

PROJECT DESCRIPTION

RESEARCH OBJECTIVE

The intent of the proposed research is to integrate existing new technologies in autonomous underwater vehicles and an automated, optical-based HAB detection instrument to provide an adaptive survey scheme that will expand the spatial and temporal boundaries for bloom detection and tracking. The system shall consist of a Webb Research Corporation autonomous glider and a WHOI REMUS adapted to accommodate a HAB optical discriminator. The shipboard system shall be a portable instrument to be used on ships involved in harmful algal bloom (HAB) research throughout the Gulf of Mexico and the Eastern U.S. coast. Our efforts will also be to refine our ability to qualitatively/quantitatively identify HAB species in laboratory cultures and field populations. To this end, we will incorporate and refine existing hardware and newly developed numerical methods.

HAB BACKGROUND & IMPACT

HABs occur worldwide, producing direct health problems along with 'trickle down' economic effects on the impacted coastal communities and potentially entire nations through reduction in wild fish stocks and losses in natural and aquacultured fisheries and tourism. Anderson et al. (2000) estimated that national economic losses due to harmful algal blooms between 1987 and 1992 were on the order of \$450,000,000. In the western waters off Florida, blooms of the toxic dinoflagellate *Gymnodinium breve* have occurred annually in the Gulf of Mexico (25 out of 26 years since records have been kept; K. Steidinger, pers. comm.) and the geographic extent of the blooms appears to have increased in recent years. These blooms have resulted in fish kills and reduced commercial seafood production and recreational fishing success along the coastlines of Alabama, Florida, Louisiana, Mississippi, North Carolina and Texas. 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 (Habas and Gilbert, 1974). A bloom of *G. breve* in 1987-88, transported from Florida to North Carolina coastal waters, led to economic losses of 25 million dollars. Similarly the outbreak of brown tides (*Aureococcus Anophagefferans*, Sieburth et al. 1988) in the Northeast have effectively decimated much of the Long Island shell-fish fisheries in the early 1980 (cf. Cosper et al. 1989). It has been suggested that HABs are increasing in frequency in the coastal oceans due to human induced changes in nutrient supply ratios (Smayda 1990). For example, in Tolo harbor, Hong Kong, red-tides have increased in frequency by 8-fold mirroring a 6-fold increase in the human population within the watershed (Lam and Ho 1989). Therefore there is a critical need to document noxious algal blooms over ecologically relevant spatial/temporal scales. This will require long-term monitoring efforts which can delineate the frequency of both phytoplankton and HABs.

Traditional monitoring programs generally detect HAB by visual confirmation (water discoloration and fish kills), illness to fish consumers (Carder and Steward, 1985; Riley et al., 1989; Pierce et al., 1990) and chemical analysis for toxin levels in shellfish samples (Schulman et al., 1990; c.f. Trainor and Baden, 1990) and mouse bioassays (McFarren et al., 1965). In a national plan identifying research and information needs for biotoxins and harmful algae, Anderson et al., (1993) suggested that more effective sampling approaches should involve analyses providing more phytoplankton-specific information allowing for the delineation potentially toxic taxa. Biological oceanographers over the last decade have developed optical instrumentation which can collect data in a non-intrusive manner over biologically relevant spatial/temporal scales in a nonintrusive manner (cf. special optics issues Limnology and

Oceanography 1989: v. 34(8) and Journal of Geophysical Research 1995: v. 100(C7)). *Applying these approaches for the detection of HABs will require that 1) both the sensitivity and spectral resolution of currently available instrumentation is increased at low cost and 2) algorithms which can deconvolute phytoplankton community composition from bulk optical techniques are improved. Using a suite of novel optical instrumentation we propose to assess the degree to which noxious algal blooms can be estimated from a combination of hyperspectral absorption and fluorescence data.*

TECHNICAL & METHODOLOGICAL GOALS

1) To develop in situ and shipboard absorption meters incorporating the new, highly-sensitive Very-Long Pathlength Capillary (VLPC) waveguide flow cell, thereby providing a means for detection (and potential 'early warning') of HABs in coastal waters.

Absorption spectra generated by the existing VLPC technology (World Precision Instruments, Inc, see below) will be validated using an existing suite of more traditional (albeit state-of-the-art) optical sensors, including laboratory spectrophotometers (scanning UV/vis Aminco Dw-2 and Varian DMS-80 spectrophotometers) and submersible field instrumentation (Dual-Path Wavelength Absorption and Attenuation meter, ac-9 which measures spectral absorption/attenuation at 9 wavelengths in the visible spectrum). Laboratory spectrophotometers and the ac-9 are already owned by the PIs. The application of the VLPC for ship-based measurements will provide a sensitivity for absorption spectra that is presently not available even in laboratory spectrophotometers and should increase our ability to deconvolute phytoplankton community composition.

CONCEPTUAL FRAMEWORK

Phytoplankton photopigments and *in vivo* absorption spectra have been used to characterize microalgal biomass, composition, and physiological state (Demers *et al.*, 1991; Johnsen and Sakshaug, 1993; Kroon *et al.*, 1993; Johnsen *et al.*, 1994a; 1994b; Kroon 1994; Millie *et al.*, 1995; Tester *et al.*, 1995). These analyses are complementary to microscopic analyses and are well-suited for assessment programs because they facilitate rapid processing of large numbers of samples acquired over diverse spatial and temporal scales. Combining discrete point (profile) measurements with data acquired from satellite/air-borne sensors also offers the potential for synoptic characterization of HABs. This multi-platform sampling perspective, if incorporated into routine assessment programs (Volent and Johnsen, 1993), could provide an objective early warning for alerting water quality managers to HAB initiation and distribution.

A remarkable confluence of established collaborators exists, centered on an continuing effort to produce an automated, *in situ* instrument to detect the presence of HABs in Florida coastal waters. Research conducted over the past four years by scientists at Mote Marine Laboratory, Rutgers University, the Agricultural Research Service-USDA, and the National Marine Fisheries Service-NOAA (see Millie *et al.*, 1997) has led to an assessment technique that uses the light absorption spectrum of *Gymnodinium breve* to discriminate its presence in mixed phytoplankton populations; methodology which has been applied to recent natural blooms with promising results (Kirkpatrick *et al.* 2000) (see Species Discrimination section and Figure 2 below). A grant by the State of Florida to Mote Marine Laboratory provides for a limited effort to produce a prototype instrument that employs this discrimination technique in an automated mode. The design employs miniature fiber-optic spectrometers manufactured by Ocean Optics,

Inc. (Dunedin, FL) in a capillary liquid waveguide (World Precision Instruments, Inc. and Ocean Optics, Inc.) (D'Sa et al. 1997, Kirkpatrick et al. 2000).

Measurement of Particulate Absorption Spectra

Techniques used presently to determine particulate absorption spectra include the filter pad technique, sample absorption using a spectrophotometer, and an absorption meter using an internally reflecting tube (Zaneveld *et al.*, 1990). In the filter pad technique, the absorption amplification factor for the filter used must be determined with reasonable accuracy and is time consuming. Measurements with the spectrophotometer or the absorption meter suffer from loss of scattered light resulting in the measured absorbance being higher than the true absorbance. Absorption measurements with the spectrophotometer are limited by the short path length of the sampling cell. Increasing the light path length allows detection of samples with low concentrations and low light absorbance (low extinction coefficient). We propose to incorporate and test two technologies for measuring absorbance spectra that will reduce the complications of inadequate pathlength and loss of scattered light. These two technologies are the integrating sphere and the VLPC wave-guide flow cell.

Very-Long Pathlength Cell (VLPC) Absorption Measurement: Until recently, aqueous liquid core waveguide technology has not been successful as no material was available to construct a capillary tube with a refractive index lower than that of water (Dasgupta 1984, Fujiwara 1988). Coating of the internal walls of a “long capillary cell” with a silver metal mirror, Dasgupta reported nearly a million fold loss of light intensity in a glass capillary cell 10 cm long. WPI recently patented the VLPC, which is an ‘aqueous waveguide’ used to measure spectral absorption using a waveguide action through a rigid capillary having a refractive index less than that of water. In the VLPC, the excitation light is axially introduced into a capillary flow cell via a fiber optic with the light being constrained within the VLPC by the waveguide action. At the other end of the waveguide, a detection fiber conducts the light that is not absorbed by the aqueous medium to a CCD detector (Ocean Optics, Inc.). The length of the waveguide will be determined by the highest sensitivity required for the application. A prototype waveguide of 0.5 m has been tested in the laboratory and has been developed for applications in chromatography. Recently, laboratory experiments in pure water from different sources and of seawater has indicated that the VLPC has potential applications in water quality management and seawater analysis. A trial study with a sample of *Gymnodinium breve* showed a remarkably good similarity between the VLPC and the standard quantitative filter-pad technique (QFT) especially considering the optical resolution of the VLPC was not optimized for comparison with the QFT (Figure 1). Based on light loss estimates, a waveguide of up to 50 m length is feasible. The present technology used in the VLPC (Liu 1996, 1995a, 1995b) is ideally suited for *in situ* seawater and/or freshwater analysis of bio-optical properties. Some important advantages of absorption measurements using the VLPC include: 1) high sensitivity enabling *in situ* spectral absorption measurements at very low pigment concentrations, 2) due to the waveguide action, forward scattered light will be measured by the detector thus minimizing scattered losses in presence of particulate matter, 3) the development of an *in situ* VLPC will eliminate the need to take water samples for laboratory analysis and enable rapid shipboard mapping of large coastal areas for detection of harmful algal blooms in the presence of mixed phytoplankton populations, and 4) the measurements are instantaneous across the spectra.

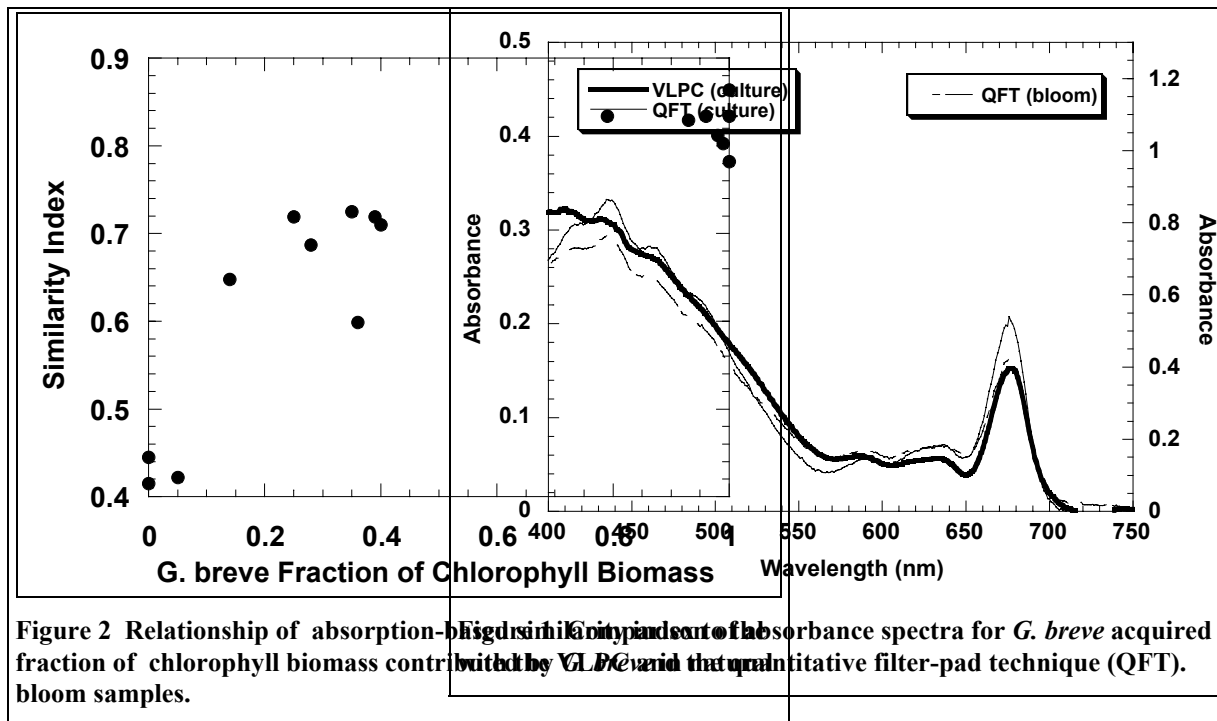


Figure 2 Relationship of absorption-based similarity index and absorbance spectra for *G. breve* acquired fraction of chlorophyll biomass contributed by VLPC and quantitative filter-pad technique (QFT) bloom samples.

Absorption Spectra Based Species Discrimination

Identification of phytoplankton on the basis in vivo absorption spectra depends on the pigment composition which is variable due to photoacclimation and the number of wavelengths which are measured (Soohoo *et al.* 1986, Bidigare *et al.* 1987, 1989, Sathyendranath *et al.* 1987, Hoepffner & Sathyendranath 1991, Johnsen *et al.* 1994). These studies have suggested the utility of stepwise discriminant analyses and that it is possible to discriminate the three major spectral classes of phytoplankton (Johnsen *et al.* 1994). While discrimination of phycobilin and chlorophyll *b* containing algae can be achieved given a limited number of wavelengths, the spectral dependency in absorption properties of chlorophyll *c*-containing algae varies little among taxa. This makes it difficult to discern the contribution of accessory chlorophylls and carotenoids from the “background” absorption associated from other taxa. As such, the use of absorption spectra alone may not identify the contribution of a specific chlorophyll *c*-containing taxon in a mixed natural assemblage dominated by chromophytes. To exaggerate the minor differences in accessory photosynthetic pigments between chlorophyll *c*-containing algae we combined a similarity algorithm in conjunction with fourth-derivative analysis (Butler and Hopkins 1970, Bidigare *et al.* 1989; Smith and Alberte 1994, Millie *et al.* 1995) of absorption data. This technique was able to discriminate *G. breve* from natural mixed populations in Sarasota Bay, FL with promising results (Fig. 2, Millie *et al.* 1997). This suggests that absorption measurements could be incorporated into coastal monitoring programs, thereby providing the capabilities for identifying and characterizing blooms of *G. breve* and potentially, other HAB species. We wish to expand our initial study to rigorously ascertain the degree which these algorithms can extract the respective concentrations of chlorophyll *c*-containing algae in mixed laboratory and field populations.

METHODOLOGICAL APPROACH

Instrument Development

VLPC: The VLPC waveguide in its present form has only been used and demonstrated to work in the laboratory and numerous improvements in the waveguide itself need to be implemented before it can be used in the ocean environment. As currently configured the VLPC is linked to a laboratory instrument that contains the light source and the fiber-optic spectrometer. That instrument is itself connected to a desktop computer. This configuration will be appropriate for some of the laboratory investigations, but will require revision prior to field applications. The VLPC will be incorporated with an existing spectrometer/computer system (Kirkpatrick *et al.*, 1997) and a strobed fiber-optic light source to achieve a portable, field instrument. Some other anticipated refinements include: a sturdier and a larger diameter waveguide, watertight system packaging, water pumping and filtering, and provision for antifouling of the waveguide. In addition, a wound waveguide may have to be used instead of the present straight waveguide to increase pathlength will simultaneously reducing the size of the *in situ* instrument.

The effects of multiple scattering due to cell concentration and the waveguide action of the VLPC will be evaluated. The range of linear relationship between cell concentration and measured absorption will be determined using serial dilutions of laboratory cultures. This study will establish the useful upper limit of cell concentration for the VLPC as well as establish limits of detection at the lower level of cell concentrations.

Field Validation

Field studies will have two goals. The first goal is to modify current instrument prototypes for field work. Current designs must be ruggedized for field work and this will be the focus of development efforts during year one as laboratory studies are being conducted. The second goal is to assess if laboratory-derived discrimination algorithms can be applied to natural field populations. Field work will utilize small research vessels available at Mote Marine Laboratory, RSMAS and Rutgers Tuckerton Marine Field Station. Estimated absorption by the ruggedized light pipette system will be compared to traditional field instrumentation with the goal of comparing *in situ* absorption spectra over a wide spatial gradient of optical water types.

Initial field work will be conducted in the oligotrophic waters of the Gulf of Mexico. Mote Marine Laboratory currently has in place a *G. breve* monitoring program, and thus we will have the opportunity to partake in targeted field sampling for waters dominated by *G. breve*. These waters will be compared to waters where *G. breve* is a minor constituent. Field trials off the coast of New Jersey, in the Florida Straits and Florida Bay will be conducted during year 2 and 3. The transects off of New Jersey will be conducted during the summer months which is dominated by episodic upwelling. Upwelling off New Jersey has been linked to bottom water hypoxia/anoxia and preliminary pigmentation data from years 1995-1997 (Schofield unpublished data) suggest that it is time this time of the year when red and green tides are most prevalent. When possible, the Rutgers surveys will also be coordinated with routine sampling conducted by the New Jersey Department of Environmental Protection.

In situ spectral absorption and beam attenuation at 9 wavelengths will be determined with a Wetlabs Dual-Path Wavelength Absorption and Attenuation meter (ac-9). Repetitive profiles

with and without filters placed on the intakes will allow determination of the inherent optical properties for the dissolved and particulate phases. The ac-9 will undergo routine calibrations by the manufacturer. The stability of the instrument output during mounting and field operations will be monitored using air-tracking and field water calibrations according to the manufacturer's recommended protocols (WET Labs, Inc., ac-9 Protocol, 10/23/96). The relative success of deconvoluting community composition by the ac-9 is limited given that it measures only 9 wavelengths. During the transects, discrete samples will be collected by Niskin bottle for phytoplankton pigmentation analyses and microscopic determination of cell abundances. Up to eight depths will be sampled for each location where a vertical absorption profile will be taken. For microscopic enumeration, phytoplankton samples will be preserved with Lugol's iodine solution and counted with a Fisher Micromaster research microscope. The composite dataset will be used to test the utility of the species-discrimination algorithms.

MILESTONES AND RESPONSIBILITIES

Time Table	Milestone	Responsibility
First year	Miniaturize of HAB optical discriminator Adapt Webb glider for HAB discriminator Adapt REMUS for HAB discriminator Theoretical treatment of capillary liquid waveguide	Kirkpatrick Webb Research Corp. Moline Schofield
Second year	Integration of instruments for shipboard Field trials Evaluation of field trials & refinement Packaging for <i>in situ</i> deployments	D'Sa, Hitchcock, Kirkpatrick As appropriate for location All D'Sa, Hitchcock, Kirkpatrick

ANTICIPATED RESULTS

This proposal will provide a comprehensive analysis of the power of the newest hyperspectral optical instrumentation to potentially document the presence of harmful algal blooms (HABs). The proposal will rigorously test the potential of these approaches for all major classes of noxious algae (red, green, brown, and cyanobacteria) and thus results will be directly applicable to both freshwater and coastal water quality managers. Several reasons justify support to explore the potential of developing the automated optical system for HABs. The most important issue is that our ability to monitor noxious algal blooms on ecologically relevant scales is woefully inadequate. This compromises our basic understanding of the physical, chemical, and biological factors which potentially regulate the onset and persistence of these blooms. The high visibility and large economic impacts of HABs has often resulted in a public demand for the government to provide some means to mitigate the adverse impacts caused by the unexpected appearance of HABs in coastal waters. Initially the development of these technologies may provide an effective early warning tool. Though early warning may not provide the “magic bullet” that the public is demanding, it will provide better information to guide reporting and may help reduce some of the potential harm of HABs. Local governments and private enterprises along the coast will be able to reschedule workforces to optimize prevention/cleanup efforts. Control or containment efforts can be applied in a much more refined manner, minimizing the costs and undesirable environmental impacts. Fisheries (both wild stocks and aquaculture enterprises) would also benefit from the early warning of harmful algal blooms. State governments will have more timely and accurate information to allow optimal closure of shellfish harvesting. Aquaculture operations will have time to make decisions about harvesting crops and/or closing natural water intact prior to contamination. Most importantly, the development of these optical sensors and discrimination algorithms may provide central tools for documenting long term changes in the frequency of HABs if deployed into already existing water quality monitoring networks.

DATA POLICY

As a condition of receiving funding under the ECOHAB program all Principal Investigators involved in this proposal agree to follow all Coastal Ocean Processes Data Policies as they pertain to the data collected for the ECOHAB program.

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