent with previously published data. Both Fos and Jun-B, which dimerize to form an AP-1 transcription factor, followed a similar expression pattern of up regulation at the 30 and 60 minute time points, and a decrease down to basal levels at 240 minutes. Some other expected up regulated genes included pain and inflammatory pathway elements such as COX-2. Two genes involved in regulating gene transcription that were found to be down-regulated were transcription factor 7-like 2 and Janus kinase 3 (JAK3), although these genes are not known to work together. Down regulated genes comprised less than 40% of all differential expressors while up regulated genes contributed to over 60% of all differential expressors. K-means and hierarchical clustering produced groups of genes with similar expression patterns that may work in conjunction with each other.

Understanding the coordinated expression and interaction of clustered genes will most likely allow a better understanding of the mechanisms of neurotoxicity and neuroprotection.

DEVELOPMENT OF THE "SECOND GENERATION" ENVIRONMENTAL SAMPLE PROCESSOR (2G ESP) FOR REMOTE DETECTION OF HARMFUL ALGAE AND TOXINS THEY PRODUCE

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Development of instruments that enable long term, unattended, *in situ*, application of molecular probes with real-time telemetry of results will offer an exceptionally novel approach for detecting HAB species, genes they harbor and express and toxins they may produce. In a step toward reaching this goal, scientists and engineers at the Monterey Bay Aquarium Research Institute (MBARI) have developed a prototype instrument known as the Environmental Sample Processor (ESP; http://www.mbari.org/microbial/ESP). The "first generation" (1G) ESP was designed to collect discrete water samples remotely, subsurface, concentrate microorganisms and automates application of DNA (or other) molecular probes to enable identification and quantification of particular species captured. The instrument transmits results of DNA probe array assays in real-time via radio modem to a shore based location for processing and interpretation. In addition, the 1G ESP can archive discrete samples for nucleic acid, microscopic and toxin analyses for validating real-time data from the probe arrays as well as facilitating other analyses in the laboratory (such as construction of gene libraries).

The 1G ESP has worked successfully on several, limited test deployments with extensive support by the original science/engineering design team. Development of a "second generation" (2G) ESP has begun, including adding an analytical capability for detection of domoic acid. The overall goal in designing the 2G ESP is to make it much more robust and user friendly relative to the 1G prototype, and to produce a system that can be successfully deployed for a variety of applications on a routine basis by a team of trained technicians. A major effort is being made to reduce the size, complexity and power consumption of the instrument, and to take advantage of microfluidic-scale detection technologies. There will also be an effort to provide space and appropriate electrical, software and fluid connectivity to support the addition of future sample and analytical 'modules', separate devices that can be used to extend the range of sample collection and analyze detection options of the core 2G ESP. The first 2G ESP is scheduled to begin operation mid-2004. This presentation focuses on progress made to date designing and developing the 2G hardware, and emphasizes development of DNA probe array and domoic acid detection technologies.

DETECTION AND MONITORING OF HARMFUL ALGAL BLOOMS WITH SATELLITE

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Since the late 1970's, remote sensing with both ocean color and temperature has been found to have the potential to detect and monitor harmful algal blooms.[1-4] With the launch of the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) in September 1997, interest was renewed in the use of ocean color imagery. A monitoring program in the Gulf of Mexico began in September 1999 with imagery constituting a key part of that program.[5]

The efforts on *Karenia brevis* blooms in the Gulf of Mexico have revealed several potential capabilities for imagery as a part of a detection and monitoring program. While spectral methods have been presumed to be preferential, they can be problematic. *Karenia* spp. are dinoflagellates and many of the other common blooms are diatoms, which often have quite similar pigment suites.[6] In fact, many HAB species are either dinoflagellates or diatoms. Several solutions are being used for the Gulf of Mexico. The nature of these solutions may allow new options for exploring HABs in other environments.

1) Ecological detection. The primary detection method involves an ecological solution. *K. brevis* usually dominates the biomass from late summer through winter. As a result, significant new blooms are usually *Karenia*. New blooms are identified from imagery as an increase in chlorophyll-a concentration of > 1 g L^{-1}. This method is effective over 80% of the time[7], and can apply in turbid water, where most optical methods would fail.
2) Ecological discrimination. While this method is effective in Florida, resuspension events in Texas frequently lead to the appearance of new blooms. The resuspension appears to introduce benthic phytoplankton to the water quality, given the impression of a new bloom. The chlorophyll associated with resuspension can be estimated and eliminated, this allows for the potential of discriminating the presence of ambient blooms in the water column, from the resuspended "blooms". [8]
3) Optical techniques. Differences in backscatter between phytoplankton may allow discrimination that is not possible from absorption. *K. brevis* appears to have less backscatter than diatoms, allowing for discrimination.[9] The solution allows for potential separation of blooms in optically simple water.
4) Ecological associations. Blooms of *K. brevis* appear to start and intensify under upwelling favorable winds.[5] This association indicates that coastal blooms appearing under these conditions are more likely to be *K. brevis*.

These methods broaden the potential use of remote sensing and have counterparts with HABs in other regions.

(1) Steidinger, K.A. and K.D. Haddad. *Bioscience* 31: 814-819 (1981).
(2) Keafer, B.A. and D.M. Anderson. In Smayda and Shimizu (eds) *Toxic Phytoplankton Blooms in the Sea*, Elsevier Science p. 763-768 (1993).
(3) Tester, P.A. and R.P. Stumpf. *Journal of Shellfish Research* 17(5): 1469-1471 (1998).
(4) Tester, P.A,. et al. *Limnology and Oceanography*, 36(5): 1053-1061 (1991).
(5) Stumpf, R.P., et al. *Harmful Algae*, 2: 147-160 (2003).
(6) Millie, D.F., et al. *Limnology and Oceanography*, 42(5): 1240-1251 (1997).
(7) Tomlinson, M., et al. *Remote Sensing of Environment* (submitted).
(8) Wynne, T., et al. Coastal Zone 03 Proceedings of the 13th Biennial Coastal Zone Conference; http://www.csc.noaa.gov/cz2003/ (2003).

(9) Cannizzaro, J.P., et al. Abstracts Xth International Conference on Harmful Algae, http://www.xhab2002.com/ p. 43 (2002).

VALIDATING REMOTE SENSING TECHNIQUES FOR DETECTING *Karenia brevis* IN THE GULF OF MEXICO

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Since 1999, the Center for Coastal Monitoring and Assessment (CCMA) has been using ocean color imagery from SeaWiFS to detect and monitor blooms of the toxic dinoflagellate, *Karenia brevis*, in the eastern Gulf of Mexico. Anomalously high chlorophyll is used for detection, where the anomaly is defined as the difference between real-time observations and a two-month running mean of SeaWiFS-derived chlorophyll. This method has proven accurate in detecting blooms >83% of the time along the Florida Panhandle, from Tampa Bay to Cape Romano and the Florida Keys.

Although this technique is successful in detecting blooms in which concentrations exceed 10^5 cells L^{-1}, the level at which marine mammal deaths and respiratory irritation occurs in humans, other techniques may aid in detecting blooms which exceed $0.5x10^5$ cells L^{-1}. Empirical optical techniques show promise in distinguishing between various phytoplankton groups. One optical algorithm distinguishes between *Karenia brevis* and other phytoplankton (such as *Trichodesmium* spp.) by comparing particulate backscattering to chlorophyll *a*; this has potential application in water with low scattering and low colored dissolved organic matter. In addition, the CCMA is developing a technique which separates resuspended benthic algae from the water column phytoplankton. This involves identifying chlorophyll associated with resuspended sediment (as determined by the 670 reflectance), as resuspension events often produce elevated chlorophyll along the Texas coast. The methods are being evaluated to determine their accuracy and reproducibility. These methods offer the potential to expand the detection of *Karenia brevis* to various regions in the Gulf.

STUDY OF THE IMMUNE RESPONSES OF THE BIVALVES *Crassostrea virginica* AND *Argopecten irradians irradians* EXPOSED TO NATURAL AND ARTIFICIAL BLOOMS OF THE DINOFLAGELLATE, *Prorocentrum minimum*

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Under controlled laboratory conditions, juvenile oysters and scallops were exposed to bloom concentrations of a cultured dinoflagellate, *Prorocentrum minimum,* with demonstrated lethal and sub-lethal pathological effects upon these bivalves. Immune status of mollusks was assessed, using flow-cytometric hemocyte analyses for hematological characteristics and several hemocyte functions, periodically during 7 days of continuous exposure. For both oysters and scallops, *P. minimum* exposure had a significant effect upon immune profile, and this effect was dependent upon duration of exposure.

In a second experiment, oysters, *Crassostrea virginica*, from two populations, one from a coastal pond experiencing repeated dinoflagellate blooms ('native'), and the other from another site where blooms have not been observed ('non-native'), were analyzed for cellular immune-system profiles before and during natural and simulated (by adding cultured algae to natural plankton) blooms of the dinoflagellate *P. minimum*. For both populations of oysters, *P. minimum* exposure had a significant effect upon immune profile. Moreover, natural and artificial blooms of *P. minimum* triggered a similar immune response in the oysters, indicating that the artificial bloom was a good simulation of a natural bloom. We believe these are the first reports of immune-system effects of harmful algae upon grazing bivalve mollusks.