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Contributions of phytoplankton and other particles to inherent optical properties in New England continental shelf waters

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Abstract

Variability in upper ocean optical properties is often driven by changes in the particle pool. We investigated the effects of such changes by characterizing individual particles. For particles in natural assemblages, we used a combination of Mie theory and flow cytometry to determine diameter (D), complex refractive index ($n + in'$), and optical cross-sections at 488 nm. Particles were grouped into categories of eukaryotic pico/nanophytoplankton, *Synechococcus*, heterotrophic prokaryotes, detritus, and minerals to interpret variability in concurrently measured bulk inherent optical properties (IOPs) in New England continental shelf waters during two seasons. The summed contributions of individual particles to phytoplankton absorption and particle scattering were close to values for these properties measured independently using bulk methods (87% and 107%, respectively). In surface waters during both seasons, eukaryotic phytoplankton were responsible for the majority of both total particle absorption and total particle scattering. Mineral particles contributed the most to backscattering (b_b) in the spring, whereas in the summer both mineral and detrital particles were important. *Synechococcus* and heterotrophic prokaryotes never contributed more than 14% to IOPs. Our findings emphasize that the measurement of nonliving particles, including detritus and minerals, is necessary for understanding variability in b_b in the ocean, an important quantity in the interpretation of satellite ocean color.

Knowledge of particle properties is important for the interpretation of variability in the inherent optical properties (IOPs; Table 1) of the upper ocean. Models of particle IOPs will be the basis for improved algorithms for light attenuation and for deriving properties such as chlorophyll concentration from satellite ocean color. Results of previous model simulations have shown how changes in particle type can give significantly different IOPs, even at the same chlorophyll concentration (Mobley and Stramski 1997; Stramski et al. 2001). These simulations were based on assumptions about the types of particles present and the distributions of diameter (D) and complex refractive index ($n + in'$) for each particle group included in the model. Certain of these particle properties are difficult to measure, especially distributions of n and n'. By definition, n governs scattering at interfaces and n' is related to the absorption properties of a particle. Inferences of average n for a particle assemblage have been made by a variety of optical methods, in conjunction with Mie theory (e.g., Zaneveld et al. 1974; Morel and Bricaud 1986; Stramski and Morel 1990; Twardowski et al. 2001), but detailed distributions of individual particle properties (i.e., within-group variations) cannot be determined from bulk optical approaches. 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 toward determining distributions of particle D, n, and n' with application to determining particle contributions to IOPs (Ackleson and Spinrad 1988; Olson et al. 1989; Perry and Porter 1989; Olson et al. 1993; Marie et al. 1997; DuRand and Olson 1998; Claustre et al. 1999; Green et al. 2003).

Total IOPs are determined by the additive contributions of the individual constituents that absorb and scatter light within a water body. Bulk IOPs have been modeled by considering typical properties of a small number of constituents, including colored dissolved organic matter (CDOM), phytoplankton, detritus, and water (Mobley 1994). Recently, Stramski et al. (2001) extended this by simulating the contributions to IOPs of 18 planktonic components (ranging in size from viruses to microplanktonic species of 30 μm in diameter), detritus (nonliving organic particles), mineral particles, and air bubbles. To apply this type of analysis to natural assemblages, Stramski et al. concluded that an increased effort is needed to characterize the types and concentrations of particles suspended in seawater, using techniques such as flow cytometry. To this end, our goal in the present study was to apply flow cytometry to the modeling of IOPs by measuring and describing populations of natural particles that are optically important.

To simplify individual particle studies, Stramski and Mobley (1997) outlined two criteria for defining functional particle categories. First, the sum of studied particulate constituents should account for the total bulk optical properties in a water body as accurately as possible. Second, each of the particle categories should play a well-defined ecological role in the marine ecosystem to allow linkage between optical studies and ecosystem models. Our emphasis in the present work is on the first criterion, although we are also interested

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in the ecological roles of each group; further research is clearly needed, for example, into the linkage between microbial production and the presence of detrital particles. In the open ocean, the dominant paradigm is that phytoplankton and their detrital products are important in determining the absorption coefficient (a), bacteria are important contributors to the scattering coeficient (b), and particles of less than 1 μm in size, most likely detritus, determine the backscattering coefficient (b_b) (Kirk 1983; Lewis and Cullen 1991; Morel and Ahn 1991; Stramski and Kiefer 1991). In an example simulation of open ocean waters, Stramski et al. (2001) suggested, however, that minerals and detritus were the most important contributors to b, and that minerals were by far the most important contributors to b_b. The contribution of viruses to IOPs is considered negligible (Stramski and Kiefer 1991; Mobley and Stramski 1997; Stramski et al. 2001).

The Coastal Mixing and Optics experiment (CMO) presented an opportunity for determining the contributions of natural assemblages of particles to IOPs. During this experiment, we intensively sampled individual particle and bulk optical properties at a site on the New England continental shelf during summer and spring. We showed in previous work that particles (as opposed to CDOM) were the primary source of temporal and vertical variability in optical properties (Sosik et al. 2001); in the present study we determine the optical contributions of distinct particle groups. We combine Mie theory and flow cytometric (FCM) measurements to determine the particle properties and contributions to IOPs at 488 nm of eukaryotic pico/nanophytoplankton (''eukaryotic phytoplankton''), *Synechococcus*, heterotrophic prokaryotes, detritus, and minerals. We then compare the summed contribution of these particles to measured particle IOPs, and discuss seasonal and vertical variability in IOPs.

Methods

Bulk optical properties—Vertical profiles for water sampling and measurements of optical properties were made as part of CMO as described by Sosik et al. (2001). The CMO site was located on the southern New England shelf, south of Martha's Vineyard (40°30′N, 70°30′W), in a region known as the ''Mud Patch'' and with a water depth of ~70 m. Data were collected during two 3-week cruises in the late summer of 1996 aboard the R/V *Seward Johnson* (cruise SJ9610, 17 August–7 September) and spring of 1997 aboard the R/V *Knorr* (cruise KN150, 24 April–13 May). Profiles were carried out approximately three times per day during daylight hours at the main CMO site. IOPs were measured in situ with absorption and attenuation meters (ac-9, WetLabs) designed to measure a and beam attenuation coefficients (c) in 9 spectral bands (412, 440, 488, 510, 532, 555, 650, 676, and 715 nm). During both cruises, ac-9 meters were mounted on a profiling package, and unfiltered and filtered (<0.2 μm) seawater was pumped through two separate meters to determine particle absorption (a_p) and attenuation (c_p), CDOM absorption (a_{CDOM}), and particle scattering ($b_p = c_p - a_p$). Additionally, optical measurements (i.e., spectrophotometry and flow cytometry) were made aboard ship on water samples collected from six depths throughout the water column using a conductivity–temperature–depth profiler/rosette system equipped with sampling bottles. Absorption coefficients for particulate material collected on GF/F filters (nominal pore size of 0.7 μm) were determined spectrophotometrically (using a Cary 3E dual beam ultraviolet/visible spectrophotometer). Subsequent to the initial optical density measurements, filters were extracted in methanol and reanalyzed to determine the residual particulate absorption (a_{dm}; detritus + minerals) (Kishino et al. 1985); the absorption coefficient due to methanol-extractable phytoplankton pigments (a_{ph}) was estimated by difference between the initial and postextraction measurements.

Flow cytometry—An Epics V flow cytometer (Coulter Electronics) interfaced with a Cicero acquisition system (Cytomation) was used to measure forward light scattering (FLS, ~3–19° at 488 nm), side light scattering (SSC, ~54–126° at 488 nm), red fluorescence from chlorophyll (CHL, 660–700 nm), orange fluorescence from phycoerythrin (560–590 nm), green fluorescence from nucleic acid stain (515–545 nm), and the concentration of particles. The dynamic range of FLS, SSC, and CHL measurements was increased by splitting the optical signals and independently detecting and amplifying them with separate photomultipliers and 3-decade logarithmic amplifiers. For each property, the relative sensitivities of the two measurements were adjusted to overlap by one decade, and thus the potential measurement range was expanded to five orders of magnitude. Particles were injected into a saline sheath flow and illuminated by an argon ion laser beam linearly polarized parallel to the fluid stream. The samples were delivered with a peristaltic pump (Harvard Apparatus), and cell concentration was determined from pump flow rate and sample analysis time. Three types of reference particles were measured, including polystyrene microspheres of various sizes (0.57–6.2 μm YG beads; Polysciences), a silica bead of 1.58 μm (Duke Scientific), and oil suspensions (heptane, nonane, and dodecane; Sigma Chemical). For populations of beads, arithmetic means of FLS, SSC, and CHL were computed after transformation of distributions to linear values. All FCM measurements were referenced to the 2.14-μm bead.

FCM measurements were made of four particle groups: eukaryotic phytoplankton, *Synechococcus*, nonphytoplankton, and heterotrophic prokaryotes. Phytoplankton and nonphytoplankton of ~0.75 μm to 50 μm in diameter were analyzed at sea in 538 and 344 samples in summer and spring, respectively. In general, 4.5 ml of sample were analyzed at 0.5 ml min^{-1}. Eukaryotic phytoplankton populations were discriminated from nonphytoplankton particles on the basis of their red fluorescence, and *Synechococcus* cells were discriminated on the basis of their orange fluorescence. The nonphytoplankton group contains several types of particles, including detritus, minerals, and microheterotrophs such as ciliates and flagellates. Selected 1-ml samples (from midday casts) were preserved in glutaraldehyde (0.1%) and frozen in liquid nitrogen; after the cruises, these samples were analyzed for heterotrophic prokaryotes on a flow cytometer in the laboratory (146 samples in summer and 185 in spring). Heterotrophic prokaryotes (which include both bacteria and archaea) were enumerated by staining samples

with a nucleic acid stain (SYBR Green I, Molecular Probes), following the protocol of Marie et al. (1997). All FCM data were saved as two-dimensional histograms and listmodes, which were analyzed using a modified version of ''CYTOWIN'' software (originally written by D. Vaulot; http://www.sb-roscoff.fr/Phyto/cyto.html). All subsequent data analysis was performed using the MATLAB software package (The MathWorks). FCM counts for each particle group in a sample were large (on the order of 10^4–10^5), and measurements of FLS, SSC, and CHL were binned to 32 logarithmically spaced divisions to minimize computation time.

FCM–Mie method—A combination of FCM measurements and Mie theory was used to determine individual particle properties (referred to as the ''FCM–Mie method''; for details *see* Green et al. 2003). We applied the FCM–Mie method to the analysis of eukaryotic phytoplankton, *Synechococcus*, heterotrophic prokaryotes, and nonphytoplankton in each sample. As described in Green et al. (2003), to apply Mie theory to FCM measurements, an optimization was performed between theory and measurements using calibration particles of known D, n, and n'. Two different optimizations were used, one for phytoplankton and nonphytoplankton particles and a second for heterotrophic prokaryotes for which FCM settings were changed to enumerate smaller particles (Green 2002). FCM–Mie analysis was limited to particles of less than 10 μm in diameter, because solutions are often not unique for larger particles. As well, an FLS and SSC correction was applied to measurements for phytoplankton cells to account for deviations of cells from the Mie theory assumptions that particles are spherical and homogenous (Green et al. 2003). Particle properties (D, n, and n') and optical cross-sections for absorption (σ_a), scattering (σ_b), and backscattering (σ_{bb}) at 488 nm were determined from the FCM–Mie method by comparison of FCM FLS, SSC, and CHL to values in a Mie-based lookup table. Optical cross-sections in the lookup table were calculated directly from D, n, and n' according to Mie theory. In our previous work with the FCM–Mie method (Green et al. 2003), we have shown that particle size can be determined accurately for cultured cells grown under both high- and low-light conditions, and that derived values of n' for phytoplankton are correlated with intracellular chlorophyll concentration; n estimated from the method can be used to distinguish organic from inorganic particles, although variability within phytoplankton was not well resolved.

Using FCM–Mie values of n, nonphytoplankton were separated into detrital and mineral components. Previous studies have shown phytoplankton n to be in the range of 1.02–1.10 (Aas 1996), and mineral n in the range of 1.14–1.26 (Lide 1997) (all refractive indices will be discussed relative to seawater, for which $n = 1.339$ relative to a vacuum). On the basis of these findings, we defined detritus (organic particles) as nonphytoplankton with $n \leq 1.10$ and minerals (inorganic particles or organic–inorganic complexes) as nonphytoplankton with $n > 1.10$. The lower diameter limit for FCM measurement (0.75 μm) was used for minerals. However, we used a minimum diameter of 1.2 μm for detritus, because detrital particles smaller than this size were in a region of FCM measurements that was not well fit by the Mie theory calibration.

Distributions of particle properties were determined for each particle group. For eukaryotic phytoplankton and *Synechococcus*, we used the FCM–Mie method to analyze each cell in a sample and created distributions of D, n, n', σ_a, σ_b, and σ_{bb}. For nonphytoplankton and heterotrophic prokaryotes, absorption could not be determined directly from FCM measurements, so we used the FCM–Mie method to create distributions of D, n, σ_a, σ_b, and σ_{bb} by assuming a constant value of n'. For heterotrophic prokaryotes, n' was assumed to be 5×10^{-4} at 488 nm on the basis of previously published values (Morel and Ahn 1990; Stramski and Mobley 1997). For nonphytoplankton, n' was determined by considering particles of 0.1–50 μm in diameter (see below) and selecting a value of n' that minimized differences between the estimated total nonphytoplankton absorption, a_{dm}, and the value independently determined by spectrophotometry. Mean property values were calculated by considering all particles in a sample. Mean daily values were calculated by first averaging replicates from the same sample, followed by averaging all samples from the same day and the same depth bin (0–20 m, 20–40 m, or 40–65 m).

Calculation of constituent contributions to IOPs—For particles of 0.1–50 μm in diameter, IOPs were calculated as the sum of particles analyzed with the FCM–Mie method plus particles from outside the FCM–Mie range that had to be analyzed using different methodology. For eukaryotic phytoplankton and nonphytoplankton outside the range of FCM measurement (i.e., <0.75 μm in diameter) and the FCM–Mie method (i.e., particles >10 μm in diameter and detritus <1.2 μm in diameter), contributions to IOPs were estimated as follows.

The contribution of small detritus (0.1–1.20 μm) and minerals (0.1–0.75 μm) was determined using Mie theory and extrapolated size distributions. The extrapolations were made from size distributions determined separately for detritus and minerals in the diameter range of the FCM–Mie method. Size distributions were described by a Junge-type model (or hyperbolic fit) and, as in previous work (Harris 1977; McCave 1983; Risović 1993), a segmented fit with two different slopes was used. For our results, we found that two slopes, above and below 3.5 μm (referred to as the ''<3.5 and >3.5 μm Junge slopes''), adequately described the size distributions. In an effort to keep our assumptions regarding description of these size distributions as simple as possible, we used a single diameter value for the slope change and 3.5 μm was chosen as the midpoint in a range of values that gave the smallest overall residuals when considering all data for detritus and minerals in both seasons. The sensitivity of our results to this selection is addressed below. We calculated contributions of small detritus and minerals to IOPs from Mie theory with inputs of the size distribution extrapolated with the <3.5 μm Junge slopes, a mean value of n from particles measured with the FCM–Mie method, and the nonphytoplankton n' estimated as described above.

The contributions of larger particles (between 10 μm and 50 μm) to IOPs were calculated with Mie theory and em-

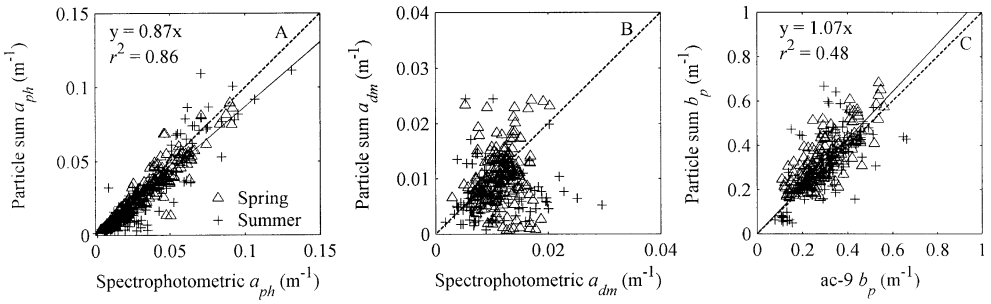

Fig. 1. Comparison of particle sum and bulk (A) phytoplankton absorption, a_{ph}, (B) nonphytoplankton absorption, a_{dm}, and (C) particle scattering, b_p, at 488 nm for the summer and spring. To estimate particle sum a_{dm}, n' was assumed equal to 0.001 for all nonphytoplankton particles. Particle sum b_p is the sum of scattering by all 0.1–50-μm particles, including eukaryotic phytoplankton, *Synechococcous,* heterotrophic prokaryotes, detritus, and minerals. The 1 : 1 line (dashed lines) and least-squares regression between particle sum and bulk inherent optical properties (solid lines) are shown.

pirically derived *D.* For both eukaryotic phytoplankton and nonphytoplankton, *D* was determined from FCM FLS and an empirical relation with Coulter Counter diameter determined in the laboratory (Green et al. 2003). For eukaryotic phytoplankton, n' was calculated from *D* and σ_a obtained from an empirical relation between FCM CHL and spectrophotometric σ_a for phytoplankton cultures (Green et al. 2003). For >10-μm eukaryotic phytoplankton and nonphytoplankton, *n* was assumed to be the same as the mean value determined for each group in the size range of the FCM–Mie method. In addition, the fractions of >10-μm nonphytoplankton present as detritus and minerals were assumed to be the same as determined for FCM–Mie nonphytoplankton. *D*, *n*, and n' were used as inputs to Mie theory to determine the contributions to *a*, *b*, and b_b of particles >10 μm in diameter.

We computed mean depth profiles of *a*, *b*, and b_b at 488 nm for seawater constituents in the summer and spring. We used constant values of IOPs for pure seawater (Morel 1974; Pope and Fry 1997) and a_{CDOM} as measured with the filtered ac-9. Total IOPs for each particle group were computed by summing the respective optical cross-sections for all particles in the group from a known sample volume. Mean particle IOPs were calculated in the same way as mean particle properties (*see above*), except that 10-m depth bins were used. For lack of precise information about the composition of detrital particles, we have assumed that heterotrophic prokaryotes are included in our extrapolation to submicron detritus. For this reason, the contributions of heterotrophic prokaryotes derived from analysis of postcruise stained samples were subtracted from the initial estimates of detrital IOPs.

We used Mie theory to evaluate the sensitivity of our IOP estimates to observed within-group changes in particle properties. First, we used the theory and inputs of all four observed properties (concentration, *D*, *n*, and n') to determine our best estimates or ''full model'' values of IOPs. Next, we estimated ''limited model'' IOPs, with observed inputs for only three properties and with the remaining property held constant at its mean. For heterotrophic prokaryotes, detritus, and minerals, n' was always held constant. Additionally, the sensitivity of detrital and mineral contributions to changes in *n* could not be evaluated because a mean *n* was used for much of the size distribution; the same mean *n* was assumed in the sensitivity analyses for these particles.

Results and discussion

Comparison of particle sum and bulk approaches—We compared phytoplankton absorption, a_{ph}, estimated from the particle sum method with spectrophotometrically determined values of a_{ph}. Particle sum a_{ph} was calculated as the sum of absorption by eukaryotic phytoplankton and *Synechococcus.* In comparison to spectrophotometric a_{ph}, particle sum a_{ph} was 87% of measured values, considering both seasons (Fig. 1A), and a high percentage of the variance was explained by a linear relation (r^2 = 0.86). Particle sum a_{ph} may be a slight underestimate because absorption by cells larger than 50 μm was not considered. Phytoplankton cells within the range of FCM–Mie analysis (0.75–10 μm in diameter) accounted for the majority of particle sum a_{ph} in both the summer (95%) and spring (80%).

We evaluated the particle sum determination of nonphytoplankton absorption, a_{dm}, by comparing values with independent measurements of a_{dm}. As described above, a_{dm} was determined by assuming a constant n' for all nonphytoplankton particles that gave the best overall agreement between particle sum and spectrophotometric a_{dm}. In the spring, all depths were used in the comparison. In the summer, we limited the comparison to the top 30 m, because a significantly higher n' was needed to reproduce the spectrophotometric a_{dm} below the mixed layer. Focusing this analysis on surface layer particles was not a major limitation since Mie calculations showed that errors in the choice of n' for nonphytoplankton below 30 m had little effect on their contribution to *b*. Assuming a value for n' of 0.001 gave the best agreement between particle sum and spectrophotometric a_{dm} for the two seasons, but the regression was not significant (Fig. 1B). Unexplained variance in the relation between particle sum and spectrophotometric a_{dm} is likely caused by variability in the absorption properties of nonphytoplankton due to their complex composition (e.g., heterotrophic organisms,

dead cells, fecal material, or minerals). Our value for nonphytoplankton n' is slightly higher than a previously proposed value of 3×10^{-4} at 488 nm (Stramski et al. 2001; accounting for typographical error in their equation on p. 2935, Stramski [pers. comm.]) determined from analysis of the microphotometric data of Iturriaga and Siegel (1989) for particles between ~9 and 27 μm in diameter from the Sargasso Sea. The difference between these two estimates may reflect differences in the composition of nonphytoplankton particles between our study site and the Sargasso Sea and between the size ranges of particles analyzed. We used the value of n' we determined for nonphytoplankton for Mie calculations of b and b_b. Because of the problems with estimating a_{dm} with the FCM–Mie method, spectrophotometric values were used for IOP budgets.

We compared total scattering by all particles to bulk (ac-9) measurements of b_p. In the summer, particle sum b_p was significantly lower than bulk b_p below 30 m presumably because the particle sum method did not include nonphytoplankton >50 μm in diameter and resuspension was active (Agrawal and Traykovski 2001; Boss et al. 2001; Hill et al. 2001). Summer samples below 30 m were therefore not included in this comparison with bulk b_p. In situ bulk measurements were collected not more than 5 h (average 1.7 h) from the time of water sampling for FCM measurements. Linear regression between particle sum and bulk b_p gave a slope of 1.07 ($r^2 = 0.48$), considering both seasons (Fig. 1C). Given that particles >50 μm in diameter were not considered in the particle sum estimates, we expected the particle sum b_p to be less than the measured bulk b_p. With the present data we cannot be sure of the absolute accuracy of the ac-9 measurements or the particle sum b_p. There are potential errors in both approaches. For instance, deviation of particles from the Mie assumptions of homogenous spheres may affect results of the particle sum approach for nonphytoplankton particles, as was shown for phytoplankton (Green et al. 2003), and uncertainty in calibration and scattering corrections can affect ac-9 data (Pegau et al. 1995). The generally small differences between particle sum and bulk optical properties gave us confidence in applying the particle sum approach to interpretation of variability in IOPs for both seasons.

Particle properties and seasonal variability—The water columns of the summer and spring exhibited different physical and optical properties. The water column was well stratified in the summer (before the passage of Hurricane Edouard), with depleted nutrient levels in the surface mixed layer, a subsurface chlorophyll maximum between 20 and 30 m, and midwater column maxima in a_p and b_p (Sosik et al. 2001). In the spring, the water column was less stratified; stratification increased over the 3-week sampling period, leading to a phytoplankton increase in surface waters, an associated decrease in nutrient levels, and increased a_p and b_p (Sosik et al. 2001). In the spring, the highest values of chlorophyll *a*, a_p, and b_p consistently occurred in the top 25 m of the water column. The relation between b and chlorophyll concentration indicated that during spring and part of the summer we sampled Case 1 water (Gordon and Morel 1983; Loisel and Morel 1998). For some of the summer period, particle scattering relative to chlorophyll was higher, indicating Case 2 conditions.

Table 1. Notation.

IOP	Inherent optical property
FCM	Flow cytometric
FLS	Forward angle light scattering, 488 nm, bead units
SSC	Side angle light scattering, 488 nm, bead units
CHL	Red chlorophyll fluorescence, 680 nm, bead units
CDOM	Colored dissolved organic matter
D	Diameter
n	Real refractive index, relative to seawater (n=1.339 relative to vacuum)
n'	Imaginary refractive index, relative to n of seawater
a	Absorption coefficient, m^{-1}
b	Scattering coefficient, m^{-1}
c	Beam attenuation coefficient, m^{-1}
b_b	Backscattering coefficient, m^{-1}
a_x, b_x, c_x, b_{bx}	Optical coefficients where x is a particular seawater constituent, e.g., a_p is particulate absorption and $b_{b,det}$ is detrital backscattering, m^{-1}
σ_a, σ_b, σ_{bb}	Optical cross-sections, m^2
$\tilde{b}_b$	Backscattering ratio, b_b/b

The seasonal differences in stratification and nutrient distributions were reflected in the phytoplankton cell properties. In surface waters (0–20 m), eukaryotic phytoplankton were significantly more abundant in the spring ($P < 0.05$), whereas *Synechococcus* were more abundant in the summer ($P < 0.001$; Table 2). Lower *Synechococcus* concentrations measured in the spring are consistent with observations of Waterbury et al. (1986), who documented a relation between water temperature and *Synechococcus* abundance in New England coastal waters. For both eukaryotic phytoplankton and *Synechococcus*, mean distributions of D, n, and n' in surface waters were unimodal in both seasons (Fig. 2A–F), and distributions of n and n' were in the ranges expected for phytoplankton, 1.01–1.10 and 0.002–0.02, respectively (Aas 1996; Stramski et al. 2001). Cells were significantly smaller in summer surface waters ($P < 0.01$) and had higher values of n ($P < 0.01$) than in spring, but n' was not significantly different between seasons (Table 2). Higher values of n suggest that cells had higher intracellular carbon content in summer surface waters, which may have been caused by changes in species composition, an increase in the carbon per cell for the same species, or rearrangements in internal structures (or all three). Despite observed changes in n, our previous work with laboratory cultures suggests that further work is needed to improve measurement of and to accurately interpret changes in n (Green et al. 2003).

Phytoplankton cell properties also varied with depth. Eukaryotic phytoplankton and *Synechococcus* decreased in abundance with depth in both seasons (Table 2). The amplitude of the change was greater in the summer, consistent with a larger change in nutrient availability with depth than in the spring (Sosik et al. 2001). Cells increased in size with depth in the summer, in contrast to the spring when cells decreased in size with depth (Table 2). In the summer, high nutrient levels below the mixed layer may have allowed larger cells to grow there, while in the spring similar nutrient

Table 2. Mean particle properties, including concentration (ml^{-1}), D (μm), n (488 nm), n' (488 nm), <3.5 Junge slope, and >3.5 Junge slope for different particle groups. Mean properties were computed for both seasons in the three depth ranges of 0–20 m, 20–40 m, and 40–65 m. As justified in the text, constant values of n' were assumed for both heterotrophic prokaryotes and nonphytoplankton (both detritus and minerals) of 5×10^{-4} and 1×10^{-3}, respectively.

		Summer			Spring		
Particle type	Property	0–20 m	20–40 m	40–65 m	0–20 m	20–40 m	40–65 m
Eukaryotic phytoplankton	Concentration	1.84×10^4	1.44×10^4	0.18×10^4	2.46×10^4	1.60×10^4	0.532×10^4
	D	2.05	2.26	2.28	2.31	2.23	2.05
	n	1.066	1.073	1.073	1.059	1.064	1.067
	n'	0.0051	0.0139	0.0165	0.0057	0.0089	0.0100
Synechococcus	Concentration	5.94×10^4	9.14×10^4	0.847×10^4	0.776×10^4	0.672×10^4	0.515×10^4
	D	1.09	1.16	1.23	1.38	1.35	1.34
	n	1.067	1.068	1.069	1.054	1.056	1.057
	n'	0.0022	0.0071	0.0093	0.0024	0.0033	0.0043
Heterotrophic prokaryotes	Concentration	1.06×10^6	1.22×10^6	0.941×10^6	1.48×10^6	1.30×10^6	1.18×10^6
	D	0.460	0.453	0.454	0.463	0.455	0.454
	n	1.079	1.077	1.077	1.081	1.081	1.078
Detritus	Concentration	16.1×10^7	8.68×10^7	4.81×10^7	0.727×10^7	0.312×10^7	2.04×10^7
	<3.5 Junge slope	4.82	4.58	4.36	3.61	3.45	4.08
	>3.5 Junge slope	1.93	2.24	2.22	2.22	2.25	2.42
	n	1.064	1.064	1.067	1.070	1.072	1.072
Minerals	Concentration	1.12×10^7	1.28×10^7	0.916×10^7	0.866×10^7	0.817×10^7	0.668×10^7
	<3.5 Junge slope	4.37	4.25	3.81	4.00	3.98	3.68
	>3.5 Junge slope	1.62	1.61	1.67	2.54	2.58	2.52
	n	1.16	1.18	1.19	1.19	1.19	1.19

levels existed throughout the water column and light level may have had a larger effect on cell size. The presence of larger cells at high light levels in spring surface waters is supported by previous studies using phytoplankton cultures, which have suggested that nutrient-replete cells are generally larger at high light levels than at low light levels (Falkowski et al. 1985; Sakshaug et al. 1987; Sosik et al. 1989). In addition, species differences could have contributed to changes in D with depth. Values of n and n' generally increased with depth in both seasons. Lower values of n' are expected in surface waters since cells have less photosynthetic pigment per cell under conditions of high light and low nutrients (e.g., Mitchell and Kiefer 1988; Sosik et al. 1989; Sosik and Mitchell 1991). The fact that similar values of n' were observed in summer and spring surface waters may have been caused by enhanced absorption by photoprotective accessory pigments in the summer. As well, mean n' for *Synechococcus* was lower than for eukaryotic phytoplankton, presumably because the phycoerythrin in coastal *Synechococcus* does not absorb at 488 nm as strongly as the accessory pigments of eukaryotic phytoplankton (Olson et al. 1990).

Seasonal and vertical variability was observed in heterotrophic prokaryotic properties, primarily in concentration and the shape of size distributions. Heterotrophic prokaryotes in summer surface waters were significantly less abundant than in spring ($P < 0.005$; Table 2). The highest concentrations of both heterotrophic prokaryotes and eukaryotic phytoplankton occurred in spring surface waters, consistent with a previous suggestion of a link between the abundances of the two microbial groups (Azam et al. 1983). Previous studies have documented decreasing bacterial concentrations with depth, as we observed in the spring, and concentrations similar to ours ($\sim10^6$ ml^{-1}) have typically been found for the upper ocean (Cho and Azam 1990; Koike et al. 1990; Sieracki and Viles 1992; Noble and Fuhrman 1998; Kuipers et al. 2000). Unimodal distributions were observed for D in the summer and n in both seasons (Fig. 2G, H). In contrast, bimodal size distributions were observed in the spring and were most likely caused by the presence of different species (Zubkov et al. 2001). Values of D and n changed little with depth, except after increased stratification in the spring when n was higher in surface waters.

For bacterial cells, our mean D estimates of $\sim$0.45 μm are within the range of previous findings (Fuhrman 1981; Lee and Fuhrman 1987; Stramski and Kiefer 1990). Our mean n values of $\sim$1.079 are slightly higher than those of Stramski and Kiefer (1990), which were between 1.042 and 1.068, but are within the range of plausible values (Aas 1996). We did observe some unexpectedly high values of n (>1.10). This may be an indication that the FCM–Mie method underestimates D and overestimates n for heterotrophic prokaryotes, which may require scattering corrections similar to those applied to phytoplankton to account for deviation of cells from homogenous spheres. It is also possible that elevated n values resulted from scattering artifacts associated with preservation of these cells (Vaulot et al. 1989).

Nonphytoplankton particles in summer surface waters differed significantly from those in deeper waters in the summer and all depths in the spring. In both seasons, Junge slopes for >3.5 μm and <3.5 μm nonphytoplankton were within the range of values (2–5, with 3–4 typical) previously reported for marine particle size distributions (Table 2; refs. in Mobley [1994]). In our observations, <3.5 μm Junge slopes were always higher than >3.5 μm Junge slopes (Table 2; Fig. 2J). Although many studies have documented

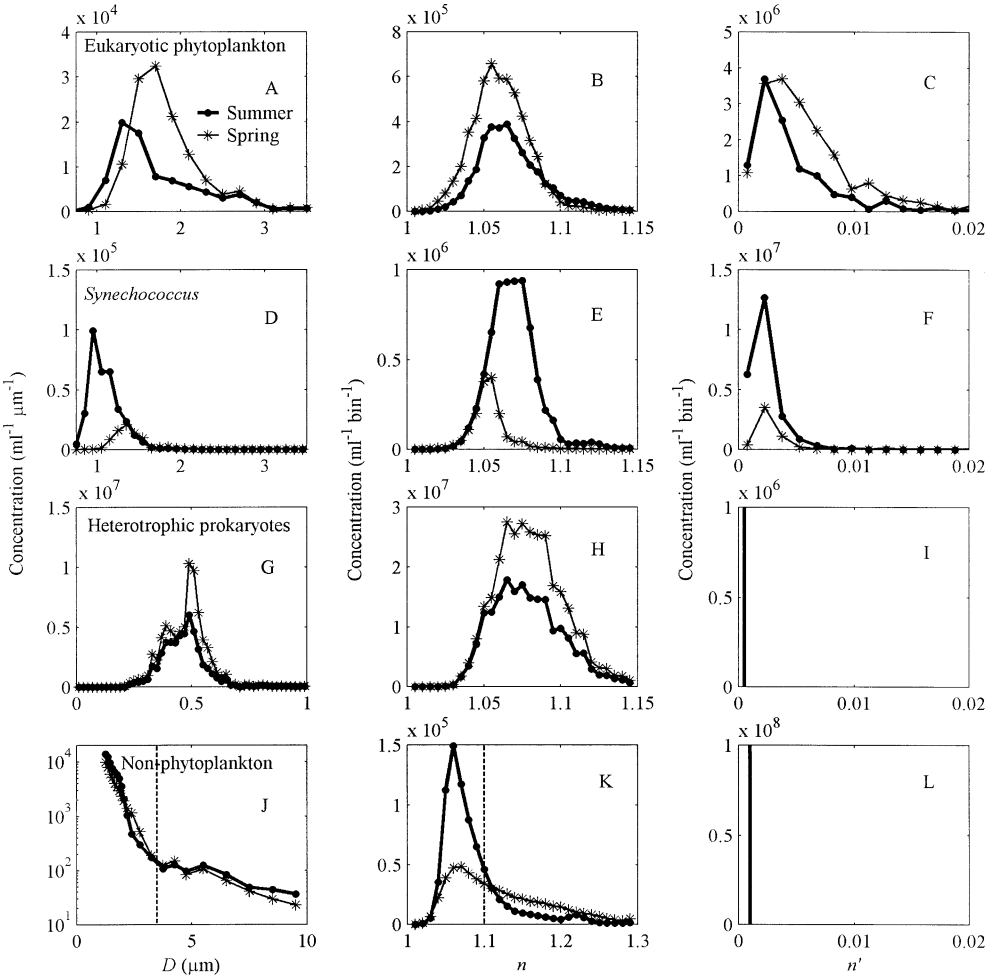

Fig. 2. Mean property distributions of D, n (488 nm), and n' (488 nm) in summer and spring surface waters (0–20 m) for eukaryotic phytoplankton (A–C), *Synechococcus* (D–F), heterotrophic prokaryotes (G–I), and nonphytoplankton (J–L). Values of n' for heterotrophic prokaryotes (I) and nonphytoplankton (L) were assumed constant at the values 5×10^{-4} and 1×10^{-3}, respectively. A dashed line was drawn at 3.5 μm on the nonphytoplankton size distributions (J) to indicate the cutoff between >3.5 μm and <3.5 μm Junge slopes. A dashed line was also drawn at 1.10 on nonphytoplankton n distributions (K) to mark the cutoff between detritus and minerals. Note the change in abscissa scale in (G), (J), and (K).

lower slopes for the smallest particles in marine size distributions, both higher and lower slopes have previously been observed for small size classes (e.g., McCave 1983; Risović 1993). Because the size distributions we report here are only for detrital and mineral particles, and specifically do not include phytoplankton, it is difficult to compare our findings with previous work that considered all particles. The highest <3.5 μm Junge slopes were in summer surface waters and were responsible for our finding that nonphytoplankton were significantly more abundant in summer surface waters, compared to the spring ($P < 0.001$). Considering both seasons, mean distributions of n for nonphytoplankton were peaked around 1.065, and values of n occurred most frequently in the range of 1.02–1.25 (Fig. 2K). Nonphytoplankton in summer surface waters, compared to the spring, had significantly lower values of n ($P < 0.001$).

Differences in detrital particle concentrations were primarily responsible for variability in nonphytoplankton properties. The highest Junge slopes observed for both detritus and minerals were for small detrital particles in summer surface waters (Table 2). Nonphytoplankton particles were more abundant in summer surface waters, compared to the spring, because detritus was significantly more abundant ($P < 0.001$; Fig. 3A). In contrast, minerals were not significantly different in concentration in surface waters between summer and spring (Fig. 3B). The percent of nonphytoplankton that was detritus in the FCM–Mie diameter range (1.2–10 μm) generally decreased with depth in both seasons, and the difference between the mixed layer and below the mixed layer was greater in the summer (Fig. 4A, B). Considering both seasons, detritus had mean n in the range of 1.064–1.072, similar to values determined for phytoplankton and heterotrophic prokaryotes; minerals had mean n in the range of 1.16–1.19, which is reasonable for minerals (Grim 1953;

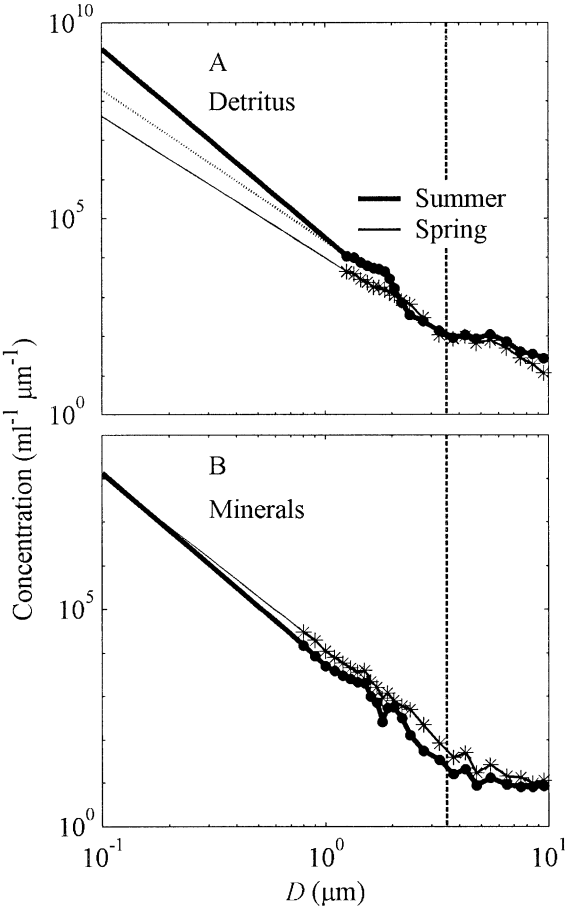

Fig. 3. Mean size distributions in summer and spring surface waters (0–20 m) for (A) detritus and (B) minerals. Points in the distributions indicated by symbols were determined from the FCM–Mie method and correspond to 1.2–10 μm for detritus and 0.75–10 μm for minerals. Below these size ranges, distributions were determined from an extrapolation of the size distribution using a Junge fit to FCM–Mie points of <3.5 μm in diameter (3.5 μm is indicated by the dashed line). The Junge fit for detritus in the summer was the most sensitive to the choice of diameter for the slope change (*see text* for details), as can be seen by comparing the extreme case of extrapolating on the basis of observations ≤ 2 μm (dotted line).

Lide 1997). Nonphytoplankton had the lowest values of n in summer surface waters because of the higher abundance of detritus.

Submicron detrital particles were responsible for the higher abundances of nonphytoplankton 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 summer surface waters, compared to spring, in contrast to submicron mineral particles, which were not significantly different in abundance between the two seasons ($P < 0.001$). One hypothesis for the higher concentrations of small detritus in the late summer is that the preceding several months of high productivity led to the accumulation of a type of organic particle that is not readily useable by heterotrophic prokaryotes. It has previously been suggested that small detrital particles may be created as a result of phytoplankton and bacterial growth, protozoan egestion of picofecal pellets, or viral lysis of bacteria (Koike et al. 1990; Sieracki and Viles 1992; Nagata and Kirchman 1996; Shibata et al. 1997; Yamasaki et al. 1998). In support of our hypothesis, microbial processes, such as bacterial feeding, have been found to alter the structure of labile submicron particles, resulting in the production of semilabile and refractory particles that are relatively resistant to decomposition (Nagata and Kirchman 1996; Ogawa et al. 2001). Two 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 because of mixing and transport to deeper layers (Carlson et al. 1994; Williams 1995). As well, the accumulation of submicron detritus in summer surface waters may be enhanced by a decrease in bacterial activity (i.e., uptake of labile submicron particles) due to ultraviolet radiation (Herndl et al. 1993).

Constituent contributions to IOPs—Phytoplankton and CDOM were the main contributors to a in surface waters (Fig. 5A, B), with CDOM slightly more important in the summer, and eukaryotic phytoplankton more important in the spring. In summer surface waters, mean contributions to a at 488 nm were 31% by CDOM, 28% by eukaryotic phytoplankton, 22% by water, 10% by nonphytoplankton, 7% by *Synechococcus*, and 2% by heterotrophic prokaryotes. In spring surface waters, mean contributions to a were 39% by eukaryotic phytoplankton, 28% by CDOM, 20% by water, 10% by nonphytoplankton, 2% by heterotrophic prokaryotes, and 1% by *Synechococcus*. Absorption by heterotrophic prokaryotes was not important in either season. The greater importance of absorption by phytoplankton compared to that by nonphytoplankton particles has been observed in other coastal systems (Roesler et al. 1989; Cleveland 1995; Sosik and Mitchell 1995). Between particle groups, values of D and n' were more important than numerical concentration in determining contributions to a_p in both seasons. For example, eukaryotic phytoplankton were the least abundant of all groups but were the most important contributors to a_p because of their large D and high n' (Table 2). Moreover, heterotrophic prokaryotes were the least important contributors to a_p even though they were higher in abundance than eukaryotic phytoplankton.

All particle groups were important contributors to b in both seasons, except for *Synechococcus* and detritus in the spring (Fig. 5C, D). In summer surface waters, mean contributions to b at 488 nm were 44% by eukaryotic phytoplankton, 17% by detritus, 14% by *Synechococcus*, 13% by minerals, 11% by heterotrophic prokaryotes, and 1% by water. In spring surface waters, mean contributions to b were 61% by eukaryotic phytoplankton, 23% by minerals, 14% by heterotrophic prokaryotes, 2% by *Synechococcus*, 1% by water, and negligible by detritus. Submicron detritus contributed ~33% and 7% to total scattering by detritus (b_{det}) in summer and spring surface waters, respectively, and submicron minerals contributed ~55% to total scattering by minerals (b_{min}) in both seasons. Ratios of the optical cross-sections, σ_b/σ_a, were responsible for the observed differences in particle group contributions to b_p compared to a_p. Notably, heterotrophic prokaryotes were more important contributors

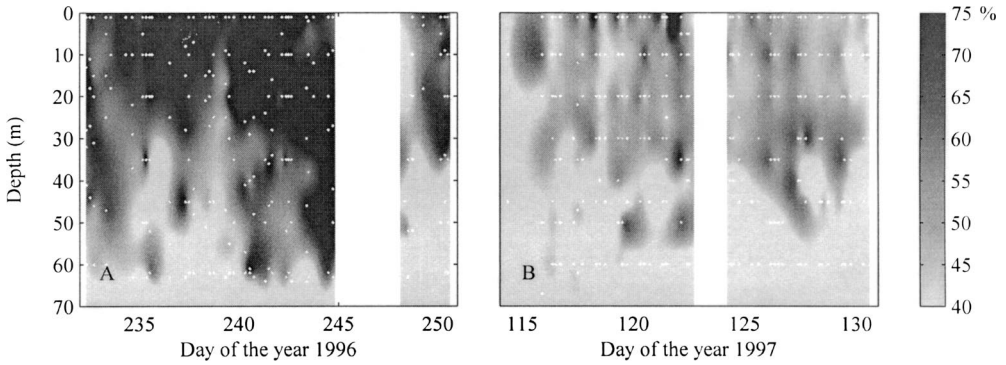

Fig. 4. Percent organic composition of nonphytoplankton for the (A) summer and (B) spring. Results are based on nonphytoplankton particles analyzed with the FCM–Mie method in the range of 1.2–10 μm. The period of no data collection between days 245 and 247 in the summer corresponds to the passage of Hurricane Edouard through the study site. Discrete sample positions are indicated (white dots).

to b_p than to a_p, because their mean σ_b/σ_a was ~31 considering both seasons, whereas the ratio for eukaryotic phytoplankton and *Synechococcus* was ~6 (Table 3). This was a direct result of heterotrophic prokaryotes having lower values of n' than phytoplankton, but similar values of n. Similar to findings in the equatorial Pacific for Case 1 waters (DuRand and Olson 1996; Claustre et al. 1999), in the present study (which includes both Case 1 and Case 2 waters), eukaryotic phytoplankton and nonphytoplankton were the most important contributors to beam attenuation by particles, $c_p = a_p + b_p$, with *Synechococcus* and heterotrophic prokaryotes being relatively less important optically. In simulations of oligotrophic waters, Stramski and Kiefer (1991) and Stramski et al. (2001) found that heterotrophic prokaryotes and nonphytoplankton were more important contributors to b than phytoplankton. This difference occurred because we observed higher concentrations of phytoplankton and lower ratios of both heterotrophic prokaryotes and nonphytoplankton to phytoplankton concentrations in continental shelf waters than were assumed for the open ocean simulations.

Seasonal variability in the particulate scattering to absorption ratio, $b_p : a_p$, was caused mainly by differences in the contributions of nonphytoplankton. Measurements of $b_p : a_p$, which are increasingly made in ocean optical studies, may provide information about the types of particles in the water. Variability in this ratio is difficult to interpret, however, without measurements of individual particles. For the top 10 m, the higher $b_p : a_p$ ratio we observed in summer (~13) compared to spring (~8) reflects the increased contribution of nonphytoplankton, and specifically detritus (Fig. 5E, F). Eukaryotic phytoplankton also contributed to high ratios in summer surface waters because of decreased cellular absorption as a result of photoacclimation. Sosik et al. (2001) proposed that high surface ratios of $b_p : a_p$ in the summer could not be explained solely on the basis of phytoplankton and were more likely caused by weakly absorbing particles such as heterotrophic prokaryotes. Heterotrophic prokaryotes did have the highest ratios of $b : a$ of all particle groups with a mean value of ~33 for both seasons compared to values of ~14 for nonphytoplankton, ~12 for *Synechococcus*, and ~8 for eukaryotic phytoplankton. Their contribution to b was low, however, compared to nonphytoplankton (Fig. 5C, D).

The main contributors to b_b were detritus and minerals in the summer and minerals alone in the spring (Fig. 6A, B). Nonphytoplankton contributed ≥50% to total b_b in both seasons. In summer surface waters, mean contributions to b_b were 33% by minerals, 31% by detritus, 25% by water, 7% by heterotrophic prokaryotes, and 2% each by eukaryotic phytoplankton and *Synechococcus*. In spring surface waters, mean contributions to b_b were 52% by minerals, 32% by water, 13% by heterotrophic prokaryotes, 3% by eukaryotic phytoplankton, and <1% by *Synechococcus* and detritus. Submicron mineral particles contributed ~72% of backscattering by minerals ($b_{b,min}$) in both seasons, and submicron detritus contributed ~85% and 31% of backscattering by detritus ($b_{b,det}$) in summer and spring surface waters, respectively. In agreement with our findings, it has previously been proposed that b_b is mainly determined by submicron nonphytoplankton particles and that microbes are of little importance (Morel and Ahn 1991; Stramski and Kiefer 1991; Stramski et al. 2001). In simulations of open ocean waters, Stramski et al. (2001) found that minerals can be the most important contributor to b_b. Although we found this to be the case in spring surface waters, detritus and minerals were both important contributors to b_b in the summer. Our results show that seasonal variability in the concentration of detrital particles needs to be considered in models of b_b for New England shelf waters and similar coastal regimes. Additionally, submicron detritus and mineral particles played an important role in determining variability in b_b, which emphasizes the need for better measurements of this size fraction.

High backscattering ratios ($\tilde{b}_b = b_b/b$ or σ_{bb}/σ_b) were observed for minerals in both seasons and for detritus in the summer. Notably, minerals were more important contributors to b_b than to b in both seasons because of their high values of n (Table 3). In both seasons, the mean ratio of $\tilde{b}_b$ in surface waters was 3.6% for minerals, 1.1% for heterotrophic prokaryotes, 0.2% for *Synechococcus*, and 0.07% for eukaryotic phytoplankton (Fig. 6C, D). The high abundance of submicron detritus in summer surface waters resulted in high

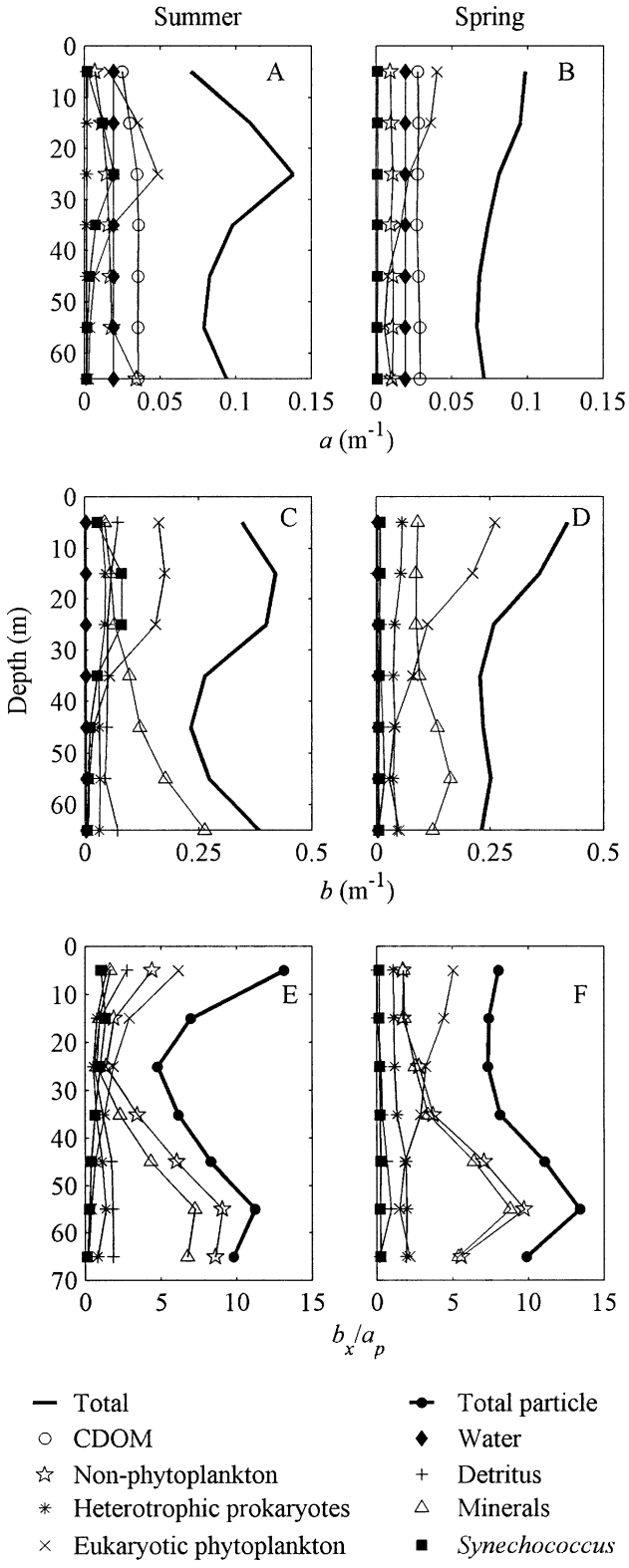

Fig. 5. Depth profiles of constituent contributions to (A, B) absorption, (C, D) scattering, and (E, F) the ratio of constituent scattering to particle absorption, $b_x : a_p$, at 488 nm in the summer and spring. In the term $b_x : a_p$, x denotes the particle group and ''total particle'' is the sum of all particle groups. Nonphytoplankton (detritus + minerals) has been plotted to emphasize the similarity with depth of the ratio for total particles and nonphytoplankton. Note that a subset of the legend symbols are used in each plot.

$\tilde{b}_b$ for detritus (2.3%) and $\tilde{b}_b$ for all particles that was higher in the summer (1.3%) than in the spring (0.9%). Considering the particle assemblage as a whole, our range of values for $\tilde{b}_b$ of 0.9–2.1% is within the range of previous measurements of 0.3–4.4% (Petzold 1972; Twardowski et al. 2001).

Particle group contributions to IOPs exhibited variability with depth, reflecting the degree of water column stratification in each season (Figs. 5A–D and 6A, B). For all microbial groups (eukaryotic phytoplankton, heterotrophic prokaryotes, and *Synechococcus*), profiles of *a* and *b* exhibited subsurface maxima in the summer at ~25 m and surface maxima in the spring. The contributions to IOPs of microbial groups decreased below 20–30 m in both seasons, mainly due to a decrease in microbial concentrations with depth (Table 2). The contribution of eukaryotic phytoplankton to *b* in the top 30 m of the water column was about sevenfold and fourfold higher than below in the summer and spring, respectively. Similarly, the concentrations of eukaryotic phytoplankton changed by about eightfold and threefold in the summer and spring, respectively. The larger vertical change in the concentration and contribution to IOPs of eukaryotic phytoplankton in the summer was associated with a higher degree of water column stratification. In contrast to microbes, the contribution to IOPs of minerals generally increased below 20–30 m in both seasons, and the contribution of detritus to IOPs was relatively constant with depth. The increased contribution of minerals with depth was most evident in the summer and was caused by mineral particles that were larger in size and had higher *n* (Table 2), indicating the effects of sediment resuspension as previously described (Boss et al. 2001).

An alternative to determining distributions of particle optical cross-sections with the FCM–Mie method would be to use particle concentrations determined from flow cytometry in conjunction with average optical cross-sections of selected species, such as those from the laboratory culture study of Stramski et al. (2001). For the different groups of natural particles we observed, the ranges of optical cross-sections encompassed the corresponding values determined by Stramski et al., but in some cases the mean values were quite different; for example, Stramski et al.'s values for heterotrophic prokaryotes and *Synechococcus* differed from our estimates by factors of 0.3 to 1.6. These differences could reflect strain-specific differences between the organisms tested in the laboratory and those present at our site or they could be due to effects of different growth conditions in the laboratory compared to the natural system.

Within-group variability in IOPs—We performed sensitivity analyses to evaluate which particle properties exhibited variability that is important for estimating IOPs (*see Methods* for details). If the observed variability in a particular property (e.g., *D*, *n*, or *n′* for a particle group) does not have a large effect on an IOP, then IOP values estimated with the mean property (i.e., the limited model) will be well correlated with and have similar amplitude to full-model IOP values, which are calculated with the observed property variability (i.e., values will fall tightly along the 1 : 1 lines in Fig. 7). If the observed mean for a property is not adequate for estimating IOP values, then there will be a bias or high

Table 3. Mean particle optical cross-sections for absorption, σ_a (m^2), scattering, σ_b (m^2), and backscattering, σ_{bb} (m^2) at 488 nm for each particle group. As in Table 2, mean properties were computed for both seasons in the three depth ranges of 0–20 m, 20–40 m, and 40–65 m.

		Summer			Spring		
Particle type	Property	0–20 m	20–40 m	40–65 m	0–20 m	20–40 m	40–65 m
Eukaryotic phytoplankton	σ_a	1.07×10^{-12}	2.85×10^{-12}	2.85×10^{-12}	1.44×10^{-12}	1.67×10^{-12}	1.32×10^{-12}
	σ_b	9.01×10^{-12}	9.25×10^{-12}	8.14×10^{-12}	1.05×10^{-11}	0.93×10^{-11}	0.67×10^{-11}
	σ_{bb}	6.58×10^{-15}	7.20×10^{-15}	7.63×10^{-15}	5.53×10^{-15}	5.40×10^{-15}	5.67×10^{-15}
Synechococcus	σ_a	0.59×10^{-13}	2.24×10^{-13}	3.19×10^{-13}	1.17×10^{-13}	1.53×10^{-13}	1.86×10^{-13}
	σ_b	0.67×10^{-12}	0.89×10^{-12}	1.11×10^{-12}	1.16×10^{-12}	1.16×10^{-12}	1.08×10^{-12}
	σ_{bb}	1.76×10^{-15}	1.91×10^{-15}	2.62×10^{-15}	1.87×10^{-15}	1.93×10^{-15}	1.84×10^{-15}
Heterotrophic prokaryotes	σ_a	1.14×10^{-15}	1.09×10^{-15}	1.11×10^{-15}	1.15×10^{-15}	1.09×10^{-15}	1.08×10^{-15}
	σ_b	3.82×10^{-14}	3.33×10^{-14}	3.39×10^{-14}	3.83×10^{-14}	3.49×10^{-14}	3.21×10^{-14}
	σ_{bb}	4.27×10^{-16}	3.79×10^{-16}	3.79×10^{-16}	4.45×10^{-16}	4.18×10^{-16}	3.77×10^{-16}
Detritus	σ_a	0.98×10^{-15}	1.64×10^{-15}	2.74×10^{-15}	6.09×10^{-14}	8.30×10^{-14}	0.57×10^{-14}
	σ_b	1.22×10^{-15}	1.56×10^{-15}	2.36×10^{-15}	4.21×10^{-14}	6.26×10^{-14}	0.52×10^{-14}
	σ_{bb}	2.17×10^{-17}	2.50×10^{-17}	3.34×10^{-17}	3.12×10^{-16}	4.39×10^{-16}	0.56×10^{-16}
Minerals	σ_a	0.70×10^{-15}	0.73×10^{-15}	3.66×10^{-15}	0.98×10^{-15}	0.95×10^{-15}	2.44×10^{-15}
	σ_b	4.06×10^{-15}	3.95×10^{-15}	8.74×10^{-15}	0.95×10^{-14}	0.70×10^{-14}	1.27×10^{-14}
	σ_{bb}	1.82×10^{-16}	1.89×10^{-16}	3.55×10^{-16}	2.45×10^{-16}	2.47×10^{-16}	3.81×10^{-16}

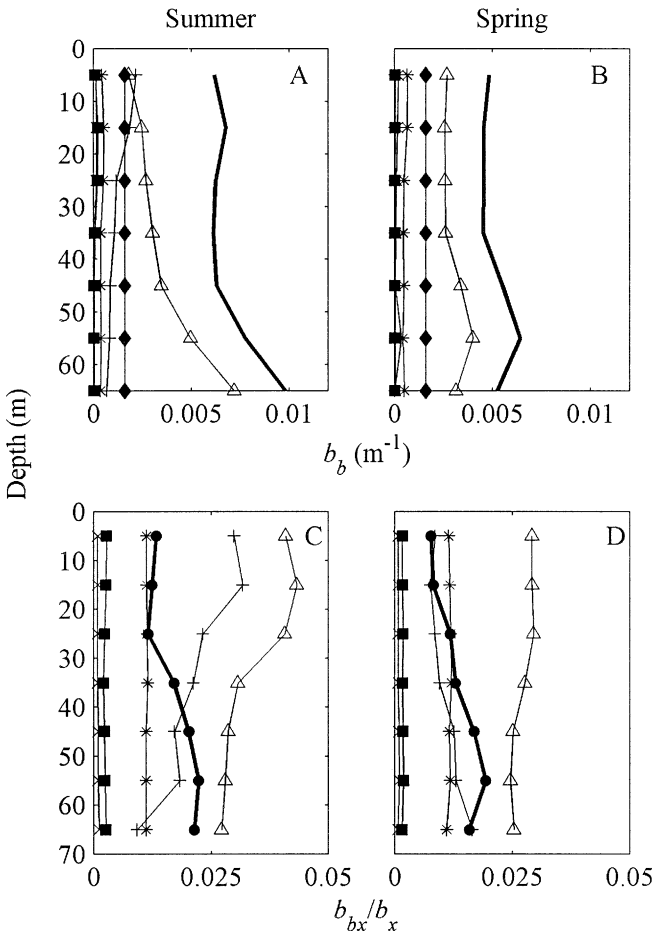

Fig. 6. Depth profiles of constituent contributions to (A, B) backscattering and (C, D) the ratio of constituent backscattering to constituent scattering, $b_{bx}:b_x$, at 488 nm in the summer and spring. In the term $b_{bx}:b_x$, x denotes the particle group and ''total particle'' is the sum of all particle groups. The legend is the same as in Fig. 5.

variance (or both) between the different IOP estimates. For eukaryotic phytoplankton in the spring, the correlations between limited-model and full-model results indicated that holding concentration constant had the largest effect on determining a_{euk} and b_{euk}, diameter had some effect, and n and n' had little effect (Fig. 7A–H). Concentration was the major determinant of variability in IOPs for eukaryotic phytoplankton, *Synechococcus*, and heterotrophic prokaryotes. Diameter and n' had secondary effects in determining IOP variability in eukaryotic phytoplankton and *Synechococcus*, and n never had a major effect on the determination of IOPs for microbes. For modeling of spatial and temporal variability in IOPs, this analysis shows that concentration is the most important property to resolve for microbes, given mean values of D, n, and n'. In contrast, between-group variability (e.g., eukaryotic phytoplankton vs. minerals) is highly dependent on n, n', and D, as well as concentration. For example, when the bulk n in spring surface waters ($\sim$1.13) was assumed to apply for eukaryotic phytoplankton, $b_{b,euk}$ was on average 11$\times$ higher than expected values.

We analyzed the sensitivity of nonphytoplankton optical contributions to changes in the extrapolation of the submicron size distribution. As discussed in the Methods, Junge size distributions were adequately described by two slopes, above and below 3.5 μm, but the choice of the cutoff size is somewhat arbitrary so it is important to know how much our estimates of total nonphytoplankton b and b_b depend on the choice. We compared other scenarios to the 3.5 μm case, including extrapolations using points in the range of 2–5 μm (outside of this range two slopes poorly described the size distributions). We found minimal differences ($\leq$5%) in contributions to b and b_b when cutoffs ranging from 2 to 5 μm were compared with the 3.5 μm case for detritus and minerals in the spring and minerals in the summer. The contribution of detritus in summer surface waters, however, was affected by changes in the extrapolated submicron size distribution. We found the most extreme difference with a cut-

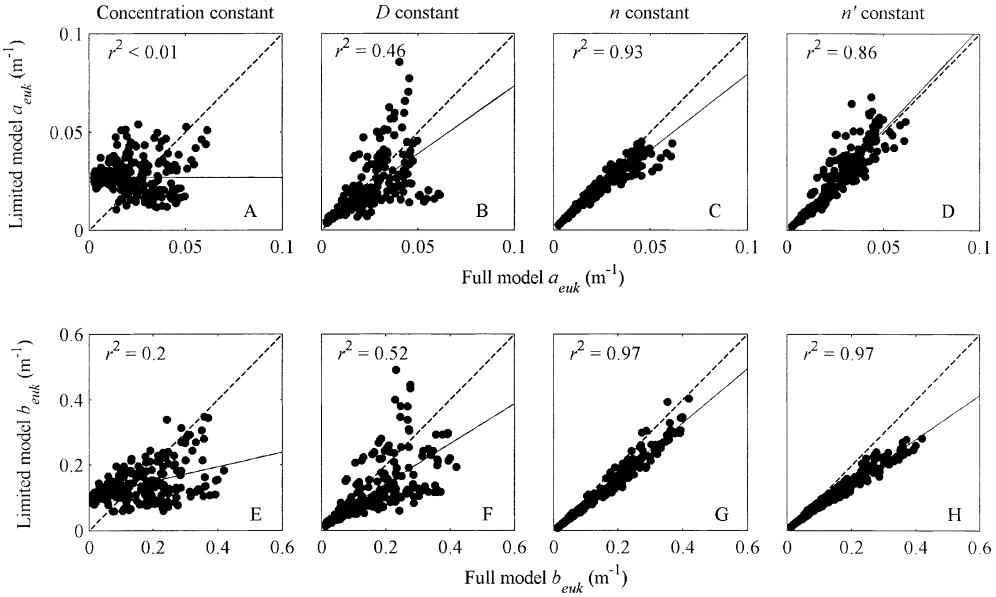

Fig. 7. Sensitivity of (A–D) eukaryotic phytoplankton absorption, a_{euk}, and (E–H) scattering, b_{euk}, at 488 nm in the spring to variability in concentration, *D*, *n* (488 nm), and n' (488 nm). Full-model values of a_{euk} and b_{euk} were calculated using Mie theory and observed values for all four properties (concentration, *D*, *n*, and n'). Limited-model values of a_{euk} and b_{euk} were calculated using Mie theory and observed values for three of the properties; the remaining property was held constant at its mean observed value. The effects of variability on b_b were not included because eukaryotic phytoplankton were not important contributors to b_b. The 1 : 1 line (dashed line) and least-squares regression between full- and limited-model values (solid line) are shown on each plot.

off of 2 μm (Fig. 3A), in which case detritus contributed 22% to *b* (compared to 25% with 3.5 μm) and 18% to b_b (compared to 31% with 3.5 μm). Even these differences, however, do not change our overall conclusion that both detrital particles and minerals were important particulate contributors to b_b in summer surface waters, whereas minerals alone were most important to b_b in the spring.

For nonphytoplankton, we also evaluated the sensitivity of b_b to changes in concentration and the shape of the size distribution. In both seasons, particle concentration had the largest effect on variability in $b_{b,det}$ and $b_{b,min}$, although changes in the shape of the size distribution were also important (Fig. 8A–D; spring data not shown). For modeling of variability in IOPs, our results show that variability both in concentration and in the shape of the size distribution must be well characterized for detritus and minerals in the ocean. To further emphasize this point, for mineral size distributions in the summer, we estimated b_b values from our measured nonphytoplankton particle concentrations but assuming a typical Junge slope observed in the ocean of 4 (Mobley 1994). This resulted in $b_{b,min}$ values that were on average underestimated by 42%, and emphasizes that lack of knowledge of the shape of the particle size distributions can lead to large uncertainties.

The combined use of Mie theory and individual particle measurements has provided an improved understanding of the roles of different particle groups in determining IOPs in New England continental shelf waters. Eukaryotic phytoplankton were the most important particle contributors to both *a* and *b* in surface waters during both seasons. Variability in cell properties, including concentration, *D*, *n*, and n', and contributions to IOPs were associated with seasonal differences in light and nutrient availability. Although *Synechococcus* and heterotrophic prokaryotes were numerically abundant and are known to play important roles in microbial ecosystems, they were not important determinants of IOPs. Nonphytoplankton were the most important source of b_b at all depths and of both *a* and *b* below the mixed layer.

Particle budgets like the one in this study, which include both microbes and nonphytoplankton, have previously only been attempted in simulations of open ocean waters. Stramski et al. (2001) used a combination of optical and ancillary measurements on many plankton cultures with Mie theory and example values of expected concentrations for various particle groups to calculate their contributions to IOPs in oligotrophic waters. Their example simulations showed that this approach could be a powerful research tool permitting analysis of IOPs. However, those simulations of a limited number of particle composition cases could not provide generalized conclusions about the roles played by various particles in the optics of diverse marine environments. We have built on Stramski et al.'s approach by using detailed individual particle measurements for natural assemblages to account for seasonal and vertical variability in measured bulk optical properties.

An improved understanding of natural variability in b_b is an important contribution of the present study. In a recent review, Morel and Maritorena (2001) concluded that interpretation of ocean color remains problematic because of insufficient knowledge of the phase function and backscatter-

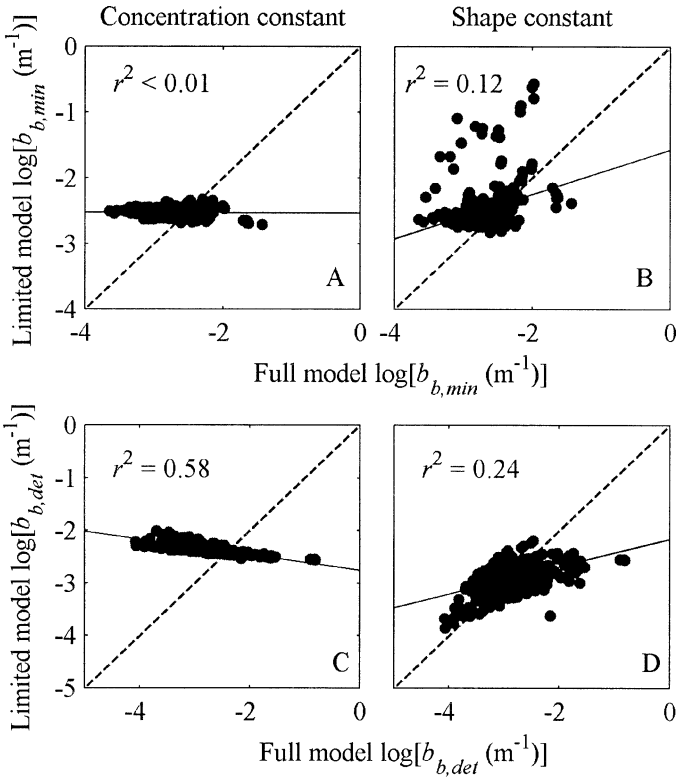

Fig. 8. Sensitivity of (A, B) backscattering by minerals, $b_{b,min}$, and (C, D) backscattering by detritus, $b_{b,det}$, at 488 nm in the summer to variability in concentration and the shape of the size distribution. Full-model values of $b_{b,min}$ and $b_{b,det}$ were calculated using Mie theory and observed concentrations and size distribution shapes; n and n' were held constant at their mean observed values. Limited-model values of $b_{b,min}$ and $b_{b,det}$ were calculated using Mie theory and either observed size distribution shapes with the mean value of particle concentration (A, C) or the mean size distribution shape and observed values of particle concentration (B, D). The 1 : 1 line (dashed line) and least-squares regression between full- and limited-model values (solid line) are shown on each plot.

ing efficiency of oceanic particles. In New England shelf waters, we found that b_b was determined by the properties of nonphytoplankton particles, including their size distribution and organic/inorganic content (indicated by n). Minerals were important in determining b_b at all depths in both seasons. The important contribution of detritus in summer surface waters was a result of their high concentrations at submicron sizes. Our results suggest a seasonal model for the contribution of nonphytoplankton in which minerals are important in determining b_b throughout the year, and detritus is important only in the summer when concentration increases because of the effects of biological productivity. In the winter, it seems likely that detritus will be a less important contributor to b_b in surface waters, because of export to deep waters during increased mixing (Carlson et al. 1994; Williams 1995). It is clear that measurements of the size distributions and optical properties of nonphytoplankton, including separation of detrital and mineral components, are necessary to explain changes in IOPs, especially b_b, in the ocean.

Further particle studies are necessary to determine how broadly these findings apply, and for some water types new approaches will be needed. These include waters such as those with bloom conditions characterized by high concentrations of large particles (>10 μm) for which Mie theory cannot be inverted unambiguously. In general, there is a need for new particle approaches that overcome the limitations of Mie theory, are spectrally resolved, and incorporate measurements of the submicron fraction.

References

AAS, E. 1996. Refractive index of phytoplankton derived from its metabolite composition. J. Plankton Res. **18:** 2223–2249.

ACKLESON, S. G., AND R. W. SPINRAD. 1988. Size and refractive index of individual marine particulates: A flow cytometric approach. Appl. Opt. **27:** 1270–1277.

AGRAWAL, Y. C., AND P. TRAYKOVSKI. 2001. Particles in the bottom boundary layer: Concentration and size dynamics through events. J. Geophys. Res. **106:** 9533–9542.

AZAM, F., T. FENCHEL, J. G. FIELD, J. S. GRAY, L. A. MEYER-REIL, AND F. THINGSTAD. 1983. The ecological role of water column microbes in the sea. Mar. Ecol. Prog. Ser. **10:** 257–263.

BOSS, E., AND OTHERS. 2001. Spectral particulate attenuation and particle size distribution in the bottom boundary layer of a continental shelf. J. Geophys. Res. **106:** 9509–9516.

CARLSON, C. A., H. W. DUCKLOW, AND A. F. MICHAELS. 1994. Annual flux of dissolved organic carbon in the northwestern Sargasso Sea. Nature **371:** 405–408.

CHO, B. C., AND F. AZAM. 1990. Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. Mar. Ecol. Prog. Ser. **63:** 253–259.

CLAUSTRE, H., AND OTHERS. 1999. Variability in particle attenuation and chlorophyll fluorescence in the tropical Pacific: Scales, patterns, and biogeochemical implications. J. Geophys. Res. **104:** 3401–3422.

CLEVELAND, J. S. 1995. Regional models for phytoplankton absorption as a function of chlorophyll a concentration. J. Geophys. Res. **100:** 13333–13344.

DURAND, M. D., AND R. J. OLSON. 1996. Contributions of phytoplankton light scattering and cell concentration changes to diel variations in beam attenuation in the equatorial Pacific from flow cytometric measurements of pico-, ultra- and nanoplankton. Deep-Sea Res. II **43:** 891–906.

———, AND ———. 1998. Diel patterns in optical properties of the chlorophyte *Nannochloris* sp.: Relating individual-cell to bulk measurements. Limnol. Oceanogr. **43:** 1107–1118.

FALKOWSKI, P. G., Z. DUBINSKY, AND K. WYMAN. 1985. Growth-irradiance relationships in phytoplankton. Limnol. Oceanogr. **30:** 311–321.

FUHRMAN, J. A. 1981. Influence of method on the apparent size distribution of bacterioplankton cells: Epifluorescence microscopy compared to scanning electron microscopy. Mar. Ecol. Prog. Ser. **5:** 103–106.

GORDON, H. R., AND A. MOREL. 1983. Remote assessment of ocean color for interpretation of satellite visible imagery. A review, p. 1–114. *In* R. T. Barber, C. N. K. Mooers, M. J. Bowman, and B. Zeizschel [eds.], Lecture notes on coastal and estuarine studies. Springer.

GREEN, R. E. 2002. Scale closure in upper ocean optical properties: From single particles to ocean color. Ph.D. thesis, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution.

———, H. M. SOSIK, R. J. OLSON, AND M. D. DURAND. 2003. Flow cytometric determination of size and complex refractive

index for marine particles: Comparison with bulk measurements. Appl. Opt. **42:** 526–541.

GRIM, R. E. 1953. Clay mineralogy. McGraw-Hill.

HARRIS, J. E. 1977. Characterization of suspended matter in the Gulf of Mexico—II, particle size analysis of suspended matter from deep water. Deep-Sea Res. **24:** 1055–1061.

HERNDL, G. J., G. MÜLLER-NIKLAS, AND J. FRICK. 1993. Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean. Nature **361:** 717–719.

HILL, P. S., G. VOULGARIS, AND J. H. TROWBRIDGE. 2001. Controls on floc size in a continental shelf bottom boundary layer. J. Geophys. Res. **106:** 9543–9549.

ITURRIAGA, R., AND D. A. SIEGEL. 1989. Microphotometric characterization of phytoplankton and detrital absorption properties in the Sargasso Sea. Limnol. Oceanogr. **34:** 1706–1726.

KIRK, J. T. O. 1983. Light and photosynthesis in aquatic ecosystems. Cambridge Univ. Press.

KISHINO, M., N. TAKAHASHI, N. OKAMI, AND S. ICHIMURA. 1985. Estimation of the spectral absorption coefficients of phytoplankton in the sea. Bull. Mar. Sci. **37:** 634–642.

KOIKE, I., S. HARA, K. TERAUCHI, AND K. KOGURE. 1990. Role of sub-micrometre particles in the ocean. Nature **345:** 242–244.

KUIPERS, B., G. J. VAN NOORT, J. VOSJAN, AND G. J. HERNDL. 2000. Diel periodicity of bacterioplankton in the euphotic zone of the subtropical Atlantic Ocean. Mar. Ecol. Prog. Ser. **201:** 13–25.

LEE, S., AND J. A. FUHRMAN. 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl. Environ. Microbiol. **53:** 1298–1303.

LEWIS, M. R., AND J. J. CULLEN. 1991. From cells to the ocean: Satellite ocean color, p. 325–337. *In* S. Demers [ed.], Particle analysis in oceanography. Springer.

LIDE, D. R. (ED.). 1997. Physical and optical properties of minerals, p. 4130–4136. *In* CRC handbook of chemistry and physics. CRC Press.

LOISEL, H., AND A. MOREL. 1998. Light scattering and chlorophyll concentration in case 1 waters: A reexamination. Limnol. Oceanogr. **43:** 847–858.

MARIE, D., F. PARTENSKY, S. JACQUET, AND D. VAULOT. 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl. Environ. Microbiol. **63:** 186–193.

MCCAVE, I. N. 1983. Particulate size spectra, behavior, and origin of nepheloid layers over the Nova Scotian continental rise. J. Geophys. Res. **88:** 7647–7666.

MITCHELL, B. G., AND D. A. KIEFER. 1988. Chlorophyll *a* specific absorption and fluorescence excitation spectra for light-limited phytoplankton. Deep-Sea Res. **35:** 639–663.

MOBLEY, C. D. 1994. Light and water; radiative transfer in natural waters. Academic.

———, AND D. STRAMSKI. 1997. Effects of microbial particles on oceanic optics: Methodology for radiative transfer modeling and example simulations. Limnol. Oceanogr. **42:** 550–560.

MOREL, A. 1974. Optical properties of pure water and pure seawater, p. 1–24. *In* N. G. Jerlov and E. S. Nielsen [eds.], Optical aspects of oceanography. Academic.

———, AND Y. H. AHN. 1990. Optical efficiency factors of free-living marine bacteria: Influence of bacterioplankton upon the optical properties and particulate organic carbon in oceanic waters. J. Mar. Res. **48:** 145–175.

———, AND ———. 1991. Optics of heterotrophic nanoflagellates and ciliates: A tentative assessment of their scattering role in oceanic waters compared to those of bacterial and algal cells. J. Mar. Res. **49:** 177–202.

———, AND A. BRICAUD. 1986. Inherent optical properties of algal cells including picoplankton: Theoretical and experimental results, p. 521–559. *In* T. Platt and W. K. W. Li [eds.], Photosynthetic picoplankton. Can. Bull. Fish. Aquat. Sci. 214.

———, AND S. MARITORENA. 2001. Bio-optical properties of oceanic waters: A reappraisal. J. Geophys. Res. **106:** 7163–7180.

———, ———, ———, and S. MARITORENA. 2001. Bio-optical properties of oceanic waters: A reappraisal. J. Geophys. Res. . **106:** 7163–7180.

NAGATA, T., AND D. L. KIRCHMAN. 1996. Roles of submicron particles and colloids in microbial food webs and biogeochemical cycles within marine environments. Adv. Microb. Ecol.. **15:** 81–103.

NOBLE, R. T., AND J. A. FUHRMAN. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. **14:** 113–118.

OGAWA, H., Y. AMAGAI, I. KOIKE, K. KAISER, AND R. BENNER. 2001. Production of refractory dissolved organic matter by bacteria. Science **292:** 917–920.

OLSON, R. J., S. W. CHISHOLM, E. R. ZETTLER, M. A. ALTABET, AND J. A. DUSENBERRY. 1990. Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep-Sea Res. **37:** 1033–1051.

———, E. R. ZETTLER, AND O. K. ANDERSON. 1989. Discrimination of eukaryotic phytoplankton cell types from light scatter and autofluorescence properties measured by flow cytometry. Cytometry **10:** 636–643.

———, ———, AND M. D. DURAND. 1993. Phytoplankton analysis using flow cytometry, p. 175–186. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Aquatic microbial ecology. Lewis.

PEGAU, W. S., AND OTHERS. 1995. A comparison of methods for the measurement of the absorption coefficient in natural waters. J. Geophys. Res. **100:** 13201–13220.

PERRY, M. J., AND S. M. PORTER. 1989. Determination of the cross-section absorption coefficient of individual phytoplankton cells by analytical flow cytometry. Limnol. Oceanogr. **34:** 1727–1738.

PETZOLD, T. J. 1972. Volume scattering functions for selected ocean waters. Univ. Calif. Scripps Inst. Oceanogr. Ref. 72-28.

POPE, R. M., AND E. S. FRY. 1997. Absorption spectrum (380–700 nm) of pure water. II Integrating cavity measurements. Appl. Opt. **36:** 8710–8723.

RISOVIĆ, D. 1993. Two-component model of sea particle size distribution. Deep-Sea Res. I **40:** 1459–1473.

ROESLER, C. S., M. J. PERRY, AND K. L. CARDER. 1989. Modeling in situ phytoplankton absorption from total absorption spectra in productive inland marine waters. Limnol. Oceanogr. **34:** 1510–1523.

SAKSHAUG, E., S. DEMERS, AND C. M. YENTSCH. 1987. *Thalassiosira oceanica* and *T. pseudonana*: Two different photoadaptational responses. Mar. Ecol. Prog. Ser. **41:** 275–282.

SHIBATA, A., K. KOGURE, I. KOIKE, AND K. OHWADA. 1997. Formation of submicron colloidal particles from marine bacteria by viral infection. Mar. Ecol. Prog. Ser. **155:** 303–307.

SIERACKI, M., AND C. VILES. 1992. Distributions and fluorochrome-staining properties of sub-micrometer particles and bacteria in the North Atlantic. Deep-Sea Res. **39:** 1919–1929.

SOSIK, H. M., S. W. CHISHOLM, AND R. J. OLSON. 1989. Chlorophyll fluorescence from single cells: Interpretation of flow cytometric signals. Limnol. Oceanogr. **34:** 1749–1761.

———, R. E. GREEN, W. S. PEGAU, AND C. S. ROESLER. 2001. Temporal and vertical variability in optical properties of New England shelf waters during late summer and spring. J. Geophys. Res. **106:** 9455–9472.

———, AND B. G. MITCHELL. 1991. Absorption, fluorescence and quantum yield for growth in nitrogen limited *Dunaliella tertiolecta.* Limnol. Oceanogr. **36:** 910–921.

———, AND ———. 1995. Light absorption by phytoplankton, photosynthetic pigments and detritus in the California Current system. Deep-Sea Res. I. **42:** 1717–1748.

STRAMSKI, D., A. BRICAUD, AND A. MOREL. 2001. Modeling the inherent optical properties of the ocean based on the detailed composition of the planktonic community. Appl. Opt. **40:** 2929–2945.

———, AND D. A. KIEFER. 1990. Optical properties of marine bacteria, p. 250–268. *In* M. A. Blizard [ed.], Ocean optics X. Society of Photo-Optical Instrumentation Engineers.

———, AND ———. 1991. Light scattering by microorganisms in the open ocean. Prog. Oceanogr. **28:** 343–383.

———, AND C. D. MOBLEY. 1997. Effects of microbial particles on oceanic optics: A database of single-particle optical properties. Limnol. Oceanogr. **42:** 538–549.

———, AND A. MOREL. 1990. Optical properties of photosynthetic picoplankton in different physiological states as affected by growth irradiance. Deep-Sea Res. **37:** 245–266.

TWARDOWSKI, M. S., E. BOSS, J. B. MACDONALD, W. S. PEGAU, A. H. BARNARD, AND J. R. V. ZANEVELD. 2001. A model for estimating bulk refractive index from the optical backscattering ratio and the implications for understanding particle composition in case I and case II waters. J. Geophys. Res. **106:** 14129–14142.

VAULOT, D., C. COURTIES, AND F. PARTENSKY. 1989. A simple method to preserve oceanic phytoplankton for flow cytometric analyses. Cytometry **10:** 629–635.

WATERBURY, J. B., S. W. WATSON, F. W. VALOIS, AND D. G. FRANKS. 1986. Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*, p. 71–120. *In* T. Platt and W. K. W. Li [eds.], Photosynthetic picoplankton, Can. Bull. Fish. Aquat. Sci. 214.

WILLIAMS, P. J. 1995. Evidence for the seasonal accumulation of carbon-rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net C/N assimilation ratios. Mar. Chem. **51:** 17–29.

YAMASAKI, A., H. FUKUDA, R. FUKUDA, T. MIYAJIMA, T. NAGATA, H. OGAWA, AND I. KOIKE. 1998. Submicrometer particles in northwest Pacific coastal environments: Abundance, size distribution, and biological origins. Limnol. Oceanogr. **43:** 536–542.

ZANEVELD, J. R. V., D. R. ROACH, AND H. PAK. 1974. The determination of the index of refraction distribution of oceanic particulates. J. Geophys. Res. **79:** 4091–4095.

ZUBKOV, M. V., B. M. FUCHS, P. H. BURKILL, AND R. AMANN. 2001. Comparison of cellular and biomass specific activities of dominant bacterioplankton groups in stratified waters of the Celtic Sea. Appl. Environ. Microbiol. **67:** 5210–5218.

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