

Project Title: Response of Particulate Optical Properties to Coastal Mixing Processes: Analysis and Interpretation of Particle Light Scattering as Measured by Flow Cytometry

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Educational Accomplishments

The primary objective of this project was to support the involvement of a Ph.D.-level graduate student in the on-going Coastal Mixing and Optics (CMO) Research Initiative. Rebecca Green, a Ph.D. candidate in the MIT/WHOI Joint Program in Oceanography and Oceanographic Engineering, was selected for participation and her graduate studies and research program were supported by this grant for the last three years.

Rebecca has successfully completed the requirements of graduate study for the first three years in the MIT/WHOI Joint Program in Oceanography. She completed course work requirements during her first two years (including completion of the Optical Oceanography course at Friday Harbor Laboratories), and passed her written general exams at the end of this period. In June 1999, she gave an oral defense of her thesis proposal titled "Interpretation of Variability in Water Column Optical Properties through Individual Particle Analysis". This research is being done with the supervision of her principal advisor Sosik and her thesis committee, Olson, Penny Chisholm (MIT), Curtis Mobley (Sequoia Scientific, Inc.), and Andrew Solow (WHOI). Rebecca is expected to complete her research project and to defend her Ph.D. thesis in July 2001.

Through the support of this grant, Rebecca made significant progress towards completion of the research for her dissertation. She participated in collecting data on three CMO cruises, in August/September 1996, January 1997, and April/May 1997. We have analyzed data from these cruises in order to interpret optical variability of both single particle and bulk optical properties. Rebecca presented initial results of this research at the 1998 AGU Ocean Sciences Meeting. These results included how variability in bulk optical properties, including diffuse attenuation and remote sensing reflectance, were forced by physical events such as differences in stratification and the passing of a hurricane, and ultimately by changes in single particle optical properties. During the last year, Rebecca also co-authored an extended abstract for Ocean Optics

XIV and a paper submitted for publication in the Journal of Geophysical Research. In addition, she participated in the interpretation and integration of optical and physical data during the last CMO Workshop in Keystone, Colorado (September 1998) and will be presenting new research results at the upcoming Ocean Sciences 2000 meeting in San Antonio, TX. These opportunities for interaction with colleagues have not only enhanced her research, but have been an important component of her career preparation.

Research Results

We have used concurrent measurements of single particle and bulk optical properties collected during the summer 1996 and spring 1997 CMO cruises in order to interpret the effects of changes in single particle optical properties on bulk optical variability, both temporally and spatially. Downwelling spectral irradiance and upwelling radiance were collected using a Satlantic Profiling Multichannel Radiometer. Profiles of total and dissolved absorption and scattering were measured with two WetLabs ac-9s (one filtered) on a profiling package. In addition, discrete water samples were collected for spectrophotometric, chlorophyll, and single particle flow cytometric analysis.

Differences in inherent optical properties were observed between the two seasons and were caused by such physical forcing events as changes in stratification and the passing of storms (e.g. Hurricane Edouard). Absorption due to dissolved materials was found to be low and fairly constant during both cruises, and so particles were the main determinant of changes in optical variability. Maxima in diffuse attenuation in the water column were related to peak concentrations of chlorophyll, with a subsurface chlorophyll maximum observed in the late summer and a surface maximum observed in the spring (Sosik et al., submitted).

Since particle variability was found to be especially important in determining differences in the optical properties of the water column, focus was placed on characterization of individual particles. We have used shipboard flow cytometry to measure the scattering and fluorescence properties of phytoplankton and non-phytoplankton particles between about 0.3 and 40 micrometers in diameter. Individual particle measurements included forward scattering and side scattering at 488 nm and orange (575 nm, from phycoerythrin) and red (680 nm, from chlorophyll) fluorescence. Concentrations of *Synechococcus* and nano/microphytoplankton were highest subsurface during the later summer cruise. In the spring, peak concentrations of *Synechococcus* and nano/microphytoplankton were seen in the surface layer, although *Synechococcus* were approximately two orders of magnitude lower in concentration than in late summer. Concentrations of non-phytoplankton particles were generally higher than those of phytoplankton, but in contrast were found to be fairly constant with depth and season. Thus, vertical distributions of apparent and inherent optical were generally driven by changes in phytoplankton (Sosik et al, submitted). Currently we are in the process of enumerating heterotrophic bacteria in preserved samples using the nucleic acid stain SYBR Green I. Measurements of bacterial concentration and scattering properties will be of particular importance to our understanding of particle contributions to total scattering.

A major focus of this research is the application of Mie theory to understanding and interpreting flow cytometric light scattering measurements. Our primary application of the theory is toward inversion of these light scattering measurements to infer both particle size and real refractive index. The assumptions of Mie theory are approximately met by our measurement setup, including use of a collimated beam of monochromatic light (ours is 1° from collimated) and nearly spherical particles which are embedded in a sheath of non-absorbing medium, in this case filtered seawater. As in previous work, we have used measurements of oil suspensions of known refractive index and varying droplet size to test the application of Mie theory toward estimation of refractive index. In addition, we have also compared measurements of cell cultures of known size and polystyrene beads of known size and refractive index to Mie theory with some success (Figure 1).

To use Mie theory for modeling the optical properties of particles, the imaginary index of refraction for each particle is needed. The imaginary index depends on the absorption properties of the particle. Our data show a good correlation ($r^2 \approx 0.98$) between absorption at 488 nm and flow cytometric fluorescence for the phytoplankton cultures measured, including two types of prokaryotic picoplankton (*Synechococcus* sp.) and six types of eukaryotic phytoplankton. Phytoplankton absorption measurements estimated in this way for the late summer and spring cruises compare well with total phytoplankton absorption measured spectrophotometrically. These results show that the majority of phytoplankton absorption is accounted for by cells less than 30 μm in size (Figure 2), a finding that is corroborated by spectrophotometric absorption measurements of phytoplankton in different size fractions (unpublished data of Roesler).

Using these individual particle methods, we have been able to show that eukaryotic phytoplankton and non-phytoplankton particles contribute roughly equally to scattering in surface waters during both seasons, while non-phytoplankton particles were the main light scatters in near-bottom waters (Figure 3). Changes in mean cellular scattering and absorption cross-section were observed both vertically and temporally. Whether the variability that we have observed in bulk optical properties is caused by changes in particle concentration, diameter, or refractive index will be determined based on continuing work with application of Mie theory and flow cytometry.

Publications and Presentations

Sosik, H.M., R.E. Green, W.S. Pegau and C.S. Roesler, Temporal and vertical variability in optical properties of New England shelf waters during late summer and spring, Submitted to *J. Geophys. Res.* (CMO special issue).

Sosik, H.M., R.E. Green and R.J. Olson. 1998. Optical variability in coastal waters of the northwest Atlantic. In: *Ocean Optics XIV*, S.G. Ackleson (ed.). 14 pp.

Green, R.E. and H.M. Sosik. 1998. Vertical and temporal variability in apparent optical properties during the coastal mixing and optics experiment, Abstract AGU/ASLO Ocean Sciences 1998, San Diego, CA.

Green, R.E., H.M. Sosik, M.D. DuRand and R.J. Olson. 1999. Toward closure between single particle and bulk optical properties during the coastal mixing and optics experiment, Abstract AGU Ocean Sciences 2000, San Antonio, TX.

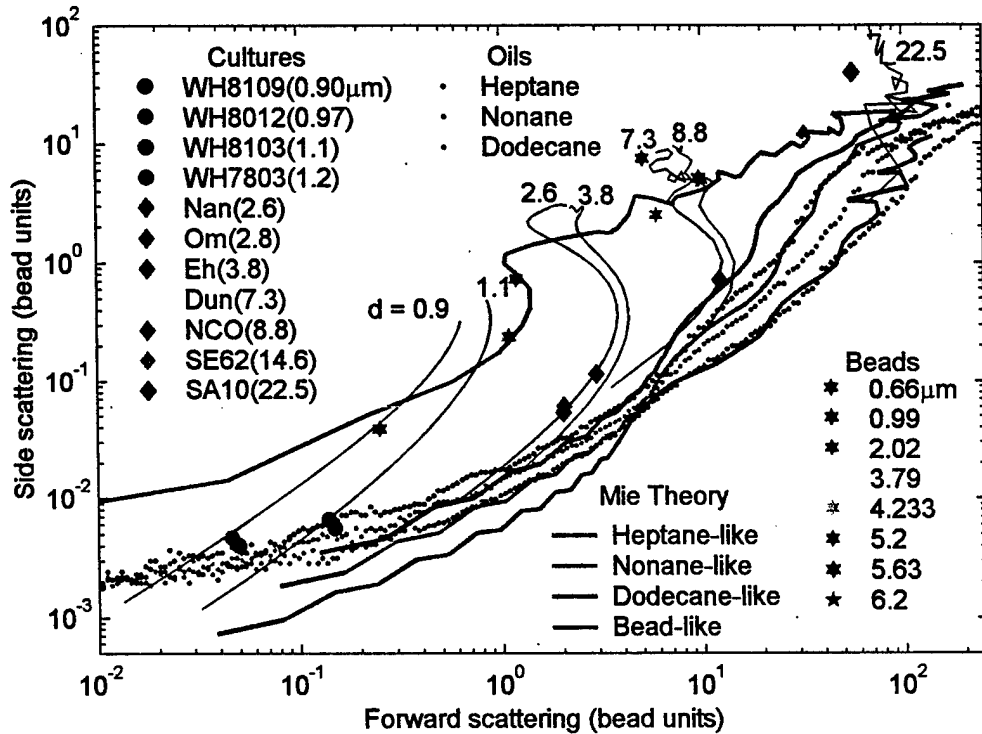


Figure 1. The application of Mie theory for interpretation of flow cytometry scattering measurements was checked with oil suspensions of known refractive index and varying droplet size, cell cultures of known size and polystyrene beads of known size and refractive index. The black lines represent the scattering relationship predicted by Mie theory for particles of constant size but varying real refractive index; to determine each of these lines, the imaginary refractive index was set to the values observed for the culture of corresponding size. The colored lines represent the theoretical relationship for particles with constant refractive index but varying size, and have been calculated for the refractive index of polystyrene beads, and also for the refractive index of the three oils.

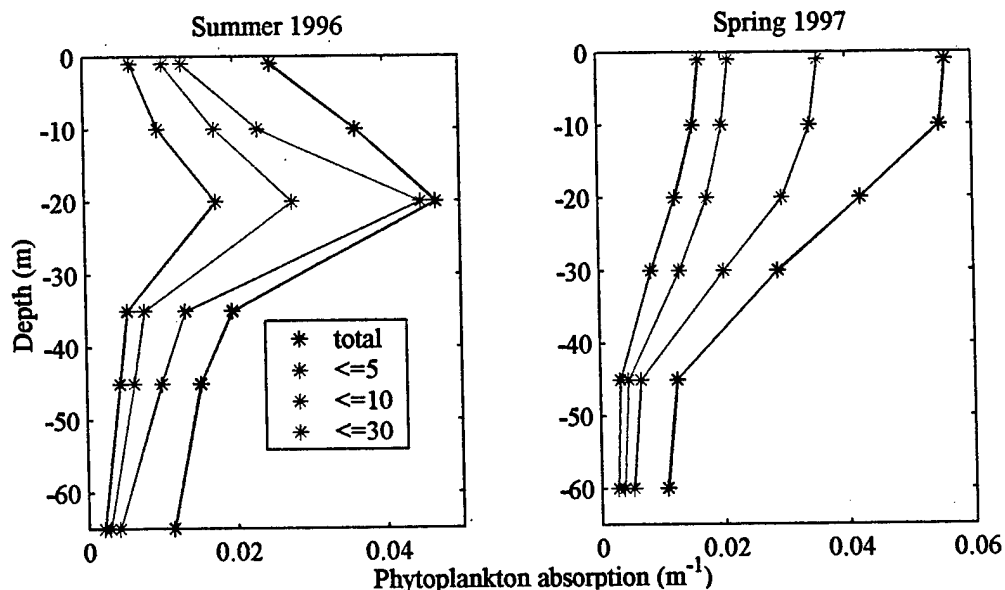


Figure 2. Mean profiles of phytoplankton absorption, including total (measured independently by spectrophotometry) and the <5 , <10 , and <30 μm size fractions (estimated flow cytometrically) for both the late summer (left panel) and spring (right panel) cruises. For all size fractions, maximal absorption is seen subsurface in the summer and in the surface layer in the spring corresponding to differences in stratification. The <30 μm size fraction is seen to account for most of the phytoplankton absorption.

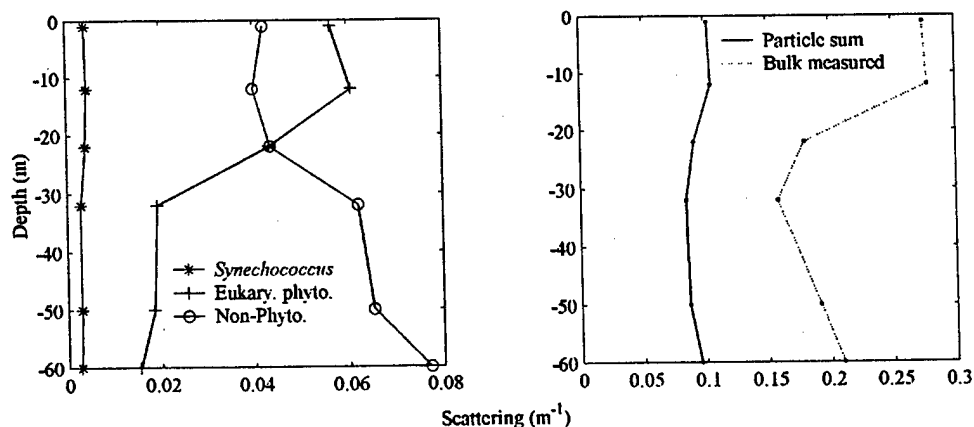


Figure 3. Example of Mie theory used to estimate the contributions of three particle classes (*Synechococcus*, eukaryotic phytoplankton and non-phytoplankton) to total scattering for a depth profile measured during the spring cruise (left panel). Theory-based calculations of total scattering cross-section were made using fluorescence (to estimate absorption) and forward and side angle light scattering for each particle. Both eukaryotic phytoplankton and non-phytoplankton particles are important contributors to scattering in the surface layer, while non-phytoplankton are the main scatterers in near bottom waters. Light scattering estimates for particles measured using shipboard flow cytometry account for $\sim 40\%$ of total scattering measured (independently) in the water column (right panel). This difference is most likely caused by particles that have not been included in the flow cytometric analysis. Of particular importance are bacterial particles, which we are currently working to enumerate and characterize.

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13. Abstract During the period of this AASERT award we successfully participated in the Coastal Mixing and Optics Research Initiative with the award supporting training of Rebecca Green, a PhD candidate in the MIT/WHOI Joint Program in Oceanography and Oceanographic Engineering. Rebecca made substantial progress towards receiving her degree, participated in three program research cruises and made valuable contributions to our research objectives. These objectives included quantifying relationships between particles, bulk water optical properties (both inherent and apparent) and physical processes that are important on the continental shelf of the US east coast. Rebecca's primary contribution has been to develop methods for the derivation of bulk optical properties from individual particle measurements based on flow cytometric analysis. This has included separation of the particle pool into different functional classes and characterization of the size-dependent contributions to light absorption and scattering. Since particles, and especially phytoplankton, are the greatest sources of optical variability in many oceanic and coastal systems, this type of investigation is important for understanding spatial and temporal changes in bulk properties such as ocean color and visibility.			
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