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# THE CONTRIBUTION OF PHYTOPLANKTON AND NON-PHYTOPLANKTON PARTICLES TO INHERENT AND APPARENT OPTICAL PROPERTIES IN NEW ENGLAND CONTINENTAL SHELF WATERS

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## ABSTRACT

Flow cytometric (FCM) measurements of optically important particle groups were used to interpret variability in bulk optical properties in New England continental shelf waters during two seasons. A combination of Mie theory and flow cytometry was used to determine the diameter ($D$), complex refractive index ($n+in'$), and optical cross-sections of individual particles. The summed contributions of particles in the size range of 0.1-50 µm to absorption ($a$) and scattering ($b$) were approximately equal to total particle $a$ and $b$ measured independently using bulk methods. In surface waters during both seasons, the large $D$ and high $n'$ of eukaryotic phytoplankton caused them to be the main particle contributors to both $a$ and $b$. Mineral particles were the main contributor to backscattering ($b_b$) in the spring, whereas in the summer both mineral and detrital particles contributed to $b_b$. *Synechococcus* and heterotrophic bacteria contributed ≤13% each to attenuation in surface waters. The role of seawater constituents in determining remote sensing reflectance, $R_{rs}$, was determined using radiative transfer theory. Seasonal differences in the spectral shape of $R_{rs}$ were contributed to by eukaryotic phytoplankton $a$, colored dissolved organic matter (CDOM) $a$, and non-phytoplankton $b_b$. In addition, we used bio-optical models based on chlorophyll concentration to simulate optical properties and interpreted deviations of our measurements from the model results.

## INTRODUCTION

Knowledge of particle properties is important for the interpretation of variability in the inherent optical properties (IOPs) and apparent optical properties (AOPs) of the upper ocean. Models of IOPs are the basis of bio-optical algorithms for deriving chlorophyll concentration from satellite ocean color. Model simulations have shown how changes in particle type can give significantly different IOPs and AOPs, even at a constant chlorophyll concentration (Mobley and Stramski 1997; Stramski et al. 2001). These simulations were based on assumptions about the types and concentrations of particles present and the distributions of $D$, $n$, and $n'$ for each particle group included in the model, rather than direct measurements of particles. One approach for determining distributions of particle properties for natural populations is through the rapid measurement of individual particles using flow cytometry. Flow cytometry has been used to enumerate and distinguish specific groups of particles, and advances have been made towards determining distributions of particle $D$, $n$, and $n'$ with application to determining particle contributions to IOPs (e.g., Ackelson and Spinrad 1988; DuRand and Olson 1998; Green et al. 2002a). Our goal in the present study was to apply flow

cytometry to the modeling of IOPs by measuring and describing populations of natural particles which are optically important.

## METHODS

*Bulk Optical Properties* - Vertical profiles for water sampling and measurements of IOPs and AOPS were made as part of the Coastal Mixing and Optics experiment (CMO) (Sosik et al. 2001). The CMO site was located on the southern New England shelf (40° 30′ N, 70° 30′ W) where the water depth is ~70 m. Data were collected during two 3-week cruises in the late summer of 1996 (17 August - 7 September) and spring of 1997 (24 April - 13 May). Profiles of particle absorption ($a_p$), $a_{CDOM}$, and particle scattering ($b_p = c - a$) were determined from in situ ac-9 measurements (Wetlabs, Inc.) at 9 wavelengths (412, 440, 488, 510, 532, 555, 650, 676, and 715 nm). Diffuse attenuation ($K_d$) and remote sensing reflectance ($R_{rs}$) were determined from measurements made with a profiling spectral radiometer (SPMR system, Satlantic, Inc.) at 7 wavelengths (412, 443, 490, 510, 555, 665, and 683 nm). Additionally, optical measurements were made on board ship on water samples collected from six depths throughout the water column, including absorption coefficients for phytoplankton ($a_{ph}$) and residual material ($a_{dm}$; detritus + minerals) by spectrophotometry, chlorophyll *a* concentrations, and of individual particles by flow cytometry.

*Flow Cytometry and Mie theory* - Particle contributions to IOPs were calculated as a sum of contributions from particles of 0.1-50 μm in diameter (for details see Green et al. 2002b). Particles measured by flow cytometry were assigned to one of five groups: eukaryotic pico/nanophytoplankton ("eukaryotic phytoplankton"), *Synechococcus*, heterotrophic bacteria, detritus, or minerals. For particles of ≤10 μm in diameter, a combination of flow cytometric (FCM) measurements and Mie theory (referred to as the "FCM-Mie" method; Green et al. 2002a) was used to determine particle $D$, $n$, and $n'$ and optical cross-sections for absorption ($\sigma_a$), scattering ($\sigma_b$), and backscattering ($\sigma_{bb}$) at 488 nm. For heterotrophic bacteria, $n'$ was assumed to be $5 \times 10^{-4}$ at 488 nm (Morel and Ahn 1990; Stramski and Mobley 1997). For small detritus (0.1 - 1.2 μm) and minerals (0.1 - 0.75 μm), optical contributions were calculated by extrapolating size distributions to 0.1 μm. The optical properties of eukaryotic phytoplankton, detritus, and minerals of 10-50 μm in diameter were determined using a combination of Mie theory and an empirical relationship for $D$ based on FCM measurements. For non-phytoplankton, n′ was determined by considering particles of 0.1-50 μm in diameter and iteratively changing the value of $n'$ until total non-phytoplankton absorption, $a_{dm}$, was approximately equal to spectrophotometric $a_{dm}$. Particle sum contributions to $a$, $b$, and $b_b$ at 488 nm were calculated by summing over the optical cross-sections of all particles in a known volume.

*Constituent Contributions to IOPs and AOPs* - Total IOPs were defined as a sum over the constituents of seawater. The total spectral absorption coefficient, $a_{total}(\lambda)$, was calculated as a sum of absorption by each of the constituents of pure seawater ($a_w(\lambda)$), CDOM ($a_{CDOM}(\lambda)$), eukaryotic phytoplankton ($a_{euk}(\lambda)$), *Synechococcus* ($a_{syn}(\lambda)$), heterotrophic bacteria ($a_{bact}(\lambda)$), and non-phytoplankton ($a_{dm}(\lambda)$; detritus+minerals). The

total scattering and backscattering coefficients, $b_{total}(\lambda)$ and $b_{b,total}(\lambda)$, were defined as sums of contributions by pure seawater and each particle group. Constant values of IOPs for pure seawater were assumed (Morel 1974; Pope and Fry 1997), and $b_{b,w}(\lambda)$ was assumed to be half of $b_w(\lambda)$. Mean depth profiles of $a$, $b$, and $b_b$ at 488 nm were computed for seawater constituents in the summer and spring. Mean IOPs for particle groups were calculated by averaging all samples from the same day and the same depth bin.

A radiative transfer model, Hydrolight (version 4.1), was used to generate values of $K_d$ and $R_{rs}$ for three examples in each season. The following boundary conditions were used: a wind speed of 5 m/s, a semi-empirical sky model, a solar zenith angle of 0° (sun directly overhead), and a bottom depth of 70 m. The spectral contributions of particles to IOPs were calculated using properties at 488 nm combined with Mie theory. Inputs to Mie theory included the particle size distribution from 0.1 µm to 50 µm, a wavelength-independent $n$ equal to the FCM-Mie mean $n$ for each particle group, and a wavelength-dependent $n'$. Mean values of $n'$ at 488 nm for eukaryotic phytoplankton and *Synechococcus* were determined from the FCM-Mie method, and the value of $n'$ at 488 nm for detritus and minerals was calculated as described above. The spectral shapes of $n'$ for phytoplankton and non-phytoplankton were based on average spectrophotometric measurements of $a_{ph}(\lambda)$ and $a_{dm}(\lambda)$ for the depth range of 0-20 m (Sosik et al. 2001). Spectral $n'$ for heterotrophic bacteria was assumed equal to values presented in Stramski and Mobley (1997). Total $a$, $b$, and $b_b$ for each particle group was calculated using the above inputs to Mie theory, with the exception of values for $a_{dm}$ for which spectrophotometric values were used.

## RESULTS

*Particle Properties and IOPs at 488 nm* - We compared IOPs calculated from the particle sum method to independent measurements at 488 nm. Particle sum $a_{ph}$ ($a_{euk}$ + $a_{syn}$) was 87% of spectrophotometric $a_{ph}$, considering both seasons (Fig. 1A), and a high percentage of the variance was explained by a linear relationship ($r^2 = 0.86$). Assuming a value for $n'$ of 0.001 gave the best agreement between particle sum and spectrophotometric $a_{dm}$ for the two seasons with a slope close to 1, but the regression was not significant (Fig. 1B). Unexplained variance in the fit between particle sum and spectrophotometric $a_{dm}$ is likely caused by variability in the absorption properties of non-phytoplankton due to their complex composition. The value of $n'$ that we determined for non-phytoplankton was used in Mie calculations of $b$ and $b_b$. Because of their higher degree of accuracy, spectrophotometric values were used for $a_{dm}$. Total scattering by all particles, $b_p$, was compared to bulk (ac-9) measurements of $b_p$. Linear regression between particle sum and bulk $b_p$ gave a slope of 1.07 ($r^2 = 0.48$), considering both seasons (Fig. 1C). The generally good comparison between particle sum and independent methods gave us confidence in applying the particle sum approach to interpretation of variability in IOPs for both seasons.

Differences in stratification between the summer and spring were reflected in particle properties. The water column was well stratified in the summer (prior to the passage of Hurricane Edouard), with depleted nutrient levels in the surface mixed layer

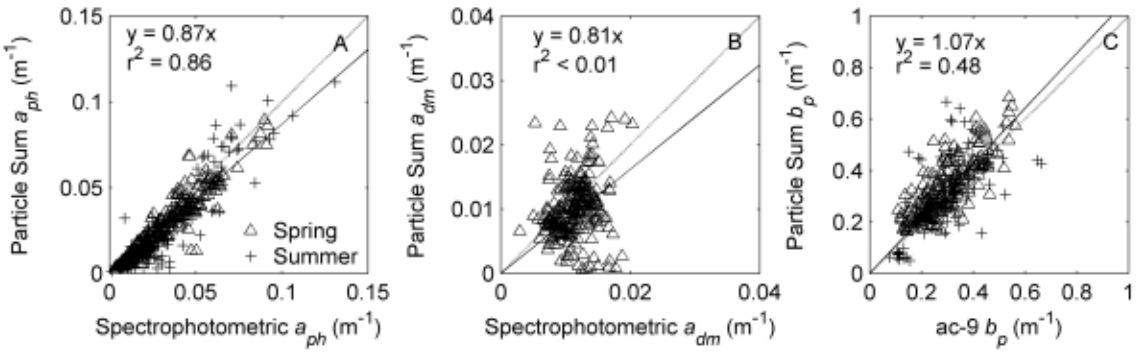


***Figure 1. Comparison of particle sum and bulk (A) phytoplankton absorption, $a_{ph}$, (B) non-phytoplankton absorption, $a_{dm}$, and (C) particle scattering, $b_p$, at 488 nm for the summer and spring. Particle sum $a_{dm}$ was calculated using a value for n′ of 0.001. Particle sum $b_p$ is a sum of scattering by all particles, including eukaryotic phytoplankton, Synechococcous, heterotrophic bacteria, detritus, and minerals, from 0.1-50 μm in diameter. The 1:1 line (dotted) and least squares regression between particle sum and bulk IOPs (solid line) are shown on each plot.***

and mid-water column maxima in chlorophyll concentrations and IOPs (Sosik et al. 2001). In the spring, the water column was less stratified, with higher nutrient concentrations than in summer and surface maxima in chlorophyll concentrations and IOPs. The highest concentrations of both heterotrophic bacteria ($1.5 \times 10^6$ ml$^{-1}$) and eukaryotic phytoplankton ($2.5 \times 10^4$ ml$^{-1}$) occurred in spring surface waters (0-20 m), consistent with previous suggestion of a link between the abundances of the two microbial groups (Azam et al. 1983). In contrast, *Synechococcus* were more abundant in the summer ($5.9 \times 10^4$ ml$^{-1}$; $p<0.001$) when water temperature was higher. Mean *D* for eukaryotic phytoplankton, *Synechococcus*, and heterotrophic bacteria were 2.17, 1.24, and 0.46 μm, respectively, in surface waters, and values of *n* were between 1.054 - 1.081, within the range expected for cells (Aas 1996). Values of *n′* were higher for eukaryotic phytoplankton (0.0051-0.0165) than for *Synechococcus* (0.0022-0.0093) and, for both groups, were always lower in surface waters than deeper in the water column. Detritus was significantly more abundant in summer surface waters, compared to the spring ($1.6 \times 10^8$ ml$^{-1}$ vs. $0.87 \times 10^7$ ml$^{-1}$; $p<0.001$). In contrast, minerals were not significantly different in concentration in surface waters between seasons (~$1.0 \times 10^7$ ml$^{-1}$). Considering both seasons, detritus had mean *n* in the range of 1.064 - 1.072; minerals had mean *n* in the range of 1.16-1.19.

Submicron detrital particles were responsible for the higher abundances of non-phytoplankton in the summer, and it seems likely that these particles have seasonally-dependent sources and sinks. Submicron detrital particles were ~18 times more abundant in surface waters in summer than in spring. One hypothesis for the higher concentrations of small detritus in the late summer is that the preceding several months of increased productivity led to the accumulation of a type of organic particle that is not readily useable by heterotrophic bacteria. In support of our hypothesis, it has been found that microbial processes, such as bacterial feeding, can alter the structure of labile submicron particles, resulting in the production of semi-labile and refractory particles that are

relatively resistant to decomposition (e.g., Ogawa et al. 2001). Previous studies have documented a seasonal dependence of submicron particle concentration in which particles accumulated in the summer and decreased in concentration in the winter due to mixing and transport to deeper layers (e.g., Williams 1995).

The most important determinants of IOPs were eukaryotic phytoplankton, CDOM, detritus, and minerals. The main contributors to absorption, $a$, in surface waters were eukaryotic phytoplankton and CDOM in both seasons (Figs. 2A-B), with CDOM slightly more important in the summer, and eukaryotic phytoplankton more important in the spring. Eukaryotic phytoplankton contributed the most to $b$ in surface waters, and minerals contributed the most to $b$ deeper in the water column (Figs. 3A-B). *Synechococcus* and heterotrophic bacteria contributed ≤13% each to attenuation in surface waters. The main contributors to backscattering, $b_b$, were detritus and minerals in the summer and minerals alone in the spring (Figs. 4A-B). Submicron mineral particles contributed ~72% of all backscattering by minerals ($b_{b,min}$), considering both seasons, and submicron detritus contributed ~87% of backscattering by detritus ($b_{b,det}$) in summer surface waters. Our findings thus agree with previous proposals that $b_b$ is mainly determined by submicron non-phytoplankton particles and that microbes are of little importance (Morel and Ahn 1991; Stramski and Kiefer 1991; Stramski et al. 2001). The highest backscattering ratios ($\tilde{b}_b = b_b/b$) were observed for minerals in both seasons and for detritus in the summer. In both seasons, the mean $\tilde{b}_b$ in surface waters was 3.6% for minerals, 1.1% for heterotrophic bacteria, 0.2% for *Synechococcus*, and 0.07% for eukaryotic phytoplankton. The high abundance of submicron detritus in summer surface waters resulted in a high $\tilde{b}_b$ for detritus of 2.3% and a $\tilde{b}_b$ for all particles which was higher in the summer (1.3%) than in the spring (0.9%).

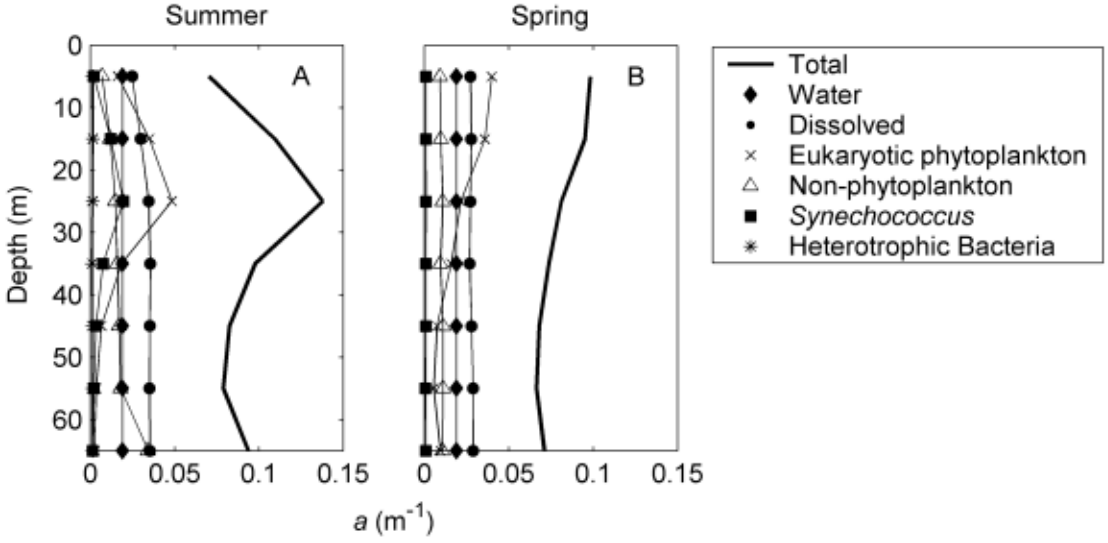


***Figure 2. Depth profiles of constituent contributions to absorption at 488 nm in the (A) summer (B) and spring.***

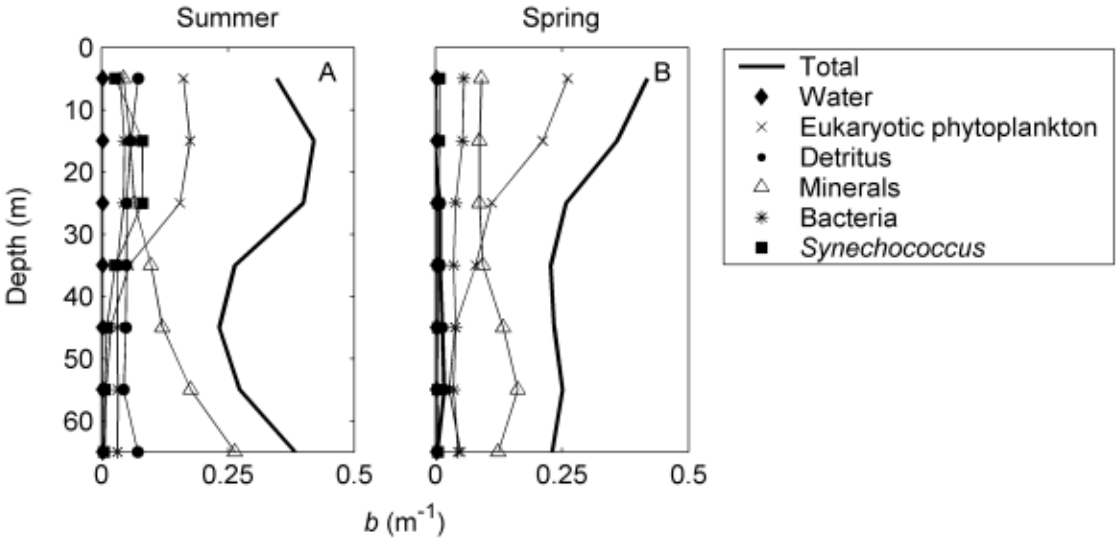


***Figure 3. Depth profiles of constituent contributions to scattering at 488 nm in the (A) summer and (B) spring.***

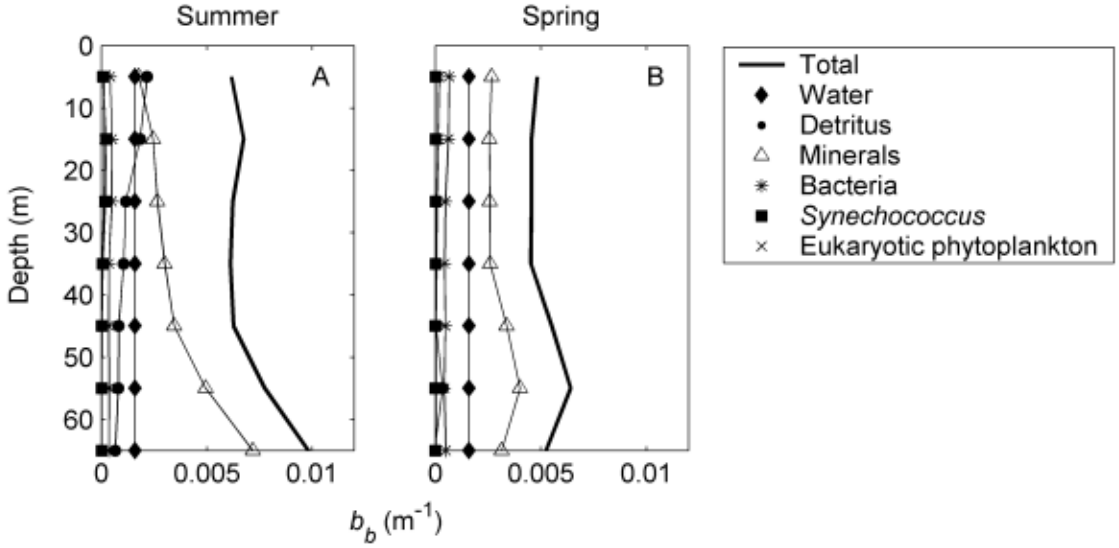


***Figure 4. Depth profiles of constituent contributions to backscattering at 488 nm in the (A) summer and (B) spring.***

*Seasonal Variability in Spectral AOPs* - Values of AOPs, modeled using Hydrolight with spectral IOPs as input, compared well with measured values of $K_d$ (Fig. 5A-F) and $R_{rs}$ (Fig. 6A-F) in both magnitude and spectral shape. The difference between modeled and measured values of $K_d$ and $R_{rs}$ were ≤14%, considering both seasons and all wavelengths. The higher magnitude of $K_d$ at all wavelengths in the spring was caused primarily by higher $a_{euk}$ and secondarily by higher $a_{CDOM}$ especially at 412 nm. There was a spectral shift toward enhanced blue relative to green attenuation in the spring primarily because phytoplankton absorb more at blue than at green wavelengths. Higher values of both $a_{euk}$ and $a_{CDOM}$ in the spring, compared to summer, were enough to account for a ratio of $R_{rs}(440):R_{rs}(555)$ which was <1 in spring and >1 in summer. Spectral differences in particle backscattering, $b_{b,p}$, also contributed to summer values of $R_{rs}$ which were higher at blue wavelengths and lower at green wavelengths. Contributions to seasonal differences in the shape of $R_{rs}$ were 48% by $a_{euk}$, 29% by $a_{CDOM}$, and 23% by $b_{b,det}$, and $b_{b,min}$. The magnitudes of $b_{b,p}$ were similar across seasons, however, spectral $b_{b,p}$ had a higher inverse wavelength dependence in the summer than in the spring, due to the contribution of submicron detrital particles in the summer (Fig. 7A). As well, higher ratios of $\tilde{b}_{b,p}$ at all wavelengths were observed in the summer (Figs. 7B).

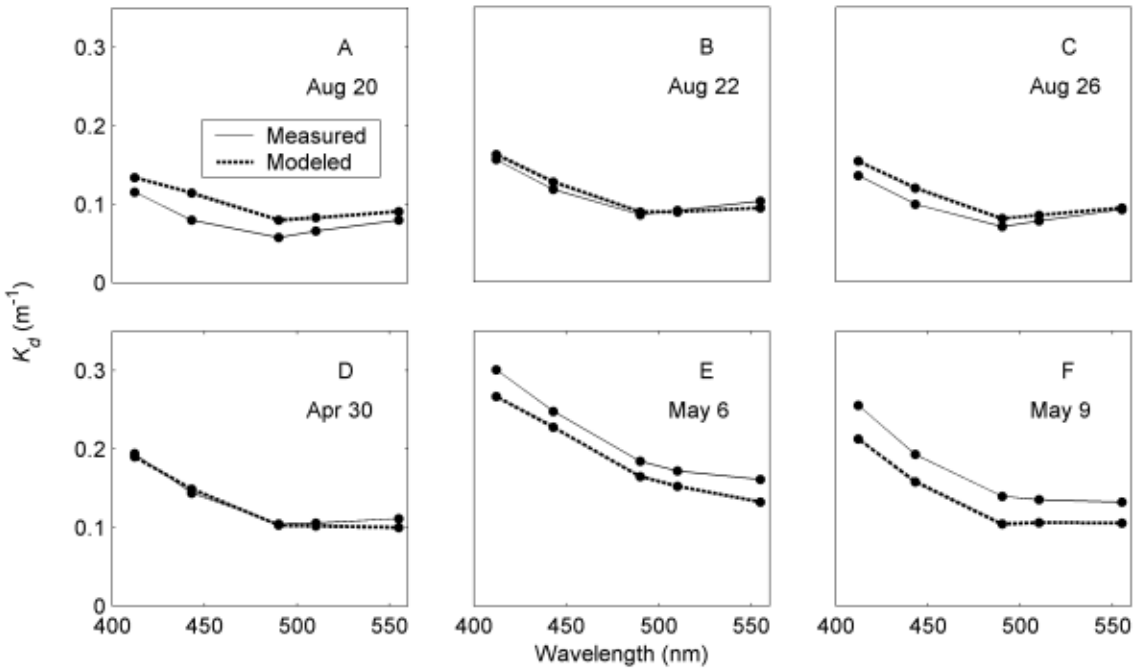


***Figure 5. Spectra of measured and modeled diffuse attenuation, $K_d$, for (A-C) the summer examples and (D-F) the spring examples.***

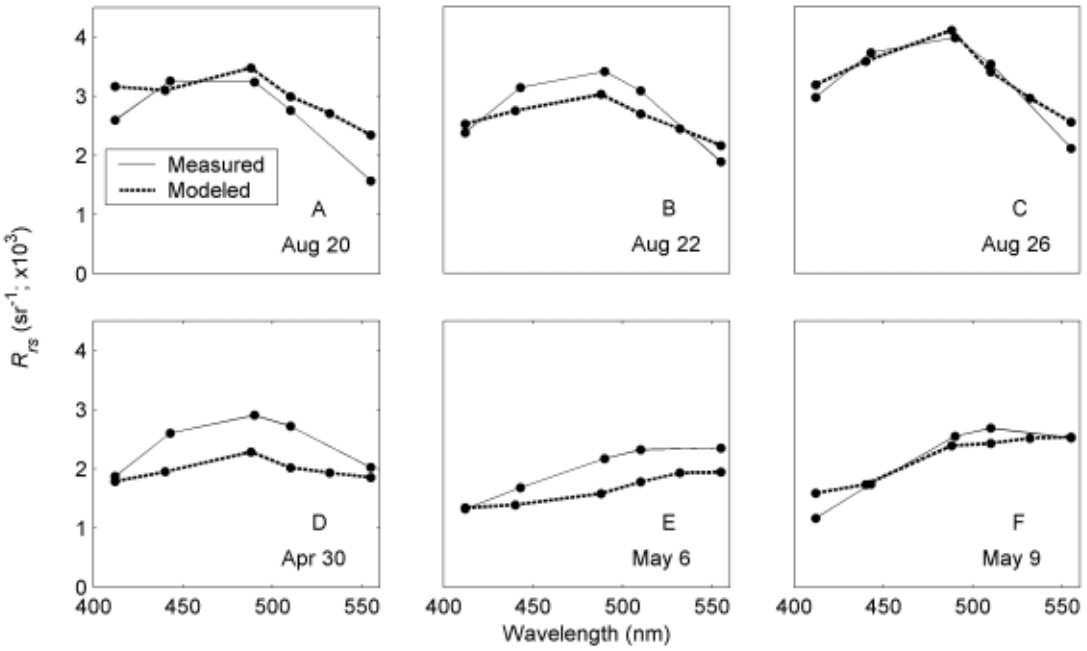


***Figure 6. Spectra of measured and modeled remote sensing reflectance, $R_{rs}$, for (A-C) the summer examples and (D-F) the spring examples.***

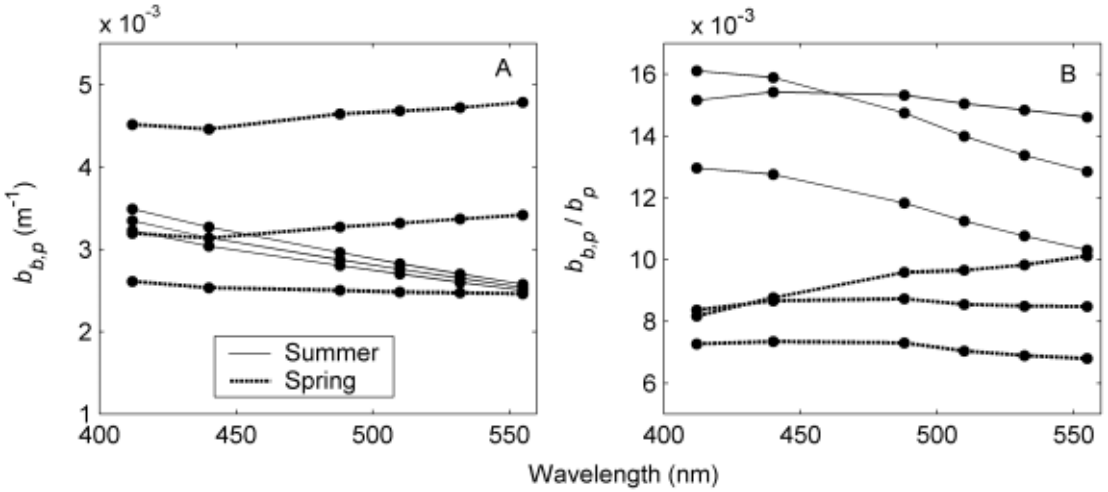


***Figure 7. Summer and spring spectra of (A) particle backscattering, $b_{b,p}$, and (B) the ratio of particle backscattering to particle scattering, $\tilde{b}_{b,p}$. The three examples shown for each season are the same as those in Figures 5 and 6. Steeper negative slopes of $b_{b,p}$ are caused by size distributions with relatively more small particles, as occurred in the summer relative to the spring. Higher values of $\tilde{b}_{b,p}$ in the summer were caused by lower values of $b_p$ in the summer, with values of $b_{b,p}$ similar between the two seasons.***

We applied the bio-optical model of Morel and Maritorena (2001), developed for Case 1 waters. Compared to our estimates, their model underestimated $b_b$ by ~40% at 488 nm in the summer and overestimated $b_b$ by 16% in the spring (Fig. 8A). The underestimation of $b_b$ in the summer was caused both by higher backscattering efficiencies and higher scattering for non-phytoplankton than were assumed by the model. Ratios of $b_b(443):b_b(555)$ in both the summer and spring were close to modeled values (Fig. 8B). Ratios of $R_{rs}(443):R_{rs}(555)$ estimated from the model were higher than measured ratios in the spring and summer by 45% and 20%, respectively, mainly because of the underestimation of the contributions by CDOM (Fig. 9). In the model, $a_{CDOM}(440)$ was assumed to be a constant fraction (0.2) of the absorption due to both water and particles. In contrast, measured values of this fraction were higher during both seasons, with means of 1.22 in the summer and 0.65 in the spring.

## CONCLUSIONS

The combined use of Mie theory and individual particle measurements provided a detailed understanding of the roles of different particle groups in determining IOPs and AOPs in New England continental shelf waters. An important step in our approach was the validation of particle sum methods by comparison with concurrently measured bulk IOPs and AOPs. A novel aspect of our study was the measurement of optical contributions by both phytoplankon and non-phytoplankton particles. It is clear that measurements of the size distributions and optical properties of non-phytoplankton particles, including both detrital and mineral components, are necessary to understand changes in IOPs, especially $b_b$, in the ocean and to explain deviations from current bio-optical models.

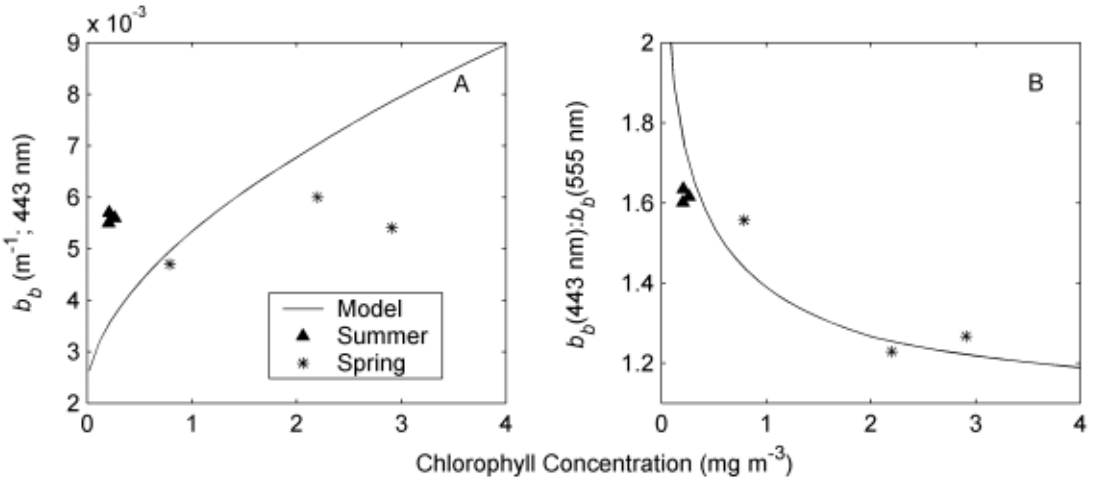


***Figure 8. Comparison of model estimates and summer and spring values of (A) $b_b(443)$ and (B) $b_b(443):b_b(555)$ as derived from our FCM-Mie method.***

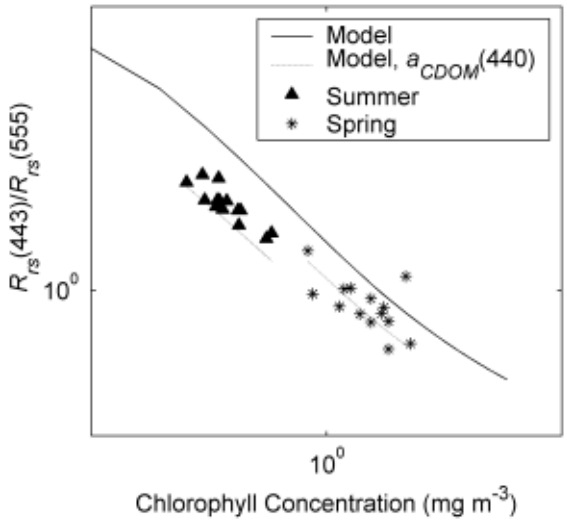


***Figure 9. Comparison of the reflectance ratio, $R_{rs}(443):R_{rs}(555)$, at different chlorophyll concentrations measured during the summer and spring and estimated using a bio-optical model. Results are shown both for the typical bio-optical model ("Model"), with its assumption of $a_{CDOM}(440)$ equal to 20% of the sum of absorption by water and particles, and for the bio-optical model with the measured percentages of $a_{CDOM}(440):(a_w(440)+a_p(440))$ in the summer and spring substituted into the model ("Model, $a_{CDOM}(440)$").***

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