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DEVELOPMENT OF A MULTI-SENSOR IN SITU FIBER OPTIC FLUOROMETER

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ABSTRACT

As part of the Department of Energy (DOE) Ocean Margins Program (OMP), we have developed an autonomous multi-sensor in situ fiber optic fluorometer for estimating the distribution and abundance of particulate pigment biomass. The instrument is designed to sample and store in vivo strobe-stimulated fluorescence data at multiple depths and high frequencies (1 Hz). It is unique in that it uses fiber optic technology to increase vertical resolution. The design of the instrument is such that high frequency, high vertical resolution pigment fluorescence data can be achieved at relatively low cost as compared to the deployment of multiple commercial instruments. In addition, the small size and flexibility in positioning of the fiber optic sensors permit fluorescence measurements to be made with a high degree of spatial resolution and minimal interference with other sensors. This makes the instrument particularly well suited for characterizing gradients in pigment fluorescence in the benthic boundary layer, where significant lateral transport of particulate organic matter may occur (Walsh et al., 1988). Initial results from two separate deployments have revealed highly variable and vertically heterogeneous near-bottom pigment distributions in coastal waters of the Gulf of Mexico and the U.S. eastern inner continental shelf near Cape Hatteras. This information will be related to measurements of the current velocity structure to enable determinations of transport of pigments in the benthic boundary layer.

SCOPE OF INVESTIGATION

Goals of the Department of Energy's (DOE) Ocean Margins Program (OMP) include quantification of primary production and flux of phytoplankton biomass on the continental shelf and determination of controlling mechanisms. To accomplish these objectives, high resolution time series observations of the distribution and abundance of particulate pigment biomass will be required. Discrete shipboard sampling is inadequate to fully resolve variations in particulate pigment biomass related to changing environmental conditions. Many important physical variables have time scales ranging from minutes to hours. Hence, the coarse temporal resolution provided by discrete sampling techniques may lead to aliasing (e.g., Dickey et al., 1991; Marra, 1994). Furthermore, shipboard studies have limited duration and may miss episodic (e.g. weather related) as well as seasonal scale events.

The utility of high resolution time-series measurements of pigment distributions for characterizing transport of pigments on the northeast U.S. continental shelf has been demonstrated in several previous studies (Falkowski et al., 1988; Walsh et al., 1988; SEEP II papers in press). Based on moored fluorometer data as well as discrete shipboard measurements during spring of 1984, Falkowski et al. (1988) observed that phytoplankton pigments were higher in shelf waters than offshore and chlorophyll

concentrations show an increase typical of the spring bloom in this region (Malone et al., 1983). Vertical distributions of chlorophyll tended to be homogeneously distributed following storms, but showed an accumulation near the bottom in inner and mid-shelf waters between periods of strong winds and storms. Falkowski et al. (1988) concluded that transport of chlorophyll from shelf to slope was not significant in the northeastern Mid-Atlantic Bight. However, observations of an accumulation of phytoplankton pigments in a near bottom nepheloid layer as the spring bloom season progressed indicated the likelihood of sinking of water column pigments to the bottom. Furthermore, Walsh et al. (1988) argued in favor of a significant seaward export of chlorophyll and carbon off the shelf with as much as 50% of this flux associated with lateral injections of near-bottom particles originating on the inner shelf. Yoder & Ishimaru (1989) cited evidence for advection of phytoplankton biomass along the bottom off the southeastern U.S. continental shelf in association with upwelling and subsequent advection of cooled upwelled water off the shelf. Recent observations of vertical profiles of chlorophyll fluorescence in shelf waters off Cape Hatteras during summer 1993 have provided further evidence for lateral transport of pigment maxima associated with a benthic boundary layer (M. Wood, personal communication and EOS 75:324, 1994).

The previous studies leave many unanswered questions regarding the sources of pigments in the benthic boundary layer, the mechanisms for transport in association with near-bottom flows, and the quantitative significance of carbon export off the shelf via lateral transport along the bottom. Previous time-series observations of near bottom fluorescence may have lacked the necessary resolution to adequately describe vertical gradients in pigments and their temporal and spatial relationships to the current regime. For example, Thomsen et al. (1994) observed a gradient in chlorophyll *a* in a Barents Sea benthic boundary layer. Measurements of this gradient in conjunction with current measurements provided evidence for dramatic changes in horizontal flux of chlorophyll with varying distance from the bottom.

PROGRESS TO DATE

Major goals of this 3 year project have included the following:

- 1) to develop a multi-sensor fiber optic fluorometer capable of autonomous collection of data at a moored location for long term (weeks to months) deployments, and,
- 2) to deploy the instrument in the benthic boundary layer with the purpose of examining the hypothesis that temporally variable vertical gradients exist near the bottom in coastal waters.

In Year 1, a PC-interfaced single sensor unit was developed and used to optimize

hardware and sensor design and to evaluate fiber performance and instrument detection limits. A prototype multi-sensor autonomous version was built and tested in Year 2. Results from these earlier efforts have been described in previous progress reports and provided the basic knowledge required to develop a working instrument. In Year 3, an improved version of the multi-sensor fiber optic fluorometer was constructed and deployed in the benthic boundary layer in coastal waters of the northern Gulf of Mexico and inner shelf of U.S. eastern coastal waters near Cape Hatteras. In this progress report, we have described the most recent instrument design and its performance. We have then discussed field results that were obtained with the instrument, as well as data obtained by other methods, that support the view that near bottom pigment distributions are vertically heterogenous and temporally variable. We have also examined the relationships among pigment concentrations, pigment fluorescence and particulate organic carbon distributions in an effort to determine the potential for using fluorescence to trace distributions of particulate organic carbon.

Autonomous Multi-sensor Fiber Optic Fluorometer

Description of the instrument: The current version of the multi-sensor fiber optic fluorometer is capable of autonomously measuring and storing fluorescence data at eight different depths. A schematic of the fluorometer is shown in Fig. 5 of Appendix I, and illustrates the optical, electronic and mechanical systems. The optical system is comprised of 4 strobes, 2 photomultiplier tubes (PMTs) and 8 optical sensors. Each strobe (EG&G) has a front end lens and filter assembly, that is coupled to a connector with a pair of excitation fibers each leading to an optical sensor. The detection fibers convey the fluorescence emission from the optical sensors to one of the two PMTs (Hamamatsu HC-125). The intensity of the strobe was monitored with a phototransistor. Fiber lengths from the sensors to the instrument casing can be varied over depth ranges of up to 70 m. The optical fiber used in the fluorometer is a 600 μm silica core/15 μm silica clad step-index fiber (3M FT-600-UMT) with broad spectral transmission characteristics. Fiber optic penetrators were designed specifically for this application to provide an optical path between the inside of the pressure casing and the external environment (i.e., from the sensors to the strobes and the PMTs). The penetrators use a 600 μm optical fiber as an integral waveguide within a metal housing. Interior and exterior SMA fiber optic couplings provide mounting capability to the fibers. Laboratory tests have shown optical coupling losses of the penetrator. These have been reduced by using index matching liquid (Norland P/N 9000) to fill the gap between the fiber tips and the penetrator optics. An O-ring sealed connector and receptacle was used to make the penetrator optics watertight. The instrument pressure housing is constructed of standard 6061 Al alloy cylindrical tubing (dimensions: 40 inches length and 6 inches diameter) and has flat end plates fitted

with pressure rated double 'O' ring type seals.

The system is capable of autonomous operation controlled by a Tattletale 7 microcomputer with 213 Mb hard disk drive for data storage. The fluorometer can be deployed for up to 120 days using internal batteries and on-board data acquisition and control. The data acquisition system has a 500 kHz analog-to-digital converter (Ocean Optics PC1000) that digitizes the fluorescence signal from the 2 PMTs. Control software for initiating the strobes, multiplexing the PMTs and the data stream, signal pre-processing, and data recording was developed in 'Aztec-C' language. Capabilities of the current version of the software include reading of the "background" signal prior to excitation, thus correcting for ambient light effects and instrument noise and adjustable signal averaging.

Sensor Design: The optical sensor for the fluorometer is made up of a pair of bare polished fiber ends of the excitation and emission fibers that are oriented at a 30° angle to each other. The active sampling volume is a cone defined by the numerical aperture and the fiber core diameter (see Appendix I for further discussion). The fiber optic sensor, as currently designed, has a detection volume of about 1 mm³. We have developed a flowthrough sensor housing (Fig. 1) that serves both to protect the fibers from impact as well as to eliminate ambient light effects.

Filter Selection: Various combinations of both excitation and emission filters have been examined in order to achieve maximal reduction in backscattered excitation while at the same time optimizing the transmission of the desired fluorescence signal. A blue (455 nm) interference filter (Omega 455DF70) with 10 nm bandwidth [full width at half-maximum (FWHM) intensity] was used at the strobe. At the detector end, a combination of a red (675 nm) interference filter (Edmond G43140) also with a FWHM bandwidth of 10 nm and a 665 nm high pass filter (Rolyn RG665) was used.

Instrument Calibration and Performance: The instrument was calibrated at different concentrations with a laboratory phytoplankton culture (*Nannochloris atomis* Butcher, clone GSB Nanno from the Bigelow Laboratories Culture Collection of Marine Phytoplankton). The calibration was performed using a series of dilutions in artificial seawater. The initial chlorophyll concentration of the culture was determined by the fluorometric method of Holm-Hansen et al. (1965). The calibration results differed between the sensors (Figs. 2a and 2b) for various reasons including differences in sensor construction, differential losses along the light path, and differences in PMT performance.

PMT performance was found to have a large impact on sensor response. Though the PMTs were selected to have high current amplification, variations from unit to unit did occur. The PMT for sensors 1-4 had a gain factor of 270,000 while that for sensors 5-8 had a gain factor of 600,000 (the PMT gain factor is the ratio of

anode luminous sensitivity/cathode luminous sensitivity). It is apparent from Fig. 2b that the PMT for sensors 5-8 saturated at high chlorophyll concentrations due to its higher gain. The dynamic ranges for sensors 1-4 were apparently greater (Fig. 2a), but detection limits for sensors 1-4 (Fig. 3a) were higher than those for sensors 5-8 (Fig. 3b). Planned modifications in future versions of the instrument will provide for both lower detection limits and greater dynamic range. Detection limits will be reduced by increasing excitation throughput using strobes (Hamamatsu) equipped with elliptical reflectors. The use of programmable gain as a function of concentration will expand dynamic range without compromising detection limits (see accompanying renewal proposal for further discussion). Additional adjustments to gain may be made electronically to the PMT amplifier circuit. Such adjustments can modify the frequency bandwidth of the PMT response. For our application, we have found that use of a smaller bandwidth (*e.g.*, 20 KHz) optimized the signal to noise ratio for our application.

Additional laboratory experiments are currently being conducted to evaluate various aspects of instrument performance. The stability of instrument calibrations over time has been examined by comparisons of periodic laboratory calibrations. Preliminary results indicate excellent reproducibility of calibrations under laboratory conditions. However, factors such as long-term effects of seawater on fiber and sensor performance are still being evaluated. Other work is being conducted to determine the relative impact of factors contributing to variations in fluorescence yield (*R*, the ratio of the fluorescence signal to the chlorophyll concentration within the fluorometer detection volume). Variations in fluorescence yield have been attributed to the following (*cf.* Falkowski and Kiefer, 1985; Strass, 1990 and references within):

- Species composition
- Light history and photoadaptive state
- Nutrient supply
- Physiological state and endogenous rhythms

Besides these factors related to phytoplankton physiology, fluorescence yield may be sensitive to variations of the irradiance within the detection volume, which is subject to changes in particle concentrations and size distributions (Morel and Bricaud, 1981; Baker and Lavelle, 1984; Collins et al., 1985). In addition, fluorescence yield will be affected by the presence of fluorescent substances other than chlorophyll *a*, such as phaeopigments (*e.g.*, Herbrand, 1978; 1988).

Efforts are continuing to evaluate the relative importance of each of these factors in influencing fluorescence yield and instrument response. A preliminary evaluation of species dependence of calibrations was made using log phase batch cultures of 3 phytoplankton species including *N. atomis* (chlorophyte), *Thalassiosira* sp. (diatom), and *Amphidinium carterae* (dinoflagellate). Calibration curves were found to be quite similar, although variances were larger for the diatom and dinoflagellate

(data not shown) possibly due to the larger cell size. Further laboratory evaluations will involve studies of the potential magnitude of algal physiological effects and the dependence of instrument response on particle size and concentration, sampling rate and signal averaging (see *Additional Activities in Year 3*). The relationship between fluorescence yield and presence of pigments other than chlorophyll *a* will be examined by comparison of *in situ* fluorescence measurements made in the field and HPLC pigment analyses of associated water samples.

Field Deployments of the Multi-sensor Fiber Optic Fluorometer

Gulf of Mexico: The multi-sensor fiber optic fluorometer was deployed in May 1994 during a cruise on the R/V *Pelican* to investigate temporal variability in near bottom pigment gradients. The fluorometer was attached to an aluminum frame and lowered to the sea bottom in 10 m waters off the Louisiana shelf near the Mississippi River plume (29° 13.68'N, 89° 40.04'W). The sensors were mounted at different depths from the sea bottom and 10 point averages of fluorescence (~1 Hz sampling rate) were recorded at 5 minute intervals over a period of 8 h. The time series (Fig. 10 in Appendix I) revealed large variations in pigment fluorescence that included the appearance of a distinct localized maximum about 1.6 m off the bottom. Vertical profiles of density (σ_t), fluorescence and beam attenuation (Fig. 11 in Appendix I) revealed a strong density gradient near this depth coinciding with a maximum in beam transmission. However, the profiling SeaTech fluorometer did not show a peak at this depth and we have no clear explanation for this. The multi-sensor fluorometer was calibrated before and during the cruise and found to be stable. The possibility that the signal was due to an artifact of high turbidity was investigated subsequently in the laboratory. Tests indicated only a marginal effect on the instrument response in the presence of nonfluorescing particulate matter. One explanation may be that the dimensions of the layer and the sampling rate of the SeaTech fluorometer were such that the feature went undetected. The ability of a fiber optic sensor to measure fine structure of the water column may have been required to resolve such a feature. Alternatively, the high turbidity of the layer may have interfered with the SeaTech's ability to detect a signal. At present, these ideas are only speculative and further evaluation of the performance of both instruments is required.

Chlorophyll Fluorescence of the Benthic Boundary Layer at Cape Hatteras: The multi-sensor fluorometer was deployed in conjunction with a BASS tripod (in collaboration with Sandy Williams and Jim Churchill, WHOI) at a 20 m site off Duck, NC (36° 11.93'N, 75° 42.25'W) (Fig. 4). The deployment began on 23 July 1994 (year day 204). Background-corrected 10 point averages were acquired every 40 min for each sensor. The data record stopped after 30 d due to battery limitations. The instrument was recovered on 1 Sep 1994 (year day 244). Sensors were inspected for fouling.

While there was some fouling on the external sensor housings, there was minimal fouling visible on the fiber optic faces. For this deployment, no treatment for fouling was used. Based on these observations, we believe that chemical treatment (*e.g.*, Whitley & Wirick, 1983; Dickey et al., 1991) of the external sensor housing and internal surfaces of the sensor (with the exception of the fiber faces) should be effective at controlling biofouling.

Time-series of near-bottom pigment concentrations (Fig. 5) revealed transient episodes of very high pigment concentrations and vertically heterogeneous distributions in the lower water column. Following year day 219, a large signal was detected by the upper three sensors, but was smaller or absent at the lower depths. The analysis of the time-series data is currently in progress. Preliminary results are briefly summarized below. The autocorrelation of each sensor time-series for a 1 point lag (40 min time period) was highly significant ranging from 0.811-0.875 (Table 1); this was an indication that temporal variations were adequately resolved by this sampling rate. Planned modifications in instrument design (see accompanying proposal) should reduce power demands and this, along with an anticipated increase in battery storage capacity, will allow us to increase the sampling rate and thereby resolve higher frequency variations. Autocorrelations were positive for time lags of up to 10 h for most sensors, and for some sensors for up to 20 h (Table 1). Cross correlations at zero point lags between adjacent sensors were positive and significant (Table 2); results ranged from $r=0.243$ for the 443 and 341 cm sensors to $r=0.533$ for the 116 and 58 cm sensors. Even for the larger separation distances between the 443, 189 and 14 cm sensors, cross correlations were found to be positive and significant. However, the value of r was generally higher for more closely spaced sensors. From this, we inferred that variations at adjacent sampling depths were affected to some degree by common factors, but similarities decreased with increasing distance between sensors. Power spectra (Fig. 6) were examined for 4 of the 8 sensors and were characterized by several peaks around 1 cpd, suggesting a diurnal component to the observed variation. Additional peaks were also observed at higher and lower frequencies.

Factors that could introduce periodic variations in pigment concentrations include tidal motions as well as growth and grazing of phytoplankton (*e.g.*, Whitley & Wirick, 1986). Variations in fluorescence yield, R , must also be considered (see discussion in Instrument Calibration and Performance).

Table 1. Autocorrelations of pigment fluorescence time-series for different time lags. Sampling rate was every 40 min. All values of r were greater than zero at a probability level of $P=0.05$, with the exception of values marked with "*".

Distances from the Bottom (cm)	Time Lag	r	SE	N
443	40 min	0.875	0.031	1021
443	10 h	0.336	0.098	1007
443	20 h	0.271	0.110	992
341	40 min	0.838	0.031	1021
341	10 h	0.251	0.090	1007
341	20 h	0.152*	0.097	992
256	40 min	0.854	0.031	1021
256	10 h	0.170	0.086	1007
256	20 h	0.137*	0.088	992
189	40 min	0.837	0.031	1021
189	10 h	0.245	0.087	1007
189	20 h	0.269	0.095	992
116	40 min	0.868	0.031	1021
116	10 h	0.345	0.102	1007
116	20 h	0.345	0.116	992
58	40 min	0.873	0.031	1021
58	10 h	0.306	0.094	1007
58	20 h	0.198	0.100	992
27	40 min	0.844	0.031	1021
27	10 h	0.364	0.102	1007
27	20 h	0.290	0.114	992
14	40 min	0.811	0.031	1021
14	10 h	0.481	0.099	1007
14	20 h	0.472	0.129	992

Table 2. Cross-correlations (zero point lags) of pigment fluorescence time-series between selected depths (N = 1022).

Distances from the Bottom (cm)		r	SE
Upper	Lower		
443	341	0.243	0.031
341	256	0.273	0.031
256	189	0.342	0.031
189	116	0.518	0.031
116	58	0.533	0.031
58	27	0.381	0.031
27	14	0.477	0.031
443	189	0.194	0.031
443	14	0.176	0.031
189	14	0.345	0.031
116	14	0.467	0.031

Further analysis of our data in conjunction with those collected by other investigators will help in understanding the causes of the observed variations in our data. Comparisons of the fluorometer time-series with the BASS data will allow us to identify relationships between the pigment variations and wind- and wave-induced currents and tidal motion. Quantification of the horizontal transport of pigments in the benthic boundary layer can be achieved by comparison of vertical gradients in fluorescence with gradients in velocity obtained from the BASS. In addition, it will be possible to examine the relative importance of sediment resuspension events in contributing to variations in near-bottom pigment concentrations and horizontal transport. Information obtained by other investigators during this same time period may also prove valuable in interpreting our results. For example, a preliminary comparison was made of our time-series measurements with dissolved oxygen and salinity time-series obtained by Creighton Wirick at a 25 m site (36° 15.0'N, 75° 35.36'W) close to our location. From visual inspection of plotted data, it was apparent that periods of high dissolved oxygen and low salinity coincided with episodes of high fluorescence. This led to speculation that Chesapeake Bay outflow may have been

a factor in the variations we observed. Very high values of pigment concentrations have been observed previously in Chesapeake Bay waters (*e.g.*, Flemer, 1969).

Water Column Pigment, Fluorescence and POC Relationships at Cape Hatteras: Vertical profiles of density (σ_t), beam c , fluorescence, extracted pigments and particulate organic carbon (POC) at the BASS site showed substantial temporal variability. Relatively low pigment concentrations with no distinct subsurface vertical features were observed at the time of deployment (23 July, Fig. 7). On recovery (1 September), distinct subsurface pigment maxima were observed and vertical distributions changed over a 19 h period (*cf.* Figs. 8 and 9). HPLC pigment analyses indicated that the dominant accessory pigment was fucoxanthin (indicative of diatoms) on 23 July (Fig. 10a) and peridinin (indicative of dinoflagellates) on 1 September (Figs. 10b and 10c). Diver observations on 1 September corroborated the presence of a thin (5-10 cm) layer of particles at the thermocline, apparently dinoflagellates.

These observations provide evidence to support the presence of high and temporally variable concentrations of pigments as estimated from the multi-sensor fluorometer time-series (Fig. 5). Measured concentrations of pigments in the dinoflagellate layer (Fig. 9) exceeded $90 \mu\text{g l}^{-1}$. Because water samples were collected with 10 l Niskin bottles, the thin dinoflagellate layer would have been substantially diluted in the bottle before the analysis. Therefore, it is likely that *in situ* concentrations were higher. Another potential source of pigments to the benthic boundary layer was from the sediments. A high concentration of pigments was found in surficial sediments collected by divers on 23 July (Table 3). This possibly originated from either phytoplankton sinking or benthic primary production. Surficial sediment concentrations were considerably lower on 1 September 1994 (Table 3). The decrease in sediment pigment content from 23 July to 1 September 1994 may have resulted from resuspension and redistribution of pigments. Alternatively, pigments may have been consumed by benthic animals or degraded by microbial activity. Chlorophyll/phaeopigment ratios were consistently low in the sediments, in contrast to the high ratios associated with the subsurface pigment maxima (Fig. 11). Thus, it is unlikely that resuspended sediment pigments were a significant proportion of the pigment features observed in the water column profiles (Figs. 8-9). However, our observations of large and variable amounts of pigments present in the sediments support arguments that periodic resuspension events (*e.g.*, associated with storms) may contribute significantly to horizontal transport of pigments along the shelf. More extensive characterization of sediment pigment distributions and composition, including analysis of core sections, would provide useful information for efforts to determine the importance of resuspension as a source of pigments into the benthic boundary layer.

Table 3. Pigment and POC Inventories at the BASS Site.

Table 3. Pigment and POC Inventories at the BASS Site.								
Bottom	Lat	Lon	Water Column			Sediments (upper 3 cm)		
Depth	N	W	Pigments	POC	POC/Pigment	Pigments	POC	POC/Pigment
(m)			mg m ⁻²	mg m ⁻²		mg m ⁻²	mg m ⁻²	
			R/V Cape Hatteras, 7-23-94					
20	36.197	75.710	121	9000	74.4	210	53350	254
25	36.251	75.589	47.2	5072	107	261	38400	147
			R/V Endeavour, 9-01-94					
20	36.199	75.704	86.1	5841	67.8	54.5	31184	572
20	36.196	75.711	268	13634	51	-	-	-

Because the goals of the DOE Ocean Margins Program center on understanding processes influencing carbon transformation and transport, we examined relationships between particulate organic carbon (POC) and pigment concentrations in an effort to determine the potential for using pigment fluorescence to trace distributions of POC. In principle, pigment concentrations can be assessed using fluorescence, recognizing that variations in fluorescence yield, *R*, can occur as discussed previously. We found that extracted pigments were strongly correlated with *in situ* fluorescence measured with the SeaTech profiling fluorometer for both recovery and deployment cruises (Fig. 12). Because different profiling fluorometers were used on the deployment and recovery cruises, it was not appropriate to compare fluorescence yields between cruises. For future operations, fluorescence yield will be evaluated using a Turner Designs Model 10 calibrated to a stable reference. POC concentrations were analyzed by filtration of water samples (1-2 l) onto 0.45 μ m 25 mm silver filters (Poretics). The filters were dried and subsequently analyzed with a Carlo-Erba Carbon/Nitrogen Analyzer. We found POC concentrations to be significantly correlated with extracted pigment concentrations (Fig. 13a) even when the very high concentrations associated with the dinoflagellate subsurface maximum were excluded (Fig. 13b). Suspended particulate matter (SPM) concentrations were less strongly related to POC concentrations. The strong correlation observed between POC and extracted pigments is an encouraging observation for prospects of using fluorescence as a tracer of POC distributions and transport. An approach for dynamic calibration of the multi-sensor

fluorometer with POC is proposed through the simultaneous deployment of an *in situ* particle/POC sampler (see accompanying proposal).

Additional Activities in Year 3

Data analysis and publication: The analysis of data obtained from field operations in both the Gulf of Mexico and off Cape Hatteras is in progress and will continue throughout the current budget period. Collaboration with the WHOI BASS investigators is planned to identify relationships between the pigment variations and wind- and wave-induced currents and tidal motion. Additional objectives will include quantification of the horizontal transport of pigments in the benthic boundary layer by comparison of vertical gradients in fluorescence with gradients in velocity obtained from the BASS. In addition, the importance of sediment resuspension events in contributing to variations in near-bottom pigment concentrations and horizontal transport will be examined. Information obtained by other investigators during this same time period may also prove valuable in interpreting our results and such interactions will be explored.

In addition to information already published (D'Sa et al., 1994, Appendix I), manuscripts describing the multi-sensor fiber optic fluorometer and results obtained will be prepared and submitted for publication. In addition, results will be presented at scientific meetings.

Further Evaluation of Instrument Performance: During the remainder of this current budget period, we will continue to evaluate various aspects of instrument performance. We are currently conducting experiments to determine the inherent stability of the instrument through comparisons of periodic calibrations. Another question currently being evaluated is the effect of particle size on the fluorescence signal. The high resolution of the sensor is an advantage for fine scale characterizations of pigment gradients. However, the small detection volume raises the question of a possible dependence on changes in particle concentrations and size distributions. To evaluate any potential dependence of fluorescence measurements on particle size and detection volume, we plan to take advantage of the flexibility of the fiber optic technology. The system will allow us to increase the effective detection volume by ganging multiple fibers within a single sensor. The signal derived from individual fibers can be compared to the integrated signal received by the complete set of fibers. Assuming that the larger volume represents the "ideal" response, the differences between the two measures can be investigated in the laboratory as a function of particle size and concentration (using different algal species and fluorescent beads), sampling rate and signal averaging. The empirical data will be compared with Monte Carlo simulations of effects of particle size on probability of detection.

Improvements to Instrument Design: Various improvements to instrument design will be examined and tested. Specific details are given in the accompanying renewal proposal.

ACTIVITIES OF PRINCIPAL INVESTIGATORS

S. E. Lohrenz (USM)

Dr. Lohrenz is co-principal investigator on the project and primary point of contact for the contract. He has been responsible for administrative operations. He has been directly involved in consultations and decisions regarding design options of the instrument. He also served as the project representative at program planning meetings. Dr. Lohrenz has had extensive experience in working with marine algal cultures, photosynthetic pigment analyses, and in the measurement of in vivo fluorescence in natural populations. He will oversee evaluation of instrument performance and calibration procedures in collaboration with Dr. V. L. Asper. Dr. Lohrenz has and will continue to devote 20% of his time to this project.

V. L. Asper (USM)

Dr. Asper is the other co-principal investigator. He has been directly involved in consultations and decisions regarding design options and participated in field trials of instruments. Dr. Asper has had extensive experience in constructing and deploying oceanographic instrumentation in various mooring configurations. He will oversee evaluation of instrument performance and calibration procedures in collaboration with Dr. Lohrenz. Dr. Asper has and will continue to spend about 10% of his time on this project.

Mr. M. J. Morris (contractor, OOI)

Mr. Morris is president of Ocean Optics, Inc., the agency contracted to construct the lab prototype and field instruments. He has had extensive experience in the design of fiber optic based sensors and has been directly involved in consultations and decisions regarding design options of the prototype. He will provide the evaluation and approval of design revisions. Dr. Morris has and will continue to devote 20% of his time on this project.

Dr. R. A. Walters (contractor, OOI)

Dr. R. Walters has been primarily responsible for construction of the instrument and implementation of design and construction options selected by the principal investigators. He is an optical engineer with extensive research and design experience with fiber optical instruments. He has participated in field operations involving the instrument and attended planning meetings. Dr. Walters has and will continue to devote 20% of his time on this project.

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ESTIMATE OF UNOBLIGATED BALANCES

At the present time, we estimate that there will be no unobligated balances of funds from this project.

FIGURE LEGENDS

Figure 1. Design of the fiber optic sensor housing. The v-groove construction allows for free flow of water through the sensor, while minimizing ambient light effects.

Figure 2. Calibration curves of the multi-sensor fiber optic fluorometer determined using various dilutions of a culture of *Nannochloris atomus* Butcher in artificial seawater. Counts represent background-corrected 10 point averages of fluorescence peak heights sampled by a 500 kHz 12 bit A/D converter (maximum counts, 4096) with the exception of sensors 5 and 7 where counts represent area integrated under the fluorescence peak. In cases where a nonlinear relationship was observed due to saturation, data were fit using a nonlinear curve fitting routine (Systat). a) sensors 1-4; b) sensors 5-7.

Figure 3. Plots of the lower range of the calibration described in Fig. 2. Detection limits for individual sensors were estimated as 3 standard deviations above the mean blank signal. a) sensors 1-4; b) sensors 5-7.

Figure 4. Location of the BASS time-series site off Duck, NC. See text for additional information.

Figure 5. Time-series of pigments estimated by the multi-sensor fiber optic fluorometer at different distances from the bottom. The fluorometer was mounted on the WHOI BASS tripod (Williams and Churchill) at 20 m depth off Duck, NC (Fig. 4). Pigment concentrations were estimated from the calibration curves given in Fig. 2. In some cases, values had to be extrapolated beyond the limits of the calibration. Sensors at 189 and 14 cm (corresponding to sensors 5 and 7 respectively in Fig. 2) saturated leading to a truncated shape of peaks in the signal. a) 443-189 cm; b) 116-14 cm.

Figure 6. Power spectral densities (variance/cpd) as estimated by Welch's method from selected depths of the pigment time-series given in Fig. 5. Only the first half of the 30 d data record was analyzed. A 512 point fast Fourier transform was applied to the linearly detrended signals with no overlapping using the psd function of MatLab. The vertical line corresponds to a frequency of 1 cycle per day (cpd).

Figure 7. Vertical profiles of water column data collected at the 20 m BASS site at 1044 UT on 23 July 1994 from the R/V Cape Hatteras. The location was 36.1967N, 75.7100W. a) CTD data obtained with a Sea Bird SBE 911plus system; b) profile data obtained with a Sea Tech fluorometer and Sea Tech transmissometer and concentrations of extracted pigments determined for water samples collected with 30

1 Niskin bottles on a rosette.

Figure 8. Vertical profiles of water column data collected at the 20 m BASS site at 0208 UT on 1 September 1994 from the R/V Endeavour. The location was 36.2507N, 75.5893W. a) CTD data were obtained with a Sea Bird SBE 911plus system; b) profile data obtained with a Sea Tech fluorometer and Sea Tech transmissometer and concentrations of extracted pigments determined for water samples collected with 10 l Niskin bottles on a rosette.

Figure 9. As for Fig. 8, but at 2112 UT on 1 September 1994. Location was 36.1961N, 75.7113W.

Figure 10. High pressure liquid chromatogram showing elution times of various photosynthetic pigments extracted from water samples obtained at the BASS site during the R/V Cape Hatteras and R/V Endeavour cruises. The methods of Wright et al. (1991) used. a) Results obtained from the 23 July profile shown in Fig. 7. Note the relatively high value of fucoxanthin, an accessory pigment indicative of diatoms. b) Results from the 1 September profile shown in Fig. 8 and c) Fig. 9, where peridinin is the dominant accessory pigment, indicative of dinoflagellates.

Figure 11. Chlorophyll/phaeopigment ratios for water samples at the BASS site.

Figure 12. The relationship between extracted pigment concentrations and *in situ* fluorescence as determined with the Sea Tech profiling fluorometer.

Figure 13. The relationship between particulate organic carbon (POC) and extracted pigment concentrations, and between particulate organic carbon and suspended particulate matter (SPM) concentrations. a) all water column data; b) data for the large subsurface dinoflagellate peak seen in Fig. 9 were excluded.

Fig. 1

Fiber optic fluorescence sensor

Fig. 2a

Fig. 2b

Fig. 3a

Fig. 3b

Fig. 4

Fig. 5a

Fig. 5b

Fig. 6

Fig. 7a

Fig. 7b

Fig. 8a

Fig. 8b

Fig. 9a

Fig. 9b

Fig. 10a

SEQ INT 1/ 1 SYSTEM[1]
 DATA FILE: fd1-1.
 ORIG. 13:01-- 09/13/1994
 CURR. 11:08-- 09/16/1994

CTD # 1 , 15.39 m

Fig. 10b

GRAPH KEY
75 MV 100 %
20.00 MIN.
%B @A C/
%C

BASS Retrieval Cruise
1 Sept 1994
02:08 Cast
15.4 m

SEQ INJ 1/ 1 SYSTEM[1]
DATA FILE: fr1-5.
ORIG. 13:39-- 09/14/1994
CURR. 10:13-- 09/16/1994

Fig. 10c

SEQ INJ 1/ 1 SYSTEM[1]
DATA FILE: fr2-7.
ORIG. 13:50-- 09/15/1994
CURR. 09:42-- 09/16/1994

Fig. 11

20 m BASS site

— — 23 Jul 94 10:44
— 1 Sep 94 2:08
..... 1 Sep 94 21:12

Fig. 12

BASS Deployment 23 Jul 94

BASS Recovery 1 Sep 94

Fig. 13a

BASS Deployment 23 Jul 94 (o)
 BASS Recovery 1 Sep 94 (+)

Fig. 13b

BASS Deployment 23 Jul 94 (o)
BASS Recovery 1 Sep 94 (+)